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Common variants in and near *IRS1* and subclinical cardiovascular disease in the Framingham Heart Study

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Abstract

Objective—Common variants at the 2q36.3-*IRS1* locus are associated with insulin resistance (IR), type 2 diabetes (T2D) and coronary artery disease (CAD) in large-scale association studies. We tested the hypothesis that variants at this locus are associated with subclinical atherosclerosis traits.

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Appendix A. Supplementary data: Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.atherosclerosis.2013.03.037>.

Methods—We studied 2740 Framingham Heart Study participants (54.9% women; mean age 57.8 years) with measures of coronary artery or abdominal aortic calcium, internal and common carotid intima-media thickness, and ankle-brachial index (ABI). We tested 1) four SNPs previously shown to be associated with IR (rs2972146, rs2943650), T2D (rs2943641) or CAD (rs2943634) and 2) any SNP at 2q36.3-*IRS1*, for association with subclinical atherosclerosis traits, adjusting for atherosclerosis risk factors. We set type 1 error rate for test 1) as $0.05/5 \text{ traits} = P < 0.01$, and for test 2) as 0.05 divided by the effective number of independent tests, divided by 5 for the number of traits analyzed.

Results—We found no association between the four known SNPs and subclinical atherosclerosis, but identified one SNP (rs10167219, r^2 with rs2943634 = 0.07) at 2q36.3 that was significantly associated with ABI (corrected $P = 0.009$). However, rs10167219 was not associated with ABI ($P = 0.70$) in 35,404 participants in a published ABI association study.

Conclusion—Common variants at the 2q36.3-*IRS1* locus were not associated with subclinical atherosclerosis traits in this study which was adequately powered to find associations with moderate effect size. Although IR and T2D may be mechanistically linked to CAD via subclinical atherosclerosis, an alternate mechanism for the IR-T2D-CAD associations at 2q36.3-*IRS1* must be postulated.

Keywords

IRS1; 2q36.3; Genetic association; Subclinical atherosclerosis; Ankle-brachial index

1. Introduction

Cardiovascular disease, principally coronary artery disease (CAD) is the leading cause of preventable death worldwide [1]. A major reason for this trend is the ongoing epidemic of type 2 diabetes (T2D) and obesity-induced insulin resistance (IR) [2]. Substantial evidence shows that IR is associated with CAD risk factors and is likely a common ground for the diabetic atherogenic milieu [3]. Further, IR and T2D are thought to be mechanistically linked to CAD *via* subclinical atherosclerosis [4].

Among many genes associated with T2D, *IRS1* is one of the most interesting candidate genes at the center of cardiometabolic genetic risk. Recent large scale association studies suggest that there may be a genetic basis linking IR, T2D and CAD [5–7]. Common variants at the chromosome 2q36.3-*IRS1* locus, an intergenic region 500 kb from the gene encoding insulin receptor substrate-1, have been associated with IR [8,9], adiposity [10], T2D [8,11], triglyceride (TG) and high density lipoprotein (HDL)-cholesterol concentrations [12], and CAD [5,6]. Thus, several lines of recent evidence support that the chromosome 2 locus, particularly near *IRS1*, has multiple associations with cardiometabolic risk.

Recently, several noninvasive techniques, including measurement of coronary artery calcium (CAC) and abdominal aortic calcium (AAC), ankle-brachial index (ABI), and carotid intima-media thickness (IMT) have been developed for precise evaluation of subclinical atherosclerosis in asymptomatic individuals [13,14]. Metabolic syndrome and T2D with IR are associated with an increased prevalence of these traits, even accounting for concomitant risk factor levels [4,14]. Thus, IR, metabolic syndrome and T2D are commonly

accompanied by subclinical atherosclerosis, which independently predicts CAD events in addition to the traditional atherosclerosis risk factors.

Given the association of common variants at chromosome 2q36.3-*IRS1* locus with IR, T2D and CAD, we hypothesized that common variants at chromosome 2q36.3-*IRS1* would be associated with measures of subclinical atherosclerosis, suggesting a common genetic basis mechanistically linking IR and T2D with CAD through subclinical atherosclerosis. We tested 1) four SNPs (rs2943634, rs2943641, rs2972146 and rs2943650), which have been shown to be associated with IR, T2D or CAD [6,8,10], and 2) any SNP at 2q36.3-*IRS1* for association with five subclinical atherosclerosis traits, measured in the Framingham Heart Study (FHS). We then tested significant associations for replication in the large published Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium GWAS of subclinical atherosclerosis traits [15].

