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ORIGINAL ARTICLE

# Type 2 Diabetes Modifies the Association of CAD Genomic Risk Variants With Subclinical Atherosclerosis

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**BACKGROUND:** Individuals with type 2 diabetes (T2D) have an increased risk of coronary artery disease (CAD), but questions remain about the underlying pathology. Identifying which CAD loci are modified by T2D in the development of subclinical atherosclerosis (coronary artery calcification [CAC], carotid intima-media thickness, or carotid plaque) may improve our understanding of the mechanisms leading to the increased CAD in T2D.

**METHODS:** We compared the common and rare variant associations of known CAD loci from the literature on CAC, carotid intima-media thickness, and carotid plaque in up to 29 670 participants, including up to 24 157 normoglycemic controls and 5513 T2D cases leveraging whole-genome sequencing data from the Trans-Omics for Precision Medicine program. We included first-order T2D interaction terms in each model to determine whether CAD loci were modified by T2D. The genetic main and interaction effects were assessed using a joint test to determine whether a CAD variant, or gene-based rare variant set, was associated with the respective subclinical atherosclerosis measures and then further determined whether these loci had a significant interaction test.

**RESULTS:** Using a Bonferroni-corrected significance threshold of $P<1.6\times10^{-4}$, we identified 3 genes (*ATP1B1*, *ARVCF*, and *LIPG*) associated with CAC and 2 genes (*ABCG8* and *EIF2B2*) associated with carotid intima-media thickness and carotid plaque, respectively, through gene-based rare variant set analysis. Both *ATP1B1* and *ARVCF* also had significantly different associations for CAC in T2D cases versus controls. No significant interaction tests were identified through the candidate single-variant analysis.

**CONCLUSIONS:** These results highlight T2D as an important modifier of rare variant associations in CAD loci with CAC.

**Key Words:** atherosclerosis ■ coronary artery disease ■ diabCs, type 2 ■ genetics ■ risk factors

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## Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **CAC** | coronary artery calcification |
| **CAD** | coronary artery disease |
| **CIMT** | carotid intima-media thickness |
| **GWAS** | genome-wide association study |
| **SNV** | single-nucleotide variant |
| **T2D** | type 2 diabetes |

Coronary artery disease (CAD) remains the leading cause of death among individuals with type 2 diabetes (T2D). Both T2D and CAD are complex disease traits, with both inherited and environmental causes, making the presentation of T2D a unique risk factor for CAD. Several studies have examined the shared genetic pathways between T2D and CAD with limited insights.[1–5] Additional measures of atherosclerosis exist and precede a clinical CAD event. These measures of subclinical atherosclerosis, including coronary artery calcification (CAC), carotid intima-media thickness (CIMT), and carotid plaque, predict future coronary events independent of known risk factors.[6,7] Furthermore, measures of subclinical atherosclerosis relate more closely to the underlying casual mechanisms leading to a CAD event.[8,9] This is especially true for CAC, which is highly correlated with incident CAD and is included in CAD risk assessment guidelines, especially for individuals with T2D.[10] Individuals with T2D have an increased risk of atherosclerosis, but additional investigation is warranted as to the biologic interdependence of these traits.[11–16]

While genome-wide association studies (GWASs) have identified hundreds of genetic loci associated with CAD, fewer GWAS-based discoveries have been observed for subclinical atherosclerosis measures despite their notable heritability and high genetic correlation with CAD.[17–25] Continuous subclinical atherosclerosis measures, such as CAC and CIMT, are particularly valuable in GWAS for measuring the early progression of atherosclerosis with greater statistical power than incident CAD. Furthermore, many studies have not considered the role of T2D in their analyses, which may differentially influence the way loci impact the development of atherosclerosis. A study by Lu et al[26] conducted a GWAS of subclinical atherosclerosis limited to individuals with T2D and subsequently evaluated whether 161 known CAD loci were significantly associated with the development of subclinical atherosclerosis in individuals with T2D. While they successfully identified 3 CAD loci that were significantly associated with CAC and CIMT in those with T2D, the study did not formally evaluate the differential associations of CAD loci in T2D compared with normoglycemic controls. Accounting for such differences by evaluating T2D-by-single-nucleotide variant (SNV) interaction terms may improve the power to detect CAD loci that have not previously been associated with subclinical atherosclerosis in the context of T2D.[27]

Moreover, rare variants play a unique role in the development of complex diseases, often having larger effects on disease than individual common variants.[28,29] At least 9 genes have been associated with CAD risk through aggregation of rare genetic variants, specifically in genes involved in regulating cholesterol levels.[23,30] Previous studies have not yet evaluated whether T2D may also modify the association of rare genetic variants in the development of atherosclerosis.

