HHS Public Access

Author manuscript

Acta Diabetol. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

Acta Diabetol. 2015 August ; 52(4): 743–751. doi:10.1007/s00592-015-0720-5.

Analysis of a Cardiovascular Disease Genetic Risk Score in the Diabetes Heart Study

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Abstract

Aims—It remains unclear whether the high cardiovascular disease (CVD) burden in people with type 2 diabetes (T2D) is associated with genetic variants that contribute to CVD in general populations. Recent studies have examined genetic risk scores of single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) for their cumulative contribution to CVD-related traits. Most analyses combined SNPs associated with a single phenotypic class, e.g. lipids. In the present analysis, we examined a more comprehensive risk score comprised of SNPs associated with a broad range of CVD risk phenotypes.

Methods—The composite risk score was analyzed for potential associations with subclinical CVD, self-reported CVD events, and mortality in 983 T2D-affected individuals of European descent from 466 Diabetes Heart Study (DHS) families. Genetic association was examined using marginal models with generalized estimating equations for subclinical CVD and prior CVD events

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Conflict of Interest

None.

Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

and Cox proportional hazards models with sandwich-based variance estimation for mortality; analyses were adjusted for age and sex.

Results—An increase in genetic risk score was significantly associated with higher levels of coronary artery calcified plaque ($p=1.23 \times 10^{-4}$); however, no significant associations with self-reported myocardial infarction and CVD events and all-cause and CVD mortality were observed.

Conclusions—These results suggest that a genetic risk score of SNPs associated with CVD events and risk factors does not significantly account for CVD risk in the DHS, highlighting the limitations of applying current genetic markers for CVD in individuals with diabetes.

Keywords

Type 2 diabetes; mortality; coronary artery calcification; genetic risk score

Mortality risk from cardiovascular disease (CVD) is increased two to fourfold in individuals with type 2 diabetes (T2D), increasing interest in CVD risk prediction for this population [1]. Identifying genetic variants which elevate CVD risk is one strategy for risk prediction. Recent studies have examined genetic risk scores (GRS) comprised of multiple single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) for their cumulative impact on CVD-related traits. Most analyses have combined SNPs associated with a single trait class. For example, some studies have examined risk scores of SNPs associated with lipid levels for their impact on CVD events [2,3], while other studies have examined SNPs associated with CVD risk for their ability to predict incident events [4,5] and for association with subclinical CVD [6,7]. These analyses suggest that GRS may be important tools for CVD risk prediction in the general population. It remains unclear whether the same genetic variants influence CVD burden in individuals with T2D. All CVD-associated SNPs in general populations do not associate with risk in individuals with T2D [8-11], and variants have been described that impact CVD risk only in the presence of diabetes [12,9] or whose effects are modified by factors such as glycemic control [13] and obesity [14] in diabetes patients. In an effort to gain further insight into the performance of GRS in patients with T2D, we tested a composite GRS of SNPs associated with CVD events and CVD risk factors.

The Diabetes Heart Study (DHS) is a family-based cohort enriched for T2D [15]. Prior work in the DHS investigated the associations between SNPs associated with CVD events [16], coronary artery calcified atherosclerotic plaque (CAC), a measure of subclinical CVD [17], and high-density lipoprotein (HDL) cholesterol [18] and CAC, self-reported CVD events, and mortality. In this analysis, we extended these approaches to examine whether a more extensive risk score constructed from SNPs associated with a wide range of CVD risk factors is more strongly associated with measures of CVD risk, including CAC, self-reported CVD, and myocardial infarction (MI), as well as all-cause and CVD mortality, in DHS participants with diabetes.

Methods

Study Design and Sample

The DHS recruited T2D-affected siblings without advanced renal insufficiency from 1998 through 2005 in western North Carolina. T2D was defined as diabetes developing after the age of 35 years treated with changes in diet and exercise and/or oral agents, in the absence of historical evidence of ketoacidosis or initial treatment with insulin. Fasting glucose and glycated hemoglobin (HbA_{1C}) were assessed at the exam visit. Ascertainment and recruitment have been described [15]. Analyses included 983 self-described European American individuals from 466 DHS families, all of whom were affected by diabetes.

