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Association of a 62 Variant Type 2 Diabetes Genetic Risk Score with Markers of Subclinical Atherosclerosis: A Transethnic, Multicenter Study

Running title: *Dauriz et al.; T2D genetics and subclinical atherosclerosis*

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Abstract:

Background - Type 2 diabetes (T2D) and cardiovascular disease (CVD) share risk factors and subclinical atherosclerosis (SCA) predicts events in those with and without diabetes. T2D genetic risk may predict both T2D and SCA. We hypothesized that greater T2D genetic risk is associated with higher extent of SCA.

Methods and Results - In a cross-sectional analysis including up to 9,210 European Americans, 3,773 African Americans, 1,446 Hispanic Americans and 773 Chinese Americans without known CVD and enrolled in the FHS, CARDIA, MESA and GENOA studies, we tested a 62 T2D-loci genetic risk score (GRS_{62}) for association with measures of SCA, including coronary artery (CACS) or abdominal aortic calcium score, common (CCA-IMT) and internal carotid artery intima-media thickness, and ankle-brachial index (ABI). We used ancestry-stratified linear regression models, with random effects accounting for family relatedness when appropriate, applying a genetic-only (adjusted for sex) and a full SCA risk factors adjusted model (significance = $p<0.01 = 0.05/5$, number of traits analyzed). An inverse association with CACS in MESA Europeans (fully-adjusted $p=0.004$) and with CCA-IMT in FHS ($p=0.009$) was not confirmed in other study cohorts, either separately or in meta-analysis. Secondary analyses showed no consistent associations with β-cell and insulin resistance sub-GRS in FHS and CARDIA.

Conclusions - SCA does not have a major genetic component linked to a burden of 62 T2D loci identified by large genome-wide association studies. A shared T2D-SCA genetic basis, if any, might become apparent from better functional information about both T2D and CVD risk loci.

Keywords: genetic association, risk assessment, subclinical atherosclerosis risk factor, type 2 diabetes mellitus, cardiovascular disease

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Introduction

Type 2 diabetes (T2D) and cardiovascular disease (CVD) are clinically associated in adults[1] and are an increasing public health and economic scourge in the US[2, 3] and worldwide[4, 5]. Better prevention strategies require comprehension of risk factors and mediators underlying T2D and CVD[6]. T2D and CVD share a common metabolic milieu that triggers metabolic and vascular dysfunction starting at subclinical disease stages[7] due to genetic and non-genetic risk factors. In particular, many recently identified common genetic variants increasing T2D risk also are associated with increased CVD risk[8, 9] and so might confer risk for subclinical atherosclerosis (SCA)[10].

Recently, a set of 36 single nucleotide polymorphisms (SNPs) previously identified in large genome-wide association studies (GWAS) as affecting T2D risk were associated with an increased risk of cardiovascular complications in T2D patients[8]. We have also shown that an additive genetic risk score (GRS_{62}) comprised of 62 validated T2D-associated SNPs[11-14] predicts incident T2D in European and African Americans[15, 16].

The present work sought to investigate whether the T2D genetic burden, as represented by the polygenic T2D GRS_{62}, is associated in cross-sectional analyses with variation in SCA measures, including coronary artery (CACS) or abdominal aortic calcium score (AACS), internal (ICA-IMT) or common carotid artery intima-media thickness (CCA-IMT), and ankle-brachial index (ABI).

To maximize our sample size we conducted a multicenter transethnic association study in large population samples from four studies currently ongoing across the US: the Framingham Heart Study (FHS), the Coronary Artery Risk Development in Young Adults (CARDIA)[17], the Multi-Ethnic Study of Atherosclerosis (MESA)[18, 19] and the Genetic Epidemiology Network of

Arteriopathy (GENOA)[20, 21].

Methods

1. Population

The Offspring Cohort of the Framingham Heart Study

Analyses were conducted for each measured SCA trait on a range of 1,111 up to 2,822 participants of European ancestry from the Offspring Cohort of the FHS[22]. These subjects pertained the same cohort used to validate the predictability of incident T2D by GRS_{62}[19]. Outcomes of interest and clinical characteristics were obtained at Offspring examination cycles 6 (for analyses of ICA-IMT and CCA-IMT) and 7 (for CACS and AACS). ABI was measured between the two examinations and covariates included in ABI analyses were from the closest examination to ABI evaluation date. More details have been published previously[22, 23].

The CARDIA Study

Analyses were conducted for the available SCA traits on 816 African Americans and 1,635 European Americans[17]. Only participants with complete genotype and clinical information for all predictors of interest were included in the analyses. We used data on SCA from follow-up visit at years 20 (ICA-IMT and CCA-IMT) and 25 (CACS).

The MESA Study

The MESA Study was designed to prospectively evaluate the development and progression of atherosclerotic disease[19]. The selection included individuals from the resident list of adults from the urban areas of the recruiting centers with emphasis on ethnic diversity. The present study included up to 2,526 participants of European ancestry, 1,611 African Americans, 773 Asian Americans and 1,446 Hispanic Americans from examination year 1 (2000-2001).

The GENOA Study

The longitudinal Genetic Epidemiology Network of Arteriopathy (GENOA) Study is one of four networks in the NHLBI Family-Blood Pressure Program and aims to elucidate the genetics of target organ complications of hypertension[21]. GENOA recruited European and African American sibships with at least 2 individuals with clinically diagnosed essential hypertension before age 60 years. European Americans were recruited from the Rochester, MN Field Center and African Americans were recruited from the Jackson, MS Field Center. Current analyses were conducted on CACS measures and genotypes available for 969 European Americans and 535 African Americans.

In all study cohorts, participants with a personal history of CVD defined as myocardial infarction, stroke, coronary angioplasty and/or amputation not due to injury, when applicable, were excluded from the analyses.

2. Assessment of subclinical atherosclerosis

SCA measures were determined in a similar fashion in all studies by carotid ultrasonography intima-media thickness, subcategorized for common and internal carotid (CCA-IMT, ICA-IMT), computed tomography scan for CACS and AACS, and ABI for peripheral artery disease[22, 24-27]. All five SCA traits were measured in FHS and MESA participants. ICA-IMT, CCA-IMT[28] and CACS[29] measurements were available in CARDIA. Evaluation and interpretation of CACS measures in MESA were conducted as published elsewhere[29, 30]. In GENOA, CACS was measured in European Americans with an Imatron C-150 electron beam CT scanner (Imatron Inc., South San Francisco, CA)[31]. In GENOA African Americans, CACS was measured with standard scanning protocols developed as part of the NHLBI's MESA and CARDIA studies[29].

3. Genotyping

Genotyping in FHS was conducted using the Affymetrix GeneChip Human Mapping 500K Array supplemented with the Affymetrix 50K array. CARDIA and MESA Studies used the Affymetrix Genome-Wide Human SNP Array 6.0 (Santa Clara, California)[32]. GENOA Study used the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, 2007) or the Illumina Human 1M-Duo BeadChip (Illumina, 2010) at the Mayo Clinic, Rochester, MN. Quality control and imputation for missing genotypes were previously detailed[16, 32-34] for FHS and CARDIA. Complete information on genotyping and imputation quality of MESA and GENOA are available in the Supplementary Material.

Ethics Statement

Local Institutional Review Boards approved FHS, CARDIA, MESA and GENOA study protocols and all participants provided written informed consent.

Statistical Analysis

The GRS in FHS and CARDIA European Americans was calculated by summing the number of risk alleles (0, 1, or 2) at each locus, weighted by its published effect-size (natural log-transformed)[11]. For CARDIA African Americans and for each MESA and GENOA ethnic group we used an unweighted GRS, calculated by summing the risk alleles across the loci. We used an unweighted GRS for non-European ancestry cohorts because most of the T2D SNPs come from GWAS conducted among people of European ancestry. However, weighting has little effect on the GRS[35], makes models fit slightly better, but does not change the ranking of individuals from low to high risk[15, 35].

ICA-IMT, CCA-IMT, AACS, CACS, fasting insulin, triglycerides and HDL-cholesterol were log-transformed to reduce skewness. Descriptive data were expressed as mean±SE, if not

otherwise indicated. We used multivariable linear regression models for CARDIA and MESA cohorts and similar models in FHS and GENOA with a random effect accounting for family relatedness to test the association of an additive 62 T2D SNPs GRS (Supplemental Table 1) with measures of SCA.

