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Multiethnic Genome-wide Association Study of Subclinical Atherosclerosis in Individuals with Type 2 Diabetes

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Supplemental Materials:
Supplementary Methods
Supplemental Tables I–IX
Supplemental Figures I–VIII
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Abstract

Background -—Coronary artery calcification (CAC) and carotid artery intima-media thickness (cIMT) are measures of subclinical atherosclerosis in asymptomatic individuals and strong risk factors for cardiovascular disease (CVD). Type 2 diabetes (T2D) is an independent CVD risk factor that accelerates atherosclerosis.

Methods -—We performed meta-analyses of genome-wide association studies (GWAS) in up to 2,500 T2D individuals of European ancestry (EA) and 1,590 T2D individuals of African ancestry (AA) with or without exclusion of prevalent CVD, for CAC measured by cardiac computed tomography, and 3,608 EA and 838 AA with T2D for cIMT measured by ultrasonography within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.

Results -—We replicated two loci (rs9369640 and rs9349379 near *PHACTR1* and rs10757278 near *CDKN2B*) for CAC and one locus for cIMT (rs7412 and rs445925 near *APOE-APOC1*) that were previously reported in the general EA populations. We identified one novel CAC locus (rs8000449 near *CSNK1A1L/LINC00547/POSTN* at 13q13.3) at $P=2.0\times10^{-8}$ in EA. No additional loci were identified with the meta-analyses of EA and AA. The expression QTL analysis with nearby expressed genes derived from arterial wall and metabolic tissues from GTEx pinpoints *POSTN*, encoding a matricellular protein involved in bone formation and bone matrix organization, as the potential candidate gene at this locus. In addition, we found significant associations ($P<3.1\times10^{-4}$) for three previously reported coronary artery disease loci for these subclinical atherosclerotic phenotypes (rs2891168 near *CDKN2B-AS1* and rs11170820 near *FLJ12825* for CAC, and rs7412 near *APOE* for cIMT).

Conclusions -—Our results provide potential biological mechanisms that could link CAC and cIMT to increased CVD risk in individuals with T2D.

Keywords

coronary artery calcification; carotid intima-media thickness; population genetics; Genome Wide Association Study; type 2 diabetes mellitus; Genetic; Association Studies; Coronary Artery Disease; Diabetes; Type 2

Introduction

Cardiovascular diseases (CVD) remain a leading cause of mortality and morbidity among adults in developed countries[1]. The presence of subclinical atherosclerosis in individuals without clinically evident CVD is associated with an increased risk of developing clinical CVD, independent from traditional risk factors[2–5]. Individuals with type 2 diabetes (T2D) tend to have higher levels of coronary artery calcification (CAC) and common carotid intima-media thickness (cIMT)[6, 7] and are at increased risk for CVD compared to those without T2D. Subclinical atherosclerosis can be directly visualized through imaging of CAC by cardiac computed tomography and cIMT by carotid B-mode ultrasound, both of which are highly heritable clinical phenotypes[8–12]. Although CAC and cIMT may have distinct genetic and biological determinants, genetic studies of both subclinical atherosclerotic phenotypes may allow the identification of genetic factors underlying atherosclerosis and CVD. Genome-wide association studies (GWAS) conducted by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and others have identified 4 loci for CAC (*APOB* at 2p24.1[13], *PHACTR1* at 6p24.1[13–15], *CDKN2B* at

9p21.3[13–15] and *APOE* at 19q13.32[13]) and 11 loci for cIMT (*LINC01717* at 1q32.2[16], *ATP6AP1L* at 5q14.2[16], *AIG1* at 6q24.2[16], *PIK3CG* at 7q22.3[16], *MCPH1* at 8p23.1[16], *SGK223* at 8p23.1[16], *PINX1* at 8p23.1[10, 16], *ZHX2* at 8q24.13[10, 16], *VTI1A* at 10q25.2[16], *CBFA2T3* at 16q24.3[16] and *APOE* at 19q13.32[10, 13, 16]) in the general populations. However, none of these variants reached genome-wide significance levels in a recent study of CAC in T2D individuals of African ancestry[17]. Additional genetic loci for these subclinical atherosclerotic phenotypes remain to be identified based on their high heritability. T2D is an independent risk factor for CVD[18], and is typically accompanied by increased adiposity, hyperglycemia, dyslipidemia, and high blood pressure that accelerates atherosclerosis and leads to the development of coronary artery disease (CAD)[19–21]. Emerging evidence also suggests that genetic perturbations in glutamic acid metabolic pathways among T2D individuals may specifically predispose to increased CAD risk[22]. In the present study, we performed a multi-ethnic GWAS of CAC and cIMT in individuals with T2D. We also explored whether known CAD risk variants[23] may exert their effects through development of subclinical atherosclerosis in these populations.

Methods

The study methods are provided in Supplementary Materials. This study was approved by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Research Committee. Each study received institutional review board approval, participants provided written informed consent, and respective governing ethics committees approved each study. All relevant summary-level data in the manuscript will be deposited in the CHARGE shared website or the NIH dbGaP. Because of the sensitive nature of the data collected for each of the included studies, requests to access the individual dataset from qualified researchers trained in human subject confidentiality protocols may be sent to the principal investigators of the corresponding cohorts to apply for access in accordance with their data access policies.

Results

Study Participants

Twelve cohorts participated in the meta-analyses of CAC and cIMT. The clinical characteristics of each cohort are summarized in Supplementary Tables 1 and 2. A total of 2,500 EA diabetic individuals and 1,590 AA diabetic individuals were genotyped and measured for CAC; and a total of 3,608 EA diabetic individuals and 838 AA diabetic individuals were genotyped and measured for cIMT.