Participant examinations were conducted in the General Clinical Research Center of Wake Forest Baptist Medical Center. Examinations included interviews for medical history and health behaviors, anthropometric measures, resting blood pressure, electrocardiography, fasting blood sampling for laboratory analyses, and spot urine collection. Data on prior MI and CVD events was self-reported by participants and non-adjudicated. Participants reporting history of a CVD event including MI, angina, or stroke, history of vascular procedures including coronary angioplasty, coronary artery bypass graft, or endarterectomy, or with Q wave abnormalities indicative of prior MI were defined as having prior CVD. Estimated glomerular filtration rate (eGFR) was computed using the CKD-EPI equation [19]. Low-density lipoprotein (LDL) cholesterol concentration was calculated using the Friedewald equation, and LDL concentrations were considered valid for subjects whose triglycerides were less than 400 mg/dL. CAC was assessed using computed tomography (CT), summing the left main, left anterior descending, circumflex, posterior descending, and right coronary arteries. CT scans were performed on multi-detector CT scanners with cardiac gating in chest scans. CAC scores were measured as previously described and validated [20,21]. Not all measures were available in all DHS participants.

Mortality was assessed using the National Social Security Death Index. For deceased participants, length of follow-up was determined from the date of initial study visit to date of death. For all other participants the length of follow-up was determined from the date of the initial study visit to December 31, 2013. When possible, copies of death certificates were obtained from county or state Vital Records Offices to determine cause of death. Cause of death was categorized based on death certificates as CVD mortality (MI, congestive heart failure, cardiac arrhythmia, sudden cardiac death, peripheral vascular disease, and stroke) or as mortality from cancer, infection, end-stage renal disease, accidental, or other causes (including obstructive pulmonary disease, pulmonary fibrosis, liver failure and Alzheimer's disease). Cause of death could not be obtained for 14 participants; these participants were excluded from all analyses of CVD mortality.

All study protocols were approved by the Institutional Review Board at Wake Forest School of Medicine, and all participants provided written informed consent.

SNP Selection

SNPs selected for the risk score were genome-wide significant (p-value $<5 \times 10^{-8}$) in large GWAS studies from the NHGRI GWAS catalog in populations of European descent. For

SNPs in linkage disequilibrium ($r^2 > 0.1$) within risk scores for each trait, only one SNP was included. SNPs included in the risk score are listed in Supplementary Table 1, including SNPs associated with blood pressure traits, body mass index, CAC, coronary artery disease (CAD), C-reactive protein (CRP), electrocardiogram traits, fasting plasma glucose, eGFR, HDL cholesterol, LDL cholesterol, total cholesterol, triglycerides, stroke, and T2D. SNP positions are for the GRCh37/hg19 reference genome.

Genotyping

Genotyping was completed using an Illumina Infinium HumanExome BeadChip array (Exome array), an Affymetrix Genome-Wide Human SNP Array 5.0 (GWAS array), imputation of 1000 Genomes project SNPs from this array using IMPUTE2 and the Phase I v2, cosmopolitan (integrated) reference panel, build 37 [22], and direct genotyping using the Sequenom platform (Supplementary Table 1). The array based sources of genotype data and imputation in the DHS have been described previously [18]. 983 European American individuals with T2D from the DHS had both GWAS array and Exome array data and were included in our GRS analysis. Some SNPs not available from the array-based datasets were directly genotyped using the MassARRAY SNP Genotyping System [23,24]. In addition to the 375 SNPs included in our risk scores, an additional 30 SNPs met our criteria for inclusion but were not available.

All SNPs included in the GRS were required to have a minimum call rate of 95% and a Hardy Weinberg equilibrium p-value>1×10^{-6}. Unweighted GRS for all traits were derived by adding the number of risk alleles across each SNP, including the composite GRS, which was derived by adding the risk alleles across 375 SNPs (Supplementary Table 1). For individuals missing genotype data for a particular SNP, the mean genotype calculated in the DHS for that given SNP was assigned [25]. Scores for the composite GRS ranged from 343 to 421 (382.3 ± 13.4, mean ± SD). All risk scores were coded so an increase in risk score would be expected to correlate with an increase in CVD risk.