For each SCA trait we applied models adjusted for sex (genetic-only model) and for a comprehensive set of SCA risk factors (full model), as shown in Supplemental Table 2. Principal components were included in GENOA and MESA models to control for population stratification in each ethnic group. The fully-adjusted model included: sex, age, waist circumference, body mass index (BMI), triglycerides, HDL-cholesterol, LDL-cholesterol, fasting insulin, fasting glucose, systolic blood pressure (SBP), hypertension/diabetes and/or lipid medication, physical activity, smoking, family history of T2D and/or CVD. SBP was excluded in the analysis for ABI since ABI is calculated from SBPs at ankle and arm.

We also conducted subsidiary analyses of two subsets of the 62 T2D SNPs comprised of 20 tag-SNPs thought to be associated with β-cell function (GRS_{β}) or 10 associated with insulin resistance (GRS_{IR})[16] in the FHS and CARDIA cohorts to further elucidate possible mechanistic pathways, testing the hypothesis that genetic risk for IR in particular would be associated with SCA.

The rationale behind computing a GRS to account for the cumulative burden of a genetic exposure stands upon prior literature[36, 37]. Indeed, using GRS allows to carry out association analyses by treating the genetic exposure as a whole, irrespective of the number of SNPs comprised in the score.

Post-hoc power calculations using QUANTO 1.2 software showed that for a sample size of 1,835 individuals, we had 80% power to detect association of GRS_{62} explaining 0.64% of the

variance in SCA traits with type 1 error rate set at $p<0.01$ ($p=0.05$ divided by the number of traits (5) analyzed). We had 80% power to detect association of GRS_{β} and GRS_{IR} each explaining 0.73% or more of the variance in SCA traits with type 1 error set at $p<0.005$ (0.05/[5 traits x 2 GRS]).

In order to replicate the primary FHS analyses in European Americans and to verify whether they might be extended to different ancestral groups, we conducted association analyses of GRS_{62} with CACS, ICA-IMT and CCA-IMT in CARDIA, MESA and GENOA cohorts separately within each ethnicity. Then, association results from each cohort were meta-analyzed using a fixed effect approach, separately for European and African Americans, with a two-sided $p<0.01$ as threshold for significance.

All statistical analyses were carried out with SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and R 2.9.2 (http://www.r-project.org).

Results

We analyzed up to 7,952 European Americans, 2,124 Africans Americans, 773 Asian Americans and 1,446 Hispanic Americans from four cohort studies. Clinical and anthropometric features and measures of SCA traits are shown for each study cohort in Supplemental Table 3 and Supplemental Table 4.

Overall, study participants were of a wide age and BMI range. Prevalence of diabetes and abdominal obesity was much higher in African Americans than in other ethnicities. Participant characteristics within each ethnic group were comparable across all cohorts with the proportion of males and females being equally distributed, except in GENOA African Americans where women comprised 74.2% of the participants. The T2D GRS_{62} was approximately normally distributed with a range from 48.3 to 83.3 in European Americans and from 46.8 to 83.2 in

African Americans over all cohorts. African Americans in CARDIA had higher mean GRS than European Americans, while the opposite was the case in MESA and GENOA cohorts. In MESA Asian and Hispanic Americans the T2D GRS_{62} spanned, respectively, from 48.1 to 73.6 and from 48.5 to 79.6 (Supplemental Figure 1 and Supplemental Figure 2). GRS_{β} and GRS_{IR} were normally distributed and ranged from 12.2 to 31.9 and from 3.3 to 18.0 in FHS and CARDIA European Americans, respectively, while in CARDIA African Americans the GRS_{β} ranged from 13.2 to 28.4 and the GRS_{IR} ranged from 5.0 and 16.9 (Supplemental Figure 2).

The primary analyses in FHS showed an inverse association between GRS_{62} and CCA-IMT (p=0.009, full model), which was not replicated in CARDIA or MESA (Table 1) European Americans. In the MESA European Americans, there was a significant inverse association between GRS_{62} and CACS (p=0.004, full model), which was not replicated in other cohorts (Table 1). Lack of a significant association between GRS_{62} and SCA was confirmed by meta-analyses of up to 12,983 individuals from four cohorts for the available SCA traits, i.e. CACS, CCA-IMT and ICA-IMT (Figure 1).

Supplemental analyses showed that ICA-IMT was negatively associated with GRS_{β} in FHS in the full model (p=0.007, Supplemental Table 5), but this finding was not replicated in CARDIA European Americans. The GRS_{IR} was not associated with any SCA trait in any of the models in either the FHS cohort or in either CARDIA ethnic group (Supplemental Table 6).

Discussion

The primary finding of our study was the absence of a significant association between the genetic burden for T2D, based on a 62 SNPs GRS, and a wide set of SCA traits in a large US adult population. Results were consistent for all four ancestral groups studied. An inverse association of the GRS_{62} with CCA-IMT in FHS was not confirmed in two other cohorts either in

replication comparisons or meta-analysis. A 10 SNP GRS and a 20 SNP GRS representing variants associated, respectively, with β-cell function or insulin resistance also showed no significant association with SCA.

A wide literature has validated the approach of using a T2D GRS in diverse populations as a robust predictor of T2D, whether alone or considering clinical variables[16, 17, 35, 38-41]. Prior studies also used the T2D GRS to predict vascular disease[8, 42-44], showing that T2D susceptibility variants are able, cumulatively, to predict coronary heart disease (CHD) events. As single genetic variants only explain a very small proportion of the variation in T2D risk, we did not expect individual SNPs to achieve study-wide significance for association with SCA traits, especially with the present sample size. To overcome weak effects of individual variants, we therefore applied the widely used strategy of expressing overall T2D genetic risk burden as a GRS, to test for association with SCA. Indeed, a recent analysis of the cumulative effect of common genetic variation associated with BMI- and fasting insulin loci showed that a higher burden of variants was associated with metabolic syndrome traits, IR and CHD events, but not SCA[45].

Starting with the plausible hypothesis that T2D genetic risk would be associated with SCA, we used a rigorous approach including conservative correction for multiple trait tests, replication studies in separate cohorts (thereby reducing type 1 error) and meta-analysis of a large sample size (increasing power). We therefore can conclusively state that a measure of T2D genetic risk is not associated with higher indices of SCA in these cohorts.

These results can be compared with other recent studies. As recently reviewed[9], the genetic signatures of T2D, CHD and glycemic quantitative traits seem to overlap only at chromosomes 2q36.3 and 9p21.3. Notably, a major proportion of fasting insulin-associated loci have shown directionally consistent associations with T2D risk and CVD quantitative risk factors

(i.e. adverse lipid profile and abdominal adiposity) but none of the glycemic quantitative traits has been directly associated with CVD-risk. In this context, Qi et al.[8] showed that the genetic risk of T2D, as represented by a 36 T2D SNPs GRS, was associated with an increased risk of CVD in European Americans affected by T2D. Additionally, while Doria et al.[46] showed that the effect of genotype at 9p21.3 locus on CVD events was raised only in persons with T2D with poor glycemic control, Rivera et al.[47] found that, compared to non-T2D individuals, the genetic variation at 9p21.3 locus was associated with a higher severity of coronary artery disease comorbid with T2D. On the other hand, a recent analysis in the FHS[22] specifically pointing to the genetic variation at candidate 2q36.3-*IRS1* locus failed to identify an etiological link between SCA and T2D.

It might therefore be argued that distinct mechanisms lead T2D and non-T2D subjects to CVD events, and that within T2D cases hyperglycemia might act as permissive environment leading to the full expression of CVD-risk genetics. These data, together with the null results of our present study, both with our T2D GRS_{62} and with the two sub-scores (GRS_{IR} and GRS_{β}), collectively suggest that in the general population T2D and CVD are not genetically linked together through SCA, the association of T2D genetics being so far observed only with CVD events but not with early subclinical disease.

Our analysis plan was designed to specifically test the impact of a comprehensive T2D genetic risk burden on SCA risk. We therefore created a basic, purely genetic, model by including as exposure both GRS and sex, as sex is 100% genetically determined and is also associated with T2D risk. Then, we added covariates like age and other confounders/mediators not completely genetically determined but potentially related to a pro-atherosclerotic, pro-diabetic phenotype. In particular, we did not specifically aim to mechanistically unravel the

pathobiology of atherosclerosis. Instead, we adjusted for sex in the genetic-only model to simply address the question of whether the known spectrum of established genetic determinants of T2D (including sex chromosome) is associated with measures of SCA.