CAC Association

Association analyses were conducted that included all participants (model 1) and with prevalent CVD cases excluded (model 2 as sensitivity analysis) for EA and AA diabetic individuals separately and jointly through meta-analysis. No significant inflation was observed in the respective association statistics (Supplementary Figure 1 and 2). The top loci with lead variants associated with CAC are listed in Table 1. Two and twelve lead variants are marginally associated with CAC with $P < 1.0 \times 10^{-6}$, near but above genome wide

significance of $P < 5 \times 10^{-8}$, based on analyses in model 1 and model 2, respectively (Table 1). The variant rs8000449 near *CSNK1A1L* at 13q13.3 was genome-wide significantly associated with CAC at $P = 2.02 \times 10^{-8}$ in EA diabetic populations after excluding individuals diagnosed with CVD (Table 1, Figures 1 and 2, and Supplementary Figure 3). This variant was also marginally associated with CAC in analyses including all EA diabetic individuals ($P = 5.2 \times 10^{-7}$, Table 1); however, the allelic effect size was attenuated (Figure 2 and Supplementary Figures 4) after including individuals diagnosed with CVD. There was no discernable association observed in AA populations at this region (Supplementary Figures 5 and 6). There was no evidence of heterogeneity in effect size of rs8000449 among cohorts ($P > 0.05$, Table 1). The mean imputation quality for rs8000449 was 0.99 over eight EA cohorts (Table 1; See supplementary Notes for details) included in the CAC analyses.

cIMT Association

No inflation was observed in association statistics for cIMT analyses (Supplementary Figure 7). The top loci with lead variants marginally associated with cIMT among all participants are listed in Table 1. However, none of the eleven variants reached the genome-wide significance level of 5×10^{-8}.

Comparison to known variants associated with subclinical atherosclerotic phenotypes in general populations

We assessed the associations for 8 variants at 4 loci previously reported in the general population to be associated with the subclinical atherosclerotic phenotype CAC (Supplementary Table 3)[13, 14]. Six variants were available in the present study and had consistent effect directions as previously reported in EA populations (Supplementary Table 4). Three variants reached Bonferroni corrected significance levels[24] with $P < 6.2 \times 10^{-3}$: rs9369640 and rs9349379 near *PHACTR1* at 6p24.1 and rs10757278 near *CDKN2B-AS1* at 9p21 in the meta-analyses including all EA (model 1, Supplementary Table 4). No additional variants were associated with CAC in EA populations after excluding individuals diagnosed with CVD (model 2, Supplementary Table 5). None of these variants were associated with CAC in AA populations (Supplementary Tables 4 and 5).

We assessed the associations for 14 variants at 11 loci previously reported in the general population to be associated with the subclinical atherosclerotic phenotype cIMT (Supplementary Table 3)[10, 13, 16]. Fourteen variants were available in the present study and thirteen of them had consistent effect directions as previously reported in EA populations (Supplementary Table 6). Two variants reached Bonferroni corrected significance levels with $P < 3.5 \times 10^{-3}$: rs7412 and rs445925 near *APOE* at 19q13 in our meta-analyses of EA populations (Supplementary Table 6). None of these variants were associated with cIMT in the AA populations (Supplementary Table 6).

Subclinical atherosclerotic phenotype associations for known variants associated with CAD risk

We further tested for associations of 161 variants reported to be associated with CAD in predominantly EA populations[23] for association with CAC and cIMT in our study. Fifty-nine variants were associated with subclinical atherosclerotic measures with $P < 0.05$

and forty-four variants had consistent effect directions for higher subclinical atherosclerotic phenotypes and increased risk of CAD (Supplementary Table 7). The variants rs2891168 near *CDKN2B-AS1* at 9p21 for CAC, rs11170820 near *FLJ12825* at 12q13.13 for CAC and rs7412 near *APOE* at 19q13.32 for cIMT were associated with subclinical atherosclerotic phenotypes at Bonferroni corrected significance levels of $P < 3.1 \times 10^{-4}$ in the EA populations (Supplementary Table 7)[24].

Discussion

In our GWASs of CAC in 4,090 and cIMT in 4,446 EA and AA participants with T2D, respectively, within the CHARGE consortium, we identified a genome-wide significant variant rs8000449 near *CSNK1A1L* at 13q13.3 for association with CAC. We confirmed 2 loci (rs9369640 and rs9349379 near *PHACTR1* at 6p24.1 and rs10757278 near *CDKN2B* at 9p21.3) for CAC and one locus for cIMT (rs7412 and rs445925 near *APOE-APOC1* at 19q13.32) previously reported in general EA populations in our T2D individuals. The specific *APOB* association reported earlier for CAC was not replicated here most likely because the Old Order Amish were not included in the present study and they have the highest frequency of this rare variant that is associated with CAC[13]. We also did not replicate the *APOE* association for CAC[13] due to relatively small sample size of our T2D individuals. In addition, we found significant associations for three coronary artery disease loci on these subclinical atherosclerotic phenotypes (rs2891168 near *CDKN2B-AS1* at 9p21 and rs11170820 near *FLJ12825* at 12q13.13 for CAC; rs7412 near *APOE* at 19q13.32 for cIMT). Overall, these analyses provide potential biological mechanisms that could link CAC and cIMT to CVD risk.

Our novel finding in those with T2D was the association between rs8000449 at 13q13.3 and CAC. Variants at 13q13.3 region were previously reported to be associated with bone mineral density[25], but the index variant rs556429 is not in linkage disequilibrium with rs8000449 (D' = 0.09 and R^2 = 0.002 of 1000 Genome phase 3 EUR populations)[26]. The CAC reducing C allele of rs8000449 was associated with increased expression of *POSTN* in the aorta artery ($P = 4.4\times10^{-4}$, Supplementary Table 8)[27]. The SNP rs8000449 is annotated as a potentially functional variant that overlaps the enhancer histone markers in osteoblast primary cells (Supplementary Table 9). *POSTN* is expressed in multiple tissues with the highest expression in arteries (Supplementary Figure 8)[27]. Periostin encoded by *POSTN*, is a matricellular protein involved in bone formation and bone matrix organization, particularly in the bone modeling response to mechanical stimulation and parathyroid hormone[28, 29]. High serum periostin levels are associated with increased fracture risk in postmenopausal women[30–33] and in those with newly diagnosed multiple myeloma.[34] High circulating periostin levels are correlated with reduced bone formation and increased bone resorption[34]. Although recent epidemiological studies indicate that decreased bone mineral density is associated with increased CVD burden[35, 36], the underlying mechanism linking increased *POSTN* expression with reduced calcification in coronary arteries warrants further investigation[37, 38]. The 4 loci previously identified for CAC (*APOB* at 2p24.1[13], *PHACTR1* at 6p24.1[13, 14], *CDKN2B* at 9p21.3[13, 14] and *APOE* at 19q13.32[13]) were all associated with coronary artery disease risk, confirming increased CAC as a biomarker of coronary artery disease risk. Although the T allele of rs8000449 was nominally associated with

increased risk of acute myocardial infarction and subsequent myocardial infarction in the UK Biobank cohort (odds ratio [95% confidence interval] = 1.0007 [1.0001-1.001] and 1.0003 [1.0002-1.0005]; $P = 9.0 \times 10^{-3}$ and 1.2×10^{-4}, respectively), it was not associated with CAD risk in the recent largest meta-analysis of GWAS of CAD (odds ratio [95% confidence interval] = 1.00 [0.99-1.02] and $P = 0.5$ with 122,733 CAD cases and 424,528 controls)[23]. This suggests that genetic determinants of CAC may not always overlap with those of CAD.