Thus, the goal of this study was to test whether common and rare variants at known CAD loci depend on T2D to exert their atherogenic effects by testing associations with CAC, CIMT, and carotid plaque. We used a gene-by-environment interaction test framework, utilizing T2D as the effect modifier to identify CAD loci that are associated with subclinical atherosclerosis.

## METHODS

The study population included 29 670 participants from 12 different studies that are a part of the Trans-Omics for Precision Medicine program sponsored by the National Heart, Lung, and Blood Institute (Table S1; Figure S1). Each study obtained informed consent from participants and approval from the appropriate institutional review boards. Additional details for these studies are available in the Supplemental Material. Individual whole-genome sequence data for Trans-Omics for Precision Medicine and harmonized subclinical atherosclerosis measurements at individual sample levels are available through restricted access via the Trans-Omics for Precision Medicine dbGaP Exchange area. Accession codes for genotype and phenotype files by cohort may be found in Table S1. This study did not rely on custom code or mathematical algorithms. The full methods for this study are available in the Supplemental Material.

## RESULTS

### Study Population

The study population consisted of 24 157 normoglycemic controls and 5 513 T2D cases. Of the 29 670 participants, 15 993 had data on CAC, 13 711 had data on CIMT, and 11 922 had data on plaque (Figure S1; Table S2). In the 15 993 individuals with CAC measured, the median CAC score was 0 (interquartile interval, 0–91) in normoglycemic controls and 32.7 (interquartile interval, 0–289.8) in T2D cases. The prevalence of CAC score >0 was 26.2% and 35.0% in T2D controls versus cases, respectively. The average mean thickness between the carotid intima and media was 0.70 (SD, 0.22) mm in controls and 0.78 (SD, 0.22) mm in T2D cases. For individuals with carotid plaque measured, the presence of a carotid plaque was noted in 19% of controls and 22.7% of T2D cases.

## Candidate Variant Interaction Tests for CAC

A summary of the study design and overview is available in the Supplemental Material (Figure S2). Five candidate SNVs (rs2891168 near *CDKN2B*, rs7412 in *APOE*, rs9349379 near *PHACTR1*, rs9515203 near *COL4A1*, and rs55730499 in *LPA*; Table 1) were significant according to the joint test ($P_{joint}$<1.7×10$^{-4}$), but none had a significant interaction test. Instead, the joint associations of these variants were largely driven by their main genetic association with CAC, regardless of T2D status. All CAD variants, except rs55730499 near *LPA*, have also previously been identified as associated with CAC in published CAC GWAS.[22,25,26]

No SNVs met the Bonferroni-corrected threshold for significance in the interaction test, but 17 candidate variants were nominally significant ($P_{int}$ and $P_{joint}$<0.05). Fifteen SNVs were in loci that had not previously been identified with CAC (Table 1). More than half (59%) of the observed effect estimates in T2D cases occurred in the same direction as CAD SNVs in the literature. The SNV with the strongest evidence for interaction with T2D was rs7623687 near *RHOA* ($P_{int}$=0.0004). T2D cases with alternate allele in rs7623687 had higher odds of a CAC score >0 (odds ratio, 1.29 [95% CI, 1.08–1.53] in T2D versus 0.98 [95% CI, 0.91–1.07] in controls; Table S3). The power to detect candidate SNV for associations across various minor allele frequency thresholds for CAC is presented in Table S4.