Strengths of our study include a validated T2D GRS aligned to the current level of evidence, a detailed characterization of SCA, a comprehensive selection of covariates, and a careful control of type 1 and type 2 error by means of a large sample size from the general population and a multicenter replication strategy in different ethnicities. Additionally, given the strong age-calcification relationship across young adulthood, mid-life and older ages[48, 49], the wide range of age among our different cohorts allowed to capture the whole spectrum from early- to late-onset calcification.

However, our approach might have been weakened by multiple interactions among different SNPs within the GRS: several of the component genes in the score may be indeed associated with SCA, but the component score might not be significantly associated if the effect was diluted by the other variants. We did not perform individual SNP tests for association with SCA, as individual locus effects were not our main hypothesis and would require a vast sample size to account for the increased type 1 error rate and to identify individual locus effects, the threshold for significance being in that case $p<1.6x10^{-4}$ (i.e. 0.05/(5 SCA traits) x (62 SNPs)). Furthermore, the 62 genome-wide significant SNPs we used explained only a fraction (around 10%) of the total T2D phenotypic variance in other studies[50] and did not represent actual functional variants that have yet to be discovered. We also acknowledge that the exclusion of CVD individuals, may have resulted in a population enriched for protective factors especially among those with higher T2D GRS, which would explain the borderline negative association of the GRS_{62} with CCA IMT in FHS and CACS in MESA European Americans. However, in

sensitivity analyses conducted in FHS and CARDIA the T2D GRS_{62} allele distribution was comparable between people with positive CVD history and the population actually analyzed (data not shown). Hence, for consistency with our main focus on SCA we excluded individuals with clinical CVD. The relatively younger age of CARDIA participants offers another possible limitation, however, it is well known that SCA begins to develop in Westernized populations in early youth[51, 52]. Further, associations in CARDIA were similar to those in the other cohorts we studied. Lastly, while we could confidently use the GRS to depict the T2D genetic risk for European and Mexican Americans, and therefore reasonably claim robustness of our results, our GRS was not best tailored to African or Asian Americans. However, we have shown in several prior studies that T2D GRS based on SNPs found in European ancestry samples do predict T2D in African American samples, even accounting for clinical risk factors[16, 17, 53].

Our results have several implications and point to future directions. We provided compelling evidence that the genetic burden of T2D risk as represented by our GRS_{62} formulation was not associated with SCA. This suggests that T2D and SCA have separate genetic structures and that no large common variant genetic soil[10] underlies both T2D and CVD. However, it is possible that more complex formulation of T2D genetic risk might be associated with SCA.

T2D and SCA are linked clinically[1, 8] and the prevalence of CVD events and the burden of CVD risk factors are higher in T2D patients. Furthermore, there is evidence that screening for SCA in asymptomatic individuals at intermediate CVD risk improves the predictability of the occurrence of CVD over and above established CVD risk factors[54]. Incorporating genetic information into disease prediction models would further improve the ability to capture people with higher CVD risk at a preclinical stage. However, current polygenic scores do not

remarkably outperform clinical models[15] and functional interrogation of T2D and CVD genetics is necessary to further optimize polygenic risk prediction of either T2D or CVD or both. Therefore, despite our understanding of the genetic signature of complex traits is steadily increasing, new approaches incorporating functional, structural and/or regulatory annotation[55] into disease prediction are needed to untangle the missing link, if any, between T2D and CVD at a genetic level.

In conclusion, common polygenic T2D risk variation, as incorporated in a comprehensive and validated GRS, was not associated with any of five measures of SCA. Our study suggests that the biological and genetic relationships among T2D, CVD and SCA are probably more complex than expected. Further mechanistic investigations are needed to explore whether shared or distinct vascular disease mechanisms related to T2D might be in play. Therefore, given the global burden of T2D and CVD in the era of precision medicine and patient-oriented healthcare, it is timely to achieve a deeper understanding about the genetic determinants of T2D, CVD and intermediate risk traits, in order to improve risk prediction and the ability to discover newly targeted therapeutic molecules.

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Table 1: Association between a 62 T2D SNPs genotype risk score (GRS) and prevalent subclinical atherosclerosis measures in linear regression models of FHS, CARDIA, GENOA* and MESA cohorts.

	FHS		CARDIA				GENOA			
	European American		European Americans		African Americans		European Americans		African American*	
Basic Model	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*
CACS	-0.012±0.026	0.66	0.011±0.010	0.27	-0.007±0.016	0.64	0.003±0.017	0.88	0.025±0.029	0.40
AACS	-0.029±0.032	0.36	-	-	-	-	-	-	-	-
ICA-IMT	-0.008±0.004	0.03	0.000±0.001	0.88	0.000±0.001	0.67	-	-	-	-
CCA-IMT	-0.002±0.002	0.21	0.000±0.000	0.94	0.001±0.001	0.08	-	-	-	-
ABI	0.000±0.001	0.72	-	-	-	-	-	-	-	-
Full Model										
CACS	-0.012±0.028	0.67	0.006±0.010	0.56	-0.018±0.015	0.24	0.001±0.015	0.95	0.041±0.026	0.12
AACS	-0.017±0.033	0.61	-	-	-	-	-	-	-	-
ICA-IMT	-0.009±0.004	0.02	0.000±0.001	0.63	0.000±0.001	0.72	-	-	-	-
CCA-IMT	-0.004±0.002	**0.009**	0.000±0.000	0.54	0.000±0.001	0.47	-	-	-	-
ABI	0.001±0.001	0.13	-	-	-	-	-	-	-	-

MESA

	European Americans		Asian Americans		African Americans		Hispanic Americans	
Basic Model	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*
CACS	-0.026±0.010	0.01	-0.027±0.012	0.14	0.019±0.014	0.17	-0.004±0.014	0.76
AACS	-0.042±0.023	0.07	-0.022±0.048	0.65	0.003±0.039	0.93	0.029±0.031	0.33
ICA-IMT	-0.001±0.002	0.75	0.001±0.004	0.85	-0.001±0.003	0.65	0.006±0.003	0.02
CCA-IMT	-0.001±0.001	0.33	-0.001±0.002	0.65	-0.000±0.001	0.79	-8.98E-06±0.001	0.99
ABI	3.27E-05±0.001	0.95	0.000±0.001	0.63	-0.000±0.001	0.64	0.000±0.001	0.61
Full Model								
CACS	-0.029±0.009	**0.004**	-0.027±0.019	0.16	0.016±0.014	0.28	0.002±0.015	0.88
AACS	-0.012±0.019	0.53	-0.033±0.039	0.40	-0.027±0.035	0.44	0.035±0.028	0.20
ICA-IMT	-0.001±0.002	0.63	-0.000±0.004	0.98	-0.002±0.003	0.52	0.006±0.003	0.02
CCA-IMT	-0.001±0.001	0.30	-0.001±0.002	0.77	-0.000±0.001	0.87	9.49E-05±0.001	0.93
ABI	0.000±0.001	0.44	0.000±0.001	0.67	-0.001±0.001	0.36	0.000±0.001	0.85

CACS: coronary artery calcium score, AACS: abdominal aorta calcium score, ICA: internal carotid artery, CCA: common carotid artery, IMT: intima-media thickness, ABI: ankle-brachial index. Basic Model: SCA trait = GRS + sex + k; Full Model: fully-adjusted model (GRS + sex, age, waist circumference, body mass index, triglycerides, HDL-cholesterol, LDL-cholesterol, fasting insulin, fasting glucose, systolic blood pressure (SBP), hypertension/diabetes and/or lipid medication, physical activity, smoking, family history of T2D and/or CVD. SBP was excluded in the analysis for ABI since ABI is calculated from SBPs at ankle and arm). *African Americans in GENOA had a genetic risk score limited to 55 of 62 T2D SNPs. Data expressed as mean ± standard error. Sample sizes (*N* [min-max]): FHS N_{Eur} [1,111-2,822]; CARDIA: N_{Eur} [1,267-1,635], N_{Afr} [562-816]; GENOA: N_{Eur} =969, N_{Afr} =535; MESA: N_{Eur} [760-2,526], N_{Asi} [247-773]; N_{Afr} [343-1,611], N_{His} [496-1,446].