Recent large-scale GWAS have established 161 independent loci for CAD in primarily EA populations; however, the underlying mechanisms on CAD risk for the majority of these variants are unknown[23]. The variants at forty-four CAD loci had consistent effect directions of increased subclinical atherosclerotic phenotypes (at $P < 0.05$) and increased CAD risk, indicating these variants potentially exert their effects on CAD risk through predisposing individuals to increased atherosclerotic risk. Three CAD loci were statistically significantly associated with subclinical atherosclerotic phenotypes with Bonferroni correction for multiple testing[24]; the CAD loci at both 9p21 (near *CDKN2B-AS1*) and 19q13.32 (near *APOE*) were previously reported to affect CAC and/or cIMT[10, 13, 14, 16]. The SNP rs11170820 near *FLJ12825* at 12q13.13 was associated with CAC in EA populations (Supplementary Table 5). However, this SNP was not associated with any traditional CAD risk factors (Supplementary Table 7), suggesting that it may potentially affect CAD risk through mechanisms intrinsic to the vessel wall or other unidentified mechanisms that are shared between CAD and CAC.

Several limitations of this study should be noted. The sample sizes of these two subclinical atherosclerotic phenotypes are relatively modest limiting study power, especially in AA populations. The lack of replication in AA populations of known variants on subclinical atherosclerotic phenotypes reported previously in EA populations could be due to the lack of power; however, we cannot rule out allelic heterogeneity at each locus between the two ancestral populations. The lack of associations between the remaining CAD variants and subclinical atherosclerotic phenotypes may also be due to limited power. Most of the identified top variants on CAC or cIMT in our analyses require further replication in independent studies. In addition, we did not include diet, physical activity, and other environmental factors for adjustment in association analyses, which may confound the identified associations.

In a study of individuals diagnosed with T2D, we confirmed known variants previously reported for CAC and cIMT. Although we observed that many CAD loci potentially exert their CAD risk through the development of coronary atherosclerosis, we also identified a locus that is associated with CAC at genome-wide significance levels but not associated with CAD risk at this time. These findings may provide an improved understanding of the biological mechanisms underlying subclinical atherosclerosis and CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

AA	African ancestry
CAC	Coronary artery calcification
CAD	Coronary artery disease
cIMT	Carotid artery intima media thickness
CVD	Cardiovascular diseases
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
EA	European ancestry
GWAS	genome wide association study
SNP	Single nucleotide polymorphism
T2D	Type 2 diabetes

References:

1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN, et al.Heart disease and stroke statistics-2020 update: A report from the american heart association. Circulation. 2020;141:e139–e596. [PubMed: 31992061]
2. Baber U, Mehran R, Sartori S, Schoos MM, Sillesen H, Muntendam P, Garcia MJ, Gregson J, Pocock S, Falk E, et al.Prevalence, impact, and predictive value of detecting subclinical coronary and carotid atherosclerosis in asymptomatic adults: The bioimage study. J Am Coll Cardiol. 2015;65:1065–1074. [PubMed: 25790876]
3. Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O’Leary D, Carr JJ, Goff DC, Greenland P, Herrington DM. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA. 2012;308:788–795. [PubMed: 22910756]
4. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, Greenland P. Coronary artery calcium score and risk classification for coronary heart disease prediction. JAMA. 2010;303:1610–1616. [PubMed: 20424251]

5. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, Volcik K, Boerwinkle E, Ballantyne CM. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: The aric (atherosclerosis risk in communities) study. J Am Coll Cardiol. 2010;55:1600–1607. [PubMed: 20378078]
6. Meigs JB, Larson MG, D'Agostino RB, Levy D, Clouse ME, Nathan DM, Wilson PW, O'Donnell CJ. Coronary artery calcification in type 2 diabetes and insulin resistance: The framingham offspring study. Diabetes Care. 2002;25:1313–1319. [PubMed: 12145227]
7. Brohall G, Oden A, Fagerberg B. Carotid artery intima-media thickness in patients with type 2 diabetes mellitus and impaired glucose tolerance: A systematic review. Diabet Med. 2006;23:609–616. [PubMed: 16759301]
8. Turner ST, Peyser PA, Kardia SL, Bielak LF, Sheedy PF 3rd, Boerwinkle E, de Andrade M. Genomic loci with pleiotropic effects on coronary artery calcification. Atherosclerosis. 2006;185:340–346. [PubMed: 16054150]
9. Fischer M, Broeckel U, Holmer S, Baessler A, Hengstenberg C, Mayer B, Erdmann J, Klein G, Riegger G, Jacob HJ, et al.Distinct heritable patterns of angiographic coronary artery disease in families with myocardial infarction. Circulation. 2005;111:855–862. [PubMed: 15710764]
10. Bis JC, Kavousi M, Franceschini N, Isaacs A, Abecasis GR, Schminke U, Post WS, Smith AV, Cupples LA, Markus HS, et al.Meta-analysis of genome-wide association studies from the charge consortium identifies common variants associated with carotid intima media thickness and plaque. Nat Genet. 2011;43:940–947. [PubMed: 21909108]
11. Peyser PA, Bielak LF, Chu JS, Turner ST, Ellsworth DL, Boerwinkle E, Sheedy PF 2nd. Heritability of coronary artery calcium quantity measured by electron beam computed tomography in asymptomatic adults. Circulation. 2002;106:304–308. [PubMed: 12119244]
12. Zhao J, Cheema FA, Bremner JD, Goldberg J, Su S, Snieder H, Maisano C, Jones L, Javed F, Murrah N, et al.Heritability of carotid intima-media thickness: A twin study. Atherosclerosis. 2008;197:814–820. [PubMed: 17825306]
13. Natarajan P, Bis JC, Bielak LF, Cox AJ, Dorr M, Feitosa MF, Franceschini N, Guo X, Hwang SJ, Isaacs A, et al.Multiethnic exome-wide association study of subclinical atherosclerosis. Circ Cardiovasc Genet. 2016;9:511–520. [PubMed: 27872105]
14. O'Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, Sun YV, Province MA, Aspelund T, Dehghan A, et al.Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. Circulation. 2011;124:2855–2864. [PubMed: 22144573]
15. Wojczynski MK, Li M, Bielak LF, Kerr KF, Reiner AP, Wong ND, Yanek LR, Qu L, White CC, Lange LA, et al.Genetics of coronary artery calcification among african americans, a meta-analysis. BMC Med Genet. 2013;14:75. [PubMed: 23870195]
16. Franceschini N, Giambartolomei C, de Vries PS, Finan C, Bis JC, Huntley RP, Lovering RC, Tajuddin SM, Winkler TW, Graff M, et al.Gwas and colocalization analyses implicate carotid intima-media thickness and carotid plaque loci in cardiovascular outcomes. Nat Commun. 2018;9:5141. [PubMed: 30510157]
17. Divers J, Palmer ND, Langefeld CD, Brown WM, Lu L, Hicks PJ, Smith SC, Xu J, Terry JG, Register TC, et al.Genome-wide association study of coronary artery calcified atherosclerotic plaque in african americans with type 2 diabetes. BMC Genet. 2017;18:105. [PubMed: 29221444]
18. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, Ingelsson E, Lawlor DA, Selvin E, Stampfer M, et al.Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies. Lancet. 2010;375:2215–2222. [PubMed: 20609967]
19. Mazzone T, Chait A, Plutzky J. Cardiovascular disease risk in type 2 diabetes mellitus: Insights from mechanistic studies. Lancet. 2008;371:1800–1809. [PubMed: 18502305]
20. Merino J, Leong A, Posner DC, Porneala B, Masana L, Dupuis J, Florez JC. Genetically driven hyperglycemia increases risk of coronary artery disease separately from type 2 diabetes. Diabetes Care. 2017;40:687–693. [PubMed: 28298470]
21. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell Metab. 2011;14:575–585. [PubMed: 22055501]