2. Methods

2.1. Population samples: the Framingham Heart Study (FHS) cohort

Participants of the FHS have been examined approximately every 4–8 years (exams 1–9). We used data from examinations 6 and 7 of the FHS and the Framingham Heart Study Multidetector Computed Tomography (FHS MDCT) study (1995–2005) when measures of five subclinical atherosclerosis traits (CAC, AAC, internal and common carotid artery IMT [ICA-IMT, CCA-IMT], and ABI) were obtained [16]. The study protocols were approved by the Institutional Review Boards of the Boston University Medical Center and of the Massachusetts General Hospital and all participants provided written informed consent.

2.2. Anthropometric and biochemical parameters

Anthropometric and biochemical parameters measured at examinations 6 (for analyses of ICA-IMT and CCA-IMT traits) and 7 (CAC and AAC traits) were used in analyses. ABI was measured between two exams and the covariates used in the analysis of ABI were from the exam with the closest data to the ABI evaluation. Risk factor assessment included age, height, body weight, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, fasting glucose, TG, HDL-cholesterol, and low density lipoprotein (LDL)-cholesterol, and medications used for hypertension, diabetes, and lipids, as described previously [17]. Insulin resistance was calculated using the homoeostasis model assessment of insulin resistance (HOMA-IR) [18].

2.3. Assessment of subclinical atherosclerosis

Five traits of subclinical atherosclerosis were assessed including carotid IMTs by carotid ultrasonography, CAC and AAC by MDCT, and ABI. Detailed information of each measurement was described previously [15,16].

Briefly, IMT was used to quantify the degree of thickening of the carotid artery walls, with measures summarized into two variables: one for CCA and one for ICA. The trained sonographer was blinded to the clinical information of the participants and made IMT measurements using a single ultrasound machine (Toshiba Medical Systems). Carotid IMT

measurements were obtained at the level of the distal CCA and at the proximal ICA. At each site the maximal IMT was assessed in the right near and far walls, and in the left near and far walls, thus giving four wall-segment measurements at each site. The mean maximal CCA-IMT and mean maximal ICA-IMT were estimated from these four measurements. We have previously reported good reproducibility of these measures, with intraclass correlation coefficients in the range: 0.86–0.74 [19].

For CAC and AAC, a calcified lesion in either the coronary arteries or in the aorta was defined as an area of at least three connected pixels with CT attenuation > 130 Hounsfield Units using 3D connectivity criteria. Participants underwent a chest scan on an 8-slice MDCT scanner (LightSpeed Ultra, General Electric; Milwaukee, WI) for quantification of CAC and AAC, as previously described [20]. Briefly, 48 contiguous 2.5-mm-thick slices were acquired, and each participant underwent a second scan after briefly being repositioned on the table. By using a dedicated off-line workstation (Aquarius, Terarecon; San Mateo, CA), each image was evaluated for the presence and amount of CAC and AAC by an experienced reader. Each scan was evaluated for the presence of CAC and AAC and a modified Agatston score was determined as previously described [21].

To calculate the ABI for each leg, the systolic blood pressure (SBP) at each ankle was divided by the SBP in the higher arm. Participants with an ABI >1.40 were excluded since this high ABI may represent medial sclerosis, fibrocalcific disease secondary to diabetes mellitus, or other causes of noncompressible vessels [15].

Clinical categories of subclinical atherosclerosis were made for descriptive purposes based on cutoff values from the literature [22]: CAC score ≥ 300 on CT; CCA-IMT or ICA-IMT > 1.0 mm; or ABI < 0.9. The percent of increased CAC was 18.2% while that of decreased ABI was 3.1%. Since there is no consensus for AAC cutoff value, we set upper 10% of AAC as a significant risk category arbitrarily.