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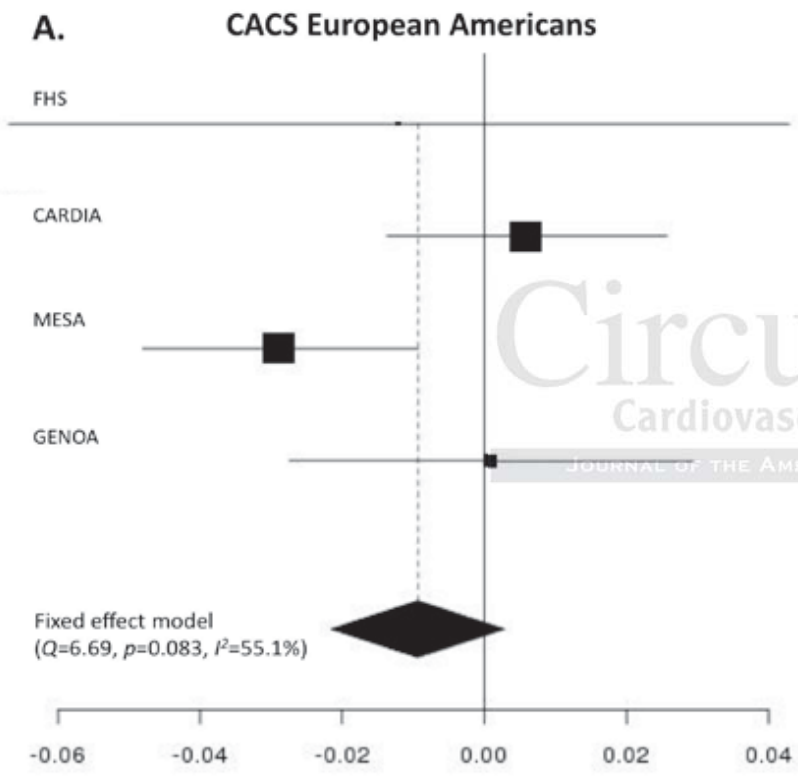
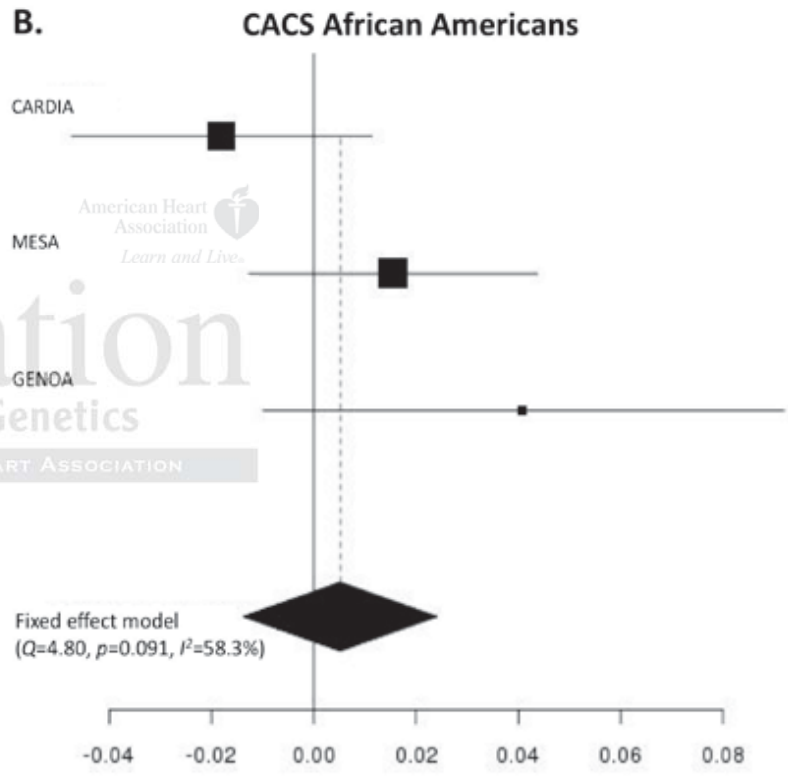
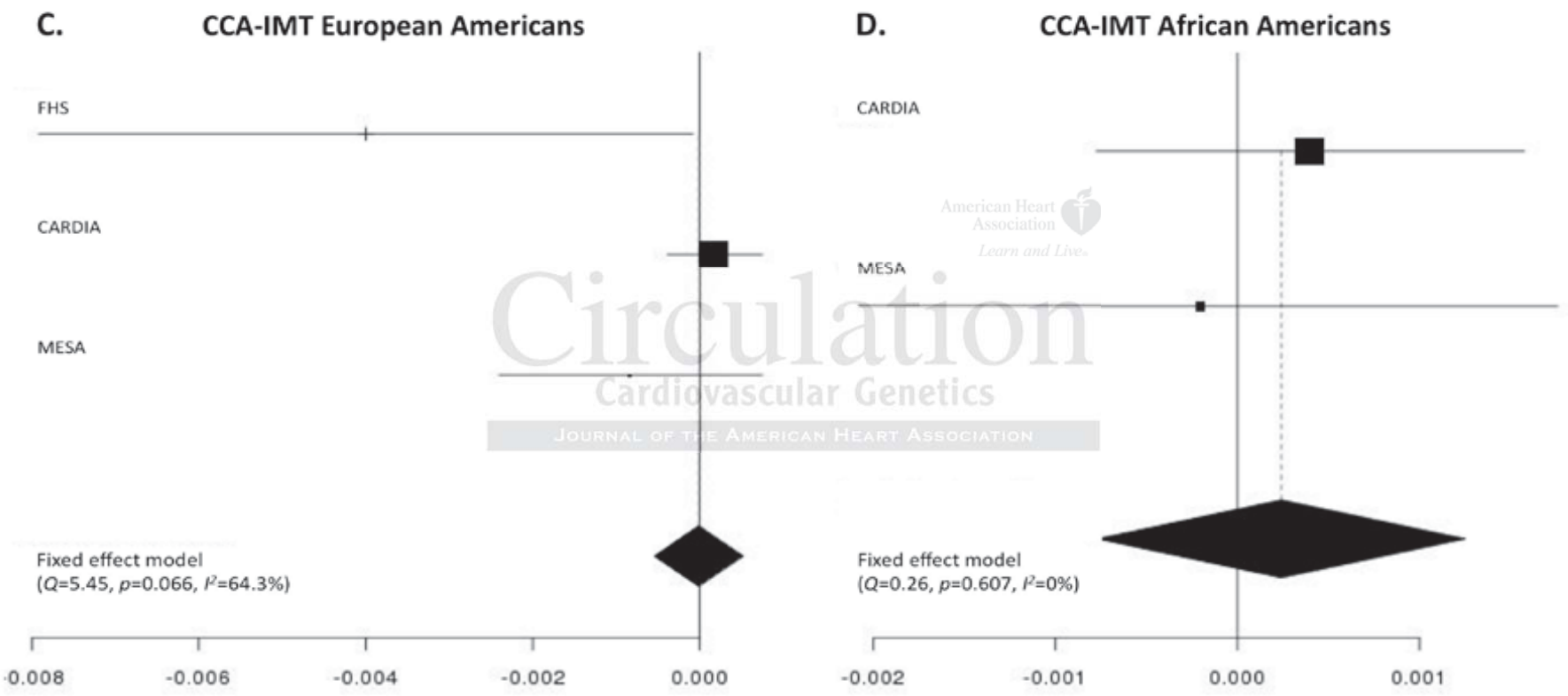
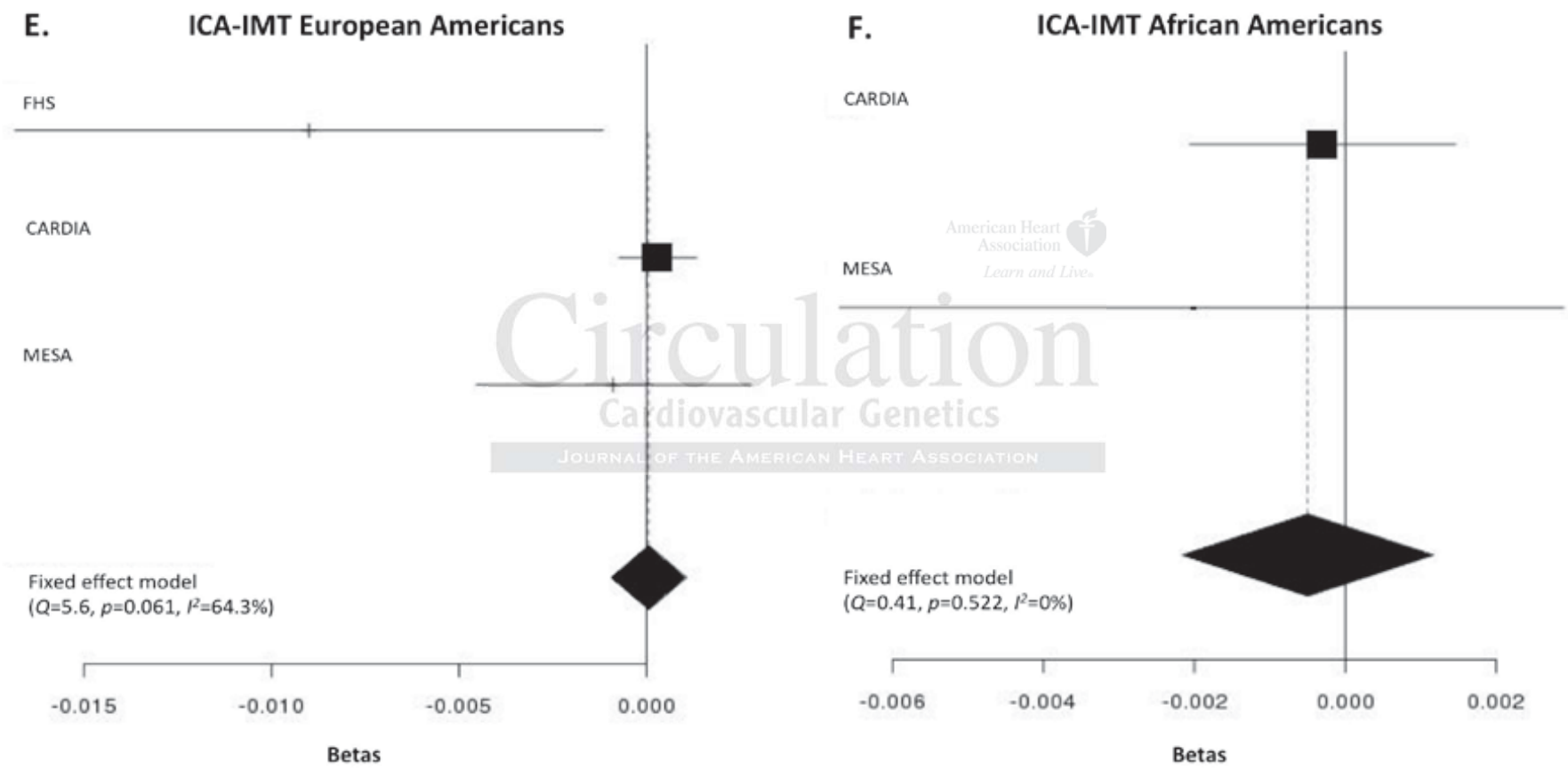
Figure Legends:

