Genetic Determinants of Diabetic Neuropathy

GENETIC DETERMINANTS OF DIABETIC NEUROPATHY

by

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I have reviewed this thesis. It represents work done by the author under my guidance/supervision.

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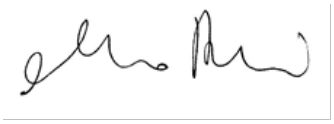

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Overview of the thesis papers

Diabetic neuropathy (DN) is a heterogeneous condition that manifests itself with diverse symptoms, variable severity, and different clinical course. While genetic factors have been postulated to explain such heterogeneity, data on genetic determinants of DN are scant in the literature. In light of this, I decided to conduct genome-wide studies (GWAS) on the two major types of DN – diabetic peripheral neuropathy (DPN) and cardiac autonomic neuropathy (CAN). The results of these studies are reported in the two papers below.

The first paper reports the identification of a previously unrecognized genetic locus on chromosome 2q24 having a strong, genome-wide significant effect on the risk of DPN among patients with type 2 diabetes from the ACCORD study. Unlike previously reported associations with DPN, our finding was replicated in an independent cohort (BARI 2D), making the 2q24 locus the only validated DPN locus identified to date. As indicated by the analysis of RNA-seq data from the Gene Expression Database, this genetic effect appears to be related to differences in the abundance of a voltage-gated sodium channel (NaV1.2) in peripheral nerves.

The second paper reports the first GWAS of diabetic CAN conducted to date, through which I have identified a genetic locus on chromosome 1p36 having a powerful, genome-wide significant effect on incident CAN. This genetic effect was independent of the interventions tested in ACCORD and was not mediated by an effect on known clinical predictors of CAN. This locus points to nearby genes that may mediate this genetic effect, including *KCNAB2* - a voltage-gated potassium channel associated with fatal arrhythmia, *RING 207* - a RING finger protein regulating heart action potential duration, and *CAMTA1*- a calmodulin binding transcription factor affecting cardiac embryonal development.

These two findings have implications for the care of diabetic patients as well as for research on diabetic neuropathy. From a clinical standpoint, determining the genotype at these loci may help clinicians identify patients at higher risk of DPN and CAN who can be the focus of especially vigorous prevention programs. From a research standpoint, studying the molecular mechanisms underlying the two loci may improve our understanding of the link between diabetes and neuropathy biology, and foster the development of new drugs to prevent these two complications.

Given the current epidemic of diabetes, the socio-economic burden caused by DN, the need for new approaches to tackle this problem, and the robustness of our data, we trust that these papers will be of great interest to the diabetes research community. We submitted the first paper, "A genetic locus on chromosome 2q24 predicting peripheral neuropathy risk in type 2 diabetes: results from the ACCORD and BARI 2D studies" to *DIABETES* – the official journal of the American Diabetes Association. The paper was well received by the editors and the reviewers, and a revised version addressing their comments has already been submitted. Submission of the second paper, "A genetic locus on chromosome 1p36 associated with cardiovascular autonomic neuropathy in type 2 diabetes" awaits replication of these findings in an independent study, as it is customary for genetic analyses. To this end, I have established a collaboration with a Danish group having access to a cohort (ADDITION-Denmark), for which rich CAN data are available. Genotyping of the top GWAS signal is underway in this cohort.

Paper 1

A genetic locus on chromosome 2q24 predicting peripheral neuropathy risk in type 2 diabetes: results from the ACCORD and BARI 2D studies.

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Abstract

Genetic factors have been postulated to be involved in the etiology of diabetic peripheral neuropathy (DPN), but their identity remains mostly unknown. The aim of this study was to conduct a systematic search for genetic variants influencing DPN risk using two well-characterized cohorts. A genome-wide association study (GWAS) testing 6.8 M SNPs was conducted among participants of the ACCORD clinical trial. Included were 4,384 White cases with type 2 diabetes (T2D) and prevalent or incident DPN (defined as a Michigan Neuropathy Screening Instrument [MNSI] clinical exam score >2.0) and 784 White controls with T2D and no evidence of DPN at baseline or during follow-up. Replication of significant loci was sought among White subjects with T2D (791 DPN-positive cases and 158 DPN-negative controls) from the BARI 2D trial. Association between significant variants and gene expression in peripheral nerves was evaluated in the GTEx database. A cluster of 28 SNPs on chromosome 2q24 reached GWAS significance ($P<5x10^{-8}$) in ACCORD. The minor allele of the lead SNP (rs13417783, minor allele frequency=0.14) decreased DPN odds by 36% (OR 0.64, 95% CI 0.55-0.74, $P=1.9x10^{-9}$). This effect was not influenced by ACCORD treatment assignments (P for interaction=0.6) nor was mediated by an association with known DPN risk factors. This locus was successfully validated in BARI 2D (OR 0.57, 95% CI 0.42-0.80, $P=9x10^{-4}$, summary P value=$7.9x10^{-12}$). In GTEx, the minor, protective allele at this locus was associated with higher tibial nerve expression of an adjacent gene (*SCN2A*) coding for human voltage-gated sodium channel NaV1.2 ($P=9x10^{-4}$). To conclude, we have identified and successfully validated a previously unknown locus with a powerful protective effect on the development of DPN in T2D. These results may provide novel insights into DPN pathogenesis and point to a potential target for novel interventions.

Diabetic peripheral neuropathy (DPN) - the most common cause of neuropathy worldwide – is a frequent complication of diabetes that significantly contributes to the increased morbidity and mortality associated with this disease(1). Up to one-fourth of the annual US expenditure on diabetes is due to DPN, a large proportion of which is secondary to type 2 diabetes (2; 3). The most common presentation of DPN is a distal symmetric polyneuropathy due to small and large fiber dysfunction leading to loss of sensory, proprioception, temperature, and pain discrimination, along with symptoms of numbness, tingling, and burning, shooting pain (1; 4). Signs and symptoms begin distally and spread proximally.

Known risk factors for DPN include duration and severity of hyperglycemia, age, dyslipidemia, hypertension, obesity, height, and smoking (1; 5-9). As these exposures fail to fully explain inter-individual differences in the risk of developing DPN, genetic factors have been postulated to be involved in the etiology of DPN (10), although formal heritability estimates such as those available for other diabetic complications, including cardiovascular autonomic neuropathy (h^2 up to 39%) (11-13), are not available for DPN. Knowledge of the genetic factors that modulate DPN risk may provide insights into the molecular pathways linking the diabetic milieu to nerve damage, which may in turn suggest new pharmacological targets for preventing or treating DPN. However, while many genetic studies have been carried out for other diabetic complications (14), data on the genetic determinants of DPN are scant. A few candidate gene studies have been performed, but these were small and not followed by replication attempts (10). A locus on chromosome 8p21.3 has been recently found to be associated with painful DPN in a genome-wide association study (GWAS), but this finding did not reach genome-wide significance and is still to be replicated (15).

Here we report the results of a GWAS for DPN conducted among participants with type 2 diabetes in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial. Our findings, which were replicated in the population of the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial, point to a locus on chromosome 2q24 as a powerful determinant of DPN risk that may act by affecting a nearby voltage-gated sodium channel, NaV1.2, expressed in peripheral nerves. This is the first GWAS of DPN yielding a genome-wide significant signal with independent replication and biological plausibility of the leading candidate gene in the associated region.

RESEARCH DESIGN AND METHODS

Study population

Discovery set: ACCORD study

The aim of the ACCORD clinical trial was to investigate whether cardiovascular event rates could be reduced by intensively targeting hyperglycemia to HbA1c < 6.0 % [42 mmol/mol], compared to a standard target of HbA1c between 7 – 7.9 % [53 - 63 mmol/mol]. For this purpose, 10,251 participants with type 2 diabetes and high cardiovascular risk were randomized in a 1:1 ratio to receive intensive or standard glycemic control therapy at 77 clinical sites across the U.S. and Canada (16) . The study also investigated the effect of intensive versus standard blood pressure control and fibrate versus placebo therapy on cardiovascular events through the ACCORD BP and Lipid sub-trials in a double 2 by 2 factorial design (16). Additionally, ACCORD had a rich follow-up of study participants and collected data on other diabetic complications, including DPN, both at baseline and during follow-up. Of the 10,251 ACCORD participants, 8,174 consented to genetic studies. To minimize confounding by race, our GWAS for discovery of genetic predictors of DPN was restricted to 5,360 whites. Among them, a total

of 5,168 participants had data on DPN collected at baseline and/or during follow-up by means the Michigan Neuropathy Screening Instrument (MNSI) clinical examination score.

Validation set: BARI 2D study

BARI 2D was a 2 by 2 factorial clinical trial enrolling 2,368 participants with both type 2 diabetes and established coronary artery disease (17) . For glycemic control, participants were randomly assigned to either insulin sensitization or insulin provision strategy to reach the same HbA1c target of less than 7.0 % [53 mmol/mol]. For coronary artery disease, participants were randomly assigned to undergo either coronary revascularization procedures (percutaneous coronary intervention [PCI] or coronary artery bypass graft surgery [CABG]) or medical therapy. The aim was to investigate the cardiovascular event reduction by both treatment strategies. As in ACCORD, other secondary outcomes were obtained in BARI 2D including DPN, which was evaluated with the same MNSI clinical exam score at baseline and follow-up (18). A total of 1,030 whites consented to genetic studies in BARI 2D; 949 had genetic data that passed internal QC and also had information about the presence of DPN at baseline and follow-up. Since participants were recruited in the two studies during the same time period (January-June 2001 and February 2003-October 2005 for ACCORD, and January 2001-March 2005 for BARI 2D) and both studies upheld participation in another clinical trial as an exclusion criterion (16; 19), the chances of a person being included in both studies were deemed to be negligible.

Outcome definition

Both the ACCORD and BARI 2D trials defined neuropathy as an MNSI clinical exam score > 2.0 (18; 20) – a criterion that has been extensively validated as being highly sensitive and specific for the diagnosis of DPN (21; 22). The MNSI clinical examination includes a focused examination of the feet to assess skin and structural abnormalities, along with assessment of

distal vibration perception with a 128 Hz tuning fork and ankle reflexes. For the purpose of the genetic association study, DPN cases were defined as participants having an MNSI > 2.0 at study entry and/or at any time during follow-up (4.9 and 5.3 years on average in ACCORD and BARI 2D, respectively), while DPN controls were defined as participants having an MNSI ≤ 2.0 at study entry and for the entire duration of follow-up.

DNA extraction and genotyping

Discovery set: ACCORD study

Detailed DNA extraction, genotyping, quality control (QC) methods and imputation in ACCORD can be found in the supplemental material of the paper in which the GWAS was first reported (23). In brief, genotyping was performed in two centers using different platforms and chips: 6,085 DNA samples (ACCORD UVA Set) were genotyped at the University of Virginia (UVA) on Illumina HumanOmniExpressExome-8 (v1.0) chips containing 951,117 SNPs; 8,174 samples (ACCORD UNC Set) were genotyped at the University of North Carolina, on Affymetrix Axiom Biobank1 chips containing 628,679 probes. The ACCORD UVA set included subjects who had given consent to genetic studies by any investigator; the ACCORD UNC included the subjects in the ACCORD UVA set plus those who had given consent only to studies by ACCORD investigators. After extensive QC of the individual genotyping sets, and harmonization of genotypes for those samples and SNPs that were in common between the two genotyping sets, data were organized in two non-overlapping datasets: ANYSET (n=5,971), including subjects from the ACCORD UVA set, genotyped at either UVA or UNC at a total of 1,263,585 individual SNPs, and ACCSET (n=2,113), including subjects from the ACCORD UNC set minus those in the ACCORD UVA set, genotyped only at UNC at a total of 572,192 SNPs. Imputation was conducted independently in the ANYSET and ACCSET datasets by means

of IMPUTEv2.3.1 (24) using the full 1000 Genomes Phase 3 (Oct 2014) panel as reference. After discarding imputed SNPs with an information content <0.3, the total (genotyped+imputed) SNPs were 25,017,489 in ANYSET and 25,012,865 in ACCSET.

Validation set: BARI 2D study

DNA was isolated and purified from whole blood using the Qiagen QIAamp DNA purification kit (Qiagen, Germantown, MD) as previously described (25). Genotyping was performed using the Infinium Multi-Ethnic Global Array (Illumina, CA, USA) as per manufacturer's instructions. After filtering out indels, SNPs with call rates < 0.99, and multiallelic sites, the samples were pre-phased with SHAPEIT and imputed towards the Phase 3 Cosmopolitan Reference panel of 1000 Human Genomes project using IMPUTE2 software. Imputed genotypes were then converted into dosages using FCGENE software. Imputation quality ranged from 85% to 100% for the 40 variants available for validation in BARI 2D (variants listed in Table 2 and Supplementary Table 6).

Data analysis

Descriptive statistics

Analyses were run in SAS v9.4 (Cary, NC). Normally distributed continuous variables were presented as mean (± standard deviation, SD) and analyzed by independent t-test for difference in means between groups. Non-normally distributed continuous variables were presented as median (inter-quartile range) values and analyzed by t-test after log transformation. Categorical variables were presented as counts (percentages) and analyzed by chi-square tests to examine differences among groups.

Genome wide association study

A GWAS for DPN was carried out in 5,168 White ACCORD participants. GWAS analyses were conducted separately in ACCSET (3,554 cases/830 controls) and ANYSET (600 cases/184 controls) using SAS v9.4 (Cary, NC) on the Harvard Medical School computing cluster, Orchestra. After filtration for minor allele frequency (MAF) ≥ 5%, 6,847,206 genotyped or imputed SNPs formed the GWAS panel in each dataset. For each common variant, minor allele dosage (ranging from 0 to 2) was inferred from the genotypes or computed from the imputed posterior probabilities. The association between minor allele dosage of each variant and DPN was tested by logistic regression, assuming an additive genetic model, and adjusting for assignment to interventions, seven clinical center networks, and top three principal components (PC1-PC3). After employing genomic control corrections (λ=1.014 and 1.032 for ANYSET and ACCSET respectively), the GWAS results from the ACCSET and ANYSET sets were meta-analyzed in METAL (Abecasis Lab, University of Michigan, MI) (26), through a fixed-effects inverse-variance approach. Genome-wide significance was defined as P value < 5×10^{-8}, suggestive (notable) significance as P<$1x10^{-6}$. Manhattan and quantile-quantile (QQ) plots were generated to illustrate the GWAS results.

Further analyses of SNPs reaching genome-wide significance were carried out by: 1. Testing whether the SNP had similar effects on prevalent DPN (MNSI > 2.0 at study entry) and incident DPN (MNSI ≤2.0 at baseline and MNSI > 2.0 during follow-up); 2. Testing for interaction with baseline characteristics or, for incident DPN, with trial treatments (intensive vs standard glycemic control, intensive vs standard blood pressure control, fenofibrate + statin vs placebo +statin for hyperlipidemia control) by adding appropriate interaction-terms; 3. Evaluating the association between SNP and baseline characteristics, individual MNSI components, and other diabetic complications, including cardiovascular autonomic neuropathy

and incident diabetic nephropathy and retinopathy; 4. Evaluating the association between DPN and SNPs in African-Americans participants; 5. Evaluating the association between DPN and genotyped low-frequency variants (MAF 0.01-0.05) placed in the LD block as the top common SNP in the GWAS. Continuous and dichotomous outcomes were tested by linear and logistic regression models, respectively. Incident outcomes were tested by means of Cox regression models.

Validation

All GWAS-significant SNPs (28 SNPs at locus 2q24), as well as lead variants at loci that reached P value < 1×10^{-5} in the discovery set (ACCORD) were tested for association with DPN in a validation set derived from the BARI 2D study. Logistic regression was used for this purpose with adjustments by the following covariates: country of origin, top three principal components, assignments to cardiovascular treatment strata (early medical therapy versus early revascularization [PCI/CABG]), and assignments to glycemic control strata (insulin sensitization versus insulin provision). Given that replication was attempted for 13 variants, the significance threshold was set to P<0.004 based on a Bonferroni correction (0.05/13). Variants that were successfully validated in BARI 2D were tested for their effects on prevalent DPN (MNSI > 2.0 at the study entry) and incident DPN (MNSI turned > 2.0 during the follow up) as described for the discovery set.

Meta-analysis of BARI 2D and ACCORD

Results in BARI 2D were meta-analyzed with those in ACCORD by means of METAL (Abecasis Lab, University of Michigan, MI) using a fixed-effects inverse-variance approach.

Power of genetic analyses

The discovery set (ACCORD) provided 80% power ($\alpha=5x10^{-8}$) to detect genetic effects corresponding to RR of 1.20 and 1.24 for effect allele frequencies of 0.50 and 0.15, respectively. The validation set (BARI 2D) provided 80% power ($\alpha=0.0038$, adjusting for the 13 loci for which replication was sought) to detect genetic effects corresponding to RR of 1.26 and 1.32 for effect allele frequencies of 0.50 and 0.15, respectively.

Expression Quantitative Trait Loci (eQTL) analysis

The top variant from the GWAS was tested for association with tibial-nerve-specific gene expression through *in-silico* analyses using the Genotype Tissue Expression (GTEx) database V7 (27). GTEx is an online database containing tissue-specific gene expression data obtained from 620 donors (34% females, 85% Whites, 68% more than 50 years of age) by means of Illumina TrueSeq RNA sequencing and the Affymetrix Human Gene 1.1 ST Expression Array, and linked to GWAS data obtained from the same individuals by whole genome sequencing, whole exome sequencing, or Illumina SNP arrays (www.gtexportal.org). eQTL analyses of genes within ±2 Mbp from the lead SNP were conducted among 361 genotyped individuals for which tibial nerve tissue expression profiles were available. Normalized effect sizes and their 95% confidence intervals were obtained from linear regression models testing the association of the minor allele of the lead SNP with gene expression adjusted by the top 3 genotyping principal components, sex, genotyping platform, and a set of other covariates described in the GTEx documentation (27).

Gene expression analysis for SCN2A

SCN2A has previously been shown to be expressed in the human peripheral nervous system in dorsal root ganglia (28). In order to quantify the level of gene expression for *SCN2A* in the tibial nerve, RNA-seq samples from several human tissues were contrasted: tibial nerve,

hippocampus, and skeletal muscle samples from the GTEx database (27), and dorsal root ganglia samples from the study by North *et al* (29). Since library preparation, mapping and relative abundance quantification (in Transcripts per Million or TPM) were performed differently for these two studies, the meta-analysis was performed as follows. Coding gene relative abundances for 18,876 quantified coding genes were extracted from the North *et al* study, and for each sample with non-zero *SCN2A* abundance, the percentile of *SCN2A* expression with respect to all coding genes in that sample were calculated. For the GTEx datasets, the uniformly processed RNA-seq abundances for coding genes were extracted from the GTEx companion website database (GTEx_Analysis_2016-01-15_v7_RNASeQCv1.1.8_gene_tpm. gct.gz), and re-normalized by constraining the TPMs of all the 19,260 coding genes quantified in the study to sum to a million. For each sample with non-zero *SCN2A* abundance, the percentile of *SCN2A* expression with respect to all coding genes in that sample was calculated.

RESULTS

Clinical Characteristics of DPN Cases and Controls

The clinical characteristics of the Discovery and Validation Sets are shown in Table 1. To minimize ethnic confounding, both sets included only self-reported Whites. The Discovery Set consisted of 5,168 ACCORD participants: 4,384 who showed signs of DPN at study entry and/or during follow-up (DPN cases) and 784 who did not show signs of DPN at any time during the study (DPN controls). As compared to the latter, DPN cases were significantly older and taller, had longer duration of diabetes, higher HbA1c, BMI, and urinary ACR, and lower HDL cholesterol, were more frequently treated with insulin, and had a higher prevalence of self-reported retinopathy. For many of these variables, differences with controls were more

pronounced for prevalent than incident cases (Supplementary Table 1). The Validation Set consisted of 949 BARI 2D participants, including 791 DPN cases and 158 DPN controls, defined as above. Overall, the clinical characteristics of the Validation Set were similar to those of the Discovery set, except for lower HbA1c and cholesterol levels (Table 1). Within the Validation Set, DPN cases had lower total, LDL, and HDL cholesterol, and were more frequently treated with insulin as compared to DPN controls.

Genome-Wide Association Study of DPN

A total of 6,847,206 common variants (MAF>0.05), genotyped or imputed, were tested for association with DPN in the Discovery Set. Of these, 28 reached genome-wide significance ($P<5x10^{-8}$), as summarized in the Manhattan and quantile-quantile plots shown in Figure 1. These variants lie in the same region of chromosome 2q24 and are in strong linkage disequilibrium with each other in Whites (Figure 2A). When the analysis was conditioned on the lead SNP (rs13417783), the effects of all the other genome-wide significant variants disappeared (Supplementary Figure 1), indicating that all 28 variants captured the same genetic effect. The lead SNP had a MAF of 0.15 and was associated with a 36% decrease in the odds of DPN per minor allele copy (OR=0.64, 95% CI 0.55-0.74; $P=1.9 x10^{-9}$) (Table 2). While rs13417783 was an imputed variant (with an excellent quality of imputation), other SNPs in the genome-wide significant cluster were genotyped (Supplementary Table 2). Effects were in the same direction in both genotype sets of ACCORD (Supplementary Table 2). Two other loci, rs13265430 on chromosome 8p23 and rs77494074 on chromosome 11q25, attained P values in the notable range ($P=1x10^{-6}$- $5x10^{-8}$) and 10 more attained P values in the $1x10^{-5}$-$1x10^{-6}$ range (Table 2). All these associations were only modestly affected by adjustment for differences in age, diabetes duration, and/or BMI (Supplementary Table 3). No evidence of association was observed with the loci on

chromosome 8 and 12 where a suggestive association with painful DPN had been detected in a previous GWAS (15) (Supplementary Table 4). Of 14 polymorphisms that had been previously reported to be associated with DPN (30) and for which data were available in the ACCORD GWAS, one (rs1963645 at the *NOS1AP* locus) showed a significant association with DPN. However, this was in the opposite direction as that previously reported (Supplementary Table 5).