2.4. Genotype source

In 2007, FHS participants underwent genotyping for the FHS-SHARe (SNP Health Association Resource) project. Genotyping was conducted using approximately 550,000 SNPs (Affymetrix 500K mapping array plus Affymetrix 50K supplemental array) in over 9300 participants from the three generations of participants (including over 1500 families). After cleaning, genotypes were available on 9274 individuals and only genotypes from 8481 individuals with good quality genotypes were included in this study. The SHARe database is housed at NCBI's dbGaP (dbGaP Study Accession: phs000342.v5.p6). In addition, over 2.5 million SNPs were imputed based on the CEU reference panel from HapMap release 2 using the software MACH [23]. Individual level phenotype and imputed or known genotype data for FHS-SHARe were used for analysis. More detailed genotyping methods are available in a previous paper [24].

1) Four SNPs associated with IR, T2D or CAD—First, we selected four known SNPs from the FHS-SHARe SNP array (rs2972146, rs2943650, rs2943634) and imputed genotypes (rs2943641) (imputation quality > 0.99) previously shown to be associated with IR, T2D or CAD [6,8,10,12] for association with subclinical atherosclerosis traits. As these

four SNPs were in high linkage disequilibrium (LD, HapMap 2, B36, $r^2 = 0.95$) [6,8,10,12], we tested each one hypothesis.

2) SNPs at 2q36.3-*IRS1*—Second, we tested whether any of 195 genotyped or imputed (imputation quality > 0.88) SNPs at 2q36.3-*IRS1* were associated with five subclinical atherosclerosis traits.

2.5. Statistical analyses

With 1) four known SNPs and 2) any SNP at 2q36.3-*IRS1*, we performed a cross-sectional study testing the association of the 1) known risk allele or 2) the minor allele for each SNP versus subclinical atherosclerosis traits. We set type 1 error rate for test 1) as $0.05/5$ traits = $P < 0.01$, and for test 2) as 0.05 divided by the effective number of independent tests, divided by 5 for the number of traits tested. The effective numbers of independent SNPs were 20 from 90 SNPs in the *IRS1* region (position 227275000–227397000, Build 36) and 18 from 105 SNPs in the 2q36.3 region (position 226708000–226852000), with the effective number of independent tests derived as in Li and Ji [25]. We also adjusted the number ($n = 5$) of subclinical atherosclerosis traits, resulting in the P values threshold for each region were $0.0005 (=0.05/(20 \times 5))$ and $0.00056 (=0.05/(18 \times 5))$, respectively. These significance thresholds are conservative given correlation among the subclinical atherosclerosis traits themselves.

We used multivariable linear regression with generalized estimating equations (accounting for family structure in the extended pedigrees) to test SNP-trait associations. To normalize the distribution, log-transformed TG/HDL-cholesterol ratio was used in the analysis. Two multivariable linear regression models were used to test the main SNP-trait association and the marginal association after accounting for major atherosclerosis risk factors: Model 1) age and sex adjusted; and Model 2) age, sex, BMI, smoking status, SBP, hypertension treatment, fasting glucose, diabetes medication, LDL-cholesterol, lipid medication, and log-transformed TG/HDL-cholesterol ratio adjusted (SBP was excluded for ABI since ABI is calculated from SBPs at ankle and arm). Using the Quanto program (Version 1.2.4, 2009) with a sample size of 2863, we had 80% power to detect association with a SNP that explained 0.52% of the total variance in a subclinical atherosclerosis trait after accounting for the effective number of independent SNPs and traits.

2.6. Validation

In the primary analysis where quality control screens included minor allele frequency 0.01, call rate 0.97, and Hardy-Weinberg P -value 0.000001, we found a SNP (rs10167219) at 2q36.3-*IRS1* that was significantly associated with ABI (corrected $P = 0.0045$). We sought validation of our significant findings in the CHARGE consortium-GWAS of ABI [15]. We also tested the association of the four known SNPs (rs2943641, rs2972146, rs2943650 and rs2943634) with ABI in the CHARGE-GWAS. The CHARGE consortium ($n = 35,404$) includes the Atherosclerosis Risk in Communities Study (ARIC, $n = 7630$), the Cardiovascular Health Study (CHS, $n = 3193$), the Framingham Heart Study (FHS, $n = 2760$), the Rotterdam Study (RS-I, $n = 5169$ and RS-II $n = 1642$) and sixteen

additional population-based cohorts. ABI was measured in similar ways to the above description in cohorts included in the CHARGE consortium, as described previously [15].