Statistical Analysis

Continuous variables were transformed as necessary to approximate normality prior to the analysis. The natural logarithm of fasting plasma glucose, total cholesterol, (CAC+1), triglyceride levels, CRP, QT interval, QRS interval, and PR interval was employed, and the square root was used for HDL and BMI. All single SNP association analyses were implemented using variance components methods in SOLAR version 6.5.8 (Texas Biomedical Research Institute, San Antonio, TX) to account for familial relationships [26]. Association was examined assuming an additive model of inheritance. Risk score associations were examined using marginal models with generalized estimating equations for subclinical CVD and self-reported CVD events, accounting for familial correlation using a sandwich estimator of the variance under exchangeable correlation. A similar approach was used to test for differences between the top and bottom 10% of the composite GRS distribution. Cox proportional hazards models with sandwich-based variance estimation were used to examine associations with all-cause and CVD mortality. Risk score analyses were performed in SAS 9.3. Single SNP and GRS analyses were adjusted for age and sex.

Analysis of the T2D risk score for association with diabetes affected status was not completed, as all individuals included in these analyses were affected by T2D.

Results

The demographic characteristics of the 983 individuals from 466 DHS families included in this GRS analysis are displayed in Table 1. All participants were affected by T2D, with average diabetes duration of 11 ± 7 (mean ± standard deviation (SD)) years. The cohort has a high prevalence of hypertension, obesity, subclinical CVD based on CAC, and prior CVD events. All-cause mortality was 31.1% over an average follow-up of 10 ± 3 years (mean ± SD).

SNPs selected for inclusion in the individual GRS are listed in Supplementary Table 1. Each SNP's association with the trait of interest for the risk score for which it was selected was assessed (Supplementary Table 2). The most significant single SNP association was for rs3764261 with HDL cholesterol ($p= 5.6 \times 10^{-5}$). Of the 375 SNPs analysed, only 42 were nominally associated ($p<0.05$) with the trait of interest in the DHS. However, when the SNPs were analyzed in combination in GRS, most risk scores were associated with the trait of interest (Table 2); the strongest association was for the 32 SNP triglyceride risk score ($p=1.75 \times 10^{-6}$) with triglyceride levels.

We also assessed the association of both single SNPs and the individual, trait-specific GRS with all-cause mortality, CVD mortality, self-reported history of MI, self-reported history of CVD, and CAC. The single SNP association results are shown in Supplementary Table 2; few SNPs showed evidence of association with these traits in single SNP association analyses, with no SNPs meeting a Bonferroni-adjusted p-value threshold of $<1.33 \times 10^{-4}$ ($\alpha=0.05$ for 375 SNPs). Few SNPs were even nominally associated with these phenotypes ($p<0.05$), with 21 SNPs nominally associated with all-cause mortality, 19 with CVD mortality, 16 with self-reported history of MI, 12 with self-reported history of CVD, and 20 with CAC. The results for the trait-specific GRS are shown in Supplementary Table 3. Most risk scores were not associated with all-cause or CVD mortality, with the exception of nominal associations between the CAD and T2D risk scores and risk of CVD mortality. No individual risk scores were strongly associated with self-reported history of CVD events, but the CAC risk score was nominally associated ($p=0.028$) with increased odds of prior CVD events. The CAD GRS was associated ($p=0.0002$) with increased odds of a history of MI; eGFR and stroke risk scores were also nominally associated with MI history. Associations with increased CAC were observed for the CAD ($p=0.0001$) and triglycerides ($p=0.007$) GRS, with nominal association with increased CAC also observed for the CAC risk score.

Next, the composite GRS derived from 375 SNPs associated with blood pressure traits, BMI, CAC, CAD, CRP, electrocardiogram traits, fasting plasma glucose, eGFR, HDL cholesterol, LDL cholesterol, stroke, T2D, total cholesterol, and triglycerides was assessed. An increase in GRS was associated with increased CAC burden ($p=1.23 \times 10^{-4}$), but significant associations with self-reported history of MI and CVD events were not observed (Table 3). Associations with all-cause or CVD mortality were also not detected (Table 3). The association with CAC is reflected by differences in the mean CAC burden for

individuals in the bottom or top 10% of the composite GRS distribution, as compared to the whole sample (Table 4). To further refine these results, a model including each trait-specific GRS as a separate variable in the model was analyzed, adjusted for age and sex, in order to determine which risk scores were driving the observed association with the composite GRS. The association of the combined GRS with increased CAC appeared to be driven mainly by the CAD SNPs (p=0.001), with the electrocardiogram trait SNPs (p=0.042) and the triglyceride SNPs (p=0.040) also nominally associated with increased CAC and contributing to the association (Supplementary Table 4).