Validation of GWAS Findings

The 13 independent loci that attained $P<1x10^{-5}$ in the GWAS were investigated for their association with DPN in the Validation Set. The effect of the top GWAS locus (2q24) was successfully replicated, with an OR of 0.57 per lead SNP (rs13417783) minor allele copy (95% CI 0.41-0.80; $P=9x10^{-4}$) (Table 2). One of the other 27 SNPs at 2q24 that were GWAS-significant in the Discovery Set (rs10200297) showed a stronger association than rs13417783 in the Validation Set (Supplementary Table 6). However, in a meta-analysis of the Discovery and Validation Sets, rs13417783 remained the lead SNP at this locus (OR=0.63, 95% CI 0.55-0.72, $p=7.9x10^{-12}$). Among the other loci with $p<1x10^{-5}$ in the Discovery Set, one (rs10555080, aka rs72397229, on 19q12) showed a nominally significant association with DPN in the Validation Set (p=0.0098), resulting into a p value in the notable range ($P=2.6x10^{-7}$) in the two sets combined. Another locus (rs201655918 on 14q24) was also nominally significant in the Validation Set, but the effect was in the opposite direction than in the Discovery Set. Four loci (rs1202660 on 7q11; rs2491019 on 10q22; rs11073752 on 15q25, rs9948095 on 18p11) showed non-significant associations in the Validation Set that were in the same direction as in the Discovery Set. One of these SNPs (rs11073752) attained a P value in the notable range ($P=6.5x10^{-7}$) in the meta-analysis of the Discovery and Validation sets.

Characteristics of the association between 2q24 locus and DPN

The magnitude of the genetic effect at 2q24 in Whites was similar if the analysis was restricted to prevalent or incident cases both in ACCORD and BARI 2D (Table 3). In ACCORD, no significant differences in the strength of the association were observed in relation to clinical characteristics, except for a significantly stronger effect among participants with self-reported diabetic retinopathy than in those without this trait (P=0.02) (Supplementary Figure 2). No interaction was found with the intervention tested in ACCORD (i.e., intensive versus standard glycemic control, intensive versus standard blood pressure control, and fenofibrate versus placebo) (Supplementary Figure 3). When the MNSI score components were analyzed individually, the strongest association was seen with alterations of foot appearance and ankle jerk (OR=0.71, $p=2x10^{-7}$, and OR=0.76, $p=2x10^{-5}$, respectively), followed by loss of vibration perception (OR=0.88, p=0.04), whereas no association could be observed with foot ulcer (OR=1.00, p=0.99). Nominally significant associations were observed between 2q24 locus and triglycerides, eGFR, and UACR at baseline (Supplementary Table 7). As reported in the Type 2 Diabetes Knowledge Portal (http://www.type2diabetesgenetics.org), an association between triglycerides and the index SNP (rs13417783) as well as the other GWAS-significant SNPs in LD with it had been previously detected in the GoDARTs study (p= 0.00011 for rs13417783). However, adjustment for this variable or for the other two associated with 2q24 (eGFR and UACR) did not attenuate the association of this locus with DPN (Supplementary Table 8). Similarly, adjustment for age and duration of diabetes, which showed significant differences between cases and controls, had no effect (Supplementary Table 8). Additionally, the association between 2q24 locus and DPN was unaffected (OR=0.64, 95% CI 0.54-0.77) by selecting controls that had a longer duration of diabetes (above the median of 7 years), and was only modestly reduced (OR=0.73, 95% CI 0.60-0.89) by selecting older controls (above the

median age of 61 years). No association was detected between 2q24 locus and risk of cardiac autonomic neuropathy, or risk of micro- and/or macro-vascular complications (Supplementary Tables 9 and 10).

2q24 locus and DPN in African-Americans

None of the 28 SNPs at the 2q24 locus showing GWAS-significant association with DPN in Whites were associated with DPN in African-American subjects from ACCORD (n=1,345), as judged on the basis of a two-sided $p < 0.05$. However, 15 of these SNPs had two-sided $p<0.20$, with effects going in the same direction as in Whites (OR=0.75-0.85) (Supplementary Table 6). The strongest association was observed with rs16852735 (OR=0.75, two-sided p= 0.069, one sided p=0.034), which in African-Americans was not in linkage disequilibrium (r^2=0.02) with the top SNP in Whites (rs13417783). The number of African-Americans in BARI 2D was too small for a meaningful analysis.

Low-frequency variants at 2q24 and DPN

Based on the findings with the common variants, five low-frequency variants (MAF 0.01-0.05), which were placed in the same LD block as the lead SNP (rs13417783) and had been genotyped in ACCORD, were tested for association with DPN among White participants. All five variants, which were in high LD with each other (r^2=0.82-1.00), showed a significant association with DPN ($p<0.01$, based on a Bonferroni adjustment for five comparisons) (Table 4). This association was somewhat attenuated but remained significant after adjustment for rs13417783 (Table 4). Conversely, adjustment for any of these low-frequency variants had minimal impact on the magnitude of the association between the common variant (rs13417783) and DPN (Table 4). Two of these variants (rs1509652 and rs6755015) were genotyped in BARI 2D and did not show any association with DPN (OR=0.94 and OR=1.07, respectively, $p>0.05$ for

both), although power for the analysis of low-frequency variants was limited in this dataset due to the smaller sample size.

Association between 2q24 Locus and Gene Expression

The common variants associated with DPN at 2q24 span 80 Kb of non-coding sequence placed between a cluster of genes coding for voltage-gated sodium channels (*SCN1A*, *SCN2A*, *SCN3A*, *SCN7A* and *SCN9A*) on the centromeric side and *XIRP2* (Xin Actin Binding Repeat Containing 2) on the telomeric side (Figure 2A). In an eQTL analysis of GTEx data concerning 16 genes placed in a 2 Mbp radius from the lead SNP, carriers of the rs13417783 minor allele had significantly higher tibial nerve expression of *SCN2A,* coding for sodium voltage-gated channel alpha subunit 2, and *AC019181.2 (LOC101929633)*, coding for an uncharacterized non-coding RNA ($P=9x10^{-4}$ and $P=6x10^{-4}$, respectively) (Figures 2B-D). A nominally significant association (P=0.03) was also detected with lower expression of the immediately adjacent *XIRP2* gene.

To gain further insight into *SCN2A* gene expression in peripheral nerves, we contrasted *SCN2A* gene expression in the hippocampus (a CNS tissue, serving as a positive control), dorsal root ganglion (DRG, which contains the cell bodies of the neurons supplying the axons to the sensory portion of the tibial nerve), tibial nerves, and skeletal muscle (an excitable non-nervous system tissue, serving as negative control). Coding gene relative abundances (in TPMs), and their corresponding percentiles are shown for all 4 tissues in Table 5 which includes samples from both sexes. Abundant RNA signal for *SCN2A* was found in both the hippocampus and in the DRG. Although at lower levels, *SCN2A* was also consistently expressed (in all but one sample) in the tibial nerve. By contrast, it was undetectable in 80% of the samples of the skeletal

muscle samples, and in the remaining 20% of the samples was among the lowest in abundance across all tissues.

DISCUSSION

Through a GWAS of the ACCORD cohort, we have identified a previously unrecognized locus on chromosome 2q24 having a powerful effect on the risk of DPN in type 2 diabetes. An association of similar strength was found between this locus and DPN in BARI 2D. In both cohorts, the 2q24 minor allele conferred protection from DPN, being more frequent in DPN-negative controls than in DPN cases and the general population. In further support of these findings, the same allele was found to be associated with higher tibial nerve expression of a nearby gene (*SCN2A*) coding for the alpha subunit of the voltage-gated sodium channel NaV1.2, providing a possible functional basis for the genetic effect observed at the population level. This is the first report of a DPN locus that reaches genome-wide significance in a GWAS and is replicated in an independent study.

The fact that almost identical effects on DPN were found for this locus in the discovery and replication sets (OR=0.63 and OR=0.57, respectively) is remarkable and makes this finding especially robust. Such high degree of concordance reflects the use of the same DPN definition (clinical exam MNSI>2) in studies and the high sensitivity, specificity, and correlation with abnormal nerve conduction characteristic of this DPN outcome (21; 22). Other contributing factors include the similar clinical characteristics of the two cohorts with regard to height, body mass index, diabetes duration, blood pressure, and smoking status, and the similar incidence of DPN despite the administration of different interventions.

There are scant data in the literature to which our findings can be compared as only one other GWAS of DPN has been published to date. That study, based on the Genetics of Diabetes Audit and Research Tayside (GoDARTS) datasets, found suggestive evidence ($P=1.8 \times 10^{-7}$) for a DPN locus on chromosome 8p21 near *GFRA2* (15). We could not replicate that finding in our study and, conversely, no significant evidence for the locus 2q24 that we identified in ACCORD and BARI 2D was reported in the GoDARTS study. In addition to the possibility of the 8p21 locus being a false-positive, given the lack of genome-wide significance and previous replication, there are important differences between the two GWAS that could explain these discrepancies. First, the outcome considered by the GoDARTS study was painful DPN, defined as a prescription history of at least one of five drugs indicated for the treatment of neuropathic pain along with a positive monofilament test for sensory neuropathy, which is known to have low specificity. By contrast, in our GWAS, DPN was defined by means of a validated instrument (clinical exam MNSI) that does not include pain in its assessment. Thus, the two studies captured different aspects of DPN, involving different components of the peripheral nervous system (predominantly small fibers in the case of painful DPN versus large fibers in the case of MNSI-diagnosed DPN). Second, both ACCORD and BARI 2D were clinical trials, with close follow-up, standardized outcome acquisition by trained professionals, and strict adjudication, whereas the GoDARTS study was based on medical record data collected as part of routine medical care. Finally, ACCORD and BARI 2D participants were characterized by a long duration of diabetes (15 years on average by the end of the study), which helped minimize misclassification of controls – an essential feature for the identification of protective effects on DPN such as that associated with the 2q24 locus. While diabetes duration was not specified in

the GoDARTS report, it was likely to be shorter owing to the population-based nature of that cohort.

The DPN locus that we have identified may allow clinicians to focus prevention efforts on patients at higher risk of DPN. However, the real relevance of this finding lies in the great potential for improving our understanding of DPN biology and for identifying novel targets for preventive interventions. It is noteworthy in this regard that the newly identified DPN locus is located in close vicinity to a cluster of genes coding for the alpha subunits of voltage-gated sodium channels (NaV), which are responsible for multiple aspects of neuron excitability including thresholds for depolarizing responses and the amplitude of action potentials (31). From the most proximal to the most distal from the top variant, these genes include *SCN7A*, *SCN9A*, *SCN1A*, *SCN2A*, and *SCN3A*, which code for NaVx, NaV1.7, NaV1.1, NaV1.2, NaV1.3, respectively. In the GTEx database, the protective allele of the 2q24 locus was significantly associated with higher expression of one of these genes (*SCN2A)* in the tibial nerve, supporting the hypothesis that increased activity of the corresponding sodium channel (NaV1.2), which is expressed in DRG and tibial nerve, may guard against the detrimental effects of the diabetic milieu on peripheral nerves. *SCN2A* mRNA is expressed by human and murine DRG neurons but not by Schwann cells or other cell types that are found in the tibial nerve (mousebrain.org), which suggests that the *SCN2A* mRNA in the tibial nerve is contributed by sensory nerve axons. Rare mutations in the *SCN2A* gene have been implicated in the etiology of neurodevelopmental diseases such as autism spectrum disorder (loss of function mutations) and infantile seizures (gain of function mutations) (32), but the effect of these variants on susceptibility to DPN has never been investigated due to the their rarity, nor has the effect of NaV1.2 loss or gain of function been studied in animal models of DPN. A relationship with DPN etiology has been

instead clearly established for NaV1.3 and NaV1.7 (coded by *SCN3A* and *SCN9A*, respectively) (33-36), but no differences in their expression were found among 2q24 genotypes in the GTEx database. Whether this was due to the small size and/or the lack of exposure to diabetes of the samples in the GTEx database, or is related to the fact that NaV1.3 and NaV 1.7 are preferentially expressed in small fibers mediating pain transmission, whereas the MNSI score mainly tests large fibers mediating vibration sense and tendon reflexes, remains to be determined. Altogether, these findings highlight the need for further studies of the SCN genes in the 2q24 region as functional mediators of the DPN locus and potential targets of novel preventive interventions. These studies would include testing of the association between 2q24 genotype and the expression of these genes in peripheral nerve samples specifically collected from diabetic subjects, along with studies assessing the impact of overexpressing or knocking down these genes in peripheral nerves of animal models of DPN (37). The non-coding gene *AC019181.2 (LOC101929633),* which also showed evidence of association with the 2q24 locus in the GTEx database, should be included in these studies given the potential role of non-coding RNAs as regulators of gene expression (38; 39).

The genomic mechanisms linking this locus to differences in gene expression are unclear at this time. The index SNP and the other 27 variants reaching GWAS significance are in a non-coding region with quite subtle epigenetic marks. The index SNP was found by ENCODE to be located in a weak enhancer (as indicated by an H3K4m1 mark) in human embryonic stem cells (hESC), but whether this regulatory element is also active in neural cells remains to be determined. Of the other 27 SNPs, the two highest ranking in RegulomeDb are rs12993796 and rs16852735. The first SNP is located in a DNA segment that binds BATF (a transcription factor involved in the differentiation of immune cells) in a lymphoblastoid cell line (GM12878). The

second SNP is placed in a region showing evidence of binding to REST in a neuroectodermic cell line (PFSK-1). REST (RE1 Silencing Transcription Factor) is a transcription factor that represses neuronal genes, including voltage-gated sodium channels (40). Since REST is expressed in peripheral nerves (as shown by GTEx data) and in DRG, one can speculate that the higher expression of *SCN2A* associated with the 2q24 protective allele in these tissues may be due to decreased binding of this repressor to the DNA segment where rs16852735 is located. The other 25 SNPs showed minimal evidence of being functional in the summary provided by RegulomeDb. Re-sequencing of the LD block in which the GWAS-significant SNPs are located in large series of cases and controls, followed by *ad hoc* functional studies, will be necessary to identify the causal variant(s) and understand its (their) impact on genomic function.

While the association with DPN was strongest at 2q24, another locus, placed on chromosome 15q25, yielded notable GWAS significance in the ACCORD GWAS as well as in the meta-analysis with BARI 2D. Especially interesting is the vicinity of this locus to the *NTRK3* gene coding for the receptor of neurotrophin 3, which, by prompting the extension of fibers from proprioceptive dorsal root ganglions to the muscle spindle and the ventral horn of the spinal cord, is responsible for inducing the synaptic connection between sensory and motor neurons (41). Based on this evidence, this locus should be considered as prime candidate for future studies, although the available GTEx data do not suggest an association between the index SNP at 15q25 and *NTRK3* expression.

Strengths of our study include the advantages of clinical trials, such as the rigorous and standardized protocols for data acquisition, the regular follow-up, and access to rich phenotype data, as well as the systematic GWAS approach based on genotyped and imputed data of excellent quality and wide coverage. As discussed above, the use of a validation cohort having

very similar clinical characteristics to the discovery cohort was another critical strength. Nonetheless, some limitations should be acknowledged. Since both ACCORD and BARI 2D were designed to include participants with type 2 diabetes at high risk of CVD, whether our findings could be generalized to individuals with different characteristics or with type 1 diabetes remains to be determined. Similarly, our study was limited to White subjects and it is not known whether these findings also apply to individuals of other races, although the limited data on African-American individuals from ACCORD suggest that this might be the case. Also, our study, while larger than most of the genetic studies of DPN published thus far, was powered to detect only relatively large genetic effects and the fact that controls were slightly younger and had slightly shorter diabetes duration than cases may have biased results towards the null-hypothesis. Therefore, other loci having smaller, yet functionally relevant effects may have gone undetected. Finally, since the GWAS was limited to common polymorphisms (minor allele frequencies greater than 5%), the existence of additional genetic effects due to less frequent variants cannot be excluded.

In summary, we have identified and successfully validated a locus on chromosome 2q24 having a powerful protective effect on the development of DPN in type 2 diabetes. Tissue expression analysis suggests that this effect may be mediated by higher expression of the voltage-gated sodium channel NaV1.2 in the tibial nerve, which is known to increase neuronal excitability. These results may provide novel insights into the pathogenesis of DPN and point to a potential new target for interventions aimed at preventing or treating this complication of diabetes.

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Author Contributions

YT, HS and AD designed the study, acquired, analyzed, and interpreted the data from ACCORD, wrote the initial draft of the manuscript and revised the manuscript to its final form; PAL, HC, and SC acquired, analyzed and interpreted the data from BARI 2D , and revised the manuscript to its final form. BAP, RPB, and BC contributed to the study design, interpreted the data, and revised the manuscript to its final form. JCM, MJW, AAM-R, and JBB acquired, analyzed and interpreted the data, and revised the manuscript to its final form. PRR and TJP performed RNA-seq meta-analysis for *SCN2A* gene expression, wrote the corresponding sections of the manuscript, and revised the manuscript to its final form. HS and AD contributed equally to this work and are co-senior authors. The content of the paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other funding entities. HS and AD are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Duality of Interest

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Data Availability

The ACCORD database is available upon request from the National Heart, Lung, and Blood Institute Biologic Specimen and Data Repository (https://biolincc.nhlbi.nih.gov/studies/accord/).

The ACCORD GWAS data have been deposited in the database of Genotypes and Phenotypes (dbGAP, Study Accession: phs001411.v1.p1).

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Table 1. Characteristics of diabetic peripheral neuropathy (DPN) cases and controls

Baseline characteristic	Discovery Set (ACCORD)			Validation Set (BARI 2D)		
	DPN Cases (N=4,384)	DPN Controls (N=784)	P-value	DPN Cases (N=791)	DPN Controls (N=158)	P-value
Female	1,538 (35.1)	289 (36.9)	0.34	204 (25.8)	51 (32.3)	0.09
Age (years)	63.2 ± 6.5	61.6 ± 6.4	<0.0001	63.33 ± 8.47	62.09 ± 9.29	0.10
DM duration (years)	10.9 ±7.5	9.1 ± 6.9	<0.0001	10.5 ± 8.38	9.06 ± 8.71	0.05
BMI (kg/m^2)	33.2 ± 5.2	31.9 ± 4.8	<0.0001	31.8 ± 5.41	32.33 ± 5.97	0.27
Waist circumference (cm)	110.0 ± 12.9	105.3 ± 12.0	<0.0001	108.99 ± 13.08	108.42 ± 13.97	0.62
Height (cm)	171.7 ± 9.5	170.0 ± 9.4	<0.0001	na	na	na
HbA1c (%)	8.22 ± 0.93	8.11 ± 0.90	0.02	7.54 ± 1.54	7.66 ± 1.63	0.40
Fasting glucose (mmol/L)	9.93 ± 2.82	9.75 ± 2.76	0.09	na	na	na
SBP (mmHg)	135.2 ± 16.7	134.7 ± 16.4	0.42	131.65 ± 20.6	128.75 ± 16.32	0.05
DBP (mmHg)	74.1 ± 10.4	75.0 ± 10.7	0.03	73.82 ± 11.92	73.54 ± 11.22	0.78
LDL (mmol/L)	2.67 ± 0.84	2.68 ± 0.83	0.52	2.43 ± 0.83	2.59 ± 0.85	0.03
HDL (mmol/L)	1.04 ± 0.27	1.06 ± 0.29	0.0047	0.95 ± 0.23	0.98 ± 0.23	0.13
Women	1.16 ± 0.29	1.22 ± 0.29	0.0009	1.06 ± 0.24	1.02 ± 0.22	0.19
Men	0.97 ± 0.23	0.98 ± 0.23	0.54	0.91 ± 0.21	0.96 ± 0.24	0.02
Total cholesterol (mmol/L)	4.74 ± 1.05	4.76 ± 1.04	0.33	4.35 ± 1.05	4.56 ± 1.16	0.03
Triglycerides (mmol/L)†	1.95 (1.37 -2.81)	1.91 (1.29 -2.76)	0.14	1.76 (1.24 -2.58)	1.90 (1.28 -2.7)	0.46
eGFR (ml/min/1.73 m^2)	87.8 ± 21.7	90.4 ± 20.4	0.0016	na	na	na
UACR (mg/mmol)†	1.4 (0.7-4.2)	1.1 (0.6-2.8)	<0.0001	na	na	na
Previous CV event*	1,556 (35.5)	264 (33.7)	0.33	318 (40.2)	69 (43.7)	0.42
Report of retinopathy	439 (11.3)	40 (5.6)	<0.0001	na	Na	na
Current smoker	496 (11.3)	84 (10.7)	0.8	63 (8)	19 (12.1)	0.09
Previous smoker	2,194 (57.0)	390 (56.2)	0.8	456 (57.8)	88 (56.1)	0.69
Insulin therapy	1,598 (36.5)	220 (28.1)	<0.0001	212 (26.8)	27 (17.1)	0.01
ACCORD Glycaemia trial						
Standard	2,227 (50.8)	371 (47.3)	0.07	-	-	-
Intensive	2,157 (49.2)	413 (52.7)		-	-	-
ACCORD BP trial	1,862 (42.5)	361 (46.1)	0.06	-	-	-
Standard	926 (21.1)	182 (23.2)	0.81	-	-	-
Intensive	936 (21.4)	179 (22.8)		-	-	-
ACCORD Lipid trial	2,522 (57.5)	423 (54.0)	0.06	-	-	-
Fibrate	1,294 (29.5)	219 (27.9)	0.86	-	-	-
Placebo	1,228 (28.0)	204 (26.0)		-	-	-
BARI 2D CV intervention						
Medical therapy	-	-	-	398 (50.3)	67 (42.4)	0.07
Early revascularization	-	-	-	393 (49.7)	91 (57.6)	
BARI 2D Diabetic therapy						
Insulin provision	-	-	-	394 (49.8)	71 (44.9)	0.26
Insulin sensitization	-	-	-	397 (50.2)	87 (55.1)	

Except where noted, data are means ± SD for continuous variables and counts (%) for categorical data. †Medians (IQR). *Prior cardiovascular event: In ACCORD, this includes secondary prevention status or history of myocardial infarction, stroke, angina and/or ischemic changes (ECG) on Graded Exercise Tolerance Test or positive imaging, coronary revascularization procedures or other revascularization procedures at baseline; in BARI 2D, this includes history of myocardial infarction, congestive heart failure, stroke, or transient ischemic attack.

Table 2. Top loci ($P < 1\times10^{-5}$) associated with diabetic peripheral neuropathy: effects in ACCORD and validation in BARI 2D.