## Rare Variant Candidate Gene–Based Interaction Results for CAC

Three genes, *ARVCF*, *ATP1B1*, and *LIPG*, were significantly associated with CAC according to the gene-based joint test ($P_{joint}$<1.6×10$^{-4}$; Table 2). Furthermore, the interaction tests for *ARVCF* and *ATP1B1* were also

**Table 1. Significant and Nominally Significant Single-Variant Associations of CAD SNVs With CAC**

| SNV ID | Chromosome, Position, Reference Allele Alternate Allele* | Nearest gene | Estimated SNV effect in controls | Estimated SNV effect in T2D cases | Interaction *P* value† | Joint *P* value† | Direction of effect for SNV association in CAD‡ |
|---|---|---|---|---|---|---|---|
| Significant associations of CAD SNVs using the joint test§ | | | | | | | |
| rs2891168 | 9:22098620:A:G | *CDKN2B-AS1* | 0.19±0.03 | 0.23±0.07 | 0.43 | 1.0×10$^{-14}$ | + |
| rs7412 | 19:44908822:C:T | *APOE* | −0.26±0.04 | −0.45±0.11 | 0.10 | 6.9×10−$^{13}$ | − |
| rs9349379 | 6:12903725:A:G | *PHACTR1* | 0.18±0.03 | 0.04±0.08 | 0.08 | 4.27×10$^{-9}$ | + |
| rs9515203 | 13:110397276:T:C | *COL4A2* | −0.11±0.03 | −0.17±0.07 | 0.28 | 1.98×10$^{-5}$ | − |
| rs55730499 | 6:160584578:C:T | *LPA* | 0.25±0.06 | 0.14±0.16 | 0.44 | 9.27×10$^{-5}$ | + |
| Nominally significant associations for both joint and interaction tests‖ | | | | | | | |
| rs283485 | 2:232780981:G:A | *GIGYF2* | 0.02±0.03 | 0.19±0.06 | 0.001 | 1.96×10$^{-4}$ | + |
| rs7485656 | 12:124831101:A:G | *SCARB1* | 0.05±0.03 | 0.24±0.07 | 0.007 | 1.97×10$^{-4}$ | + |
| rs2839812 | 11:103802566:T:A | *MIR4693* | −0.06±0.03 | −0.17±0.06 | 0.019 | 2.29×10$^{-4}$ | − |
| rs7623687 | 3:49411133:A:C | *RHOA* | 0.01±0.04 | 0.30±0.08 | 4.21×10$^{-4}$ | 4.83×10$^{-4}$ | − |
| rs6909752 | 6:22612400:G:A | *HDGFL1* | 0.01±0.03 | 0.17±0.06 | 0.004 | 0.002 | + |
| rs12500824 | 4:76495474:A:G | *SHROOM3* | −0.02±0.03 | −0.16±0.06 | 0.011 | 0.004 | + |
| rs2954029 | 8:125478730:A:T | *TRIB1* | −0.03±0.03 | −0.13±0.06 | 0.016 | 0.005 | − |
| rs11591147 | 1:55039974:G:T | *PCSK9* | −0.35±0.12 | 0.17±0.33 | 0.021 | 0.01 | − |
| rs7118294 | 11:32358975:T:C | *WT1* | 0.06±0.03 | −0.12±0.07 | 0.008 | 0.01 | + |
| rs3775058 | 4:95196220:A:T | *UNC5C* | −0.07±0.03 | 0.10±0.07 | 0.017 | 0.02 | − |
| rs11099493 | 4:81665896:A:G | *HNRNPD* | −0.04±0.03 | 0.14±0.07 | 0.006 | 0.02 | − |
| rs1321309 | 6:36670859:G:A | *CDKN1A* | −0.02±0.03 | 0.20±0.07 | 0.008 | 0.03 | + |
| rs4140748 | 2:229140789:A:G | *PID1* | 0.01±0.03 | 0.15±0.06 | 0.017 | 0.03 | − |
| rs11601507 | 11:5679844:C:A | *TRIM5* | 0.04±0.05 | 0.20±0.14 | 0.033 | 0.03 | + |
| rs2067831 | 10:103883465:G:C | *OBFC1* | 0.06±0.03 | −0.08±0.08 | 0.022 | 0.03 | + |
| rs584961 | 11:75566583:A:G | *SERPINH1* | 0±0.04 | 0.18±0.11 | 0.015 | 0.04 | + |
| rs2895811 | 14:99667605:T:C | *HHIPL1* | 0±0.03 | 0.15±0.07 | 0.034 | 0.04 | + |

CAC indicates coronary artery calcification; CAD, coronary artery disease; ID, identifier; PC, principal component; SNV, single-nucleotide variant; and T2D, type 2 diabetes.
*Chromosome and position are in build hg38.
†*P* values were computed using linear mixed models accounting for age, sex, ancestry informative PC1-11, PC1-11-by-sex interaction terms, a PC1-2-by-T2D interaction term, and a T2D-by-SNV interaction term.
‡Direction of the reported SNV association with CAD was based on the most significant *P* value from the literature.
§It consists of candidate CAD SNVs that met the Bonferroni-corrected threshold of 1.7×10$^{-4}$.
‖It consists of candidate CAD SNVs that met the nominal significance level of *P*<0.05 in both joint and interaction tests.