Figure 1: Meta-analysis of GRS_{62} association tests with CACS (panel-A, N_{meta}=4,780; panel-B, N_{meta}=1,835), CCA-IMT (panel-C, N_{meta}=6,220; panel-D, N_{meta}=2,190) and ICA-IMT (panel-E, N_{meta}=5,842; panel-F, N_{meta}=2,109) measures across all study cohorts stratified by European and African Americans (GRS_{62}, genetic risk score comprised of 62 single nucleotide polymorphisms associated with type 2 diabetes; CACS, coronary artery calcium score; CCA-IMT and ICA-IMT, intima-media thickness of common and internal carotid artery).









SUPPLEMENTAL MATERIAL

Supplemental Methods

We applied a multivariable linear regression model with random effects to account for family relatedness, where appropriate, to test the association of subclinical atherosclerosis (SCA) measures with an additive genetic risk score (GRS_{62}) comprised of 62 single nucleotide polymorphisms (SNPs) known to be linked with type 2 diabetes (T2D) risk (**Table S1**)[1]. Many of them are associated with either beta-cell function or insulin resistance (IR) physiology. Therefore, as described by Vassy *et al.* [2], we used prior genetic and physiologic evidence [1, 3-6] to define a sub-GRS comprised of 20 T2D SNPs mainly associated with beta-cell function (GRS_{β}) and a sub-GRS comprised of 10 T2D SNPs associated with peripheral insulin resistance (GRS_{IR}), with each locus weighted in European Americans by the same effect size as in the GRS_{62}.

For each SCA trait we applied a genetic-only model (adjusted for sex) and a full atherosclerosis risk factors adjusted model (**Table S2**). Clinical and anthropometric characteristics of study cohorts are shown in **Table S3** and **Table S4**. The GRS_{β} and GRS_{IR} were tested only in FHS and CARDIA study samples (**Table S5-S6**).

Many of the 62 tag-SNPs associated with T2D (**Table S1**) are also known to be associated with SCA risk factors/confounders. We interrogated Genome.gov (http://www.genome.gov/), a catalog of published GWAS, and PheGenI (http://www.ncbi.nlm.nih.gov/gap/phegeni), a phenotype-oriented resource housed at the National Center for Biotechnology Information. Risk factors listed in the catalogs as being associated with one or more of the known 62 T2D loci were included, among others, in the full model (BMI, waist circumference, systolic blood pressure, fasting insulin, fasting glucose, triglycerides, HDL-cholesterol and LDL-cholesterol). Therefore, the basic model could be described as "purely" genetic, as it tested the association of a T2D GRS alone with SCA traits, after adjustment for sex, while the full model accounted for the overall spectrum of confounders, mediators and/or risk factors for SCA available in our dataset, by adjusting for a comprehensive list of atherosclerosis risk factors.

Genotyping:

MESA: Caucasian, Hispanic, and Chinese American participants were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) at the Affymetrix Research Services Lab. 6,880 samples passed initial genotyping QC. African American samples were genotyped at the Broad Institute of Harvard and MIT as part of the CARe project [7]. Affymetrix performed wet lab hybridization assay, and plate-based genotype calling using Birdseed v2. Sample QC was based on call rates and contrast QC (cQC) statistics. Broad performed similar QC for CARe sample. Additional sample and SNP QC were carried out at University of Virginia, including sample call rate, sample cQC, and sample heterozygosity by ethnicity at the sample level; outlier plates checking by call rate, median cQC or heterozygosity at plate level. Four samples were removed due to low call rate (<95%). Cryptic sample duplicates or unresolved cryptic duplicates were dropped. Unresolved gender mismatches were also dropped. At the SNP level, we excluded monomorphic SNPs across all samples; SNPs with missing rate was > 5% or observed heterozygosity > 53% were also excluded. Additional genotypes were imputed to the 1000 Genomes Phase I integrated variant set (NCBI build 37 / hg19) separately in each ethnic group using the program IMPUTEv2. We used data freezes from 23 Nov 2010 (low-coverage whole-genome) and 21st May 2011 (high-coverage exome), phased haplotypes released March 2012 (v3), and phased haplotypes for 1,092 individuals and over 39 million variants. All imputed and genotyped SNPs were aligned to the '+' strand of the human genome reference sequence (NCBI Build 37). The Affymetrix annotation file "GenomeWideSNP_6.na31.annot.csv" was used for all matching of probe set IDs with RS IDs.

GENOA: GENOA Study participants were genotyped on the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, 2007) or the Illumina Human 1M-Duo BeadChip (Illumina, 2010) at the Mayo Clinic, Rochester, MN. African American sibships for the GENOA study were identified using hypertensive participants from the Atherosclerosis Risk in Communities Study (ARIC) as probands. Genotypes were obtained for 92 additional GENOA participants who were also in the ARIC Study and who could not be genotyped on either platform using the GENOA blood sample. Genotyping for the

ARIC study was done at the Broad Institute on the Affymetrix 6.0 platform. For all genotyping platforms used, samples and SNPs with a call rate <95% were removed. Samples demonstrating sex mismatch, duplicate samples, and samples with low identity-by-state with all other samples were also removed. Imputation was performed with the single-step approach implemented in Markov Chain Haplotyper (MaCH) 1.0.16 [8]. The reference panel was composed of the HapMap phased haplotypes (release 22). Imputation was performed separately for participants genotyped on the Affymetrix 6.0 as part of the GENOA study, participants genotyped on the Illumina Human 1M-Duo BeadChip, and participants genotyped on the Affymetrix 6.0 as part of the ARIC Study. Since only a small number of directly genotyped SNPs overlap on the Affymetrix and Illumina platforms, imputed dosages were used for all.

In GENOA African Americans the GRS was limited to 55 SNPs due to poor imputation quality for 7 SNPs.

FHS and CARDIA genotyping and imputation strategy have been previously detailed elsewhere[2, 7, 9].

Supplemental Table 1 – 62 independent loci and relative tag-SNPs associated with Type 2 Diabetes from DIAGRAMv3 [1].