				Discovery Set (ACCORD)					Validation Set (BARI 2D)				ACCORD + BARI 2D*	
SNP	**Position‡**	**Closest gene**	**MA****	**MAF¶ 1000G**	**MAF§ cases**	**MAF§ controls**	**OR (95% CI)**	**P**	**MAF cases**	**MAF controls**	**OR (95% CI)**	**P**	**OR (95% CI)**	**P**
rs13417783	2:167629849	*XIRP2*	T	0.14	0.14	0.20	0.64 (0.55-0.74)	1.9×10^{-9}	0.14	0.20	0.57 (0.41-0.80)	0.0009	0.63 (0.55-0.72)	7.9×10^{-12}
rs12988669	2:240275570	*HDAC4*	C	0.15	0.16	0.20	0.71 (0.62-0.82)	2.7×10^{-6}	0.16	0.13	1.22 (0.83-1.78)	0.32	0.76 (0.66-0.87)	5.1×10^{-5}
rs60770880	3:8037416	*LOC101-927394*	A	0.21	0.21	0.26	0.75 (0.66-0.84)	5.0×10^{-6}	0.20	0.21	0.93 (0.68-1.26)	0.62	0.77 (0.68-0.86)	1.0×10^{-5}
rs11932946	4:45140214	*GUF1*	G	0.12	0.12	0.16	0.69 (0.59-0.82)	9.6×10^{-6}	0.13	0.11	1.01 (0.68-1.49)	0.98	0.73 (0.63-0.85)	4.5x10-5
rs1202660	7:70658177	*WBSCR17*	T	0.22	0.20	0.25	0.74 (0.65-0.84)	6.2×10^{-6}	0.23	0.26	0.81 (0.60-1.09)	0.16	0.75 (0.67-0.85)	2.5×10^{-6}
rs13265430	8:4165607	*CSMD1*	A	0.09	0.09	0.13	0.64 (0.52-0.76)	1.0×10^{-6}	0.11	0.09	1.35 (0.84-2.15)	0.21	0.70 (0.59-0.83)	4.3×10^{-5}
rs2491019	10:70776987	*KIAA1279*	A	0.45	0.47	0.40	1.31 (1.17-1.47)	4.4×10^{-6}	0.45	0.46	1.02 (0.79-1.32)	0.86	1.25 (1.13-1.39)	2.0×10^{-5}
rs77494074	11:132794801	*OPCML*	T	0.08	0.07	0.10	0.61 (0.50-0.74)	1.0×10^{-6}	0.07	0.06	1.28 (0.71-2.29)	0.41	0.66 (0.54-0.79)	1.3×10^{-5}
rs201655918	14:76791306	*ESRRB*	C	0.28	0.26	0.32	0.75 (0.66-0.85)	6.9×10^{-6}	0.30	0.24	1.41 (1.02-1.96)	0.04	0.81 (0.72-0.91)	5.3×10^{-4}
rs11073752	15:88423051	*NTRK3*	C	0.33	0.32	0.38	0.76 (0.67-0.85)	2.1×10^{-6}	0.33	0.36	0.80 (0.61-1.05)	0.11	0.76 (0.68-0.85)	6.5×10^{-7}
rs9948095	18:12018665	*IMPA2*	C	0.14	0.15	0.20	0.71 (0.61-0.82)	3.6×10^{-6}	0.14	0.16	0.88 (0.63-1.24)	0.46	0.73 (0.64-0.84)	5.4×10^{-6}
rs10555080 (aka rs72397229)	19:32043170	*THEG5*	A	na	0.36	0.30	1.32 (1.17-1.49)	6.9×10^{-6}	0.38	0.32	1.46 (1.10-1.94)	0.0098	1.34 (1.20-1.50)	2.6×10^{-7}
rs34948558	21:42825856	*MX1*	A	0.28	0.27	0.32	0.76 (0.67-0.85)	4.7×10^{-6}	0.30	0.31	1.01 (0.77-1.33)	0.95	0.79 (0.71-0.88)	3.0×10^{-5}

In ACCORD, the primary model was adjusted by assignment to interventions, 7 clinical center networks, and top 3 principal components. In BARI 2D, adjustments included assignment to interventions, country of origin, top 3 principal components. *Meta-analysis of results in the Discovery and Validation Sets. ‡Position is chromosome:bp according to the National Center for Biotechnology Information assembly build GRCh37/hg19. **MA is minor or effect allele. §MAF in the Discovery Set is the average of minor allele frequencies in the two ACCORD genotyping sets (ANYSET and ACCSET). ¶MAF in 1000 genomes Project Phase 3 EUR populations was derived from Ensembl GRCh37 Release 93 (http://grch37.ensembl.org/index.html).

Table 3. Association between top loci and prevalent/incident DPN in ACCORD and BARI 2D.

	ACCORD			BARI 2D		
	N cases / N controls	OR (95% CI)	HR (95% CI)	N cases/ N controls	OR (95% CI)	HR (95% CI)
Prevalent Cases vs. Controls	2,547/784	0.64 (0.55-0.75)	-	518/158	0.59 (0.42–0.83)	-
Incident Cases vs. Controls	1,837/784	0.65 (0.56-0.77)	0.80 (0.73-0.88)	271/158	0.59 (0.40-0.87)	0.69 (0.55-0.88)

Table 4. Association between low-frequency variants at 2q24 and DPN.

					Univariable				Multivariable†			
					Low-frequency variant		rs13417783		Low-frequency variant		rs13417783	
Low-frequency variant	**position**	**Alleles***	**MAF**	**r^2‡**	**OR**	**p**	**OR**	**p**	**OR**	**p**	**OR**	**p**
rs16852465	2:167524744	G/A	0.036	0.009	1.94	3.8E-04	0.64	1.9E-09	1.80	1.7E-03	0.66	2.1E-08
rs1509652	2:167589745	C/T	0.038	0.01	1.76	1.1E-03	0.64	1.9E-09	1.63	5.3E-03	0.66	2.0E-08
rs11884905	2:167611786	C/T	0.037	0.009	1.84	6.7E-04	0.64	1.9E-09	1.70	3.1E-03	0.66	2.1E-08
rs6721669	2:167636784	T/C	0.035	0.009	1.98	2.9E-04	0.64	1.9E-09	1.84	1.3E-03	0.66	2.2E-08
rs6755015	2:167666021	T/C	0.040	0.008	1.68	2.5E-03	0.64	1.9E-09	1.56	1.0E-02	0.66	1.6E-08

*minor allele/ major allele
†Results of regression models including one of the low-frequency variants along with rs13417783 as DPN predictors.
‡r^2 with rs13417783

Table 5. Relative *SCN2A* abundance (in TPM) and corresponding percentiles across coding genes .

Tissue	**Fraction of samples with detectable (TPM > 0) *SCN2A* expression**	**Mean *SCN2A* TPM (± SD) among coding genes in samples with TPM > 0**	**Mean *SCN2A* percentile (± SD) among coding genes in samples with TPM > 0**
Hippocampus	123 out of 123	18.8 ± 15.8	71.7 ± 14.9
Dorsal root ganglion (DRG)	21 out of 21	18.1 ± 6.0	49.0 ± 6.6
Tibial nerve	413 out of 414	2.0 ± 1.8	30.8 ± 5.7
Skeletal muscle	112 out of 564	0.13 ± 0.08	22.8 ± 3.3

TPM, transcript per million

FIGURE LEGENDS

Figure 1. GWAS results. A) Manhattan plot. Each dot represents a polymorphism (SNP). The X-axis depicts each chromosome and the Y axis shows the negative $\log_{10}$ p value for association of each SNP with DPN. The red dotted line indicates the Genome-wide significance threshold of $p = 5 \times 10^{-8}$, the gray dotted line indicates the notable genome-wide significance threshold of $p = 1\times 10^{-6}$. **B)** Q-Q plot (inset). The black dashed line indicates the null hypothesis. The blue line represents the observed $\log_{10}$ p values corresponding to the expected p values. Lambda=0.994.

Figure 2. Characterization of Locus 2q24. **A)** Regional association plot of locus 2q24**.** Variants are displayed ±2 Mbp upstream and downstream of the lead SNP (rs13417783). The x-axis depicts the chromosomal positions of the SNPs as per NCBI Build 37, and the y-axis depicts the $-\log_{10}$ p values for association of these SNPs with diabetic peripheral neuropathy in ACCORD. SNPs in strong linkage disequilibrium with the lead SNP (purple) are marked in red ($r^2>0.8$). The bottom panel depicts UCSC genes within the region. The locus zoom plot was generated using LocusZoom (Abecasis Lab, University of Michigan School of Public Health) through http://locuszoom.sph.umich.edu/genform.php?type=yourdata. The reference database was hg19/1000 Genomes Nov 2014 EUR; **B)** eQTL analysis of locus 2q24 showing association between rs13417783 and tibial nerve-specific expression of neighboring genes. The eQTL analysis was conducted on data from the Genotype Tissue Expression (GTEx) database (http://www.gtexportal.org/home/). Genes were those located within ±2 Mbp from the lead SNP rs13417783. *NES is Normalized Effect size, the slope of the linear regression computed as effect of minor allele (T) of rs13417783 relative to the reference allele (C). This effect size is computed in a normalized space where magnitude has no direct biological interpretation; **C)** Tibial nerve-specific expression of *AC019181.2* stratified by rs13417783 genotypes; **D)** Tibial nerve-specific expression of *SCN2A* stratified by rs13417783 genotypes. Boxplots were generated in GTEx.

Online Supplemental Material

ACCORD

Members of the ACCORD DSMB included Antonio M. Gotto Jr. (chair), Kent Bailey, Dorothy Gohdes, Steven Haffner, Roland Hiss, Kenneth Jamerson, Kerry Lee, David Nathan, James Sowers, and LeRoy Walters. The following companies provided study medications, equipment, or supplies: Abbott Laboratories (Abbott Park, IL), Amylin Pharmaceuticals (San Diego, CA), AstraZeneca (Wilmington, DE), Bayer (Tarrytown, NY), Closer Healthcare (Tequesta, FL), GlaxoSmithKline (Philadelphia, PA), King Pharmaceuticals (Bristol, TN), Merck (Whitehouse Station, NJ), Novartis (East Hanover, NJ), NovoNordisk (Princeton, NJ), Omron Healthcare (Schaumburg, IL), Sanofi (Bridgewater, NJ), Schering-Plough (Kenilworth, NJ), and Takeda Pharmaceuticals (Deerfield, IL). None of these companies had an interest or bearing on the genome-wide analysis of the ACCORD data.

BARI 2D

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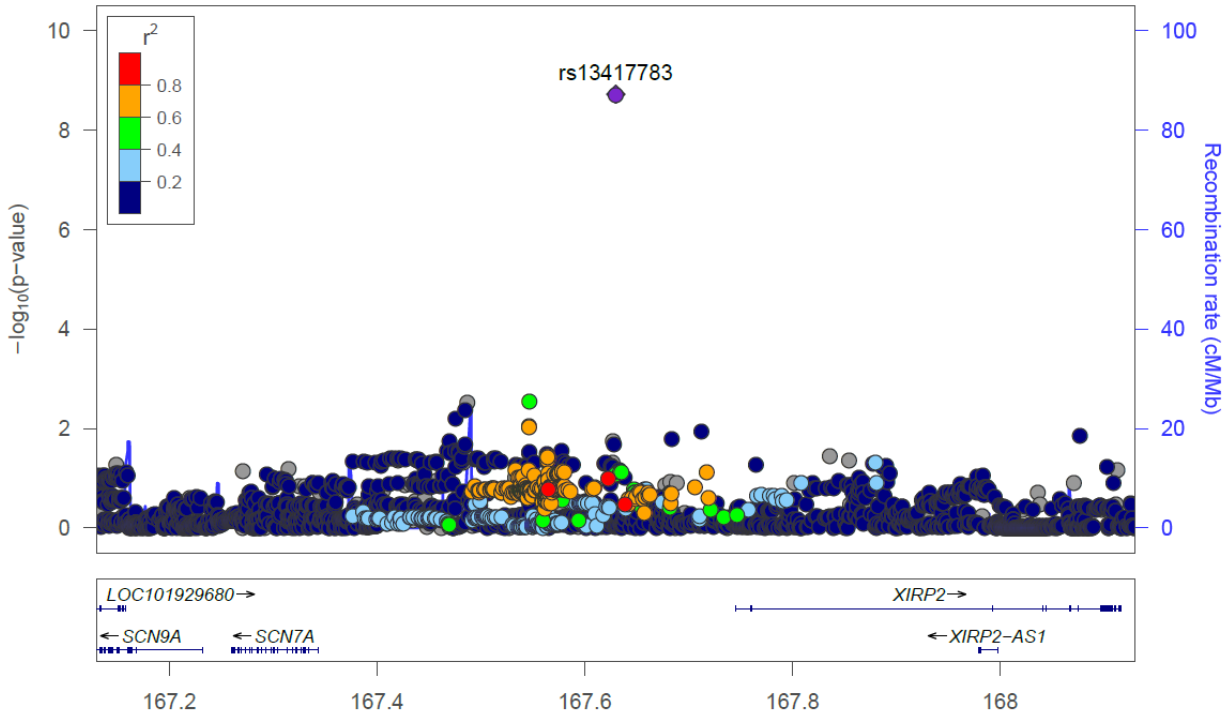
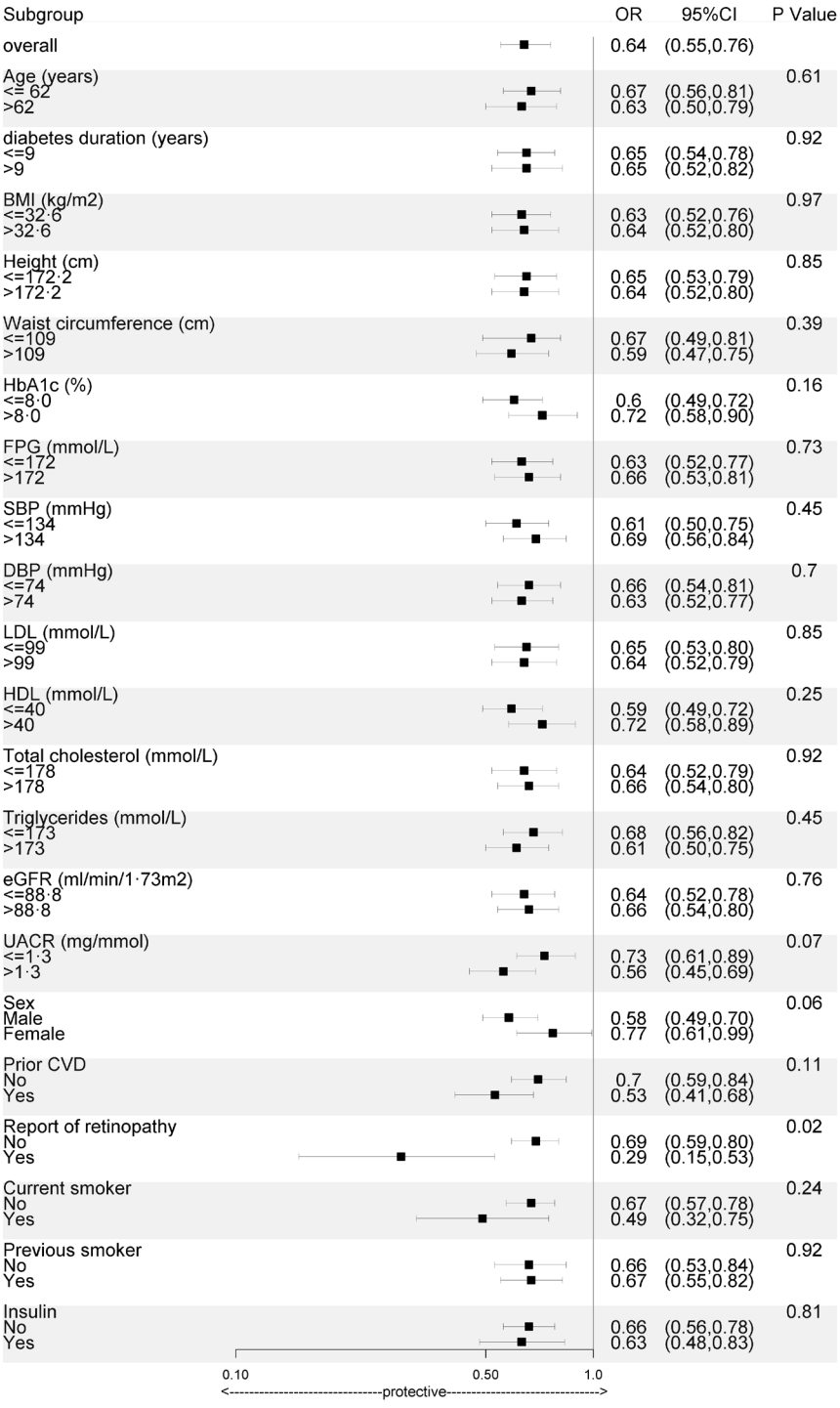
Supplementary Figure 1. Regional association plot for locus 2q24 (± 500 kbp of lead SNP, rs13417783) after conditioning on lead SNP.

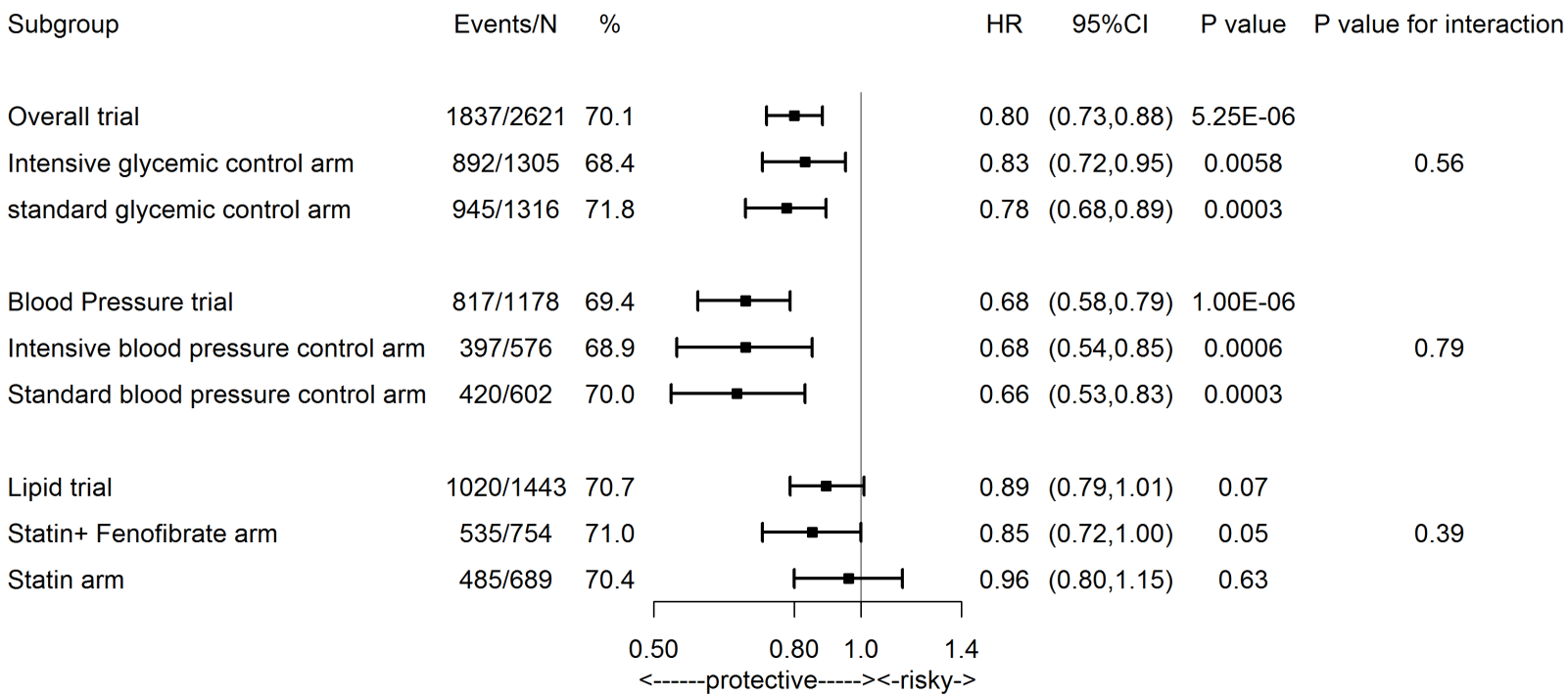


Supplementary Figure 2. Effect of the lead DPN SNP according to baseline characteristics in the Discovery Set.



For continuous variables, subgroups were defined based on median cut-offs. P values are for the interaction between clinical characteristics and SNP.

Supplementary Figure 3. Interaction between DPN-associated SNP rs13417783 and interventions tested in ACCORD.



Cox proportional regression models were adjusted by assignment to other interventions, seven clinical center networks, and top three principal components. The analysis is restricted to the prospective ACCORD cohorts – 1837 DPN cases and 784 controls. The intervention and control arms were : 1) Glycemia trial- intensive glycemic control versus standard glycemic control; 2) Blood pressure trial- intensive blood pressure control versus standard glycemic control; 3) Lipid trial: fenofibrate + statin versus placebo + statin. Hazard ratios, 95% confidence intervals and p values are provided for marginal associations of SNP with progression to DPN in each of the trial arms. P values for interaction between SNP x intervention are also provided.

Supplementary Table 1. Baseline characteristics in prevalent cases and incident cases controls in the Discovery Set (ACCORD).