**Table 2. Genes Significantly Associated With CAC Score According to the Joint Test**

| Gene | N variants | Main effect *P* value[1] | Interaction *P* value* | Joint *P* value* | Variant grouping strategy | Genome region |
|---|---|---|---|---|---|---|
| *ARVCF* | 59 | 0.050 | $9.9\times10^{-5}$ | $6.1\times10^{-5}$ | Missense | Coding |
| *ATP1B1* | 6 | 0.018 | $4.0\times10^{-5}$ | $9.9\times10^{-6}$ | Synonymous | Coding |
| *LIPG* | 371 | 0.001 | 0.004 | $6.2\times10^{-5}$ | Enhancer overlaid with DHS sites | Non-coding |

CAC indicates coronary artery calcification; DHS, DNAse I hypersensitive site; PC, principal component; and T2D, type 2 diabetes.

*P values computed using linear mixed models accounting for age, sex, ancestry informative PC1-11, PC1-11-by-sex interaction terms, PC1-2-by-T2D interaction terms, and T2D-by-gene–based aggregation units. The main effect *P* value refers to the association of the gene-based aggregation unit. Interaction *P* value refers to the association of the T2D-by-gene–based aggregation unit interaction term. The joint *P* value refers to the association of the combined test of both the T2D-by-gene–based interaction term and main effect association test.

significant ($P_{int}$=$9.9\times10^{-5}$; $P_{int}$=$4.0\times10^{-5}$, respectively). Both *ARVCF* and *ATP1B1* gene–based tests included variants in protein-coding regions. The significant *ARVCF* test included missense variants, while the significant *ATP1B1* associations were driven by synonymous variants.

Variants within each aggregation unit were evaluated for their individual variant contributions to their associated joint and interaction tests (Tables S5 and S6). For *ATP1B1*, notable changes in the joint *P* value (>100% change) were observed after the removal of 3 variants (>100% change). After excluding variant rs61742560, a nominally significant (*P*<0.05) main effect was no longer observed, but a strong contribution from the interaction test remained. After excluding either rs144621395 or rs61803314 from the analysis, the main effect *P* value remained the same with notable changes in $P_{int}$ (Table S7). We further evaluated the distribution of CAC scores in individuals who were carriers of the minor allele for the variants with the largest contribution to the significant gene-based test for *ATP1B1*. Overall, individuals with T2D who carried at least 1 of the alternate alleles of these variants had the lowest CAC scores (Figure). This is primarily driven by 2 variants: rs61742560 and rs61803314 (Figure S3). For individuals with T2D and rs144621395, the opposite association was observed, with the highest CAC scores observed in this group.

In *ARVCF*, 3 of the 59 variants within the *ARVCF* unit appeared to contribute the most to the significant association tests. Excluding either rs113625788, rs116782322 or rs76496156 notably changed the observed joint *P* values (>100% change), while the exclusion of the other variants did not (Table S8). We further evaluated 3 variants driving the significant interaction test for *ARVCF*. Individuals with T2D who carried at least 1 of the minor alleles of the 3 identified variants had the highest CAC scores (Figure; Figure S5).

## Candidate Variant Interaction Tests With CIMT and Carotid Plaque

One variant (rs7412 in the *APOE* gene) was significantly associated with CIMT using the joint test ($P_{joint}$=$2.6\times10^{-6}$) but did not have a significant interaction test. No CAD variants were significantly associated with carotid plaque. No significant interaction tests were observed for either CIMT or carotid plaque. Across both