22. Qi L, Qi Q, Prudente S, Mendonca C, Andreozzi F, di Pietro N, Sturma M, Novelli V, Mannino GC, Formoso G, et al.Association between a genetic variant related to glutamic acid metabolism and coronary heart disease in individuals with type 2 diabetes. JAMA. 2013;310:821–828. [PubMed: 23982368]

23. van der Harst P, Verweij N. Identification of 64 novel genetic loci provides an expanded view on the genetic architecture of coronary artery disease. Circ Res. 2018;122:433–443. [PubMed: 29212778]

24. Bland JM, Altman DG. Multiple significance tests: The bonferroni method. BMJ. 1995;310:170. [PubMed: 7833759]

25. Medina-Gomez C, Kemp JP, Trajanoska K, Luan J, Chesi A, Ahluwalia TS, Mook-Kanamori DO, Ham A, Hartwig FP, Evans DS, et al.Life-course genome-wide association study meta-analysis of total body bmd and assessment of age-specific effects. Am J Hum Genet. 2018;102:88–102. [PubMed: 29304378]

26. Slatkin MLinkage disequilibrium--understanding the evolutionary past and mapping the medical future. Nat Rev Genet. 2008;9:477–485. [PubMed: 18427557]

27. Human genomics. The genotype-tissue expression (gtex) pilot analysis: Multitissue gene regulation in humans. Science. 2015;348:648–660. [PubMed: 25954001]

28. Bonnet N, Garnero P, Ferrari S. Periostin action in bone. Mol Cell Endocrinol. 2016;432:75–82. [PubMed: 26721738]

29. Bonnet N, Conway SJ, Ferrari SL. Regulation of beta catenin signaling and parathyroid hormone anabolic effects in bone by the matricellular protein periostin. Proc Natl Acad Sci U S A. 2012;109:15048–15053. [PubMed: 22927401]

30. Kim BJ, Rhee Y, Kim CH, Baek KH, Min YK, Kim DY, Ahn SH, Kim H, Lee SH, Lee SY, et al.Plasma periostin associates significantly with non-vertebral but not vertebral fractures in postmenopausal women: Clinical evidence for the different effects of periostin depending on the skeletal site. Bone. 2015;81:435–441. [PubMed: 26297442]

31. Rousseau JC, Sornay-Rendu E, Bertholon C, Chapurlat R, Garnero P. Serum periostin is associated with fracture risk in postmenopausal women: A 7-year prospective analysis of the ofely study. J Clin Endocrinol Metab. 2014;99:2533–2539. [PubMed: 24628551]

32. Bonnet N, Biver E, Chevalley T, Rizzoli R, Garnero P, Ferrari SL. Serum levels of a cathepsin-k generated periostin fragment predict incident low-trauma fractures in postmenopausal women independently of bmd and frax. J Bone Miner Res. 2017;32:2232–2238. [PubMed: 28766739]

33. Yan J, Liu HJ, Li H, Chen L, Bian YQ, Zhao B, Han HX, Han SZ, Han LR, Wang DW, et al.Circulating periostin levels increase in association with bone density loss and healing progression during the early phase of hip fracture in chinese older women. Osteoporos Int. 2017;28:2335–2341. [PubMed: 28382553]

34. Terpos E, Christoulas D, Kastritis E, Bagratuni T, Gavriatopoulou M, Roussou M, Papatheodorou A, Eleutherakis-Papaiakovou E, Kanellias N, Liakou C, et al.High levels of periostin correlate with increased fracture rate, diffuse mri pattern, abnormal bone remodeling and advanced disease stage in patients with newly diagnosed symptomatic multiple myeloma. Blood Cancer J. 2016;6:e482. [PubMed: 27716740]

35. Ye C, Xu M, Wang S, Jiang S, Chen X, Zhou X, He R. Decreased bone mineral density is an independent predictor for the development of atherosclerosis: A systematic review and meta-analysis. PLoS One. 2016;11:e0154740. [PubMed: 27149062]

36. Guan XQ, Xue YJ, Wang J, Ma J, Li YC, Zheng C, Wu SZ. Low bone mineral density is associated with global coronary atherosclerotic plaque burden in stable angina patients. Clin Interv Aging. 2018;13:1475–1483. [PubMed: 30197509]

37. Hixson JE, Shimmin LC, Montasser ME, Kim DK, Zhong Y, Ibarguen H, Follis J, Malcom G, Strong J, Howard T, et al.Common variants in the periostin gene influence development of atherosclerosis in young persons. Arterioscler Thromb Vasc Biol. 2011;31:1661–1667. [PubMed: 21474826]