Baseline Characteristics	prevalent cases	incident cases	p value
	(N= 2,547)	(N= 1,837)	
Female	855 (33.6)	683 (37.2)	0.02
Age (years)	63.8 ± 6.5	62.8 ± 6.2	<0.0001
DM duration (years)	11.5 ± 7.8	10.1 ± 6.9	<0.0001
BMI (kg/m^2)	33.4 ± 5.2	32.8 ± 5.2	0.003
Waist circumference (cm)	111.0 ± 13.0	108.6 ± 12.6	<0.0001
Height (cm)	172.4 ± 9.5	170.7 ± 9.3	<0.0001
HbA1c (%)	8.22 ± 0.92	8.21 ± 0.95	0.52
Fasting serum glucose (mmol/L)	9.96 ± 2.91	9.89 ± 2.7	0.42
SBP (mmHg)	135.6 ± 16.8	134.7 ± 16.6	0.0750
DBP (mmHg)	73.6 ± 10.3	74.8 ± 10.5	0.0011
LDL (mmol/L)	2.65 ± 0.84	2.68 ± 0.83	0.21
HDL (mmol/L)	1.03 ± 0.26	1.05 ± 0.27	0.0040
Women	1.15 ± 0.28	1.17 ± 0.29	0.19
Men	0.97 ± 0.22	0.98 ± 0.23	0.15
Total cholesterol (mmol/L)	4.73 ± 1.04	4.75 ± 1.03	0.28
Triglycerides (mmol/L)†	1.97 (1.36-2.85)	1.95 (1.38-2.77)	0.71
eGFR (ml/min/1.73 m2)	87.8 ± 22.2	87.8 ± 20.9	0.94
UACR (mg/mmol)†	1.5 (0.7-5.0)	1.3 (0.7-3.2)	<0.0001
Previous cardiovascular event	939 (36.9)	617 (33.6)	0.03
Report of retinopathy	317 (13.9)	122 (7.6)	<0.0001
Current smoker	353 (13.9)	213 (11.6)	0.03
Previous smoker	1303 (58.7)	891 (54.6)	0.02
Insulin therapy	1013 (39.9)	585 (32.0)	<0.0001
ACCORD glycaemia trial			
Standard	1282 (50.3)	945 (51.4)	0.49
Intensive	1265 (49.7)	892 (48.6)	
ACCORD blood pressure trial	1045 (41.0)	817 (44.5)	0.03
Standard	506 (48.4)	420 (51.4)	0.75
Intensive	539 (51.6)	397 (48.6)	
ACCORD lipid trial	1502 (59.0)	1020 (55.5)	0.03
Fibrate	759 (51.5)	535 (52.5)	0.65
Placebo	743 (49.5)	485 (47.5)	

†median (IQR)

Supplementary Table 2. Effects of GWAS significant ($p<5x10^{-8}$) SNPs in the two ACCORD genotyping sets

				ANYSET(3,554 cases/830 controls)					ACCSET(600 cases/184 controls)					Meta-analysis of ANYSET and ACCSET				
SNP	Chr:bp	Minor /major allele	MAF	Type*	info†	effect	SE	p	type	info	effect	SE	P	beta	SE	P	Dire-ction	P value For Hetero-geneity
rs13417783	2:167629849	T/C	0.148	I	0.991	-0.50	0.08	1.38×10^{-9}	I	0.96	-0.23	0.17	0.16	-0.45	0.07	1.91×10^{-9}	--	0.16
rs145971945	2:167677755	TAC /T	0.134	I	0.988	-0.51	0.09	1.45×10^{-9}	I	0.967	-0.24	0.17	0.15	-0.46	0.08	2.00×10^{-9}	--	0.15
rs13000447	2:167717280	G/C	0.145	I	0.983	-0.48	0.08	8.32×10^{-9}	I	0.951	-0.32	0.16	0.05	-0.45	0.07	2.29×10^{-9}	--	0.37
rs10164425	2:167706169	C/T	0.136	I	0.983	-0.51	0.08	1.7×10^{-9}	I	0.961	-0.23	0.17	0.17	-0.45	0.08	2.63×10^{-9}	--	0.14
rs13392463	2:167652676	A/G	0.136	G	1	-0.51	0.08	2.09×10^{-9}	I	0.971	-0.23	0.17	0.17	-0.45	0.08	3.09×10^{-9}	--	0.15
rs13031854	2:167638666	G/C	0.134	I	0.961	-0.53	0.09	9.77×10^{-9}	I	0.922	-0.19	0.17	0.28	-0.46	0.08	3.39×10^{-9}	--	0.08
rs13421469	2:167653868	G/A	0.135	I	0.998	-0.51	0.08	2.51×10^{-9}	I	0.971	-0.23	0.17	0.16	-0.45	0.08	3.55×10^{-9}	--	0.16
rs2390487	2:167660965	C/T	0.136	I	0.999	-0.50	0.08	2.45×10^{-9}	I	0.975	-0.23	0.17	0.17	-0.45	0.08	3.63×10^{-9}	--	0.15
rs536046332	2:167681464	-/TA	0.136	I	0.993	-0.50	0.08	3.47×10^{-9}	I	0.971	-0.24	0.17	0.14	-0.45	0.08	3.98×10^{-9}	--	0.17
rs13026906	2:167682431	C/T	0.136	I	0.995	-0.50	0.08	2.88×10^{-9}	I	0.973	-0.23	0.17	0.16	-0.45	0.08	3.98×10^{-9}	--	0.15
rs13034083	2:167683463	C/T	0.136	I	0.995	-0.50	0.08	2.88×10^{-9}	I	0.973	-0.23	0.17	0.16	-0.45	0.08	3.98×10^{-9}	--	0.15
rs16852695	2:167662782	C/G	0.135	I	0.999	-0.50	0.08	3.02×10^{-9}	I	0.974	-0.23	0.17	0.17	-0.45	0.08	4.27×10^{-9}	--	0.15
rs10183769	2:167661550	T/C	0.136	I	0.999	-0.50	0.08	3.09×10^{-9}	I	0.974	-0.23	0.17	0.17	-0.45	0.08	4.37×10^{-9}	--	0.15
rs10178966	2:167642185	G/A	0.136	I	0.99	-0.50	0.09	3.39×10^{-9}	I	0.966	-0.23	0.17	0.17	-0.45	0.08	4.68×10^{-9}	--	0.16
rs10189266	2:167645439	C/A	0.136	I	0.992	-0.50	0.08	3.24×10^{-9}	I	0.967	-0.23	0.17	0.17	-0.45	0.08	4.68×10^{-9}	--	0.15
rs12993796	2:167649537	C/T	0.136	G	1	-0.50	0.08	3.63×10^{-9}	I	0.969	-0.23	0.17	0.16	-0.45	0.08	4.79×10^{-9}	--	0.16
rs35762912	2:167659769	C/CT	0.136	I	0.999	-0.50	0.08	3.47×10^{-9}	I	0.974	-0.23	0.17	0.17	-0.45	0.08	5.01×10^{-9}	--	0.16
rs12692848	2:167658191	G/A	0.136	I	1	-0.50	0.08	3.55×10^{-9}	I	0.976	-0.23	0.17	0.17	-0.45	0.08	5.13×10^{-9}	--	0.16
rs12692849	2:167658309	G/A	0.136	I	1	-0.50	0.08	3.55×10^{-9}	I	0.975	-0.23	0.17	0.17	-0.45	0.08	5.13×10^{-9}	--	0.16
rs76036953	2:167649160	T/A	0.136	I	1	-0.50	0.08	4.07×10^{-9}	I	0.97	-0.23	0.17	0.17	-0.44	0.08	5.62×10^{-9}	--	0.16
rs7558617	2:167655841	T/A	0.136	I	1	-0.50	0.08	4.07×10^{-9}	I	0.974	-0.23	0.17	0.17	-0.44	0.08	5.75×10^{-9}	--	0.16
rs34867578	2:167642067	T/C	0.136	I	0.989	-0.50	0.09	5.37×10^{-9}	I	0.966	-0.22	0.17	0.19	-0.44	0.08	8.13×10^{-9}	--	0.16
rs34148223	2:167682742	C/A	0.137	I	0.989	-0.49	0.08	5.5×10^{-9}	I	0.966	-0.22	0.17	0.19	-0.44	0.08	8.71×10^{-9}	--	0.15
rs16852735	2:167719276	T/C	0.139	I	0.986	-0.48	0.08	1.62×10^{-8}	I	0.956	-0.25	0.16	0.12	-0.43	0.08	1.29×10^{-8}	--	0.23
rs148112563	2:167657499	GGATA /G	0.133	I	0.987	-0.49	0.09	1.66×10^{-8}	I	0.967	-0.24	0.17	0.15	-0.44	0.08	1.74×10^{-8}	--	0.21
rs10200297	2:167657230	C/T	0.138	I	1	-0.48	0.08	1.38×10^{-8}	I	0.984	-0.20	0.17	0.23	-0.42	0.08	2.57×10^{-8}	--	0.14
rs10193273	2:167633880	A/C	0.257	I	0.992	-0.39	0.07	2.75×10^{-8}	I	0.96	-0.17	0.13	0.22	-0.34	0.06	4.57×10^{-8}	--	0.15
rs4629176	2:167635250	T/C	0.257	I	0.991	-0.39	0.07	2.75×10^{-8}	I	0.959	-0.16	0.13	0.22	-0.34	0.06	4.57×10^{-8}	--	0.15

*G: genotyped, I:imputed. †Info represents quality of imputation in IMPUTE V2. Values closer to 1 indicate good quality of imputation.

Supplementary Table 3. Effects of Top loci ($P < 1\times10^{-5}$) associated with diabetic peripheral neuropathy in ACCORD : Adjustment by age, duration of diabetes, BMI.

SNP	Position‡	Closest gene	MA**	Primary model*		Full model†	
				OR (95%CI)	P	OR (95%CI)	P
rs13417783	2:167629849	XIRP2	T	0.64 (0.55-0.74)	1.9×10^{-9}	0.65 (0.56-0.78)	1.2×10^{-8}
rs12988669	2:240275570	HDAC4	C	0.71 (0.62-0.82)	2.7×10^{-6}	0.70 (0.61-0.82)	3.0×10^{-6}
rs60770880	3:8037416	LOC101-927394	A	0.75 (0.66-0.84)	5.0×10^{-6}	0.75 (0.66-0.85)	1.8×10^{-5}
rs11932946	4:45140214	GUF1	G	0.69 (0.59-0.82)	9.6×10^{-6}	0.70 (0.60-0.83)	3.9 x 10-5
rs1202660	7:70658177	WBSCR-17	T	0.74 (0.65-0.84)	6.2×10^{-6}	0.73 (0.63-0.83)	2.8×10^{-6}
rs13265430	8:4165607	CSMD1	A	0.64 (0.52-0.76)	1.0×10^{-6}	0.65 (0.54-0.78)	7.6×10^{-6}
rs2491019	10:70776987	KIAA12-79	A	1.31 (1.17-1.47)	4.4×10^{-6}	1.31 (1.16-1.47)	7.1×10^{-6}
rs77494074	11:132794801	OPCML	T	0.61 (0.50-0.74)	1.0×10^{-6}	0.60 (0.48-0.78)	8.9×10^{-7}
rs201655918	14:76791306	ESRRB	C	0.75 (0.66-0.85)	6.9×10^{-6}	0.75 (0.65-0.85)	9.3×10^{-6}
rs11073752	15:88423051	NTRK3	C	0.76 (0.67-0.85)	2.1×10^{-6}	0.76 (0.67-0.86)	6.6×10^{-6}
rs9948095	18:12018665	IMPA2	C	0.71 (0.61-0.82)	3.6×10^{-6}	0.72 (0.62-0.84)	1.9×10^{-5}
rs10555080	19:32043170	THEG5	A	1.32 (1.17-1.49)	6.9×10^{-6}	1.30 (1.15-1.48)	3.5×10^{-5}
rs34948558	21:42825856	MX1	A	0.76 (0.67-0.85)	4.7 x1 0^{-6}	0.76 (0.67-0.85)	7.8×10^{-6}

* Primary model is reported in the main manuscript in Table 2: additive genetic model, adjusted by assignment to ACCORD interventions, seven clinical centers, and top three principal components.

†Primary model + age, duration of diabetes and BMI.

‡Position is chromosome:bp according to the National Center for Biotechnology Information assembly build GRCh37/hg19.

**MA is minor or effect allele.

Supplementary Table 4. Association between top loci in the GoDarts GWAS and DPN in ACCORD.

SNP	Chr:bp*	Minor/major allele	MAF	ACCORD Model		GoDarts Model**	
				OR(95%CI)	P	OR(95%Ci)	P
12:5391393	12:5391393	A/G	0.125	0.94 (0.79,1.11)	0.44	0.95 (0.81,1.12)	0.56
12:5393329	12:5393329	T/C	0.125	0.94 (0.79,1.10)	0.43	0.95 (0.81,1.12)	0.56
12:5400620	12:5400620	T/C	0.126	0.91 (0.78,1.07)	0.28	0.93 (0.79,1.09)	0.36
12:5401196	12:5401196	G/A	0.127	0.92 (0.78,1.08)	0.32	0.93 (0.79,1.09)	0.36
12:5401450	12:5401450	G/T	0.126	0.91 (0.78,1.08)	0.28	0.93 (0.79,1.09)	0.36
12:5402869	12:5402869	C/T	0.131	0.91 (0.77,1.07)	0.23	0.92 (0.79,1.08)	0.29
12:5405457	12:5405457	T/C	0.128	0.92 (0.78,1.09)	0.34	0.94 (0.80,1.10)	0.44
rs4872521	8:21707713	G/C	0.266	1.07 (0.94,1.22)	0.28	1.09 (0.96,1.23)	0.17
rs4872522	8:21707844	C/A	0.266	1.07 (0.94,1.21)	0.31	1.09 (0.96,1.23)	0.20
rs10098807	8:21708824	A/G	0.265	1.08 (0.95,1.22)	0.26	1.09 (0.96,1.23)	0.17
rs11774105	8:21710146	C/T	0.265	1.08 (0.95,1.22)	0.25	1.09 (0.97,1.24)	0.16
rs17428041	8:21711431	C/T	0.265	1.07 (0.95,1.22)	0.27	1.09 (0.96,1.23)	0.17
rs17615364	8:21711580	A/G	0.265	1.07 (0.95,1.22)	0.27	1.09 (0.96,1.23)	0.17
rs11776842	8:21711651	C/A	0.266	1.07 (0.94,1.21)	0.32	1.08 (0.96,1.22)	0.22
rs12545534	8:21712401	A/G	0.265	1.08 (0.95,1.22)	0.25	1.09 (0.97,1.24)	0.17
rs11780601	8:21717841	T/G	0.231	1.10 (0.96,1.26)	0.15	1.13 (0.99,1.29)	0.07

*Position is chromosome:bp according to the NCBI assembly build GRCh37/hg19.
** Fisher's exact test integrated in PLINK.

Supplementary Table 5. Association between significant loci in candidate gene studies and DPN in ACCORD.

Gene	Original locus associated with DPN	Reference	SNP in ACCORD	Chr	bp	Minor allele*	Major Allele	MAF	OR	95%CI	P value
ACE	I/D intron 19	Li et al, 2014	rs4341	17	61565990	C	CG	0.462	1.03	(0.92,1.15)	0.64
AKR1B1	-106 C/T	Sivenius et al., 2004	rs759853	7	134143958	A	G	0.397	1.08	(0.96,1.21)	0.19
APOE	rs429358	Tzuzuki et al., 1998	rs429358	19	45411941	C	T	0.132	0.99	(0.84,1.16)	0.88
APOE	rs7412	Tzuzuki et al, 1998	rs7412	19	45412079	T	C	0.077	0.99	(0.8,1.21)	0.89
GPX1	rs1050450	Tang et al, 2012	rs1050450	3	49394834	A	G	0.308	0.98	(0.87,1.10)	0.74
IFNG	874 A/T	Kolla et al, 2009	rs2430561	12	68552522	A	T	0.465	1.03	(0.92,1.15)	0.63
IL10	-1082 G/A	Kolla et al, 2009	rs1800896	1	206946897	C	T	0.471	1.09	(0.97,1.21)	0.13
MTHFR	677 C/T	Yigit et al, 2013	rs1801133	1	11856378	A	G	0.351	0.99	(0.88,1.11)	0.81
NOS1AP	rs1963645	Margolis et al, 2014	rs1963645	1	162333990	G	A	0.373	0.84	(0.76,0.94)	0.003
NOS1AP	rs16849113	Margolis et al, 2014	rs16849113	1	162040878	T	C	0.059	0.90	(0.72,1.13)	0.37
NOS1AP	rs6659759	Margolis et al, 2014	rs6659759	1	162037609	C	T	0.293	1.04	(0.92,1.17)	0.53
NOS1AP	rs880296	Margolis et al, 2014	rs880296	1	162128446	G	C	0.216	1.04	(0.91,1.19)	0.57
TLR4	1196 C/T	Rudofsky et al., 2004	rs4986791	9	120475602	T	C	0.061	1.06	(0.84,1.34)	0.60
UCP2	-866 G/A	Yamasaki et al., 2006	rs659366	11	73694754	T	C	0.363	1.02	(0.91,1.14)	0.73

*Effect allele

Supplementary Table 6. Association of 2q24 variants with DPN in Whites and African Americans.

	Whites								African-Americans			
			ACCORD		BARI 2D		ACCORD+BARI2D		ACCORD			
	MAF	r^2	OR	P	OR	P	OR	P	MAF	r^2	OR	P
rs13417783	0.148	-	0.64	1.9×10^{-9}	0.57	8.9×10^{-4}	0.63	7.9×10^{-12}	0.077	-	1.04	0.813
rs145971945	0.134	0.74	0.63	2.0×10^{-9}	0.58	2.6×10^{-3}	0.62	2.1×10^{-11}	0.036	0.12	0.96	0.871
rs13000447	0.145	0.66	0.64	2.3×10^{-9}	0.68	2.8×10^{-2}	0.65	2.1×10^{-10}	0.242	0.16	0.94	0.575
rs10164425	0.136	0.73	0.64	2.6×10^{-9}	0.58	2.2×10^{-3}	0.63	2.4×10^{-11}	0.144	0.31	0.83	0.132
rs13392463	0.136	0.75	0.64	3.1×10^{-9}	0.60	3.8×10^{-3}	0.63	4.6×10^{-11}	0.121	0.42	0.82	0.138
rs13031854	0.134	0.82	0.63	3.4×10^{-9}	0.61	6.2×10^{-3}	0.63	7.5×10^{-11}	0.203	0.28	0.93	0.513
rs13421469	0.135	0.74	0.64	3.6×10^{-9}	0.59	2.7×10^{-3}	0.63	3.9×10^{-11}	0.038	0.28	0.98	0.933
rs2390487	0.136	0.73	0.64	3.6×10^{-9}	0.57	1.3×10^{-3}	0.63	2.1×10^{-11}	0.145	0.32	0.85	0.178
rs536046332	0.136	0.74	0.64	4.0×10^{-9}	0.61	5.0×10^{-3}	0.63	7.5×10^{-11}	0.125	NA	0.84	0.195
rs13026906	0.136	0.75	0.64	4.0×10^{-9}	0.60	3.8×10^{-3}	0.63	5.9×10^{-11}	0.124	0.36	0.81	0.123
rs13034083	0.136	0.75	0.64	4.0×10^{-9}	0.60	3.8×10^{-3}	0.63	5.9×10^{-11}	0.124	0.39	0.83	0.148
rs16852695	0.135	0.75	0.64	4.3×10^{-9}	0.60	3.8×10^{-3}	0.63	6.2×10^{-11}	0.124	0.37	0.83	0.162
rs10183769	0.136	0.74	0.64	4.4×10^{-9}	0.60	3.8×10^{-3}	0.63	6.3×10^{-11}	0.124	0.40	0.82	0.123
rs10178966	0.136	0.75	0.64	4.7×10^{-9}	0.60	3.8×10^{-3}	0.63	6.9×10^{-11}	0.122	0.41	0.83	0.162
rs10189266	0.136	0.75	0.64	4.7×10^{-9}	0.60	4.1×10^{-3}	0.63	7.3×10^{-11}	0.122	0.41	0.81	0.105
rs12993796	0.136	0.74	0.64	4.8×10^{-9}	0.60	4.2×10^{-3}	0.63	7.6×10^{-11}	0.182	0.26	0.93	0.490
rs35762912	0.136	0.74	0.64	5.0×10^{-9}	0.60	3.8×10^{-3}	0.63	7.2×10^{-11}	0.136	0.35	0.83	0.132
rs12692848	0.136	0.74	0.64	5.1×10^{-9}	0.58	2.2×10^{-3}	0.63	4.8×10^{-11}	0.126	0.39	0.83	0.151
rs12692849	0.136	0.74	0.64	5.1×10^{-9}	0.58	2.2×10^{-3}	0.63	4.8×10^{-11}	0.127	0.38	0.84	0.174
rs76036953 (rs35714834)	0.136	0.74	0.64	5.6×10^{-9}	0.58	2.5×10^{-3}	0.63	5.8×10^{-11}	0.185	0.25	0.95	0.631
rs7558617	0.136	NA	0.64	5.8×10^{-9}	NA	NA	0.64	5.8×10^{-9}	0.185	NA	0.94	0.603
rs34867578	0.136	0.74	0.64	8.1×10^{-9}	0.59	3.4×10^{-3}	0.64	1.1×10^{-10}	0.210	0.21	0.99	0.912
rs34148223	0.137	0.74	0.64	8.7×10^{-9}	0.61	5.3×10^{-3}	0.64	1.7×10^{-10}	0.124	0.39	0.82	0.138
rs16852735	0.139	0.67	0.65	1.3×10^{-8}	0.66	2.0×10^{-2}	0.65	8.0×10^{-10}	0.083	0.02	0.75	0.069
rs148112563	0.133	0.74	0.64	1.7×10^{-8}	0.58	2.9×10^{-3}	0.64	2.2×10^{-10}	0.184	0.24	0.94	0.562
rs10200297	0.138	0.73	0.66	2.6×10^{-8}	0.55	7.1×10^{-4}	0.64	1.1×10^{-10}	0.482	0.03	1.03	0.741
rs10193273	0.257	0.48	0.71	4.6×10^{-8}	0.69	1.0×10^{-2}	0.71	1.6×10^{-9}	0.265	0.23	1.01	0.891
rs4629176	0.257	0.48	0.71	4.6×10^{-8}	0.69	1.0×10^{-2}	0.71	1.6×10^{-9}	0.265	0.23	1.01	0.891

Supplementary Table 7. Baseline characteristics of ACCORD DPN cases and controls according to rs13417783 genotypes.