Chr	SNP-risk allele	Locus	OR	Chr	SNP-risk allele	Locus	OR
1	rs2075423-G	*PROX1 or PPP2R5A*	1.07	9	rs10758593-A	*GLIS3*	1.06
1	rs10923931-T	*NOTCH2*	1.08	9	rs17791513-A	*TLE4*	1.12
2	rs10203174-C	*THADA*	1.14	9	rs2796441-G	*TLE1*	1.07
2	rs243088-T	*BCL11A*	1.07	9	rs16927668-T	*PTPRD*	1.04
2	rs13389219-C	*GRB14*	1.07	10	rs11257655-T	*CDC123/CAMK1D*	1.07
2	rs2943640-C	*IRS1*	1.10	10	rs7903146-T	*TCF7L2*	1.39
2	rs7569522-A	*RBMS1*	1.05	10	rs1111875-C	*HHEX/IDE*	1.11
2	rs780094-C	*GCKR*	1.06	10	rs12571751-A	*ZMIZ1 or PPIF*	1.08
3	rs11717195-T	*ADCY5*	1.11	10	rs12242953-G	*VPS26A*	1.07
3	rs1496653-A	*UBE2E2*	1.09	11	rs10830963-G	*MTNR1B*	1.10
3	rs4402960-T	*IGF2BP2*	1.13	11	rs1552224-A	*ARAP1 (CENTD2)*	1.11
3	rs1801282-C	*PPARG*	1.13	11	rs163184-G	*KCNQ1*	1.09
3	rs6795735-C	*ADAMTS9*	1.08	11	rs5215-C	*KCNJ11*	1.07
3	rs12497268-G	*PSMD6*	1.03	11	rs2334499-T	*DUSP8 or HCCA2 (YY1AP1)*	1.04
3	rs17301514-A	*ST64GAL1*	1.05	12	rs7955901-C	*TSPAN8/LGR5*	1.07
4	rs6819243-T	*MAEA*	1.07	12	rs12427353-G	*HNF1A (TCF1)*	1.08
4	rs4458523-G	*WFS1*	1.10	12	rs2261181-T	*HMGA2*	1.13
5	rs6878122-G	*ZBED3 or PDE8B*	1.10	12	rs10842994-C	*KLHDC5 or PPFIBP1*	1.10
5	rs459193-G	*ANKRD55*	1.08	13	rs1359790-G	*SPRY2*	1.08
6	rs7756992-G	*CDKAL1*	1.17	15	rs4502156-T	*C2CD4A or VPS13C*	1.06
6	rs3734621-C	*KCNK16*	1.07	15	rs11634397-G	*ZFAND6*	1.05
6	rs4299828-A	*ZFAND3*	1.04	15	rs12899811-G	*PRC1*	1.08
7	rs17168486-T	*DGKB*	1.11	15	rs2007084-G	*AP3S2*	1.02
7	rs10278336-A	*GCK*	1.07	15	rs7177055-A	*HMG20A*	1.08
7	rs849135-G	*JAZF1*	1.11	16	rs9936385-C	*FTO*	1.13
7	rs17867832-T	*GCC1 or PAX-4*	1.09	16	rs7202877-T	*BCAR1*	1.12
7	rs13233731-G	*KLF14*	1.05	17	rs2447090-A	*SRR*	1.04
8	rs3802177-G	*SLC30A8*	1.14	18	rs12970134-A	*MC4R*	1.08
8	rs7845219-T	*TP53INP1*	1.06	19	rs10401969-C	*CILP2*	1.13
8	rs516946-C	*ANK1*	1.09	19	rs8182584-T	*PEPD*	1.04
9	rs10811661-T	*CDKN2A/B*	1.18	20	rs4812829-A	*HNF4A*	1.06

Chr, chromosome; SNP, single nucleotide polymorphism; OR, odd ratio

Supplemental Table 2 – Outline of models applied in the association analysis of Genetic Risk Scores (GRS) with subclinical atherosclerosis traits, plus covariates.

BASIC MODEL	GRS, sex	
FULL MODEL	GRS, sex	Age BMI Waist circumference Systolic blood pressure (SBP)* Fasting insulin Fasting glucose Triglycerides HDL-Cholesterol LDL-Cholesterol Family history of T2D Family history of CVD Smoking status Physical activity Diabetes medication Hypertension medication Lipid-lowering medication

BMI, body mass index; T2D, type 2 diabetes; CVD, cardiovascular disease; ABI, ankle-brachial index.
*NOTE: SBP excluded for ABI since ABI is calculated from SBPs at ankle and arm.

Supplemental Table 3 – Subclinical atherosclerosis measures, anthropometric and clinical characteristics in FHS and CARDIA cohorts.

	FHS		CARDIA			
	Exam 6	*Exam 7*	*Exam year 20*		*Exam year 25*	
Ethnicity	**European Americans**		**African Americans**	**European Americans**	**African Americans**	**European Americans**
N (male %)	2459 (44.8%)	1111 (44.8%)	816 (38.6%)	1635 (45.9 %)	811 (38.8%)	1621 (45.9 %)
Age (yr)	57.9 ± 9.6	58.9 ± 8.9	44.4 ± 3.8	45.5 ± 3.3	49.4 ± 3.8	50.6 ± 3.3
BMI (kg/m^2)	27.6 ± 4.9	28.1 ± 4.9	31.7 ± 7.6	27.9 ± 6.7	32.2 ± 7.8	28.2 ± 6.2
Waist circumference (cm)	96.5 ± 12.7	96.5 ± 12.7	94.7 ± 15.7	89.8 ± 15.1	97.1 ± 15.8	91.6 ± 15.6
Systolic blood pressure (mmHg)	127.1 ± 18.3	124.9 ± 17.7	119.1 ± 15.5	112.2 ± 12.5	122.2 ± 14.5	114.5 ± 13.7
Fasting glucose (mg/dL)	101.3 ± 22.9	99.9 ± 18.2	102.3 ± 30.2	97.8 ± 21.2	102.1 ± 34.6	96.7 ± 20.4
Fasting insulin (pmol/L)	-	14.3 ± 8.6	17.1 ± 12.2	13.5 ± 9.1	13.39 ± 14.1	9.6 ± 7.3
Triglycerides (mg/dL)	136.1 ± 88.2	132.6 ± 86.5	96.4 ± 58.4	116.9 ± 82.4	101.4 ± 67.1	120.0 ± 86.3
HDL-cholesterol (mg/dL)	51.9 ± 16.1	53.9 ± 15.9	54.1 ± 16.3	54.4 ± 17.2	57.7 ± 17.3	58.7 ± 18.5
LDL-cholesterol (mg/dL)	127.4 ± 32.9	121.4 ± 31.3	110.4 ± 33.6	110.3 ± 30.5	109.2 ± 33.9	113.4 ± 30.9
Parental history of diabetes (%)	19.8	19.8	17.9	9.5	17.6	9.4
Parental history of CVD (%)	43.2	41.9	39.8	41.3	39.9	41.2
Diabetes (%)	7.1	6.3	10.9	3.4	13.3	6.5
Smokers (never/former/current - %)	35.8/48.9/15.2[*]	39.5/50.7/9.8[*]	59.9/40.1[†]	46.1/53.9[†]	62.2/37.9[†]	50.7/49.3[†]
Physical activity	-	-	287.5±285.4[‡]	370.3±260.8[‡]	264.4±257.5[‡]	388.0±280.9[‡]
Genetic Risk Score	66.7 ± 5.3	66.7 ± 5.2	69.2 ± 4.5	66.4 ± 5.2	69.2 ± 4.5	66.4 ± 5.2
Comorbidity status						
Diabetes medication (%)	3.3	2.9	7.9	3.4	10.7	4.6
Hypertension medication (%)	23.4	24.8	23.1	10.2	41.8	31.8
Lipid-lowering medication (%)	9.8	13.9	-	-	-	-
Subclinical atherosclerosis traits						
AACS (Agatston unit)	-	1458.6 ± 2332.3	-	-	-	-
CACS (Agatston unit)	-	229.8 ± 550.8	-	-	31.7 ± 154.6 (n=586)	49.2 ± 252.1 (n=1267)
CCA-IMT (mm)	0.5 ± 0.4 (n=2340)	-	0.7 ± 0.1 (n=617)	0.7 ± 0.1 (n=1379)	-	-
ICA-IMT (mm)	0.8 ± 1.9 (n=2035)	-	0.6 ± 0.2 (n=562)	0.6 ± 0.2 (n=1332)	-	-
ABI	1.1 ± 0.1 (n=2822)		-	-	-	-

Data expressed as mean±standard deviation, if not otherwise indicated. AACS, abdominal aorta calcium score; CACS, coronary artery calcium score; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima-media thickness; ABI, ankle-brachial index. [*]Smoking status categorized as never/former/current in FHS. [†]Smoking status categorized as never/ever in CARDIA. [‡]Physical activity is expressed as Total Intensity Score, according to the CARDIA Physical Activity History Questionnaire (Pereira MA *et al.*; PMID: 9243481).