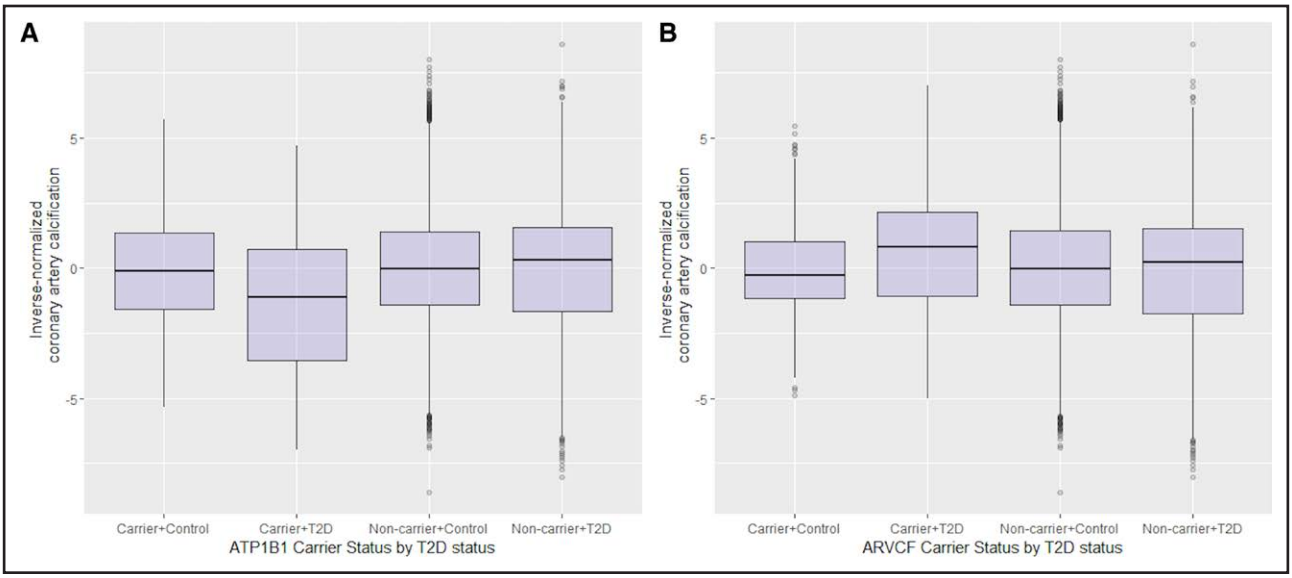


**Figure. Distribution of coronary artery calcification (CAC) score by carrier and type 2 diabetes (T2D) status.**
Data are boxplots for the distribution of CAC scores for individuals according to their carrier and T2D status. Carriers were defined as carrying at least 1 minor allele from the largest contributing variants from the respective aggregation tests. **A**, Variants from the *ATP1B1* aggregation unit. **B**, Variants from the *ARVCF* aggregation unit.

traits, 24 variants met nominal significance (14 for CIMT and 10 for carotid plaque; Table 3). The variant with the smallest interaction *P* value for CIMT was at the *SORT1* locus ($P_{int}$=0.0004; $P_{joint}$=0.002) and for carotid plaque at the *ZC3HC1* ($P_{int}$=0.006; $P_{joint}$=0.02) locus. Two nominally significant variants overlapped with the nominally significant findings from the CAC analysis (*PCSK9* in CIMT and *SCARB1* in carotid plaque).

## Rare Variant Gene–Based Interaction Tests for CIMT and Plaque

Two gene-based aggregation units (*ABCG8* with CIMT and *EIF2B2* with carotid plaque) met the Bonferroni significance threshold ($P<1.6\times10^{-4}$) according to the joint test but not according to the interaction test (Table S9). While the main effect (interaction free) *P* value for *ABCG8* met the significance threshold, the association of *EIF2B2* with carotid plaque was not significant according to the main effect (interaction free) *P* value alone. Instead, the significant association of *EIF2B2* required both the main and interaction effects to cross the Bonferroni significance threshold. Both gene-based aggregation units that were significant for the joint test included only protein-coding regions of the genome. The *ABCG8* unit consisted of putative loss-of-function variants, while the *EIF2B2* unit consisted of missense mutations.