Baseline Characteristics	rs13417783 genotype	Cases+ Controls			Cases			Controls		
		N	Mean ± SD	P	N	Mean ± SD	P	N	Mean ± SD	P
BMI (kg/m2)	CC	3736	32.93 ± 5.23	0.44	3244	33.10 ± 5.26	0.25	492	31.87 ± 4.88	0.81
	CT	1322	33.02 ± 5.09		1051	33.34 ± 5.12		271	31.77 ± 4.75	
	TT	108	33.00 ± 4.76		87	33.00 ± 4.83		21	32.96 ± 4.61	
Height (cm)	CC	3737	171.46 ± 9.40	0.71	3245	171.73 ± 9.38	0.65	492	169.73 ± 9.36	0.19
	CT	1322	171.38 ± 9.78		1051	171.69 ± 9.81		271	170.17 ± 9.57	
	TT	108	171.53 ± 8.30		87	171.66 ± 8.35		21	170.98 ± 8.30	
Waist circumference (cm)	CC	3703	109.33 ± 13	0.64	3214	109.98 ± 13.01	0.91	489	105.10 ± 12.14	0.41
	CT	1308	109.15 ± 12.73		1037	110.14 ± 12.79		271	105.37 ± 11.77	
	TT	108	108.94 ± 11.22		87	109.13 ± 11.53		21	108.12 ± 10.09	
HbA1c (%)	CC	3736	8.21 ± 0.93	0.29	3245	8.22 ± 0.93	0.82	491	8.16 ± 0.95	0.13
	CT	1319	8.20 ± 0.92		1050	8.23 ± 0.92		269	8.09 ± 0.91	
	TT	108	8.07 ± 0.9		87	8.12 ± 0.86		21	7.89 ± 1.05	
Fasting serum glucose (mmol/L)	CC	3728	9.90 ± 2.81	0.94	3239	9.93 ± 2.81	0.85	489	9.75 ± 2.78	0.74
	CT	1317	9.92 ± 2.86		1048	9.96 ± 2.87		269	9.78 ± 2.75	
	TT	107	9.74 ± 2.73		87	9.86 ± 2.78		20	9.23 ± 2.51	
SBP (mmHg)	CC	3726	135.38 ± 16.65	0.37	3236	135.42 ± 16.68	0.68	490	135.07 ± 16.45	0.39
	CT	1314	134.51 ± 16.64		1045	134.59 ± 16.71		269	134.21 ± 16.42	
	TT	108	135.31 ± 17.7		87	135.90 ± 17.96		21	132.90 ± 16.78	
DBP (mmHg)	CC	3726	74.16 ± 10.48	0.19	3236	74.06 ± 10.44	0.29	490	74.81 ± 10.7	0.72
	CT	1314	74.34 ± 10.33		1045	74.13 ± 10.21		269	75.19 ± 10.78	
	TT	108	75.09 ± 10.3		87	75.01 ± 10.3		21	75.43 ± 10.55	
LDL (mmol/L)	CC	3722	2.67 ± 0.85	0.38	3235	2.66 ± 0.85	0.69	487	2.67 ± 0.84	0.30
	CT	1318	2.68 ± 0.84		1049	2.68 ± 0.83		269	2.70 ± 0.91	
	TT	107	2.67 ± 0.72		87	2.60 ± 0.72		20	2.98 ± 0.65	
HDL (mmol/L)	CC	3723	1.05 ± 0.27	0.16	3236	1.05 ± 0.27	0.36	487	1.09 ± 0.29	0.04
	CT	1318	1.03 ± 0.26		1049	1.02 ± 0.26		269	1.05 ± 0.28	
	TT	107	1.05 ± 0.30		87	1.05 ± 0.30		20	1.06 ± 0.30	
Total cholesterol (mmol/L)	CC	3722	4.74 ± 1.07	0.09	3235	4.73 ± 1.06	0.19	487	4.75 ± 1.09	0.34
	CT	1318	4.78 ± 1.06		1049	4.77 ± 1.04		269	4.81 ± 1.14	
	TT	107	4.75 ± 0.89		87	4.67 ± 0.90		20	5.10 ± 0.80	
Triglycerides (mmol/L) †	CC	3722	1.93 (1.34-2.76)	0.01	3235	1.94 (1.36-2.76)	0.05	487	1.82 (1.25-2.68)	0.05
	CT	1318	2.02 (1.39-2.96)		1049	2.00 (1.39-2.94)		269	2.03 (1.32-2.98)	
	TT	107	1.99 (1.33-2.96)		87	1.99 (1.33-2.90)		20	1.95 (1.31-3.28)	
eGFR (ml/min/1.73 m2) †	CC	3727	88.3 (72.9-103.3)	0.03	3238	88.0 (72.1-103.2)	0.12	489	90.0 (77.2-104.4)	0.27
	CT	1317	89.8 (76.1-104.9)		1048	89.5 (75.4-104.7)		269	90.7 (77.8-105.4)	
	TT	107	87.4 (72.1-104.8)		87	87.3 (72.4-102.3)		20	98.55 (70.2-106.4)	
UACR (mg/mmol) †	CC	3594	1.4 (0.7-4.1)	0.03	3122	1.4 (0.7-4.4)	0.05	472	1.0 (0.6-2.6)	0.80
	CT	1248	1.3 (0.7-3.7)		997	1.3 (0.7-3.7)		251	1.4 (0.7-3.6)	
	TT	101	1.2 (0.7-2.8)		80	1.6 (0.8-3.1)		21	0.7 (0.5-1.0)	

Values are means ± SD or medians (IQR) (†). P values are according to an additive genetic model, adjusted by assignment to interventions, seven clinical center networks, and top three principal components.

Supplementary Table 8. Effects of lead SNP rs13417783 on DPN in ACCORD after adjustment for risk factors.

Adjusted covariates*	OR (95%CI)	P
Age	0.66 (0.57,0.76)	1.45E-08
Duration of diabetes	0.66 (0.57,0.76)	9.65E-09
eGFR	0.66 (0.57,0.76)	1.23E-08
UACR	0.65 (0.56,0.75)	1.13E-08
Triglyceride	0.65 (0.56,0.75)	5.12E-09

* In ACCORD, the primary model was adjusted by assignment to interventions, 7 clinical center networks, and top 3 principal components. These covariates were added to the primary model.

Supplementary Table 9. Effects of lead SNP rs13417783 on prevalent and incident microvascular outcomes.

Outcome	Events/ N	OR (95%CI)	HR (95% CI)	P
Neph1: Microalbuminuria (urine albumin:creatinine ratio ≥ 3.4 mg/mmol)†	1,141/3,859	0.98 (0.85,1.12)	-	0.73
Neph2: Macroalbuminuria (urine albumin:creatinine ratio ≥ 33.9 mg/mmol)†	304/4,693	0.96 (0.76,1.22)	-	0.74
Neph3: Renal failure (Initiation of Dialysis or ESRD, or renal transplantation, or rise of serum creatinine >291.72 µmol/L in absence of an acute reversible cause)‡	142/5,146	-	0.95 (0.68,1.33)	0.78
Neph4: Doubling of baseline serum creatinine or >20 mL/min per 1.73 m^2 decrease in estimated GFR‡	3,078/5,163	-	0.97 (0.91,1.05)	0.45
Neph5: Any of Neph2/Neph3/Neph4	3,204/5,159	0.95 (0.84,1.07)	-	0.39
Eye1: Photocoagulation or Vitrectomy to treat retinopathy†	647/5,168	0.95 (0.80,1.12)	-	0.52
Eye2: Surgery for Cataract Extraction†	1304/5,168	0.92 (0.81,1.04)	-	0.18
Eye3: 3-line Worsened Visual Acuity‡	2183/4996	-	1.02 (0.94,1.11)	0.66
Eye4: Severe Vision Loss (Snellen fraction <20/200)†	900/4,652	0.91 (0.78,1.06)	-	0.23
Progression to Diabetic Retinopathy in ACCORD EYE Study*	132/1707	0.99 (0.69-1.42)	-	0.97

†Prevalent + incident cases vs. controls (subjects without the outcome of interest at study entry and during follow-up).

‡Incident cases vs. controls at study entry and during follow-up. GFR estimation was done on the basis of the four variable MDRD GFR equation from Levey and colleagues.

*Progression of diabetic retinopathy defined by ≥3 steps from baseline on the ETDRS (Early Treatment Diabetic Retinopathy Study Severity Scale) or the development of diabetic retinopathy requiring laser photocoagulation or vitrectomy.

All outcomes above were predefined in ACCORD as secondary microvascular outcomes. (1; 2)

Supplementary Table 10. Effects of lead SNP rs13417783 on prevalent and incident cardiac autonomic neuropathy.

Outcome	Cases*/N	OR (95%CI)	P
CAN1	768/3,863	0.96 (0.82,1.13)	0.61
CAN2	314/3,847	1.11 (0.88,1.40)	0.37
CAN3	201/3,812	1.08 (0.82,1.44)	0.57

CAN1 is defined as the lowest quartile of standard deviation of N interval (SDNN) (<7.815 ms) and the highest quartile of QT-index (QTI) (>104.32%). Thresholds derived from the baseline electrocardiogram (ECG);
CAN2 is defined as the lowest quartile of SDNN and the highest quartiles of QTI and resting heart rate (>99bpm);
CAN3 is defined as the lowest quartile of SDNN and the highest quartiles of QTI and heart rate, in the presence of DPN (3).
*CAN1, CAN2, and CAN3 cases are those that met the criteria either at baseline or during follow-up, and controls are those that did not meet the criteria both at baseline and during follow-up.

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Paper 2

A genetic locus on chromosome 1p36 associated with cardiovascular autonomic neuropathy in type 2 diabetes.

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Abstract

Cardiovascular autonomic neuropathy (CAN) is an independent predictor of cardiovascular disease (CVD) morbidity and all-cause and CVD mortality in diabetes. In light of its significant heritability, we aimed to identify genetic predictors of CAN through an unbiased genome-wide association study (GWAS) among Whites in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) cohort, comprising individuals with Type 2 Diabetes (T2D) and high CVD risk. CAN was defined based on indices of heart rate variability derived from 10-s resting electrocardiograms, namely, as the lowest quartile of standard deviation of normally conducted R-R intervals (SDNN <7.813 ms) and highest quartile of QT index (QTI >104.32%). Applying logistic regression models, we conducted a GWAS testing 6.8 million common single nucleotide polymorphisms (SNPs), with cases (N=807) having CAN at baseline and/or during follow-up and controls (N=3,144) being without CAN at end of follow-up. The top signal, rs779142, on chromosome 1p36, was associated with 35% increased odds of CAN (OR=1.35 [1.20 – 1.51], $P=1.9 \times 10^{-7}$). Among incident cases (N=499), this SNP reached GWAS significance (OR=1.48 [1.29 – 1.69], $P=1.7 \times 10^{-8}$). In a time-to-event analysis, rs779142 was associated with increased risk of progression to CAN (HR=1.42 [1.25 – 1.61], $P=1.8 \times 10^{-8}$) – an effect that was independent of the glycemic control arm assignments within the trial (P for interaction = 0.35). Genes lying within 1Mb of the lead SNP included *CAMTA1* – a calmodulin binding transcription factor affecting cardiac embryonal development, *KCNAB2* – a potassium voltage-gated channel associated with fatal cardiac arrhythmia, and *RNF207* – a RING finger protein regulating heart action potential duration. To conclude, we have identified a locus on 1p36 having a genome-wide significant effect on the development of incident CAN in T2D. Pending further studies, these findings may provide better mechanistic insights into CAN pathophysiology as well as potential new therapeutic targets against this complication.

Introduction

Cardiovascular autonomic neuropathy (CAN) - a serious and common complication of diabetes – independently predicts cardiovascular disease (CVD) morbidity and mortality in diabetes [1-3]. Despite a prevalence as high as 60% among diabetic subjects [1], CAN is easily overlooked due to its poor correlation with specific clinical symptoms and signs, and its silent progression. When symptomatic, it commonly manifests itself as light-headedness, weakness, visual impairment upon standing, syncope, and/or palpitations in the resting state. At the subclinical stage, it can be diagnosed based on abnormally low heart rate variability (HRV) and elongation of QT corrected interval (QTc) on electrocardiogram, reflecting an imbalance between cardiac parasympathetic and sympathetic tone, which contribute to the pathogenesis of this complication [4]. Low HRV can be detected in the resting state or after maneuvers aimed at inducing variation in heart rate such as deep breathing, lying-to-standing, and Valsalva maneuver (collectively known as cardiovascular autonomic reflex tests or CARTs). Modifiable risk factors for CAN include hyperglycemia, obesity, dyslipidemia, hypertension, and smoking [5]. Targeting hyperglycemia to prevent CAN has shown inconsistent results [6][7][8], and evidence in support of a beneficial effect of targeting other risk factors is scant. Treatment is mainly focused on alleviating symptoms [1].

In clinical practice, a significant heterogeneity is observed in the occurrence and manifestation of CAN among diabetic subjects. Part of this heterogeneity is explained by variability in the occurrence of risk factors for CAN such as age, duration of diabetes, degree of hyperglycemia and presence of other diabetic complications such proliferative retinopathy and microalbuminuria [9], but based on twin studies, up to 40% of the variance in CAN occurrence (so call heritability) is accounted for by genetic factors [10]. However, data on genetic

determinants of CAN are scant in the literature. A few studies on candidate genes, such as *TCF7L2, TCFalpha*, were carried out without replication attempts [11]. While genome-wide association studies (GWAS) were done for HRV, resting heart rate (HR), and QT interval, these did not specifically target the combination of these components that defines CAN. Moreover, these studies were conducted in the general population rather than in patients with diabetes.

Here we present the results of the first GWAS specific to CAN, which we conducted in the population with type 2 diabetes of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) clinical trial. In this study, we found a strong genetic association signal at a locus on chromosome 1p36, reaching genome-wide significance for incident CAN. Nearby genes that could mediate this genetic effect include voltage-gated potassium voltage-gated channel (*KCNAB2*) , calmodulin binding transcription activator (*CAMTA1*), and RING finger protein 207 (*RNF 207*). These findings may provide novel insights into the pathogenesis of CAN and point to new pharmacological and/or and pharmacogenetic targets to prevent or treat this complication of diabetes.

Methods

Study population: ACCORD trial

The design of the ACCORD clinical trial has been previously described [12]. Briefly, ACCORD aimed to investigate whether cardiovascular event rates could be reduced by targeting three important cardiovascular risk factors - hyperglycemia, high blood pressure, and dyslipidemia. For this purpose, 10,251 participants with type 2 diabetes and high cardiovascular risk were randomized in a 1:1 ratio to receive intensive (aiming at HbA1c < 6.0 % [42 mmol/mol]) or standard (aiming at Hba1c 7- 7.9%[53- 64 mmol/mol]) glycemic control at 77

clinical sites across the U.S. and Canada [13] . At the same time, 4,733 of these participants were randomly assigned to intensive (aiming at SBP < 120 mmHg) or standard (aiming at SBP <140 mmHg) BP control and 5,518 participants were randomly assigned to treatment with simvastatin plus fenofibrate or simvastatin plus placebo. The primary outcome of ACCORD trial was the first occurrence of a major cardiovascular event, which was defined as the composite of non-fatal myocardial infarction, non-fatal stroke, or cardiovascular death. The occurrence of diabetic complications, including CAN, was monitored closely during the trial. Recruitment occurred from 2001 to 2005, and the trial ended in June 2009. 8,174 out of the 10,251 ACCORD participants consented to genetic studies, 5,360 of whom were White. To minimize confounding by race, our GWAS was restricted to White participants who had CAN evaluated at baseline (n=3,951).

Outcome definition: CAN

CAN was evaluated in ACCORD as a composite of low HRV and long QTI, both of which were derived from 12-lead digitized electrocardiograms (ECGs) recorded over 10 consecutive seconds with the patient resting supine after an overnight fast. HRV was defined as the SD of normally conducted R-R intervals (SDNN), which captures both sympathetic/parasympathetic control. QTI was defined as the time between the onset of ventricular activation and the end of repolarization, mainly capturing sympathetic input. CAN indices derived from the composite of these two measures have shown high predictive value for morbidity and mortality in large cohorts [1, 14, 15]. In ACCORD, CAN was defined as the co-occurrence of SDNN < 7.813 ms (the lowest quartile of baseline SDNN) and QTI > 104.32% (the highest quartile of baseline QTI). The combination of these two traits at study entry was associated with a 55% and 94% increase in the hazard of all-cause mortality and CVD mortality,

respectively [2]. For the purpose of the GWAS, CAN cases were defined as patients having CAN at study entry and/or at any time during the follow-up. CAN controls were defined as either SDNN ≥ 7.813ms or QTI ≤ 104.32% at study entry and each follow-up visit. In ACCORD, ECG was taken at baseline and every two years. ECG digitalization, quality control procedures, and calculations of SDNN and QTI were previously described [2].

DNA extraction and genotyping: ACCORD

Detailed DNA extraction, genotyping, quality control (QC) and imputation procedures were described by Shah et al. in the first ACCORD GWAS paper [16]. In brief, 8,174 out of the 10,251 participants in ACCORD consented to genetic studies. These included subjects who had consented to 1) genetic studies only by ACCORD investigators; and 2) genetic studies by any investigators. DNA samples from the 8,174 participants who gave either type of consent were genotyped at the University of North Carolina on Affymetrix Axiom Biobank chips with 628,679 probes (ACCORD UNC genotyping set). DNA Samples from the 6,085 participants who provided consent to studies by any investigator were genotyped at the University of Virginia on Illumina HumanOmniExpressExome-8 (v1.0) chips with 951,117 probes (ACCORD UVA genotyping set). After extensive harmonization and quality control procedures, data from the two genotyping sets were merged and organized in two non-overlapping datasets: ANYSET, including a total of 1,263,585 SNPs for each subject in the ACCORD UVA set (n= 5,971) genotyped at either UVA or UNC, and ACCSET, including a total of 572,192 SNPs for each subject in the ACCORD UNC who were not in the ACCORD UVA set (n= 2,113) genotyped only at UNC. Imputation was conducted in ANYSET and ACCSET separately by means of IMPUTE v2.3.1 [17], using the full 1000 Genomes Phase 3 (Oct 2014) as reference. Imputed

SNPs with an information content < 0.3 were discarded, resulting in 25,017,489 SNPs in ANYSET and 25,012,865 SNPs in ACCSET.

Statistical analysis

Descriptive data

All analyses were performed with SAS v9.4 (Cary, NC). CAN was coded as present (1) or absent (0) based on the SDNN and QTI values, as described above. Normally distributed continuous variables were presented as mean ± standard deviation (SD) and analyzed by independent t-test for difference in means between groups. Non-normally distributed continuous variables were presented as median (inter-quartile range) values and analyzed by t-test after log transformation. Categorical variables were presented as counts (percentages) and analyzed by chi-square tests to examine differences among groups.

Genome-wide association study

The GWAS analysis was carried out in SAS v9.4 (Cary, NC) on the Odyssey cluster supported by the FAS Division of Science, Research Computing Group at Harvard University. Among the 3,951 whites included for the GWAS of CAN in ACCORD, the genetic dataset ACCSET included 156 cases and 591 controls whereas ANYSET included 651 cases and 2,553 controls. A GWAS was conducted in the two sets separately, testing a total of 6,857,206 common SNPs (filtered for a minor allele frequency [MAF≥5%] for association between their minor allele dosages and CAN by means of logistic regression. The primary regression model was adjusted by trial assignment, seven clinical centers, and top three principal components (PC1-PC3, derived from principal component analysis of the merged data set). The indicator of trial assignments included: 1) randomization to intensive vs. standard glycemic therapy; 2)

randomization to BP vs. lipid trial; 3) randomization to intensive vs. standard BP control in the BP trial; 4) randomization to fenofibrate or placebo in the lipid trial.

Sensitivity analyses

The lead SNPs at loci reaching notable significance ($P<1\times10^{-6}$) were further evaluated by means of the following analyses: 1) Effect of SNPs after limiting CAN cases to prevalent cases (i.e., having CAN at the study entry) or incident cases (i.e., free of CAN at the study entry but developing CAN during follow-up); 2) Effect of SNPs on the individual components of CAN (SDNN < 7.813 ms or QTI > 104.32%); 3) Effect of the SNPs on CAN in different treatment or baseline strata, and effect modification of these strata by adding appropriate interaction terms; 4) Effect of SNPs on CAN after adjustment for known clinical risk factors for CAN; 5) Kaplan-Meier and Cox regression analyses of the effect of the SNPs on time to development of CAN among individuals free of CAN at baseline.

Functional analyses

Regional plots of the lead SNP were generated using LocusZoom (Abecasis Lab, University of Michigan School of Public Health) through http://locuszoom.sph.umich.edu/genform.php?type=yourdata. The reference database was hg19/1000 Genomes Nov 2014 EUR. Significant SNPs ($P<1\times10^{-6}$) from the top locus were tested in the RegulomeDB (http://www.regulomedb.org/) to annotate their regulatory elements. Source of RegulomeDB include public datasets from GEO, the ENCODE project and published literature [18]. Expression Quantitative Trait Loci (eQTL) analysis was conducted using the Genotype Tissue Expression (GTEx) database V7 [19]. GTEx is an online database containing tissue-specific gene expression data obtained from 620 donors (34% females, 85% Whites, 68% more than 50 years of age) by means of Illumina TrueSeq RNA sequencing and the Affymetrix

Human Gene 1.1 ST Expression Array, and linked to GWAS data obtained from the same individuals by whole genome sequencing, whole exome sequencing, or Illumina SNP arrays (www.gtexportal.org). In GTEx, we tested the association between the top SNP and the expression of its nearby genes (±2 Mbp) in: 1) heart atrial appendage from 264 genotyped individuals; 2) heart left ventricle from 272 genotyped individuals.

Results

Clinical Characteristics of CAN cases and controls

Of the 5,360 White participants who consented to genetic studies, 3,951 were evaluated for CAN at baseline, including 308 prevalent cases and 3,643 prevalent controls. Of the prevalent controls, 499 developed CAN during follow-up. In total, the discovery set included 807 prevalent + incident cases and 3,144 controls. The baseline characteristics of cases and controls are summarized in Table 1. Compared to the controls, cases were taller and had a longer duration of diabetes, greater waist circumference, and higher HbA1c, SBP, DBP, triglycerides, and UACR. In addition, cases were more frequently female and had a higher prevalence of retinopathy and insulin use. No significant differences were found between the incident and prevalent cases, except for a higher prevalence of prior CVD events among the former (Supplementary Table 1). The medication used at the study entry, at 12 months, and at the last visit are summarized in Supplementary Table 2.

Genome-wide association study of CAN

A total of 6,857,206 common variants were tested for association with CAN. While no SNP reached genome-wide significance ($P=5\times10^{-8}$), four SNPs at a locus on 1p36, all in strong linkage disequilibrium with each other in Whites, met the notable significance threshold

($P<1\times10^{-6}$) (Figure 1 and Supplementary Table 2). The lead SNP (rs779142, MAF=0.366) was associated with a 35% increase in the odds of CAN for each copy of the minor allele (OR = 1.35 [1.20, 1.51] $p=1.9\times10^{-7}$). Two additional SNPs at 1p36 and 12 at other locations reached p values between 1.0×10^{-5} and 1.0×10^{-6} (Table 2 and Supplementary Table 2). Of the previous loci reported to be associated with either SDNN or QTI, 12 showed nominal significance for association with CAN in ACCORD ($P<0.05$, Supplementary Table 4).