Supplemental Table 4 – Subclinical atherosclerosis measures, anthropometric and clinical characteristics in MESA and GENOA cohorts.

	MESA				GENOA	
Ethnicity	**European Americans**	**Asian Americans**	**African Americans**	**Hispanic Americans**	**European Americans**	**African Americans**[||]
N (male %)	2526 (47.7%)	773 (49.2%)	1611 (46.1%)	1446 (48.3%)	969 (40.9%)	535 (25.8%)
Age (yr)	62.7 ± 10.2	62.4 ± 10.4	62.3 ± 10.1	61.4 ± 10.3	58.9 ± 9.5	68.5 ± 7.7
BMI (kg/m^2)	27.7 ± 5.1	23.9 ± 3.3	30.2 ± 5.9	29.5 ± 5.2	30.7 ± 6.3	32.7 ± 7.2
Waist circumference (cm)	97.9 ± 14.5	87.1 ± 9.8	101.3 ± 14.7	100.7 ± 13.1	100.3 ± 16.2	101.1 ± 15.4
Systolic blood pressure (mmHg)	123.5 ± 20.5	124.6 ± 21.7	131.8 ± 21.8	126.8 ± 21.9	131.4 ± 16.8	137.5 ± 21.0
Fasting glucose (mg/dL)	91.3 ± 21.6	99.2 ± 28.6	100.3 ± 32.7	103.9 ± 39.4	104.6 ± 24.5	111.6 ± 37.7
Fasting insulin (pmol/L)	9.1 ± 5.6	9.6 ± 12.5	11.5 ± 27.5	11.8 ± 15.7	54.2 ± 40.3	80.6 ± 87.5
Triglycerides (mg/dL)	133 ± 90.1	143.1 ± 85.7	105.2 ± 70.5	158.4 ± 101.8	159.0 ± 96.9	101.0 ± 63.3
HDL-cholesterol (mg/dL)	52.4 ± 15.8	49.3 ± 12.4	52.3 ± 15.2	47.5 ± 13.1	52.4 ± 15.6	57.1 ± 16.5
LDL-cholesterol (mg/dL)	117.1 ± 30.3	115.1 ± 28.8	116.7 ± 33.3	119.9 ± 32.9	122.7 ± 32.1	114.6 ± 35.3
Parental history of diabetes (%)	-	-	-	-	29.4	40.9
Parental history of CVD (%)	44.6/33.2/2.8[*]	14.5/23.3/1.2[*]	31.9/31/7[*]	31.2/23.8/3.2[*]	57.5[†]	56.1[†]
Diabetes (%)	5.9	13.5	17.4	17.8	13.5	35.5
Smokers (never/former/current - %)	33.1/66.9[‡]	69.6/30.4[‡]	26.6/73.4[‡]	40.7/59.3[‡]	52.5/37.3/10.2	60.4/31.4/8.2
Regular physical activity (daily hours)	12.8 ± 4.9	9.9 ± 4.4	14.4 ± 7.1	11.6 ± 5.9	3.7 ± 2.5[§]	1.0 ± 1.8[§]
Genetic Risk Score	63.9 ± 4.7	61.5 ± 4.2	56.5 ± 4.7	62.7 ± 4.8	64.2 ± 4.9	57.0 ± 3.9
Comorbidity status						
Diabetes medication (%)	4.6	9.2	13.6	15.8	8.9	32.0
Hypertension medication (%)	33.3	29.1	50.3	32.9	68.4	80.8
Lipid-lowering medication (%)	18.3	14.1	15.8	13.3	27.0	40.9
Subclinical atherosclerosis traits						
AACS (Agatston unit)	1668.4 ± 2581.4 (n=760)	1044.7 ± 2015.4 (n=247)	887.2 ± 1737.7 (n=343)	1044.6 ± 1898.4 (n=496)	-	-
CACS (Agatston unit)	338.6 ± 577.2 (n=1433)	205.8 ± 374.3 (n=392)	294.0 ± 582.8 (n=714)	281.4 ± 567.2 (n=659)	201.6 ± 467.2	236.3 ± 583.0
CCA-IMT (mm)	0.9 ± 0.2 (n=2501)	0.8 ± 0.2 (n=770)	0.9 ± 0.2 (n=1573)	0.9 ± 0.2 (n=1431)	-	-
ICA-IMT (mm)	1.1 ± 0.6 (n=2475)	0.9 ± 0.5 (n=766)	1.1 ± 0.6 (n=1547)	1.0 ± 0.6 (n=1399)	-	-
ABI	1.1 ± 0.1 (n=2494)	1.1 ± 0.1 (n=768)	1.1 ± 0.1 (n=1432)	1.3 ± 0.1 (n=1430)	-	-

Data expressed as mean±standard error, if not otherwise indicated. AACS, abdominal aorta calcium score; CACS, coronary artery calcium score; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima-media thickness; ABI, ankle-brachial index. [*]CVD is categorized in MESA as myocardial infarction/stroke/amputation not due to injury. [†]Expressed as parental history of coronary heart disease in GENOA. [‡]Smoking status categorized as never/ever in MESA. [**]Smoking status categorized as never/former/current in GENOA. [§]Physical activity categorized as moderate or heavy. [||]African Americans in GENOA had an available genetic risk score limited to 55 of 62 T2D SNPs.

Supplemental Table 5 – Association between prevalent subclinical atherosclerosis measures and a T2D genotype risk score (GRS) comprised of 20 tag SNPs mostly linked with beta-cell function (GRS_β) in linear regression models of FHS and CARDIA cohorts.

	FHS		CARDIA			
	European Americans		African Americans		European Americans	
Basic Model	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*
CACS	-0.023±0.05	0.64	-0.011±0.03	0.71	0.005±0.02	0.76
AACS	-0.072±0.06	0.23	-	-	-	-
ICA-IMT	-0.016±0.01	0.01	-0.001±0.00	0.55	0.001±0.00	0.31
CCA-IMT	-0.002±0.00	0.41	-0.001±0.00	0.60	0.000±0.00	0.69
ABI	-9.33E+08±0.00	0.94	-	-	-	-
Full Model						
CACS	-0.021±0.05	0.69	-0.027±0.03	0.36	0.001±0.02	0.95
AACS	-0.012±0.06	0.85	-	-	-	-
ICA-IMT	-0.018±0.01	**0.007**	-0.001±0.00	0.68	0.001±0.00	0.15
CCA-IMT	-0.004±0.00	0.15	-0.001±0.00	0.49	0.001±0.00	0.21
ABI	0.001±0.00	0.46	-	-	-	-