We also evaluated the effect of CAC-associated genes on CIMT and carotid plaque. One variant category in *ATP1B1* and 1 variant category in *LIPG* met nominal significance ($P<0.05$) for both the joint and interaction tests in CIMT (Table S10). None of the significantly associated gene-based rare variant aggregation units with

**Table 3. Nominally Significant ($P<0.05$ in Both Joint and Interaction Tests) Associations of CAD SNVs With CIMT and Carotid Plaque**

| SNV ID | Chromosome: Position: Reference Allele: Alternative Allele* | Nearest gene | Estimated SNV effect in controls | Estimated SNV effect in T2D cases† | Interaction *P* value† | Joint *P* value† | Direction of SNV association in CAD‡ |
|---|---|---|---|---|---|---|---|
| CIMT | | | | | | | |
| rs602633 | 1:109278889:T:G | *PSRC1* | −0.003±0.003 | 0.026±0.007 | $3.62\times10^{-4}$ | 0.002 | − |
| rs668948 | 2:21068657:G:A | *APOB* | 0.01±0.003 | −0.016±0.008 | 0.029 | 0.007 | + |
| rs651007 | 9:133278431:T:C | *ABO* | −0.001±0.003 | 0.023±0.008 | 0.003 | 0.007 | + |
| rs12976411 | 19:32391114:A:T | *ZNF507/LOC400684* | 0.003±0.006 | 0.035±0.014 | 0.01 | 0.01 | + |
| rs112949822 | 5:108749489:G:A | *FER* | −0.013±0.005 | 0.025±0.012 | 0.04 | 0.02 | − |
| rs7991314 | 13:32551937:T:C | *N4BP2L2* | 0.0016±0.003 | 0.014±0.007 | 0.030 | 0.02 | + |
| rs884811 | 10:98164006:C:G | *R3HCC1L* | 0.001±0.003 | −0.016±0.007 | 0.02 | 0.02 | + |
| rs944172 | 9:107755513:C:T | *KLF4* | 0.004±0.003 | −0.019±0.007 | 0.007 | 0.02 | + |
| rs56408342 | 8:22190977:G:A | *BMP1* | −0.002±0.005 | −0.027±0.012 | 0.03 | 0.03 | + |
| rs768453105 | 19:41284181:GTTATGGTA:G | *HNRNPUL1* | 0.008±0.004 | −0.025±0.01 | 0.02 | 0.03 | + |
| rs6919211 | 6:133678730:C:G | *TARID* | −0.007±0.004 | 0.02±0.008 | 0.01 | 0.03 | − |
| rs7617773 | 3:48152025:C:T | *CDC25A* | 0.002±0.003 | 0.014±0.007 | 0.04 | 0.03 | + |
| rs11206510 | 1:55030366:T:C | *PCSK9* | −0.008±0.004 | 0.017±0.008 | 0.04 | 0.04 | + |
| Carotid plaque | | | | | | | |
| rs35879803 | 4:145861685:C:A | *ZNF827* | 0.90 (0.84–0.97) | 1.11 (0.95–1.30) | 0.01 | 0.007 | + |
| rs11057830 | 12:124822507:G:A | *SCARB1* | 1.03 (0.94–1.12) | 1.31 (1.08–1.60) | 0.01 | 0.0097 | + |
| rs17083333 | 4:53705899:G:T | *FIP1L1/LNX1* | 1.04 (0.97–1.11) | 1.25 (1.07–1.45) | 0.01 | 0.01 | − |
| rs6997330 | 8:19943018:G:C | *LPL* | 1.04 (0.90–1.20) | 0.70 (0.55–0.90) | 0.01 | 0.02 | + |
| rs7991314 | 13:32551937:T:C | *N4BP2L2* | 1.03 (0.96–1.10) | 1.22 (1.05–1.42) | 0.04 | 0.02 | + |
| rs11556924 | 7:130023656:C:T | *ZC3HC1* | 0.94 (0.87–1.02) | 1.23 (1.02–1.48) | 0.006 | 0.02 | − |
| rs3184504 | 12:111446804:T:C | *ATXN2/HNF1A* | 8.03 (1.81–35.55) | 0.39 (0.01–12.94) | 0.02 | 0.02 | + |
| rs10951983 | 7:6406396:A:G | *RAC1/DAGLB* | 1.04 (0.95–1.14) | 1.35 (1.09–1.67) | 0.03 | 0.03 | + |
| rs11663411 | 18:59293278:T:C | *CPLX4* | 1.00 (0.93–1.08) | 1.23 (1.04–1.45) | 0.02 | 0.04 | − |
| rs61797068 | 1:115359893:G:C | *NGF* | 1.05 (0.95–1.15) | 0.78 (0.61–0.98) | 0.01 | 0.046 | − |

CAD indicates coronary artery disease; CIMT, carotid intimate media thickness; ID, identifier; PC, principal component; SNV, single-nucleotide variant; and T2D, type 2 diabetes.