Association between 1p36 locus and incident CAN

The effect of the lead SNP at 1p36 (rs779142) was stronger and reached genome-wide significance if the analysis was restricted to incident as opposed to prevalent cases (OR= 1.48 [1.29, 1.69], $P = 1.7 \times 10^{-8}$ vs. OR = 1.14 [0.96, 1.36], P = 0.12) (Table 3). Accordingly, a time-to-event analysis in the incident cohort showed a genome-wide significant association of rs779142 with a diagnosis of CAN during follow-up (HR = 1.42[1.25,1.61], $P = 1.8 \times 10^{-8}$), as illustrated by the Kaplan-Meier curves in Supplementary Figure 2. Both components of CAN (SDNN < 7.813 ms and QTI > 104.32%) were individually associated with the 1p36 locus (OR=1.15, p=0.0043 and OR=1.20, p=0.0001, respectively), and similar to the analysis of CAN as a composite outcome, the association mainly concerned incident cases (Table 3). The effect of the lead SNP on incident CAN was not affected by the interventions tested in ACCORD (intensive glycemic control Vs. standard glycemic control, intensive BP control Vs. standard BP control, statin + fenofibrate Vs. statin) (Supplementary Figure 3).

Association between 1p36 locus and clinical characteristics

A nominal association was observed between 1p36 risk allele and systolic blood pressure (P = 0.03, Supplementary Table 6). However, the effect of 1p36 on CAN was not attenuated after adjustment for this variable or for other known CAN risk factors such as age, sex, duration of

diabetes, prior CVD events, and DBP, or for treatment with beta-blockers, TZD, or ACEI/ARB. No differences were observed in the effect of the 1p36 locus between participants with a prior CVD event and those without, or between strata based on other baseline characteristics (p for interaction >0.05 for all, Supplementary Figure 2).

Association between 1p36 locus and other diabetic complications

A nominal association was observed between 1p36 locus and loss of light touch, as evaluated by 10g force monofilament (OR = 1.13[1.01, 1.28], P =0.04). However, no association was found with other features of diabetic peripheral neuropathy or with other diabetic complications such as diabetic nephropathy and retinopathy (Supplementary Table 7).

Association between 1p36 locus and gene expression

The lead variant associated with CAN is located within the intron of *CAMTA1* (Figure 2) and in the vicinity of many other genes including *KCNAB2*, *RNF207*, and others. In the GTEx database, expression data concerning the heart atrial appendage and the left ventricle were available for 34 and 30, respectively, of the genes placed in a 2 Mbp radius from the lead SNP. Carriers of the rs779142 minor allele showed higher expression of *PHF13* and *RERE* in the atrial appendage, lower expression of *ESPN* in the left ventricle, and lower expression of *KLHL21* in both (Table 4). Of the 6 SNPs on 1p36 having $P<1\times10^{-5}$, rs6657847 was the highest ranking for a functional effect in the RegulomeDb, where it was shown to affect binding to *RFX3* - a ciliogenic transcription factor.

Discussion

Through the first GWAS of diabetic CAN conducted to date, we have identified a locus on chromosome 1p36 having a powerful effect on the risk of this complication among

individuals with type 2 diabetes from the ACCORD clinical trial. This genetic effect was independent of the interventions tested in ACCORD and was not mediated by effects on known clinical predictors of CAN. Pending future validation, the identification of this locus may assist in the identification of individual at high risk of CAN and may provide new insights into the pathogenesis of this complication, which may in turn point to targets for new pharmacological interventions aimed at preventing this complication of diabetes.

The genetic effect associated with the 1p36 locus was especially evident for incident CAN, reaching genome-wide significance in the analysis of this outcome. The reason for a stronger association with incident than prevalent CAN is unclear. As compared to incident cases, prevalent CAN cases were characterized by a more frequent history of CVD and more frequent treatment with diuretics. One possibility is that the more frequent CVD may have led to ECG alterations that mimicked CAN among prevalent cases, thereby leading to false positives, which might have attenuated the evidence of association with the SNP.

The SNPs associated with CAN are placed in a gene-rich region on chromosome 1p36, the deletion of which causes a syndrome (known as "1p36 deletion syndrome") characterized by neurodevelopmental defects, seizures, congenital heart defects, and cardiomyopathy [20]. The lead SNP, rs779142, lies within an intron of *CAMTA1* coding for a calmodulin-binding transcription activator, which acts as a calcium-sensitive regulator of gene expression. *CAMTA1* is expressed in the central nervous system where it is known to regulate cerebellar functions [21] and memory formation [22], but is also expressed at high levels in the embryonic heart [23] where it is required for the differentiation of stem cells into cardiomyocytes [24]. Another potential candidate gene at this locus is KCNAB2 coding for voltage-gated K+ channel beta2 subunit, which activates K+ channels by converting NADPH to NADP+ [25]. Highly expressed

in the human heart, this protein has been shown to specifically regulate potassium channel Kv 4.3 [26], which accounts for fast transient outward K+ current and controls the pace of ventricular repolarization. *KCNAB2* mutations are responsible for the Brugada syndrome - a cardiac arrhythmia associated with sudden death [26]. Finally, rs779142 is placed 600 Kb downstream from a SNP (rs846111) that was found to be significantly associated with QTI in a GWAS of the general population. While SNP rs846111 and rs779142 are not in linkage disequilibrium, they may both affect the expression of the same gene relevant to heart conduction, such as for instance *RNF207* (RING finger protein 207), which is placed near rs846111 and regulates heart action potential duration [27]. In the GTEx database, no association could be found between rs779142 and the expression of *CAMTA1*, *KCNAB2*, and *RNF207* in the heart. Rather, the SNP showed nominal associations with other genes that have not been implicated in cardiac innervation or autonomic function. However, in interpreting these data, one should consider that the gene expression data from the cardiac tissues collected in GTEx database (heart appendage and left ventricle) mostly reflect gene expression in cardiomyocytes rather than in the neural fibers innervating the heart. Furthermore, the GTEx data concern tissues collected from the general population rather than diabetic subjects and therefore cannot account for interactions that may be present between genes and the diabetic milieu. Further studies are clearly needed to understand the biological mechanisms underlying the observed genetic association with CAN.

No other GWAS of CAN has been reported in the literature, but data from GWAS on the individual traits used to define CAN in ACCORD – SDNN and QTI – are available. Some of the loci that were found to be associated with QTI (but not with SDNN) showed nominal significance for association with CAN in our study. However, while QTI reflects the function of

the autonomic system, it also depends on cardiac electrophysiology, drug treatments, and electrolyte levels. Additionally, these QTI GWAS were conducted in the general population. Thus, the relevance of these findings to diabetic CAN is unclear, although a contribution of these loci cannot be excluded.

Strengths of our GWAS include the high-quality of clinical data resulting from a clinical trial, including the frequent follow-up, standardized adjudication of outcomes, and rich data on various traits. Also, application of the GWAS approach allowed a systematic search of the entire genome for genetic determinants of CAN without the need for a priori hypotheses. However, some limitations should be acknowledged. First, CAN was defined based on two resting ECG parameters – SDNN and QTI – rather than on the diagnostic gold standard of abnormalities of dynamic cardiovascular autonomic reflex tests (CARTS) since the latter were not available in ACCORD due to the complexity of conducting CARTS in a large clinical trial. However, while the definition of CAN used in our study may have been sub-optimal, the composite SDNN/QTI variable was found to be a strong predictor of cardiovascular mortality in both type 1 and type 2 diabetes [14] as well as in the general population [15], to the point that current guidelines recommend its use to evaluate CAN in large trials [1]. Second, our study, although it included thousands of patients, was only powered to identify loci with large effects on CAN, owing to the stringent significant criteria that are applied to GWAS in order to account for multiple comparisons. Thus, smaller genetic effects may have gone undetected, especially those associated with less frequent variants. Third, to minimize the risk of population stratification, the genetic study was restricted to Whites. Whether these findings can be generalized to other population remains to be determined. Finally, even if the association with incident CAN reached

genome-wide significance, there is still the possibility of a false positive. Thus, these findings should be taken with caution until they are replicated in independent cohorts.

Conclusions

In summary, we have identified a genetic locus at 1p36 showing a strong association with the risk of CAN among patients with type 2 diabetes. This genetic marker may help clinicians identify diabetic patients at increased risk of CAN so that these can be targeted with aggressive interventions aimed at normalizing glycemic control and eliminate other modifiable risk factors. Functional studies of the mechanisms underlying this genetic effect may provide novel insights into the pathogenesis of CAN, which may, in turn, suggest molecular targets for the development of novel preventive treatments.

Author Contributions

YT, HS and AD designed the study, acquired, analyzed, and interpreted the data from ACCORD. YT and AD wrote the initial draft of the manuscript and revised the manuscript to its final form. XS analyzed data from ACCORD. BAP, RPB, and BC contributed to the study design, interpreted the data, and revised the manuscript to its final form. JCM, MJW, AAM-R, and JBB acquired, analyzed and interpreted the data, and revised the manuscript to its final form. The content of the paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other funding entities. AD is the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Y1-HC-1010. Other components of the National Institutes of Health, including the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute on Aging, and the National Eye Institute, contributed funding. The Centers for Disease Control and Prevention funded substudies within ACCORD on cost effectiveness and health-related quality of life. General Clinical Research Centers and Clinical and Translational Science Awards provided support at many sites. Portions of this research were conducted on the Orchestra High Performance Computing Cluster, supported by the Research Computing Group, at Harvard Medical School. See http://rc.hms.harvard.edu for more information.

Duality of Interest

YT, XS, and HS have nothing to disclose.
RPB reports grants from Astra Zeneca, outside the submitted work;
BAP reports grants and other from Boehringer Ingelheim, personal fees from Medtronic, personal fees from Abbott, personal fees from Insulet, personal fees from Dexcom, personal fees from Astra Zeneca, personal fees from Janssen, grants and personal fees from Bank of Montreal, personal fees from Neurometrix, outside the submitted work;
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AD reports grants from NIH, during the conduct of the study; grants from Sanofi Aventis, outside the submitted work.

Data Availability

The ACCORD database is available upon request from the National Heart, Lung, and Blood Institute Biologic Specimen and Data Repository (https://biolincc.nhlbi.nih.gov/studies/accord/). The ACCORD GWAS data have been deposited in the database of Genotypes and Phenotypes (dbGAP, Study Accession: phs001411.v1.p1).

Presentation

This study will be presented as an oral abstract at the 79th Scientific Sessions of the American Diabetes Association, San Francisco, California, 7-11 June 2018.

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Table 1. Characteristics of cardiac autonomic neuropathy (CAN) cases and controls.

Baseline characteristic	Discovery Set (ACCORD)		
	CAN Cases (N=807)	CAN Controls (N=3,144)	P-value
Female	366 (45.4)	1,093 (34.8)	<0.0001
Age (years)	62.7 ± 6.5	62.2 ± 6.2	0.07
DM duration (years)	11.8 ± 7.7	10.3 ± 7.3	< 0.0001
BMI (kg/m^2)	33.5 ± 5.2	32.9 ± 5.2	0.0012
Waist circumference (cm)	110.5 ± 13.0	109.0 ± 12.8	0.0023
Height (cm)	170.6 ± 9.6	171.5 ± 9.4	0.02
HbA1c (%)	8.29 ± 1.00	8.20 ± 0.93	0.02
Fasting glucose (mg/dL)	179.3 ± 54.2	179.3 ± 49.3	0.98
SBP (mmHg)	136.9 ± 17.9	134.8 ± 16.6	0.0028
DBP (mmHg)	75.5 ± 11.0	74.3 ± 10.2	0.0057
LDL (mg/dL)	104.0 ± 33.3	103.3 ± 33.1	0.61
HDL (mg/dL)	40.7 ± 11.0	40.4 ± 10.4	0.61
Women	44.7 ± 11.9	45.2 ± 11.0	0.43
Men	37.3 ± 8.9	37.9 ± 9.0	0.22
Total cholesterol (mg/dL)	183 (157- 210)	178 (155-207)	0.80
Triglycerides (mg/dL)†	185 (127- 266)	173 (121-251)	0.03
eGFR (ml/min/1.73 m^2)	88.7 ± 23.1	88.9 ± 21.1	0.83
UACR (mg/mmol)†	1.5 (0.7-5.3)	1.3 (0.7 – 3.4)	<0.0001
Previous CV event*	293 (36.3)	1,025 (32.6)	0.05
Report of retinopathy	110 (15.6)	257 (9.1)	< 0.0001
Current smoker	92 (11.4)	326 (10.4)	0.42
Previous smoker	396 (55.5)	1,594 (56.8)	0.42
Insulin therapy	363 (45.1)	1,012 (32.3)	<0.0001
ACCORD Glycaemia trial			
Standard	419 (51.9)	1,587 (50.5)	0.46
Intensive	388 (48.1)	1,557 (49.5)	
ACCORD BP trial	352 (43.6)	1,342 (42.7)	0.64
Standard	193 (23.9)	660 (21.0)	0.06
Intensive	159 (19.7)	682 (21.7)	
ACCORD Lipid trial	455 (56.4)	1,802 (57.3)	0.64
Fibrate	248 (30.7)	915 (29.1)	0.16
Placebo	207 (25.7)	887 (28.2)	

Except where noted, data are means ± SD for continuous variables and counts (%) for categorical data. †Medians (IQR). *Prior cardiovascular event: In ACCORD, this includes secondary prevention status or history of myocardial infarction, stroke, angina and/or ischemic changes (ECG) on Graded Exercise Tolerance Test or positive imaging, coronary revascularization procedures or other revascularization procedures at baseline.

Table 2. Association between top loci (P <1×10 $^{-5)}$ and CAN in ACCORD.

SNP	Position‡	bp	Closest gene	Minor/ Major	MAF¶ 1000G	MAF§ cases	MAF§ controls	OR (95% CI)	P
rs779142	1	6883843	CAMTA1	G/A	0.391	0.421	0.351	1.35(1.20-1.51)	1.91E-07
rs35097942	1	190245090	FAM5C	G/T	0.171	0.211	0.165	1.43(1.23-1.66)	2.09E-06
rs200687865(aka rs1320465270)	12	6378549	PLEKHG6	TT/-	NA	0.205	0.261	0.71(0.62-0.82)	2.51E-06
rs17723514	18	63554245	CDH7	G/A	0.329	0.374	0.313	1.32 (1.18-1.48)	2.69E-06
rs576246198	20	50236117	ATP9A	A/G	0.155	0.202	0.152	1.45 (1.24-1.69)	2.75E-06
rs4407861	8	56527642	XKR4 / TMEM68	T/C	0.174	0.226	0.175	1.39 (1.21-1.59)	3.16E-06
rs12682865	9	116742214	ZNF618	C/T	0.315	0.371	0.313	1.32 (1.17-1.48)	3.31E-06
rs28631956	16	22789410	MIR548AA2	T/A	0.286	0.306	0.254	1.37(1.20-1.56)	3.39E-06
rs1510021	18	1928842	METTL4	T/G	0.371	0.468	0.405	1.30(1.17-1.46)	3.63E-06
rs377096647	10	67415967	LINC01515	-/A	0.129	0.142	0.107	1.48(1.25-1.75)	4.37E-06
rs2497665	10	116362500	ABLIM1	A/G	0.046	0.077	0.049	1.72(1.36-2.18)	6.31E-06
rs200207763	5	160063990	ATP10B	-/CCAC	0.358	0.314	0.368	0.74(0.65-0.85)	8.32E-06
rs34079572	16	4271382	SRL	C/T	0.313	0.245	0.300	0.74(0.66-0.85)	8.51E-06

In ACCORD, the primary model was adjusted by assignment to interventions, 7 clinical center networks, and top 3 principal components.‡Position is chromosome:bp according to the National Center for Biotechnology Information assembly build GRCh37/hg19. §MAF is the average of minor allele frequencies in the two ACCORD genotyping sets (ANYSET and ACCSET). ¶MAF in 1000 genomes Project Phase 3 EUR populations was derived from Ensembl GRCh37 Release 93 (http://grch37.ensembl.org/index.html).

Table 3. Association between top loci and prevalent/incident CAN in ACCORD.

	CAN			SDNN < 7.813 ms (25th baseline percentile)		QTI > 104.32 % (75th baseline percentile)	
	N cases / N controls	OR (95% CI)	HR (95% CI)	OR (95% CI)	HR (95% CI)	OR (95% CI)	HR (95% CI)
Prevalent Cases vs. Controls	308/3,144	1.14 (0.96, 1.36)	-	1.00 (0.90, 1.12)	-	1.07 (0.96. 1.19)	-
Incident Cases vs. Controls	499/3,144	1.48 (1.29, 1.69)	1.42 (1.25, 1.61)	1.15(1.04, 1.28)	1.14 (1.05, 1.24)	1.22 (1.10, 1.35)	1.17 (1.08, 1.27)
Overall	807/3,144	1.35 (1.20, 1.51)	-	1.15 (1.04, 1.26)	-	1.20 (1.09, 1.32)	-

Table 4. eQTL analysis of locus 2q24 showing association between rs779142 and expression of neighboring genes (± 2Mbp) in the heart atrial appendage and left ventricle.

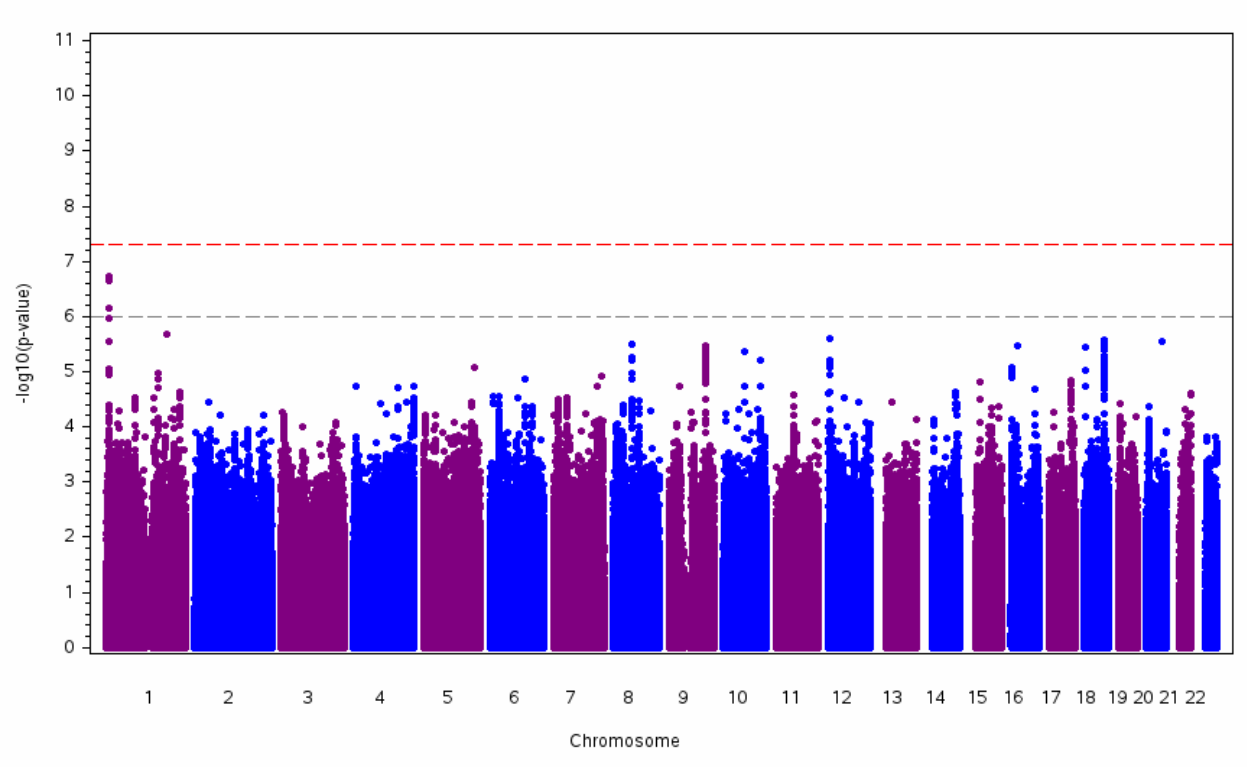
	Atrial Appendage			Left Ventricle		
Gene	**NES**	**95%CI**	**P**	**NES**	**95%CI**	**P**
ACOT7	0.028	[-0.07,0.13]	0.58	-0.058	[-0.15,0.03]	0.19
CAMTA1	0.04	[-0.03,0.11]	0.25	0.0055	[-0.08,0.09]	0.9
CHD5	-0.023	[-0.18,0.14]	0.78	NA	NA	NA
DNAJC11	-0.016	[-0.08,0.05]	0.61	-0.037	[-0.09,0.02]	0.21
ENO1	-0.011	[-0.08,0.06]	0.76	0.024	[-0.07,0.11]	0.6
ERRFI1	0.027	[-0.06,0.12]	0.55	-0.009	[-0.10,0.08]	0.85
ESPN	0.021	[-0.10,0.14]	0.74	-0.18	[-0.32,-0.04]	0.012
GPR153	0.016	[-0.13,0.17]	0.84	0.07	[-0.08,0.22]	0.35
ICMT	0.039	[-0.04,0.12]	0.32	0.016	[-0.04,0.08]	0.6
KCNAB2	0.055	[-0.05,0.16]	0.32	0.054	[-0.07,0.18]	0.4
KLHL21	-0.1	[-0.19,-0.01]	0.031	-0.17	[-0.27,-0.07]	0.0017
NOL9	-0.0039	[-0.09,0.08]	0.93	-0.077	[-0.18,0.02]	0.14
NPHP4	0.03	[-0.07,0.13]	0.56	0.0075	[-0.11,0.12]	0.89
PARK7	-0.034	[-0.09,0.02]	0.24	-0.029	[-0.09,0.03]	0.34
PER3	0.087	[-0.06,0.23]	0.23	0.12	[0.00,0.24]	0.064
PHF13	0.095	[0.01,0.18]	0.031	0.031	[-0.07,0.13]	0.54
PLEKHG5	0.0025	[-0.09,0.09]	0.96	-0.063	[-0.15,0.02]	0.14
RERE	0.072	[0.01,0.14]	0.026	-0.03	[-0.08,0.02]	0.26
RNF207	-0.046	[-0.12,0.03]	0.21	-0.011	[-0.07,0.05]	0.71
RP1-120G22.11	0.014	[-0.13,0.16]	0.85	0.025	[-0.12,0.17]	0.72
RP11-338N10.1	0.057	[-0.09,0.20]	0.44	NA	NA	NA
RP11-338N10.2	0.01	[-0.13,0.15]	0.89	0.078	[-0.07,0.23]	0.3
RP11-338N10.3	0.0042	[-0.13,0.14]	0.95	0.047	[-0.1,0.2]	0.54
RP11-431K24.1	-0.033	[-0.19,0.13]	0.69	NA	NA	NA
RP3-467L1.4	0.0067	[-0.11,0.13]	0.92	0.008	[-0.13,0.15]	0.91
RP3-467L1.6	-0.057	[-0.18,0.07]	0.37	0.035	[-0.11,0.18]	0.62
RP5-1115A15.1	0.075	[-0.08,0.23]	0.34	0.039	[-0.14,0.22]	0.66
RPL22	0.013	[-0.06,0.08]	0.72	-0.074	[-0.15,0.00]	0.064
SLC45A1	0.052	[-0.09,0.19]	0.46	0.13	[0.00,0.26]	0.059
THAP3	-0.017	[-0.11,0.08]	0.73	-0.097	[-0.19,0.00]	0.047
TNFRSF25	0.015	[-0.06,0.09]	0.68	-0.051	[-0.11,0.01]	0.097
TNFRSF9	0.049	[-0.08,0.18]	0.47	NA	NA	NA
VAMP3	0.017	[-0.08,0.12]	0.74	0.002	[-0.07,0.07]	0.96
ZBTB48	-0.032	[-0.11,0.04]	0.4	-0.048	[-0.12,0.02]	0.16

Figure 1. GWAS results. A) Manhattan plot. Each dot represents a polymorphism (SNP). The X-axis depicts each chromosome and the Y axis shows the negative $\log_{10}$ p value for association of each SNP with CAN. The red dotted line indicates the Genome-wide significance threshold of $p = 5 \times 10^{-8}$, the gray dotted line indicates the notable genome-wide significance threshold of $p = 1\times 10^{-6}$. **B)** Q-Q plot (inset). The black dashed line indicates the null hypothesis. The blue line represents the observed $\log_{10}$ p values corresponding to the expected p values.