Conclusions

This study explored potential associations with a genetic risk score created from SNPs associated with CVD events and CVD risk factors in general populations in a T2D-affected cohort with measures of CAC, self-reported CVD events, and mortality. Not surprisingly, given the prior associations reported in GWAS analyses, risk scores of SNPs selected for each individual CVD risk trait (for example lipid levels, QT interval, and CRP) tended to be associated with that trait of interest (Table 2), indicating that at least some variants identified as associated with CVD risk factors in the general population also impact risk factors in individuals with T2D. However, while the composite risk score was associated with increased CAC burden, associations with self-reported CVD events and mortality were not observed. There are a number of potential explanations; for example, analysis of a continuous trait like CAC has more power, and self-reported events and CVD mortality have risks of potential misclassification. Given these issues, the genetic association results highlight the limitations of GRS in this high CVD risk group of diabetes affected individuals and raises questions about the translational value of GWAS identified SNPs for risk prediction in community-based cohorts at high CVD risk.

The association of our GRS with CAC shows that the selected CVD-related SNPs are associated with elevated CVD burden in individuals with T2D ($p=1.23 \times 10^{-4}$, β value 0.021 for change in ln transformed CAC per one unit increase in GRS, 95% confidence interval (0.010, 0.031), corresponding to a β value of 0.275 for a one SD change in GRS). This is reflected by a lower mean CAC burden for individuals in the bottom 10% of the composite GRS distribution and higher CAC burden for those in top 10% as compared to the cohort as a whole (Table 4). CAC is a strong independent predictor of CVD events and mortality in the general population and in T2D [27-34], with individuals affected by diabetes tending to have higher CAC [35]. However, the association of the composite GRS with CAC seemed to be driven by only a few of the included risk scores; in a model including all of the individual GRS, the only associations were for the CAD, electrocardiogram traits, and triglycerides GRS (Supplementary Table 4), which likely explain much of the association of the composite risk score. A prior analysis of an unweighted risk score in the DHS of 30 SNPs associated with risk of CAD events found a strong association with CAC ($p= 7.34 \times 10^{-5}$ for unweighted risk score model adjusted for age, sex, and diabetes affected status) [16], so the association of the CAD risk score included here with CAC (p=0.0001 (Supplementary Table 3), p=0.001 in model adjusted for other risk scores (Supplementary Table 4)) is not surprising.

The lack of association between our composite risk score and all-cause and CVD mortality and self-report of prior CVD events may be explained by a number of factors. All scores analyzed were unweighted by previously reported SNP effect size; this may weaken associations, but effect size estimates would be difficult to use for weighting in this study as SNPs within scores were reported in different publications with varying sample sizes, recruitment criteria, and phenotype definitions. The lack of association with prior CVD events may be due to the availability of only self-reported, non-adjudicated event data in the DHS cohort; underreporting or overreporting of events by patients may have weakened potential associations. Inaccuracies in cause of death data from death certificates are well documented [36,37]; again, under- or overreporting of CVD causes of death by physicians may have weakened associations with CVD mortality. More broadly, GWAS studies to date have identified common variants that account for only a modest percentage of the heritability of most complex traits, with most identified variants having small effect sizes, leading to recent efforts to identify other factors that may contribute to the "missing heritability" [38]. While our individual risk scores were generally associated with the trait of interest (Table 2), for example BMI, HDL, and CRP levels, the estimated effect sizes were small. Differential effects of genetic variants in individuals affected by T2D may also contribute to these small effect sizes for the GRS, as well as the lack of association with mortality for the composite score. A number of previous studies have found different genetic contributors to CVD risk in diabetes affected individuals as compared to general population cohorts [8-10,12,11], with factors such as glycemic control [13] and obesity [14] potentially modifying the impact of genetic variants in individuals affected by T2D. Finally, Mendelian randomization studies have cast doubts that some of the risk factors included (for example HDL [3] and CRP [39]) are causally associated with risk of CVD events, which could weaken the association of our composite GRS with CVD risk, though in our analysis results were essentially unchanged when HDL and CRP associated SNPs were excluded from the composite risk score analysis.