38. Schwanekamp JA, Lorts A, Vagnozzi RJ, Vanhoutte D, Molkentin JD. Deletion of periostin protects against atherosclerosis in mice by altering inflammation and extracellular matrix remodeling. Arterioscler Thromb Vasc Biol. 2016;36:60–68. [PubMed: 26564821]

39. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Patterson N, Daly MJ, Price AL, Neale BM. Ld score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet. 2015;47:291–295. [PubMed: 25642630]
40. Morris AP, Voight BF, Teslovich TM, Ferreira T, Segre AV, Steinthorsdottir V, Strawbridge RJ, Khan H, Grallert H, Mahajan A, et al.Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012;44:981–990. [PubMed: 22885922]
41. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37Suppl 1:S81–90. [PubMed: 24357215]
42. Daniell AL, Wong ND, Friedman JD, Ben-Yosef N, Miranda-Peats R, Hayes SW, Kang X, Sciammarella MG, de Yang L, Germano G, et al.Concordance of coronary artery calcium estimates between mdct and electron beam tomography. AJR Am J Roentgenol. 2005;185:1542–1545. [PubMed: 16304010]
43. Mao SS, Pal RS, McKay CR, Gao YG, Gopal A, Ahmadi N, Child J, Carson S, Takasu J, Sarlak B, et al.Comparison of coronary artery calcium scores between electron beam computed tomography and 64-multidetector computed tomographic scanner. J Comput Assist Tomogr. 2009;33:175–178. [PubMed: 19346841]
44. Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, et al.2010 accf/aha guideline for assessment of cardiovascular risk in asymptomatic adults: A report of the american college of cardiology foundation/american heart association task force on practice guidelines. J Am Coll Cardiol. 2010;56:e50–103. [PubMed: 21144964]
45. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr., Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol. 1990;15:827–832. [PubMed: 2407762]
46. Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, Najjar SS, Rembold CM, Post WS. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: A consensus statement from the american society of echocardiography carotid intima-media thickness task force. Endorsed by the society for vascular medicine. J Am Soc Echocardiogr. 2008;21:93–111; quiz 189–190. [PubMed: 18261694]
47. Das S, Abecasis GR, Browning BL. Genotype imputation from large reference panels. Annu Rev Genomics Hum Genet. 2018;19:73–96. [PubMed: 29799802]
48. Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Magi R, Ferreira T, Fall T, Graff M, Justice AE, et al.Quality control and conduct of genome-wide association meta-analyses. Nat Protoc. 2014;9:1192–1212. [PubMed: 24762786]
49. Reilly MP, Wolfe ML, Localio AR, Rader DJ. Coronary artery calcification and cardiovascular risk factors: Impact of the analytic approach. Atherosclerosis. 2004;173:69–78. [PubMed: 15177125]
50. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;38:904–909. [PubMed: 16862161]
51. Willer CJ, Li Y, Abecasis GR. Metal: Fast and efficient meta-analysis of genomewide association scans. Bioinformatics. 2010;26:2190–2191. [PubMed: 20616382]
52. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ. Locuszoom: Regional visualization of genome-wide association scan results. Bioinformatics. 2010;26:2336–2337. [PubMed: 20634204]
53. McVean GA, Myers SR, Hunt S, Deloukas P, Bentley DR, Donnelly P. The fine-scale structure of recombination rate variation in the human genome. Science. 2004;304:581–584. [PubMed: 15105499]
54. Panagiotou OA, Ioannidis JP. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. Int J Epidemiol. 2012;41:273–286. [PubMed: 22253303]
55. Lau J, Ioannidis JP, Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med. 1997;127:820–826. [PubMed: 9382404]

56. Liu B, Pjanic M, Wang T, Nguyen T, Gloudemans M, Rao A, Castano VG, Nurnberg S, Rader DJ, Elwyn S, et al.Genetic regulatory mechanisms of smooth muscle cells map to coronary artery disease risk loci. Am J Hum Genet. 2018;103:377–388. [PubMed: 30146127]
57. Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G, Thorgeirsson G, Aspelund T, Garcia ME, Cotch MF, et al.Age, gene/environment susceptibility-reykjavik study: Multidisciplinary applied phenomics. Am J Epidemiol. 2007;165:1076–1087. [PubMed: 17351290]
58. The atherosclerosis risk in communities (aric) study: Design and objectives. The aric investigators. Am J Epidemiol. 1989;129:687–702. [PubMed: 2646917]
59. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A, et al.The cardiovascular health study: Design and rationale. Ann Epidemiol. 1991;1:263–276. [PubMed: 1669507]
60. Bowden DW, Cox AJ, Freedman BI, Hugenschimdt CE, Wagenknecht LE, Herrington D, Agarwal S, Register TC, Maldjian JA, Ng MC, et al.Review of the diabetes heart study (dhs) family of studies: A comprehensively examined sample for genetic and epidemiological studies of type 2 diabetes and its complications. Rev Diabet Stud. 2010;7:188–201. [PubMed: 21409311]
61. Higgins M, Province M, Heiss G, Eckfeldt J, Ellison RC, Folsom AR, Rao DC, Sprafka JM, Williams R. Nhlbi family heart study: Objectives and design. Am J Epidemiol. 1996;143:1219–1228. [PubMed: 8651220]
62. Dawber TR, Kannel WB. The framingham study. An epidemiological approach to coronary heart disease. Circulation. 1966;34:553–555. [PubMed: 5921755]
63. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The framingham offspring study. Am J Epidemiol. 1979;110:281–290. [PubMed: 474565]
64. Splansky GL, Corey D, Yang Q, Atwood LD, Cupples LA, Benjamin EJ, D'Agostino RB Sr., Fox CS, Larson MG, Murabito JM, et al.The third generation cohort of the national heart, lung, and blood institute's framingham heart study: Design, recruitment, and initial examination. Am J Epidemiol. 2007;165:1328–1335. [PubMed: 17372189]
65. Daniels PR, Kardia SL, Hanis CL, Brown CA, Hutchinson R, Boerwinkle E, Turner ST. Familial aggregation of hypertension treatment and control in the genetic epidemiology network of arteriopathy (genoa) study. Am J Med. 2004;116:676–681. [PubMed: 15121494]
66. Taylor HA Jr., Wilson JG, Jones DW, Sarpong DF, Srinivasan A, Garrison RJ, Nelson C, Wyatt SB. Toward resolution of cardiovascular health disparities in african americans: Design and methods of the jackson heart study. Ethn Dis. 2005;15:S6-4–17.
67. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr., Kronmal R, Liu K, et al.Multi-ethnic study of atherosclerosis: Objectives and design. Am J Epidemiol. 2002;156:871–881. [PubMed: 12397006]
68. Hofman A, Breteler MM, van Duijn CM, Janssen HL, Krestin GP, Kuipers EJ, Stricker BH, Tiemeier H, Uitterlinden AG, Vingerling JR, et al.The rotterdam study: 2010 objectives and design update. Eur J Epidemiol. 2009;24:553–572. [PubMed: 19728115]
69. Carr JJ, Register TC, Hsu FC, Lohman K, Lenchik L, Bowden DW, Langefeld CD, Xu J, Rich SS, Wagenknecht LE, et al.Calcified atherosclerotic plaque and bone mineral density in type 2 diabetes: The diabetes heart study. Bone. 2008;42:43–52. [PubMed: 17964237]
70. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr., Sidney S, Bild DE, Williams OD, Detrano RC. Calcified coronary artery plaque measurement with cardiac ct in population-based studies: Standardized protocol of multi-ethnic study of atherosclerosis (mesa) and coronary artery risk development in young adults (cardia) study. Radiology. 2005;234:35–43. [PubMed: 15618373]
71. Multi-center genetic study of hypertension: The family blood pressure program (fbpp). Hypertension. 2002;39:3–9. [PubMed: 11799070]
72. Tullos BW, Sung JH, Lee JE, Criqui MH, Mitchell ME, Taylor HA. Ankle-brachial index (abi), abdominal aortic calcification (aac), and coronary artery calcification (cac): The jackson heart study. Int J Cardiovasc Imaging. 2013;29:891–897. [PubMed: 23111408]

73. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M, Bluemke DA, et al.Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med. 2008;358:1336–1345. [PubMed: 18367736]
74. Elias-Smale SE, Proenca RV, Koller MT, Kavousi M, van Rooij FJ, Hunink MG, Steyerberg EW, Hofman A, Oudkerk M, Witteman JC. Coronary calcium score improves classification of coronary heart disease risk in the elderly: The rotterdam study. J Am Coll Cardiol. 2010;56:1407–1414. [PubMed: 20946998]
75. Odink AE, van der Lugt A, Hofman A, Hunink MG, Breteler MM, Krestin GP, Witteman JC. Association between calcification in the coronary arteries, aortic arch and carotid arteries: The rotterdam study. Atherosclerosis. 2007;193:408–413. [PubMed: 16919637]
76. O'Leary DH, Polak JF, Wolfson SK, Jr., Bond MG, Bommer W, Sheth S, Psaty BM, Sharrett AR, Manolio TA. Use of sonography to evaluate carotid atherosclerosis in the elderly. The cardiovascular health study. Chs collaborative research group. Stroke. 1991;22:1155–1163. [PubMed: 1926258]
77. Lange LA, Bowden DW, Langefeld CD, Wagenknecht LE, Carr JJ, Rich SS, Riley WA, Freedman BI. Heritability of carotid artery intima-medial thickness in type 2 diabetes. Stroke. 2002;33:1876–1881. [PubMed: 12105369]
78. Gebreab SY, Riestra P, Khan RJ, Xu R, Musani SK, Tekola-Ayele F, Correa A, Wilson JG, Rotimi CN, Davis SK. Genetic ancestry is associated with measures of subclinical atherosclerosis in african americans: The jackson heart study. Arterioscler Thromb Vasc Biol. 2015;35:1271–1278. [PubMed: 25745061]
79. Gepner AD, Young R, Delaney JA, Tattersall MC, Blaha MJ, Post WS, Gottesman RF, Kronmal R, Budoff MJ, Burke GL, et al.Comparison of coronary artery calcium presence, carotid plaque presence, and carotid intima-media thickness for cardiovascular disease prediction in the multi-ethnic study of atherosclerosis. Circ Cardiovasc Imaging. 2015;8.
80. Polak JF, Szklo M, Kronmal RA, Burke GL, Shea S, Zavodni AE, O'Leary DH. The value of carotid artery plaque and intima-media thickness for incident cardiovascular disease: The multi-ethnic study of atherosclerosis. J Am Heart Assoc. 2013;2:e000087. [PubMed: 23568342]
81. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The rotterdam study. Circulation. 1997;96:1432–1437. [PubMed: 9315528]
82. Volzke H, Alte D, Schmidt CO, Radke D, Lorbeer R, Friedrich N, Aumann N, Lau K, Piontek M, Born G, et al.Cohort profile: The study of health in pomerania. Int J Epidemiol. 2011;40:294–307. [PubMed: 20167617]
83. Ludemann J, Piek M, Wood WG, Meyer S, Greiner B, John U, Hense HW. [methods for quality assurance of medical examination in epidemiological field studies: The "study of health in pomerania" (ship)]. Gesundheitswesen. 2000;62:234–243. [PubMed: 10844821]