Figure 2. Regional association plot of locus 1p36. Variants are displayed ±2 Mbp upstream and downstream of the lead SNP (rs779142). The x-axis depicts the chromosomal positions of the SNPs as per NCBI Build 37, and the y-axis depicts the $-\log_{10}$ p values for association of these SNPs with diabetic peripheral neuropathy in ACCORD. SNPs in strong linkage disequilibrium with the lead SNP (purple) are marked in red ($r^2>0.8$). The bottom panel depicts UCSC genes within the region. The locus zoom plot was generated using LocusZoom (Abecasis Lab, University of Michigan School of Public Health) through http://locuszoom.sph.umich.edu/genform.php?type=yourdata. The reference database was hg19/1000 Genomes Nov 2014 EUR.

Figure 1.

A.


-log10(p-value)
0
1
2
3
4
5
6
7
8
9
10
11
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Chromosome

B.

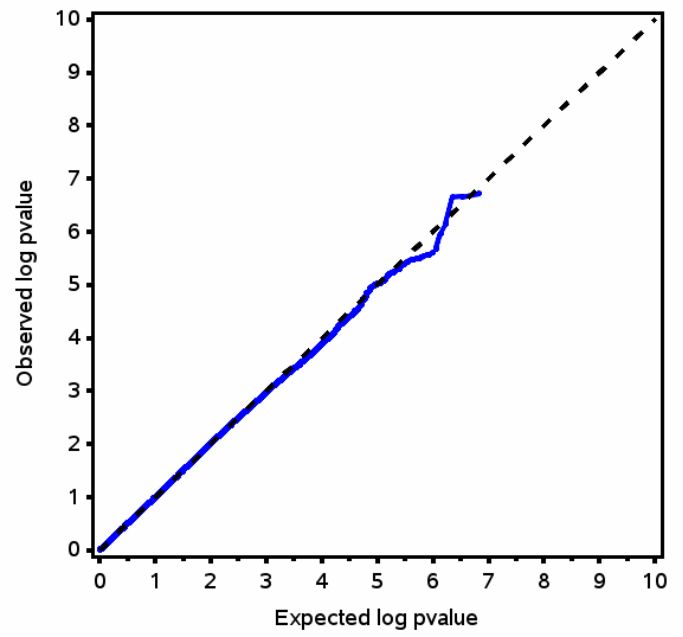
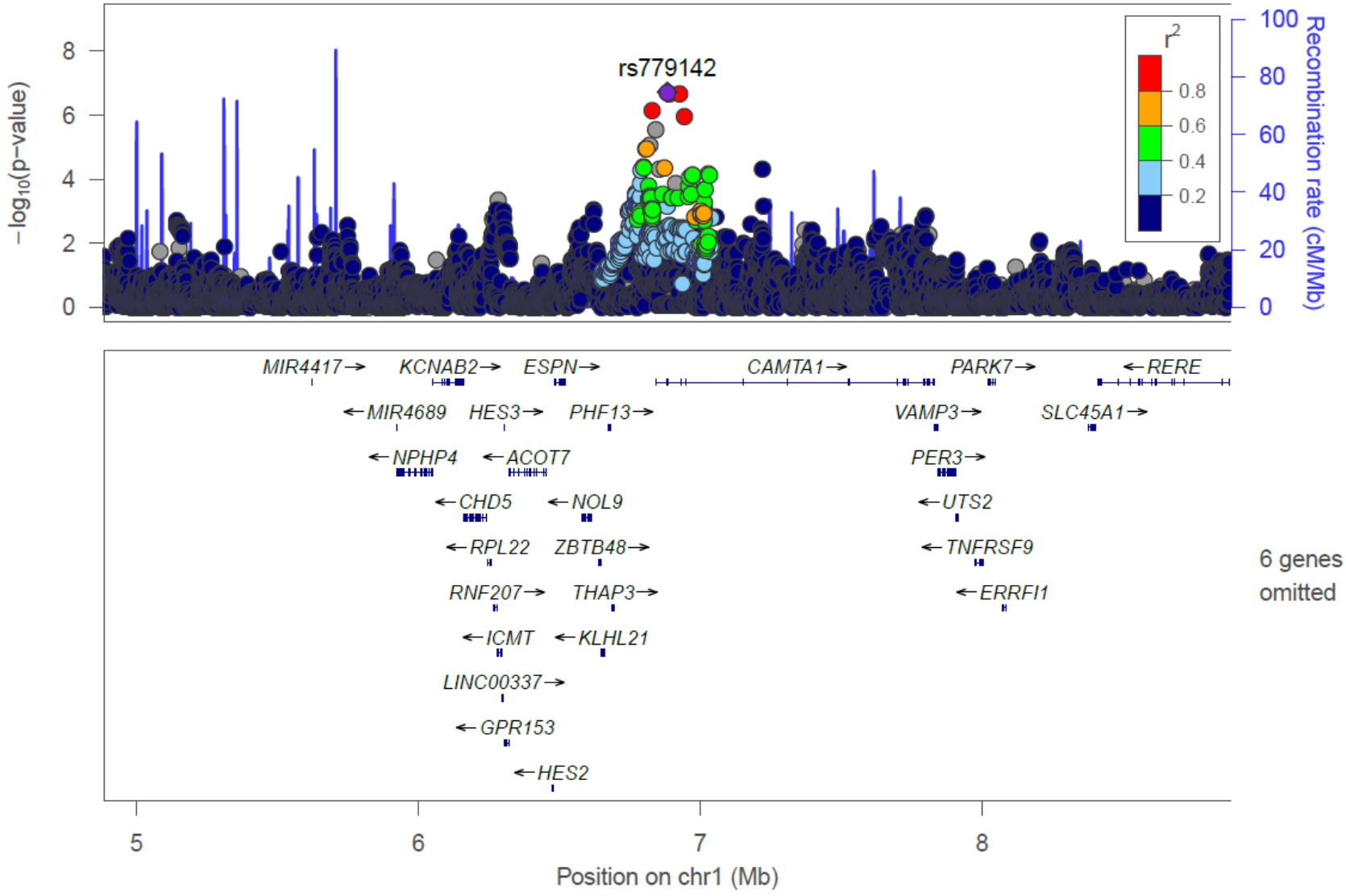

Observed log pvalue
Expected log pvalue
0 1 2 3 4 5 6 7 8 9 10

Figure 2.

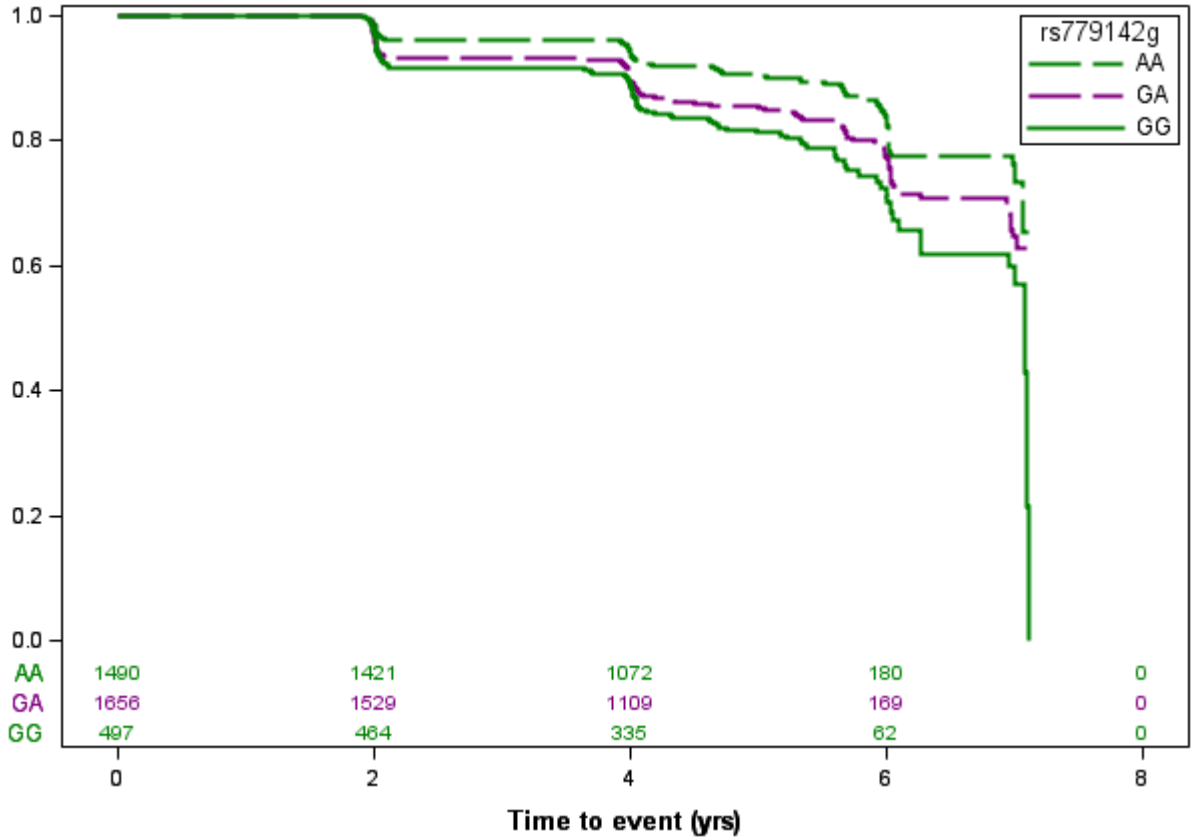

rs779142
r2
0.8
0.6
0.4
0.2
−log10(p−value)
Recombination rate (cM/Mb)
MIR4417→
KCNAB2→
ESPN→
CAMTA1→
PARK7→
←RERE
←MIR4689
HES3→
PHF13→
VAMP3→
SLC45A1→
←NPHP4
←ACOT7
PER3→
←CHD5
←NOL9
←UTS2
←RPL22
ZBTB48→
←TNFRSF9
RNF207→
THAP3→
←ERRFI1
←ICMT
←KLHL21
LINC00337→
←GPR153
←HES2
6 genes omitted
Position on chr1 (Mb)

Online Supplemental Material

ACCORD

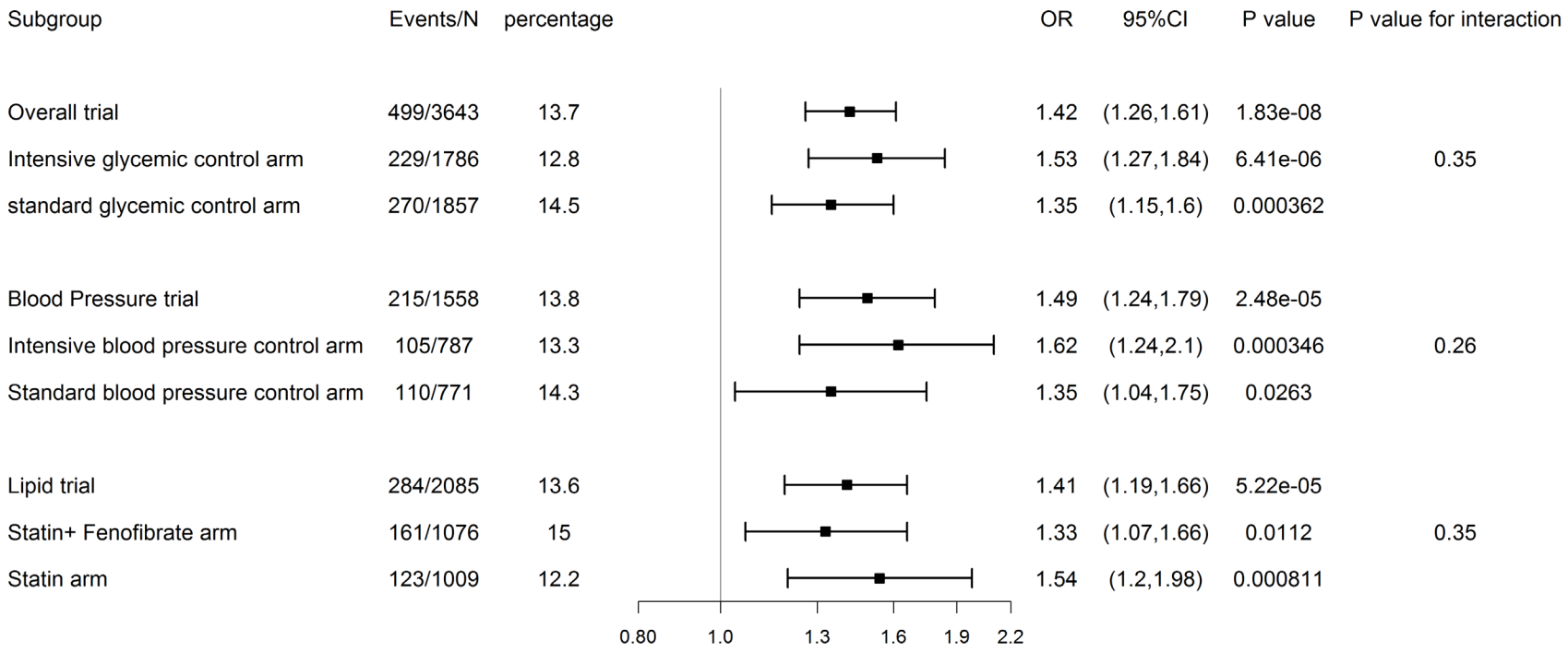
Members of the ACCORD DSMB included Antonio M. Gotto Jr. (chair), Kent Bailey, Dorothy Gohdes, Steven Haffner, Roland Hiss, Kenneth Jamerson, Kerry Lee, David Nathan, James Sowers, and LeRoy Walters. The following companies provided study medications, equipment, or supplies: Abbott Laboratories (Abbott Park, IL), Amylin Pharmaceuticals (San Diego, CA), AstraZeneca (Wilmington, DE), Bayer (Tarrytown, NY), Closer Healthcare (Tequesta, FL), GlaxoSmithKline (Philadelphia, PA), King Pharmaceuticals (Bristol, TN), Merck (Whitehouse Station, NJ), Novartis (East Hanover, NJ), NovoNordisk (Princeton, NJ), Omron Healthcare (Schaumburg, IL), Sanofi (Bridgewater, NJ), Schering-Plough (Kenilworth, NJ), and Takeda Pharmaceuticals (Deerfield, IL). None of these companies had an interest or bearing on the genome-wide analysis of the ACCORD data.

Supplementary Figure 1. Kaplan-Meier plots of CAN in ACCORD, by rs779142 genotype group.



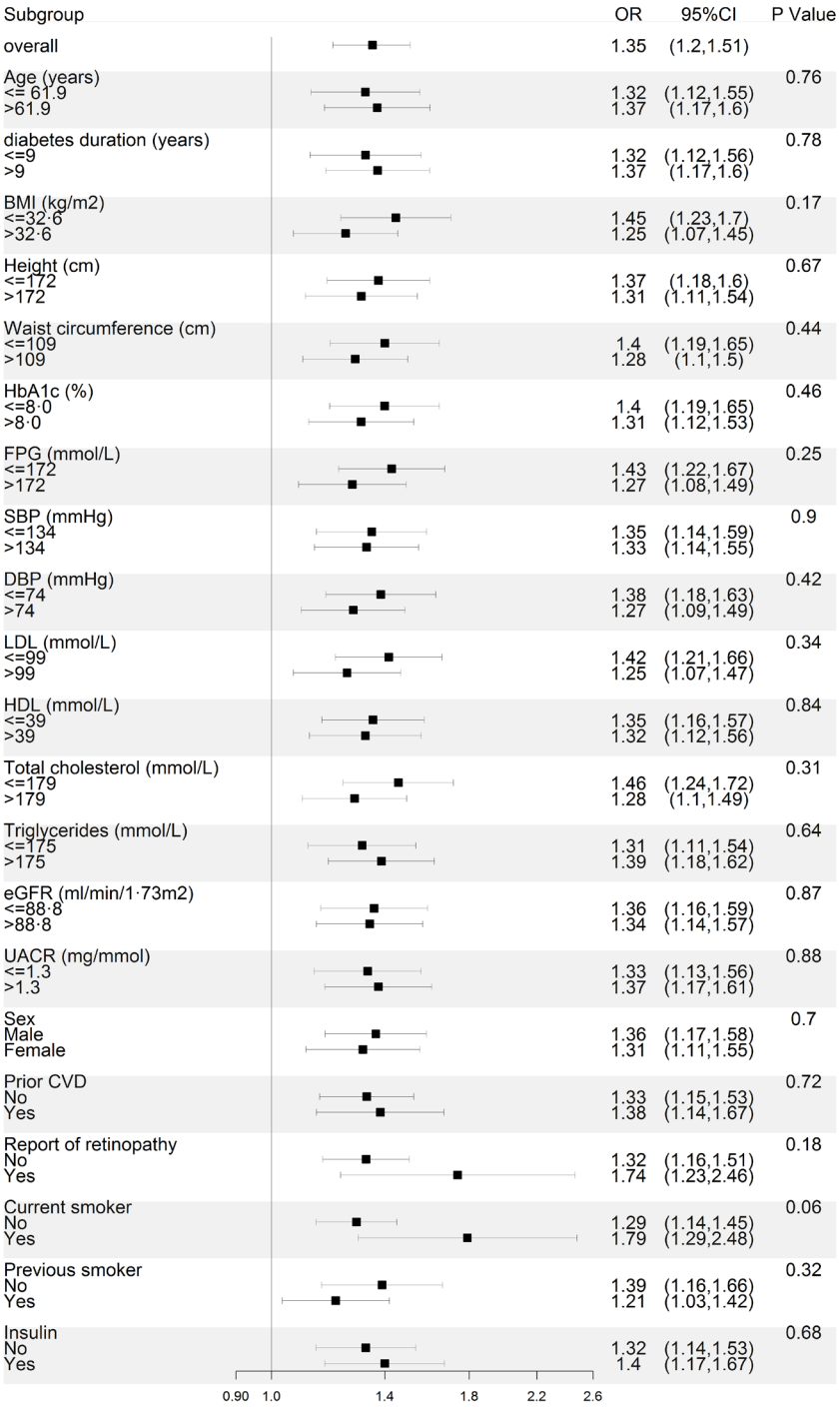
Y axis represented the patients at risk of CAN (%). X axis –numbers in colour represented number of patients at risk in terms of each genotype.

Supplementary Figure 2. Analysis if the interaction between CAN-associated SNP rs779142 and interventions tested in ACCORD.



Cox proportional regression models were adjusted by assignment to other interventions, seven clinical center networks, and top three principal components. The analysis is restricted to the prospective ACCORD cohorts – 499 CAN cases and 3,144 controls. The intervention and control arms were : 1) Glycemia trial- intensive glycemic control versus standard glycemic control; 2) Blood pressure trial- intensive blood pressure control versus standard glycemic control; 3) Lipid trial: fenofibrate + statin versus placebo + statin. Hazard ratios, 95% confidence intervals and p values are provided for marginal associations of SNP with progression to CAN in each of the trial arms. P values for interaction between SNP x intervention are also provided.

Supplementary Figure 3. Effect of the lead CAN SNP (rs779142) according to baseline characteristics in ACCORD.



For continuous variables, subgroups were defined based on median cut-offs. P values are for the interaction between clinical characteristics and SNP.

Supplementary Table 1. Characteristics of cardiac autonomic neuropathy (CAN) in prevalent and incident cases.

Baseline characteristic	Discovery Set (ACCORD)		
	Incident Cases (N=499)	Prevalent Cases (N=308)	P-value
Female	216 (43.3)	150 (48.7)	0.13
Age (years)	62.8 ± 6.5	62.5 ± 6.5	0.49
DM duration (years)	11.6 ± 7.5	12.1 ± 8.0	0.40
BMI (kg/m^2)	33.4 ± 5.4	33.8 ± 5.1	0.28
Waist circumference (cm)	110.0 ± 13.5	111.5 ± 12.2	0.11
Height (cm)	170.7 ± 9.6	170.4 ± 9.5	0.72
HbA1c (%)	8.30 ± 1.04	8.27 ± 9.43	0.63
Fasting glucose (mg/dL)	179.2 ± 52.6	179.5 ± 56.7	0.94
SBP (mmHg)	136.7 ± 18.0	137.2 ± 17.6	0.74
DBP (mmHg)	75.2 ± 11.2	76.1 ± 10.6	0.27
LDL (mg/dL)	104.7 ± 33.6	102.8 ± 32.8	0.45
HDL (mg/dL)	40.2 ± 11.1	41.4 ± 10.9	0.12
Women	44.8 ± 12.3	44.6 ± 11.5	0.88
Men	36.7 ± 8.5	38.4 ± 9.4	0.05
Total cholesterol (mg/dL)	181 (154 - 211)	186 (159 - 209)	0.67
Triglycerides (mg/dL)†	175 (125 - 261)	198 (129 – 268)	0.36
eGFR (ml/min/1.73 m^2)	88.4 ± 22.7	89.1 ± 23.9	0.69
UACR (mg/mmol)†	1.4 (0.7 – 4.9)	1.8 (0.8 – 6.1)	0.17
Previous CV event*	167 (33.5)	126 (40.9)	0.03
Report of retinopathy	69 (16.0)	41 (14.9)	0.67
Current smoker	52 (10.4)	40 (13.0)	0.27
Previous smoker	248 (55.5)	148 (55.4)	0.99
Insulin therapy	215 (43.3)	148 (48.2)	0.17
ACCORD Glycaemia trial			
Standard	270 (54.1)	149 (48.4)	0.11
Intensive	229 (45.9)	159 (51.6)	
ACCORD BP trial	215 (43.1)	137 (44.5)	0.70
Standard	110 (22.0)	83 (26.9)	0.08
Intensive	105 (21.0)	54 (17.6)	
ACCORD Lipid trial	284 (56.9)	171 (55.5)	0.70
Fibrate	161 (32.3)	87 (28.2)	0.23
Placebo	123 (24.6)	84 (27.3)	

†median (IQR)

Supplementary Table 2. Medication prescribed at baseline, 12 months, and Last visit.