*Chromosome and position are in build hg38.

†*P* values were computed using linear or logistic models mixed models accounting for age, sex, ancestry informative PC1-11, PC1-11-by-sex interaction terms, a PC1-2-by-T2D interaction term, and a T2D-by-SNV interaction term for CIMT and carotid plaque, respectively.

‡Direction of the SNV association with CAD is based on the odds ratios from the literature, where >1.0 is + and <1.0 is −.

CAC had a nominally significant association with carotid plaque (Table S11).

## DISCUSSION

Our study highlights the importance of considering T2D case-control status in the development of subclinical atherosclerosis and subsequent CAD. Rare variant gene–based interaction tests identified 2 CAD-associated genes, *ARVCF* and *ATP1B1*, whose association with CAC was modified by T2D status. Furthermore, 3 additional genes (*LIPG* with CAC, *ABCG8* with CIMT, and *EIF2B2* with carotid plaque) were significantly associated with subclinical atherosclerosis according to their respective joint tests, with nominally significant interaction tests. While the single-variant SNV-by-T2D interaction tests did not yield Bonferroni significant results for any of the subclinical atherosclerotic traits, many of the nominally significant associations were identified in CAD SNVs previously associated with lipid traits, supporting the importance of cholesterol to the underlying relationship between subclinical atherosclerosis and T2D.

Rare variants in 2 genes, *ARVCF* and *ATP1B1*, were significantly associated with CAC with significantly different associations observed in T2D cases compared with normoglycemic controls. Neither gene had previously been reported associated with CAC.[22,25,31] Furthermore, despite common variant associations near these genes with CAD, the suspected role of *ARVCF* and *ATP1B1* in the development of atherosclerosis has not been well studied. *ARVCF* is a member of the catenin family, which plays an important role in cell adhesion and communication.[32] In addition to CAD, previous studies have associated the gene with pulse pressure and platelet count.[33] Gene expression studies have shown high levels of *ARVCF* expression in arterial tissues.[34] According to our data, individuals with T2D carrying at least 1 minor allele in *ARVCF* had higher levels of CAC than noncarriers. Interestingly, normoglycemic controls carrying at least 1 of the variants had the lowest observed CAC scores. These observations suggest that, for individuals with T2D, carriers of these mutations in *ARVCF* have an excess risk of elevated CAC and potential clinical CAD compared with noncarriers. Furthermore, the effects of the mutations in *ARVCF* may only accelerate the burden of CAC in the presence of disrupted glucose metabolism such as those created by T2D. Additional studies are needed to further understand the mechanisms through which *ARVCF* increases CAC burden development in individuals with T2D.

Similarly, *ATP1B1* belongs to a subfamily of Na(+)/K(+)-ATPases responsible for establishing and maintaining the electrochemical gradients of sodium and potassium ions across the plasma membranes.[35] In addition to CAD, previous studies have shown that this gene is associated with QT interval length and venous thrombosis.[36,37] Laboratory studies in mouse models associated expression levels of *ATP1B1* with cardiac contractility and calcium homeostasis.[38,39] Our data suggest that 2 rare variants contributed the most to the observed differences in this gene between T2D status and CAC burden development. These 2 variants act in opposing directions. Interestingly, individuals with T2D and carrying the alternate allele in rs61803314 had the lowest observed CAC scores. This protective effect against excessive CAC for T2D cases is of particular interest as it may provide therapeutic insights into slowing the progression or preventing CAC buildup and subsequent CAD for such a high-risk group.

Three additional CAD genes (*LIPG* with CAC, *ABCG8* with CIMT, and *EIF2B2* with carotid plaque) were also significantly associated with subclinical atherosclerosis according to the joint test. In addition to CAD,[19,20] GWAS studies of lipid traits have identified common variant associations in *LIPG*, *ABCG8*, and *EIF2B2* with total, high-density lipoprotein, and low-density lipoprotein cholesterol levels.[40] While the interaction with T2D at each of these genes is only nominally significant, both *LIPG* and *EIF2B2* would not have reached the Bonferroni-corrected significance threshold by evaluating the main effects alone. Thus, the observed significance of the association test required the inclusion of the T2D interaction term to be discovered. This is consistent with the shared evidence related to the importance of lipid metabolism in T2D and atherosclerosis. Improving our understanding of how T2D may exacerbate the roles of *LIPG* and *EIF2B2* in their respective subclinical traits may highlight distinct pathways through which individuals with T2D experience excess risk for a CAD event.