Previous analyses of GRS have found fairly modest predictive power for these scores. A prior analysis of 102 SNPs associated with CVD events and major CVD risk factors, including LDL, HDL, triglycerides, diabetes, fasting plasma glucose, systolic and diastolic blood pressure, and CRP, in the Framingham Heart Study found a fairly modest association with high CAC (defined as an Agatston score greater than the 75th percentile in a healthy population) ($p=0.002$) [6]. In analysis of a GRS of 24 CAD SNPs, the risk score was able to explain only 2.4% of Agatston score variance in a cohort of men who were current or former heavy smokers [7]. A GRS of 101 SNPs associated with CVD or CVD intermediate phenotypes was modestly associated with increased risk of CVD events in the Women's Genome Health Study (HR 1.02 per risk allele, 95% CI (1.00-1.03)), but the GRS was no longer associated after adjustment for traditional CVD risk factors [5].

In our analysis of a GRS constructed from 375 SNPs previously associated with CVD events or risk factors, we observed modest association of this risk score with CAC, a marker of subclinical CVD risk, but observed no association with self-reported CVD events or mortality in the DHS cohort. While new GWAS meta-analyses continue to discover new variants, for example for lipid traits [40], these variants were able to be discovered only by very large recent meta-analyses due to their small effect sizes, making it unlikely that

addition of these variants to our GRS would lead to a significantly stronger association. However, ongoing analyses of lower frequency coding variants and copy number variation may identify higher impact variants that may significantly impact CVD risk in individuals in individuals with T2D. The current analysis points to the limited associations between currently identified CVD-related common genetic variants and CVD risk, in particular mortality risk, in individuals of European descent affected by T2D. This lack of utility may partly be due to differential impacts of genetic variants in individuals affected by T2D and highlights the need for further analysis of genetic contributors to CVD risk in diabetes affected cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors thank the other investigators, the staff, and the participants of the DHS study for their valuable contributions. This study was supported by the National Institutes of Health through R01 HL67348 and R01 HL092301 (to DWB), R01 AR48797 (to JJC), and F31 AG044879 (to LMR).

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Table 1

Demographic characteristics of 983 individuals from 466 families in the Diabetes Heart Study.

Phenotype	Mean (SD) or %	Median (range)
Demographics		
Age (yrs)	62.5 (9.1)	63.0 (34.2 - 86.0)
Gender (% female)	51.8%	
BMI (kg/m^2)	32.3 (6.6)	31.2 (17.1 - 58.0)
Past smoking	43.1 %	
Current smoking	16.3 %	
Medications		
Cholesterol Medication	47.8%	
Insulin	27.6%	
Oral Diabetes Medication	78.8%	
Hypertension Medication	75.9%	
Diabetes Measures		
Duration of diabetes (yrs)	11 (7)	8 (0 - 46)
Glycated hemoglobin (%)	7.6 (1.7)	7.2 (4.3 - 18.3)
Glycated hemoglobin (mmol/mol)	60 (19)	55 (23 - 177)
Fasting glucose (mg/dL)	148 (56)	135 (16 - 463)
Lipids		
Total Cholesterol (mg/dL)	185 (44)	181 (65 - 427)
HDL Cholesterol (mg/dL)	42 (12)	41(8 - 98)
LDL Cholesterol (mg/dL)	103 (33)	100 (12 - 236)
Triglycerides (mg/dL)	209 (140)	175 (30 - 1310)
Cardiovascular Disease Measures		
Coronary Artery Calcification	1888 (3382)	478 (0 - 50415)
Self-reported history of prior CVD	43.2%	
Self-reported history of myocardial infarction	21.6%	
Self-reported history of stroke	10.1%	
Systolic BP (mmHg)	140 (19)	139 (94 - 260)
Diastolic BP (mmHg)	73 (10)	72 (37 - 106)
Pulse Pressure (mmHg)	67 (17)	66 (28 - 159)
Estimated Glomerular Filtration Rate (ml/min/L73m^2)	66 (18)	64 (9 - 126)
PR interval (ms)	169 (32)	166 (0 - 420)
QRS interval (ms)	96 (18)	92 (64 - 192)
QT interval (ms)	394 (33)	392 (270 - 564)
C-reactive protein (mg/dL)	0.61 (0.97)	0.30 (0.005 - 12.7)
Mortality		
All-cause mortality	31.1%	