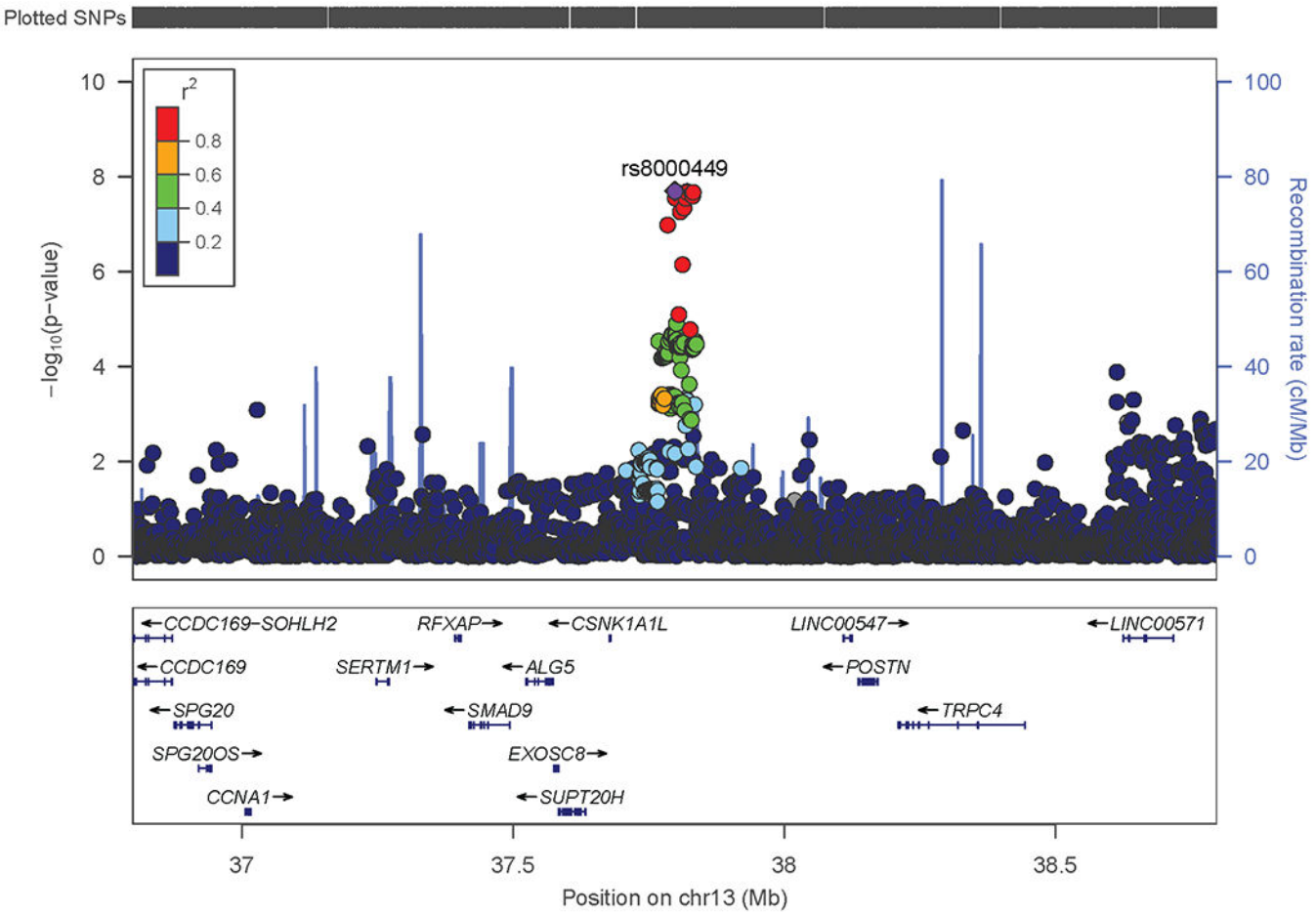

Figure 1.

Regional association plot of coronary artery calcification quantity at 13q13.3 (the *CSNK1A1L* locus) in populations of European ancestry (model 2, cardiovascular disease cases excluded). Each dot represents the P value (on a −log10 scale) of association for a SNP with CAC risk, presented according to its genomic position (NCBI Build 37). The most significantly associated SNP is represented by a purple diamond. The color of all other SNPs indicates the level of linkage disequilibrium with the lead SNP (estimated by EUR r^2 from the 1000 Genome Project data). Recombination rates were also estimated from 1000 Genomes Project data (Phase 3), and gene annotations within the 2-Mb regions centered on rs8000449 were obtained from the UCSC Genome Browser.

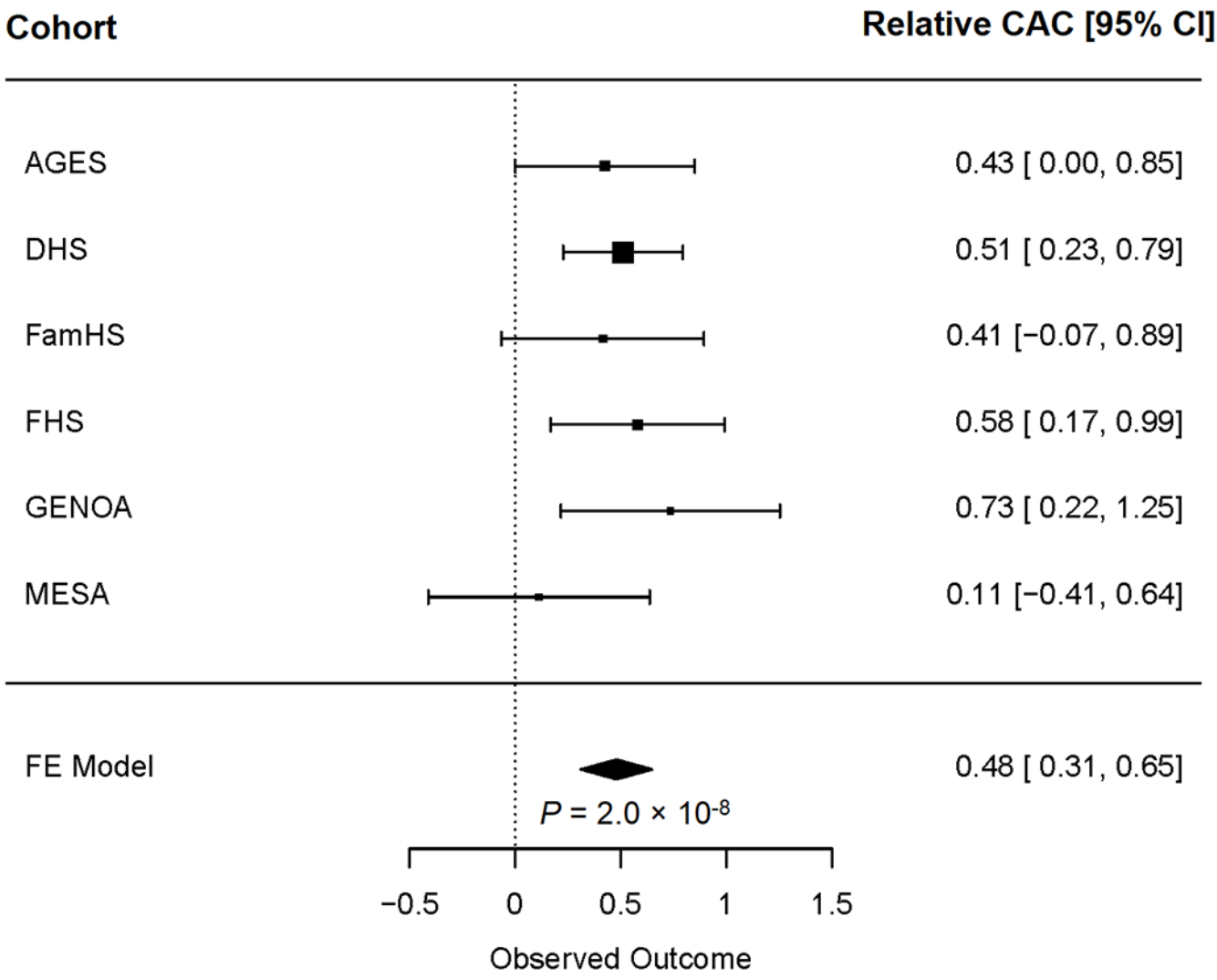

Figure 2.