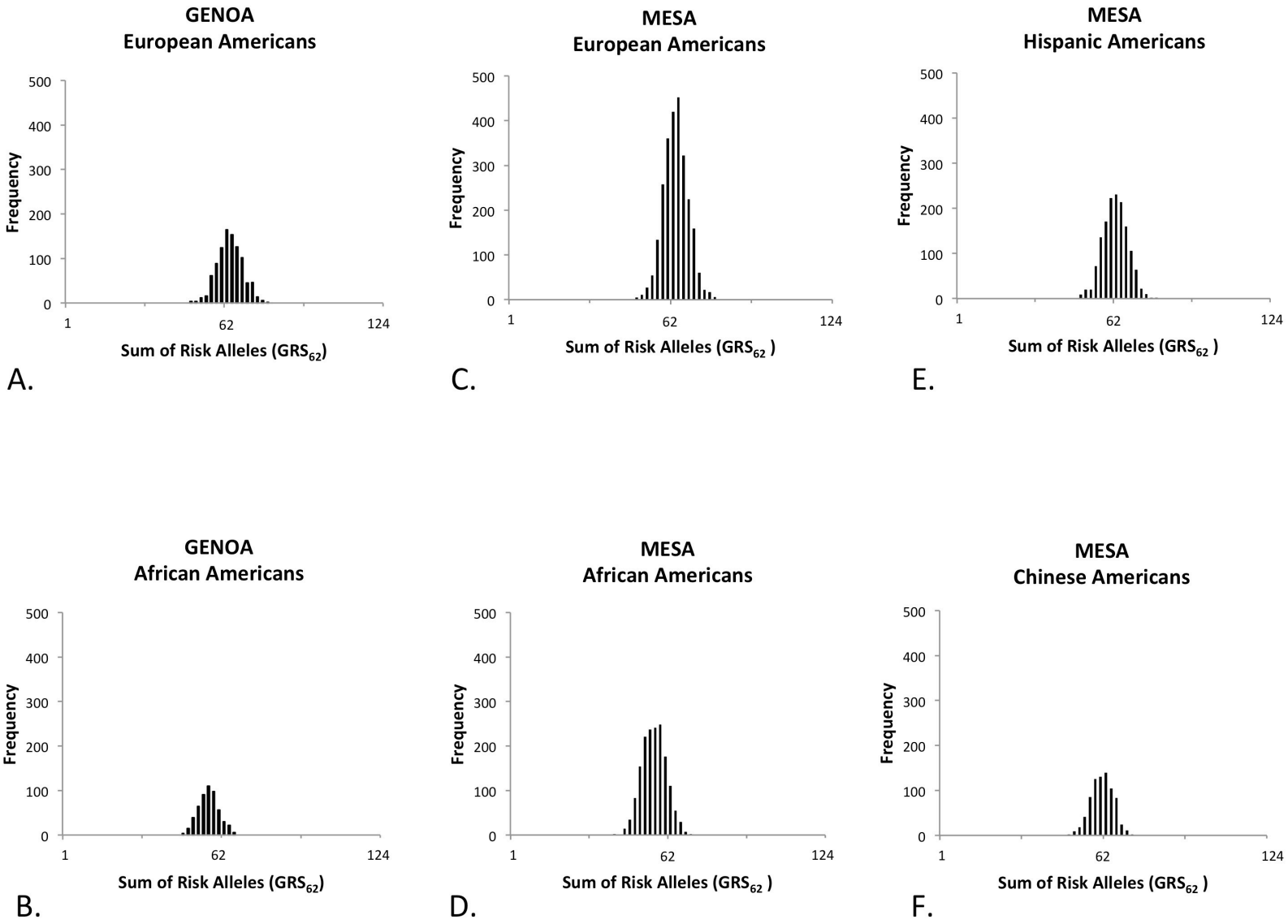
AACS, abdominal aorta calcium score; CACS, coronary artery calcium score; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima-media thickness; ABI, ankle-brachial index. Data expressed as mean±standard error.

Supplemental Table 6 – Association between prevalent subclinical atherosclerosis measures and a T2D genotype risk score (GRS) comprised of 10 tag SNPs mostly linked with insulin resistance (GRS_{IR}) in linear regression models of FHS and CARDIA cohorts.

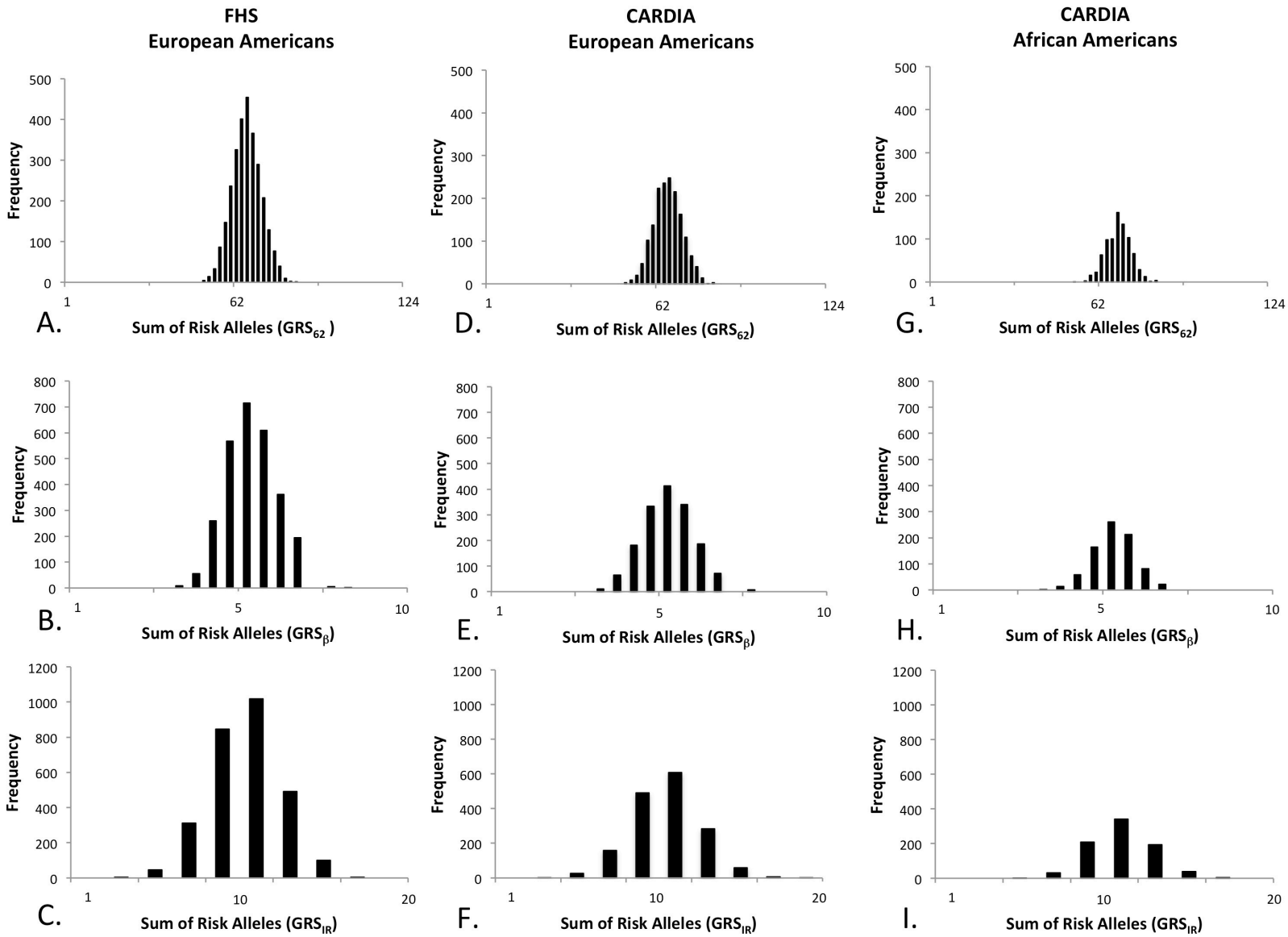
	FHS		CARDIA			
	European Americans		African Americans		European Americans	
Basic Model	*Beta±SE*	*P*	*Beta±SE*	*P*	*Beta±SE*	*P*
CACS	-0.004±0.07	0.95	-0.011±0.04	0.98	0.041±0.03	0.11
AACS	0.112±0.08	0.17	-	-	-	-
ICA-IMT	-0.003±0.01	0.77	0.000±0.00	0.93	-0.001±0.00	0.71
CCA-IMT	-0.004±0.00	0.29	0.001±0.00	0.46	-0.000±0.00	0.77
ABI	-7.74-06±0.00	0.99	-	-	-	-
Full Model						
CACS	0.036±0.08	0.65	0.005±0.04	0.89	0.025±0.03	0.32
AACS	0.056±0.09	0.57	-	-	-	-
ICA-IMT	-0.005±0.01	0.65	0.001±0.00	0.84	-0.000±0.00	0.83
CCA-IMT	-0.009±0.00	0.01	0.001±0.00	0.58	-0.001±0.00	0.63
ABI	0.001±0.00	0.56	-	-	-	-

AACS, abdominal aorta calcium score; CACS, coronary artery calcium score; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima-media thickness; ABI, ankle-brachial index. Data expressed as mean±standard error.

Supplemental Figure 1 – Distribution of the total sum of risk alleles comprised in the T2D GRS_{62} in GENOA (panel A and B) and MESA cohorts (panels C to F), stratified by ethnicity.



Supplemental Figure 2 – Distribution of the total sum of risk alleles comprised in the T2D GRS_{62}, GRS_{β} and GRS_{IR} in FHS (panel A, B, C, respectively) and in CARDIA cohorts (panel D to I), stratified by ethnicity.



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