While common candidate SNV tests were less successful at detecting novel significant associations for their respective subclinical traits, a couple of interesting observations were made. First, 2 SNVs (near *SCARB1* and *PCSK9*) were nominally significant for >1 subclinical atherosclerosis trait. Both variants are near genes with well-known roles in lipid metabolism, echoing findings from our rare variant gene–based analysis, highlighting the strong pathogenic link between lipid metabolism, glucose metabolism, and subclinical atherosclerosis. Second, for most of the variants, the direction of association with subclinical atherosclerosis in T2D cases mirrored the direction of association identified with CAD. This echoes the results from the Lu et al[26] study of subclinical atherosclerosis GWAS in T2D only, where they identified 3 significant associations (rs2891168 near *CDKN2B-AS1* at 9p21, rs11170820 near *FLJ12825* for CAC, and rs7412 near *APOE* for CIMT), concluding that some CAD loci act through subclinical atherosclerosis in individuals with T2D. Finally, while these associations were only nominally significant, the contributions from the interaction tests were the primary drivers of the nominally significant associations, suggesting that the overall fit of the model was

improved by the inclusion of the T2D interaction term. This highlights the importance of considering T2D, and perhaps other important risk factors, in understanding the genetics of subclinical atherosclerosis and CAD.

A few limitations of this study must be acknowledged. First, while representing the largest whole-genome sequence study of subclinical atherosclerosis in T2D to date, our analysis had a limited sample size. Despite this limitation, our analysis conserved power using a candidate SNV and gene approach, to identify CAD loci that rely on T2D status to associate with subclinical atherosclerosis. Similarly, we were able to use 2 continuous atherosclerotic traits in CAC and CIMT, which also conserved power and allowed for a shorter time between T2D onset and each outcome measurement. Second, we were limited to CAD SNVs primarily discovered in European and East Asian ancestries. Recent studies suggest that including populations for different ancestry populations improves fine mapping and increases the probability of identifying potentially causal loci.[41] It is possible that the reason for the lack of associations observed in our candidate single-variant analysis is because the selected variants were not representative of the true casual associations. Future studies may expand the SNV set to accommodate large CAD GWAS on individuals with African and Hispanic backgrounds. Third, while we removed individuals with prediabetes from our analysis to lower the likelihood of misclassification of T2D status in our controls, it is possible that individuals with a high risk of T2D still exist in the controls, lowering our ability to detect significant interactions, particularly in the SNV analysis. Finally, our rare variant analysis was restricted to CAD loci defined by proximity to the nearest SNV. While previous studies have also supported this approach, some of the loci included in our study may not have been the true associated CAD gene based on more advanced gene prioritization methods.

This study also has several strengths. We carefully and clearly defined our case-control groups, specifically restricting our study to include only normoglycemic controls to further improve the interpretability of our findings. We also leveraged data from multiple race and ethnicity groups to further expand the generalizability of our study. Similarly, this study did not need to rely on imputed genotypes, given the availability of whole-genome sequence data. This allowed us to use both single-variant and gene-based methods to characterize both common and rare variations. Most importantly, being able to include the T2D interaction terms provided the opportunity to identify differential associations with CAC in those with T2D and those without.

## CONCLUSIONS

We evaluated the role of common and rare genetic variation in CAD loci in the development of subclinical atherosclerosis accounting for interaction with T2D and identified genes associated with subclinical atherosclerosis of which 2 genes, *ARVCF* and *ATP1B1*, had significant gene-T2D interaction effects. While no significant CAD SNV-T2D interaction effects were detected, nominally significant associations across traits still highlighted the importance of lipid traits in the development of subclinical atherosclerosis, especially for individuals with T2D. Our results suggest that using T2D interaction terms improved our ability to detect CAD loci associated with subclinical atherosclerosis and highlight the importance of considering T2D, and other important risk factors, in understanding the genetics of subclinical atherosclerosis and CAD.

## ARTICLE INFORMATION

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## Supplemental Material

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