Forest plot of relative coronary artery calcification (CAC) quantity for rs8000449 near *CSNK1A1L* at 13q13.3. CAC quantity in the log scale for T allele carriers relative to noncarriers (in model 2 excluding individuals diagnosed with cardiovascular diseases) is displayed for all cohorts to demonstrate consistency across cohorts in populations of European ancestry. AGES, The Reykjavik study cohort of Age, Gene/Environment Susceptibility; DHS, The Diabetes Heart Study; FamHS, Family Heart Study; FHS, Framingham Heart Study; GENOA, Genetic Epidemiology Network of Arteriopathy; MESA, Multi-Ethnic Study of Atherosclerosis.

Table 1.

Top meta-analysis variant associations for subclinical atherosclerotic phenotypes (CAC, coronary artery calcification; cIMT, carotid intima-media thickness) with $P < 1 \times 10^{-6}$.

SNP	Chr	Position	Nearby genes	RA	OA	EAF	Beta	SE	*P value*	N	*P* het.	R^2	Analysis*	Populations
Loci reaching genome-wide significance with $P < 5 \times 10^{-8}$														
rs8000449	13	37,798,358	*CSNK1A1L*	T	C	0.42	0.48	0.09	2.02E-08	1,420	0.66	0.99	CACm2	EA
Loci near genome-wide significance $P < 1 \times 10^{-6}$ but $P > 5 \times 10^{-8}$														
rs142317896	4	129,462,367	*PGRMC2*	C	T	0.05	1.02	0.20	1.73E-07	1,590	0.69	0.93	CACm1	AA
rs8000449	13	37,798,358	*CSNK1A1L*	T	C	0.42	0.32	0.06	5.16E-07	2,500	0.40	0.99	CACm1	EA
rs73908993	2	2,605,393	*MYT1L*	C	T	0.92	0.91	0.18	5.13E-07	1,248	0.68	0.97	CACm2	AA
rs7559121	2	19,961,396	*FLJ12334*	T	A	0.39	0.54	0.10	6.51E-08	1,248	0.42	0.97	CACm2	AA
rs9345325	6	65,343,240	*EYS*	T	C	0.05	1.12	0.22	4.69E-07	1,248	0.90	0.98	CACm2	AA
rs9402365	6	132,229,582	*ENPP1*	G	A	0.28	0.56	0.11	9.26E-08	1,248	0.91	0.99	CACm2	AA
rs6559349	9	92,007,225	*SEMA4D*	G	T	0.67	0.53	0.11	9.19E-07	1,248	0.29	0.79	CACm2	AA
rs3895978	11	104,241,240	*PDGFD*	C	T	0.94	0.99	0.20	8.32E-07	1,248	0.60	0.99	CACm2	AA
rs11088975	21	46,407,438	*NCRNA00163*	G	A	0.45	0.50	0.10	4.13E-07	1,248	0.14	0.86	CACm2	AA
rs17178946	14	72,365,767	*RGS6*	G	A	0.10	0.74	0.15	4.19E-07	1,420	0.54	0.98	CACm2	EA
rs145388160	15	51,813,649	*DMXL2*	A	G	0.90	1.04	0.21	6.71E-07	896	0.76	0.67	CACm2	EA
rs143306427	2	232,599,797	*PDE6D*	A	G	0.17	0.53	0.11	7.11E-07	2,144	0.23	0.82	CACm2	AA_EA
rs35519714	15	53,033,919	*ONECUT1*	T	C	0.07	0.86	0.16	1.96E-07	2,012	0.72	0.72	CACm2	AA_EA
rs74064266	1	39,084,837	*RRAGC*	A	G	0.08	0.10	0.02	6.21E-07	838	0.39	0.95	cIMT	AA
rs77371030	2	40,534,703	*SLC8A1*	A	C	0.85	0.07	0.01	8.72E-07	838	0.87	0.85	cIMT	AA
rs2364693	2	209,753,095	*PTH2R*	C	T	0.13	0.08	0.02	7.26E-07	838	0.91	0.98	cIMT	AA
rs7619004	3	185,983,561	*DGKG*	T	C	0.14	0.08	0.02	6.81E-07	838	0.42	0.87	cIMT	AA
rs11951438	5	176,057,328	*SNCB*	A	G	0.22	0.07	0.01	3.80E-07	838	0.44	0.77	cIMT	AA
rs6922641	6	115,128,946	*HS3ST5*	C	A	0.21	0.06	0.01	5.96E-07	838	0.51	0.99	cIMT	AA
rs142782777	11	56,904,254	*LRRC55*	G	A	0.13	0.08	0.02	2.92E-07	838	0.98	0.72	cIMT	AA
rs2119976	4	78,480,210	*CXCL13*	A	C	0.06	0.06	0.01	4.65E-07	2,869	0.91	0.78	cIMT	EA
rs6447296	4	43,795,651	*KCTD8*	A	T	0.13	0.03	0.01	9.86E-07	4,445	0.95	0.92	cIMT	AA_EA
rs10942555	5	88,906,680	*MIR3660*	T	C	0.18	0.03	0.01	7.44E-07	4,435	0.60	0.90	cIMT	AA_EA

SNP	Chr	Position	Nearby genes	RA	OA	EAF	Beta	SE	*P value*	N	*P* het.	R^2	Analysis*	Populations
rs7337123	13	82,471,871	*SPRY2*	A	G	0.05	0.08	0.02	7.26E-07	3,127	0.59	0.45	cIMT	AA_EA

RA: reference (effect) allele; OA: other allele; EAF; effect allele frequency; *P* het: P value for heterogeneity test; R^2: mean imputation quality.

*model 1 (m1): analyses conducted with all diabetic individuals; model 2 (m2): analyses conducted in diabetic individuals excluding individuals diagnosed with cardiovascular diseases.