Phenotype	Mean (SD) or %	Median (range)
CVD mortality	14.3%	

Table 2

Individual genetic risk scores and their association with the trait of interest in the DHS. Relationships between the risk scores and the traits were examined using marginal models with generalized estimating equations. Models were adjusted for age and sex.

SNP Selection Trait	Number of SNPs	β Estimate	Standard Error	p-value	Trait in DHS
Blood pressure traits	24	0.244	0.161	0.13	Pulse Pressure
BMI	31	0.023	0.006	3.79×10^{-5}	BMI
Coronary Artery Calcification	2	0.178	0.073	0.015	Coronary Artery Calcification
Coronary Artery Disease*	28	1.10	1.03	2.43×10^{-4}	Self-reported history of myocardial infarction
C-reactive protein	18	0.077	0.019	3.32×10^{-5}	C-reactive protein
Electrocardiogram traits	22	0.003	0.001	5.82×10^{-4}	QT interval
		0.005	0.002	0.016	QRS interval
		0.002	0.002	0.226	PR interval
Fasting glucose	16	0.002	0.004	0.604	Fasting glucose
eGFR	23	−0.408	0.167	0.015	eGFR
HDL Cholesterol	40	−0.032	0.007	3.32×10^{-6}	HDL
LDL Cholesterol	41	0.794	0.279	0.004	LDL
Stroke*	5	1.04	1.09	0.643	Self-reported history of stroke
Type 2 Diabetes	47	-	-	-	Type 2 Diabetes
Total Cholesterol	46	0.006	0.002	6.74×10^{-4}	Total Cholesterol
Triglycerides	32	0.026	0.005	1.75×10^{-6}	Triglycerides

*Results shown as odds ratio.

Table 3

Associations between composite genetic risk score and prior cardiovascular disease (CVD), prior myocardial infarction (MI), and coronary artery calcification in the DHS were assessed using marginal models with generalized estimating equations. Associations between the composite genetic risk score and all-cause and CVD mortality were assessed using Cox proportional hazards models. Models were adjusted for age and sex.

Trait	β Estimate/Odds or Hazard Ratio	95% Confidence Interval		p-value
Self-reported history of MI *	1.01	1.00	1.02	0.149
Self-reported history of CVD *	1.00	0.99	1.01	0.481
Coronary artery calcification †	0.021	0.010	0.031	1.23×10^{-4}
All-cause mortality ‡	1.00	0.99	1.01	0.919
CVD mortality ‡	1.00	0.99	1.01	0.718

* Calculated using marginal models with generalized estimating equations, odds ratios shown

† calculated using marginal models with generalized estimating equations, β estimates shown

‡ Calculated using Cox proportional hazards models, hazard ratios shown

Table 4

Mean coronary artery calcification and prevalence of prior cardiovascular disease (CVD), prior myocardial infarction (MI), and all-cause and CVD mortality for all study participants, participants in the bottom 10% of the composite risk score (GRS) distribution, and participants in the top 10% of the composite GRS distribution. P-value is for the difference between the bottom and top 10% of the composite GRS.

Phenotype	All Participants-Mean (SD) or %	Bottom 10% of Composite GRS- Mean (SD) or %	Top 10% of Composite GRS-Mean (SD) or %	p-value
Self-reported history of MI	21.60%	22.68%	29.47%	0.280*
Self-reported history of CVD	43.20%	42.11%	46.88%	0.461*
Coronary artery calcification	1888 (3382)	1575 (2355)	2216 (2971)	0.009*†
All-cause mortality	31.10%	29.59%	25.77%	0.526*
CVD mortality	14.30%	17.02%	7.29%	0.051*

*Calculated using marginal models with generalized estimating equations

†for ln (coronary artery calcification + 1)