Medication	baseline			12months			Last visit		
	Prevalent case (N= 308)	Incident case (N=499)	Controls (N=3,144)	Prevalent case (N=292)	Incident case (N=494)	Controls (N=3,126)	Prevalent case (N=262)	Incident case (N=466)	Controls (N=2,977)
ACE inhibitor (ACEI)	164(53.2)	263(52.7)	1668(53.1)	169(57.9)	273(55.3)	1750(56)	137(52.3)	237(50.9)	1660(55.8)
Angiotensin-receptor blocker (ARB)	55(17.9)	82(16.4)	527(16.8)	53(18.2)	98(19.8)	636(20.3)	75(28.6)	120(25.8)	757(25.4)
ACEI or ARB	215(69.8)	333(66.7)	2148(68.3)	211(72.3)	348(70.4)	2306(73.8)	195(74.4)	338(72.5)	2282(76.7)
Diuretics	151(49)	191(38.3)	1046(33.3)	170(58.2)	256(51.8)	1489(47.6)	165(63)	251(53.9)	1595(53.6)
Beta blocker	110(35.7)	154(30.9)	916(29.1)	113(38.7)	177(35.8)	1088(34.8)	133(50.8)	226(48.5)	1247(41.9)
Calcium channel blocker	53(17.2)	106(21.2)	504(16)	60(20.5)	112(22.7)	632(20.2)	59(22.5)	128(27.5)	725(24.4)
Alpha blocker	6(1.9)	14(2.8)	69(2.2)	12(4.1)	21(4.3)	102(3.3)	22(8.4)	31(6.7)	168(5.6)
Reserpine	1(0.3)	0(0)	52(1.7)	1(0.3)	12(2.4)	55(1.8)	3(1.1)	9(1.9)	58(1.9)
Other Anti-hypertensive	8(2.6)	3(0.6)	25(0.8)	2(0.7)	4(0.8)	24(0.8)	4(1.5)	6(1.3)	20(0.7)
Insulin	148(48.1)	215(43.1)	1012(32.2)	191(65.4)	288(58.3)	1623(51.9)	195(74.4)	327(70.2)	1878(63.1)
Metformin	169(54.9)	308(61.7)	2098(66.7)	195(66.8)	371(75.1)	2534(81.1)	167(63.7)	312(67)	2205(74.1)
Any sulfonylurea	140(45.5)	265(53.1)	1687(53.7)	137(46.9)	247(50)	1694(54.2)	95(36.3)	187(40.1)	1236(41.5)
Any thiazolidinedione	76(24.7)	119(23.8)	763(24.3)	169(57.9)	255(51.6)	1771(56.7)	60(22.9)	116(24.9)	805(27)
Aspirin	180(58.4)	297(59.5)	1834(58.3)	189(64.7)	314(63.6)	2043(65.4)	174(66.4)	322(69.1)	2044(68.7)
Any platelet inhibitor (including Aspirin)	191(62)	310(62.1)	1893(60.2)	199(68.2)	328(66.4)	2104(67.3)	184(70.2)	334(71.7)	2125(71.4)
Statin	191(62)	315(63.1)	2018(64.2)	232(79.5)	377(76.3)	2521(80.6)	215(82.1)	369(79.2)	2465(82.8)

Other lipid-lowering agent	44(14.3)	62(12.4)	397(12.6)	28(9.6)	51(10.3)	291(9.3)	33(12.6)	61(13.1)	371(12.5)
Any lipid-lowering agent	207(67.2)	342(68.5)	2183(69.4)	241(82.5)	392(79.4)	2614(83.6)	227(86.6)	384(82.4)	2557(85.9)

Data were presented as N (%).

Supplementary Table 3. Effects of SNPs on 1p36 ($p<1x10^{-5}$) in the two ACCORD genotyping sets

				ANYSET (156 cases/691 controls)					ACCSET (651 cases/2553 controls)					Meta-analysis of ANYSET and ACCSET				
SNP	Chr1:bp	Minor /major allele	MAF	Type*	info†	effect	SE	p	type	info	effect	SE	P	beta	SE	P	Dire-ction	P For Heter ogene ity
rs779142	6883843	G/A	0.366	I	0.976	0.349	0.136	0.010	I	0.999	0.288	0.063	4.8E-06	0.299	0.057	1.9E-07	++	0.69
rs6657847	6927523	T/C	0.363	I	0.969	0.357	0.137	0.009	I	0.998	0.286	0.063	6.0E-06	0.298	0.058	2.1E-07	++	0.64
rs11120775	6891124	T/C	0.366	I	0.976	0.352	0.136	0.010	I	0.998	0.286	0.063	5.8E-06	0.298	0.057	2.2E-07	++	0.66
rs11122142	6829948	A/G	0.401	I	0.989	0.266	0.133	0.045	I	0.991	0.284	0.062	5.4E-06	0.280	0.057	7.1E-07	++	0.91
rs950495	6944395	A/C	0.386	I	0.946	0.312	0.139	0.025	I	0.981	0.276	0.063	1.3E-05	0.282	0.058	1.1E-06	++	0.82
rs151091772	6842974	A/-	0.386	I	0.958	0.281	0.136	0.039	I	0.969	0.267	0.063	2.5E-05	0.270	0.058	2.9E-06	++	0.93
rs34695005	6821864	T/-	0.445	I	0.873	0.344	0.141	0.015	I	0.901	0.246	0.065	1.5E-04	0.263	0.059	8.9E-06	++	0.53

* I:imputed. †Info represents quality of imputation in IMPUTE V2. Values closer to 1 indicate good quality of imputation.

Supplementary Table 4. Association between top loci of CAN in ACCORD and GWAS of QTI, SDNN in GWAS catalog (https://www.ebi.ac.uk/gwas/)?

SNPs			GWAS catalog					ACCORD	
SNP	Chr	bp	PMID	Phenotypes	BETA	Pvalue	gene mapped	OR	Pvalue
rs846111	1	6279370	19305408	QT interval	1.75[1.41-2.09]	1.00E-16	RNF207	1.23[1.09,1.4]	8.91E-04
			19305409	QT interval	1.49[1.00-1.98]	4.00E-16	RNF207		
			24952745	QT interval	1.73[1.48-1.98]	7.00E-40	RNF207		
rs735951	16	11693536	29213071	QT interval	1.55[1.12-1.98]	6.00E-13	LITAF	0.86[0.77,0.96]	1.00E-02
			24952745	QT interval	1.15[0.95-1.35]	2.00E-28	LITAF	0.86[0.77,0.96]	1.00E-02
rs7531322	1	6299823	29213071	QT interval	1.73[1.26-2.2]	1.00E-12	LINC00337 HES3	1.17[1.04,1.31]	1.05E-02
rs4725982	7	150637863	19305408	QT interval	1.58[1.23-1.92]	5.00E-16	LOC105375567 KCNH2	1.18[1.04,1.35]	1.20E-02
rs12143842	1	162033890	19305408	QT interval	3.15[2.81-3.49]	2.00E-78	LOC107985450 NOS1AP	1.17[1.03,1.33]	1.29E-02
			29213071	QT interval	3.46[2.97-3.95]	3.00E-42	LOC107985450 NOS1AP		
			19305409	QT interval	2.88[2.43-3.33]	2.00E-78	LOC107985450 NOS1AP		
			23166209	QT interval	3.14[2.38-3.90]	2.00E-15	LOC107985450 NOS1AP		
			19587794	QT interval	0.18[NR]	1.00E-83	LOC107985450 NOS1AP		
			24952745	QT interval	3.5[3.28-3.72]	1.00E-213	LOC107985450 NOS1AP		
rs35760656	7	150658678	29213071	QT interval	1.7[1.25-2.15]	4.00E-13	KCNH2	1.18[1.03,1.35]	1.35E-02
rs230014	20	49739860	23459443	QT interval (drug interaction)	.	4.00E-06	LOC105372662	1.15[1.03,1.28]	1.58E-02
rs8049607	16	11691753	19305408	QT interval	1.23[0.88-1.57]	5.00E-15	LITAF	0.88[0.79,0.98]	2.14E-02
			19305409	QT interval	1.25[0.81-1.69]	6.00E-15	LITAF		
			23166209	QT interval	1.63[0.98-2.28]	7.00E-07	LITAF		

12:20531756	12	20531756	20031603	QT interval	0.2[0.12-0.28]	1.00E-06	PDE3A	1.18[1.02,1.36]	2.45E-02
rs7616330	3	71115751	23166209	QT interval	1.72[0.99-2.45]	6.00E-06	FOXP1	1.17[1.01,1.36]	3.47E-02
rs7195486	16	4255180	24952745	QT interval	0.55[0.33-0.77]	9.00E-07	SRL	1.15[1.01,1.32]	4.17E-02
rs8045405	16	8503222	23534349	QT interval	11.38[NR]	9.00E-06	LOC105371072 LOC1001	0.87[0.75,1]	4.68E-02

Supplementary Table 5. Baseline characteristics of ACCORD CAN cases and controls according to rs779142 genotypes.

Baseline Characteristics	rs779142 genotype	Cases + controls			Cases			Controls		
		N	Mean ± SD	P	N	Mean ± SD	P	N	Mean ± SD	P
BMI (kg/m2)	AA	1611	33.04 ± 5.25	0.84	274	33.63 ± 5.07	0.4	1337	32.92 ± 5.28	0.68
	GA	1793	32.97 ± 5.16		386	33.71 ± 5.28		1407	32.76 ± 5.11	
	GG	545	33.14 ± 5.23		147	32.98 ± 5.47		398	33.2 ± 5.14	
Height (m)	AA	1611	1.71 ± 0.1	0.59	274	1.7 ± 0.1	0.68	1337	1.71 ± 0.1	0.53
	GA	1794	1.72 ± 0.09		386	1.71 ± 0.09		1408	1.72 ± 0.09	
	GG	545	1.71 ± 0.1		147	1.7 ± 0.1		398	1.71 ± 0.1	
Waist circumference (cm)	AA	1597	109.4 ± 13.09	0.67	271	111.51 ± 13.02	0.04	1326	108.97 ± 13.07	0.74
	GA	1777	109.22 ± 12.68		380	110.54 ± 12.71		1397	108.86 ± 12.65	
	GG	541	109.2 ± 12.67		146	108.69 ± 13.77		395	109.38 ± 12.25	
HbA1c (%)	AA	1609	8.22 ± 0.97	0.74	273	8.31 ± 0.99	0.64	1336	8.2 ± 0.96	0.14
	GA	1794	8.2 ± 0.95		385	8.28 ± 1.01		1409	8.18 ± 0.93	
	GG	545	8.24 ± 0.9		147	8.27 ± 0.99		398	8.23 ± 0.87	
Fasting serum glucose (mmol/L)	AA	1606	178.93 ± 51.18	0.63	273	180.34 ± 57.04	0.8	1333	178.64 ± 49.92	0.29
	GA	1788	179.58 ± 50.08		383	178.69 ± 53.01		1405	179.82 ± 49.27	
	GG	545	179.74 ± 48.47		147	178.97 ± 51.88		398	180.02 ± 47.21	
SBP (mmHg)	AA	1607	134.69 ± 16.65	0.02	274	136.38 ± 17.96	0.49	1333	134.34 ± 16.35	0.03
	GA	1785	135.3 ± 17.2		385	137.02 ± 18.03		1400	134.83 ± 16.94	
	GG	545	136.79 ± 16.61		147	137.63 ± 17.27		398	136.48 ± 16.37	
DBP (mmHg)	AA	1607	74.37 ± 10.24	0.05	274	75.53 ± 10.6	0.74	1333	74.13 ± 10.15	0.06
	GA	1785	74.49 ± 10.47		385	75.4 ± 11.41		1400	74.24 ± 10.18	
	GG	545	75.57 ± 10.41		147	75.88 ± 10.62		398	75.45 ± 10.35	
LDL (mmol/L)	AA	1606	104.29 ± 32.59	0.65	273	105.32 ± 33.45	0.54	1333	104.08 ± 32.42	0.81
	GA	1787	102.28 ± 32.57		383	103.03 ± 31.91		1404	102.07 ± 32.76	
	GG	545	104.78 ± 36.48		147	103.94 ± 36.56		398	105.09 ± 36.5	
HDL (mmol/L)	AA	1606	40.6 ± 10.74	0.63	273	41.12 ± 10.78	0.49	1333	40.5 ± 10.73	0.86
	GA	1787	40.35 ± 10.3		383	40.37 ± 11.06		1404	40.34 ± 10.09	
	GG	545	40.56 ± 10.43		147	40.55 ± 11.29		398	40.56 ± 10.11	
Total cholesterol (mg/dL)	AA	1606	186.51 ± 41.38	0.19	273	189.26 ± 39.51	0.52	1333	185.95 ± 41.75	0.19
	GA	1787	182.22 ± 40.26		383	184.67 ± 40.46		1404	181.55 ± 40.2	
	GG	545	186.37 ± 48.09		147	188.04 ± 52.36		398	185.75 ± 46.47	

Triglycerides (mg/dL) †	AA	1606	176 (124 - 259)	0.08	273	183 (129 - 284)	0.51	1333	175 (123 -256)	0.07
	GA	1787	175 (121 - 245)		383	185 (126 - 259)		1404	171.5 (119.0 - 238.5)	
	GG	545	170 (121 - 260)		147	184 (125 - 262)		398	165.5 (120.0 - 254.0)	
eGFR (ml/min/1.73 m2) †	AA	1606	89.1 (73.7 - 104.1)	0.21	273	88.9 (73.4 - 104.6)	0.66	1333	89.1 (73.8 - 104.0)	0.22
	GA	1787	88.9 (74.3 - 104.3)		383	86.7 (70.8 - 103.8)		1404	89.3 (75.3 -104.5)	
	GG	545	90.2 (76.4 - 105.0)		147	89.7 (76.7 - 106.4)		398	90.4 (75.4 - 104.6)	
UACR (mg/mmol) †	AA	1531	1.3 (0.7 - 3.8)	0.99	261	1.6 (0.7 - 5.7)	0.81	1270	1.2 (0.6 - 3.5)	0.57
	GA	1713	1.3 (0.7 - 3.6)		372	1.4 (0.7 - 5.2)		1341	1.3 (0.7 - 3.3)	
	GG	521	1.3 (0.7 -3.9)		142	1.7 (0.8 - 5.5)		379	1.3 (0.7 - 3.3)	

Values are means ± SD or medians (IQR) (†). P values are according to an additive genetic model, adjusted by assignment to interventions, seven clinical center networks, and top three principal components.

Supplementary Table 6. Effects of lead SNP rs779142 on CAN in ACCORD after adjustment for risk factors.

Adjusted covariates*	OR (95%CI)	P
Age	1.35 (1.20 – 1.51)	1.90E-07
Sex	1.34 (1.20 – 1.50)	3.63E-07
Duration of diabetes	1.34 (1.20 – 1.50)	3.48E-07
Prior CVD events	1.34 (1.20 – 1.50)	2.53E-07
SBP	1.33 (1.19 – 1.49)	4.64E-07
DBP	1.33 (1.19 – 1.49)	4.21E-07
Beta- blocker	1.34 (1.19 – 1.49)	4.22E-07
TZD	1.34 (1.20 – 1.50)	2.49E-07
ACEI/ ARB	1.34 (1.20 – 1.50)	3.47E-07

* In ACCORD, the primary model was adjusted by assignment to interventions, 7 clinical center networks, and top 3 principal components. These covariates were added to the primary model.

Supplementary Table 7. Effects of lead SNP rs779142 on prevalent and incident microvascular outcomes.

Outcome	Events/ N	OR (95%CI)	HR (95% CI)	P
Neph1: Microalbuminuria (urine albumin:creatinine ratio ≥ 3.4 mg/mmol)†	877/3,055	0.96 (0.86, 1.08)	-	0.54
Neph2: Macroalbuminuria (urine albumin:creatinine ratio ≥ 33.9 mg/mmol)†	236/3,455	0.98 (0.81, 1.20)	-	0.88
Neph3: Renal failure (Initiation of Dialysis or ESRD, or renal transplantation, or rise of serum creatinine >291.72 µmol/L in absence of an acute reversible cause)‡	117/3,938	-	1.03 (0.79, 1.34)	0.80
Neph4: Doubling of baseline serum creatinine or >20 mL/min per 1.73 m^2 decrease in estimated GFR‡	2,388/3,951	-	1.03 (0.98, 1.10)	0.17
Neph5: Any of Neph2/Neph3/Neph4	2,484/3,945	1.04 (0.94, 1.15)	-	0.41
Eye1: Photocoagulation or Vitrectomy to treat retinopathy†	493 / 3,951	1.06 (0.92, 1.21)		0.43
Eye2: Surgery for Cataract Extraction†	973/3,951	0.95 (0.85, 1.05)		0.33
Eye3: 3-line Worsened Visual Acuity‡	1,687/ 3,951		0.93 (0.87, 1.00)	0.05
Eye4: Severe Vision Loss (Snellen fraction <20/200)†	668/ 3,575	0.94 (0.83, 1.06)		0.30
Progression to Diabetic Retinopathy in ACCORD EYE Study*	106/1,390	0.96 (0.71, 1.30)		0.79
Neuro1: Michigan Neuropathy Screening Instrument score (MNSI) > 2	3,268/3,863	1.06 (0.93, 1.21)		0.35
Neuro2: Loss of vibratory sensation (tested with 128 Hz tuning fork)	1,265/3,647	1.07 (0.97, 1.18)		0.20
Neuro3: Loss of ankle jerk during Jendrassik maneuver	2,638/1,067	1.10 (0.99, 1.22)		0.09
Neuro4: Loss of light touch (10g force monofilament test)	662/3,139	1.13 (1.01, 1.28)		0.04

†Prevalent + incident cases vs. controls (subjects without the outcome of interest at study entry and during follow-up).

‡Incident cases vs. controls at study entry and during follow-up. GFR estimation was done on the basis of the four variable MDRD GFR equation from Levey and colleagues.

Progression of diabetic retinopathy defined by ≥3 steps from baseline on the ETDRS (Early Treatment Diabetic Retinopathy Study Severity Scale) or the development of diabetic retinopathy requiring laser photocoagulation or vitrectomy.

All outcomes above were predefined in ACCORD as secondary microvascular outcomes. (1; 2)

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Summary of Paper 1 and Paper 2 conclusions

For the development of DPN in type 2 diabetes, we have identified and successfully validated a locus on chromosome 2q24 having a powerful protective effect. Tissue expression analysis suggests that this effect may be mediated by higher expression of the voltage-gated sodium channel NaV1.2 in the tibial nerve, which is known to increase neuronal excitability. For the development of CAN in type 2 diabetes, we have identified a genetic locus at 1p36 showing a strong predisposing effect. These two genetic markers may help clinicians identify patients at especially high risk of developing DPN and/or CAN, may provide insights into the underlying pathogenesis mechanisms, and may point to potential pharmacologic intervention targets for DPN and CAN.

Discussion and perspectives

Evidence before these studies

We searched PubMed and Embase databases from inception up to September 1, 2018 using the search terms: “genes”, “diabetic” and “neuropathy”. We found several reports of small, under-powered candidate gene studies of DPN and CAN that yielded marginally significant associations that were not confirmed by subsequent studies. Using the search terms: “genome-wide”, “diabetic” and “neuropathy”, we found one GWAS concerning a DPN subtype (painful DPN). However, the top signal in that study did not reach genome-wide significance and replication of this finding was not sought in other populations. No GWAS on CAN was found.

Strengths of these studies

The two studies reported the first genome-wide search for genetic determinants of DPN and CAN, respectively. They were conducted within the rigorous and standardized data acquisition framework of a clinical trial (ACCORD study), using validated and highly specific instruments to define outcomes. A locus at 2q24 was found to have a powerful genetic protective effect on DPN whereas a locus at 1p36 was found to have a strong predisposing effect on CAN. The significant findings on DPN from ACCORD were successfully validated in a cohort with similar clinical characteristics (BARI 2D study). The genetic findings were supported by functional evidence from eQTL, gene expression, and DNA regulatory element analyses.

Limitations of these studies

Limitations of these studies include: 1. Power to detect only relatively large genetic effects; 2. Limited generalizability due to the source data being from cohorts including participants at high CVD risk and the genome-wide search being restricted to Whites. 3. Exclusion of low frequency variants from the analysis due to concerns about imputation quality and the limited power provided by these variants. 4. For the time being, lack of validation in another cohortof the CAN locus on 1p36.

Future directions

We are seeking validation of the CAN locus in another cohort (ADDITION-Denmark) for which rich CAN data are available. As far as generalizability is concerned, we plan to seek replication of both loci in type 1 diabetes, using the well-characterized DCCT/EDIC cohort (this study has already been approved by the DCCT/EDIC Publication Committee). In terms of understanding the mechanisms underlying the genetic associations, future studies include testing the association between genotypes and gene expression in samples specifically collected from diabetic subjects (peripheral tibial nerve for DPN, cardiac autonomic plexus for CAN), along with studies assessing the impact of overexpressing or knocking down these genes in peripheral nerves of animal models of DPN and CAN. Collaborations with Drs. Rodica Pop-Busui, Eva Feldman and Martin Myers (U. of Michigan), and Drs. Pradipta Ray and Theodore Price (U. of Texas) have been established for these purposes.

Bibliography

See “Reference” built in Paper 1 and Paper 2 (including supplementary materials).