3. Results

3.1. Baseline characteristics of participants

Table 1 shows study participants' anthropometric and clinical characteristics at examination 6. About half of study participants were women. The mean $\pm$ SD of BMI was 28.5 ± 4.3 in men and 27.3 ± 5.6 in women. There was significant positive correlation among CCA-IMT, ICA-IMT, CAC, and AAC (Supplementary Table 1). The ABI value was negatively correlated with CCA-IMT, ICA-IMT, and AAC but not with CAC.

3.2. Association of four known SNPs with five subclinical atherosclerosis traits

Associations of rs2943641, rs2972146, rs2943650 and rs2943634 with subclinical atherosclerosis traits are shown in Table 2. There was no significant association between these four SNPs and subclinical atherosclerosis traits in Model 1 or fully adjusted Model 2 (all $P > 0.05$).

3.3. Association of any SNPs at 2q36.3-IRS1 with subclinical atherosclerosis traits

In the analysis testing any SNP at 2q36.3-*IRS1* for association with five subclinical atherosclerosis traits, we found five SNPs (rs10498199, rs950503, rs11675231, rs964818, and rs10167219) which were associated with ABI with $P < 0.0005$. These five SNPs were in high LD ($r^2 = 1$) and thus represented a single signal (Fig. 1 and Supplementary Table 2). Thus, we focused on the most significant SNP rs10167219. rs10167219 was only weakly correlated with the four previously known SNPs, with $r^2 = 0.12$ (Supplementary Table 3).

SNP rs10167219 showed a significant association with ABI (corrected $P = 0.0045$) after accounting for the effective number of independent SNPs for the 2q36.3 region and the number of traits (Table 3). We further tested the association of rs10167219 with ABI in men and women separately, due to prior evidence of strong gene-by-sex interactions in the association of SNPs at 2q36.3/*IRS1* and body fat distribution and IR [26]. There was no significance in sex-specific association of rs10167219 with ABI, with similar effect sizes in men and women (Table 3). Next, to test whether there were genetic effects on ABI only among people with lesser degrees insulin resistance, we tested the association of rs10167219 with ABI stratified by the lower 75% versus the upper 25% of the distribution of homeostasis model insulin resistance HOMA-IR [18]. There was no significance in the association of rs10167219 with ABI in those with low or high HOMA-IR.

We also did the same analysis with 4 main SNPs and rs10167219 by sex and quartiles of age. We found that there was no significant association between these SNPs and ABI or other phenotypes of subclinical atherosclerosis (Supplementary Tables 4–8).

3.4. Validation

We found no evidence of association between rs10167219 and ABI in our validation analysis with the CHARGE-GWAS ($P = 0.70$) (Table 3). Further, we did not detect any

evidence of association of the four known SNPs (rs2972146, rs2943650, rs2943641, and rs2943634) with ABI in the CHARGE GWAS (Supplementary Table 9).

4. Discussion

Our candidate locus analysis conducted in the FHS found no association of four IR-T2D-CAD candidate SNPs (representing a single signal) at 2q36.3-*IRS1* with the five traits of subclinical atherosclerosis: CAC, AAC, CCA-IMT, ICA-IMT and ABI. Despite one promising new signal at 2q36.3-*IRS1* for ABI, we did not validate the initial association in a much larger published GWAS of ABI. Although the association of CAD with IR and T2D has been mechanistically linked through subclinical atherosclerosis [27], the data suggest that these relationships are not due to a common genetic association at ch2q36.3 with subclinical atherosclerosis, IR, T2D and CAD.

In this study, we tested the hypothesis that four known SNPs (rs2943641, rs2972146, rs2943650 and rs2943634) would be associated with subclinical atherosclerosis traits since previous studies showed association of these SNPs with IR, T2D or CAD [6,8,10,12]. Among many genes related to IR or T2D, the *IRS1* locus was shown in large scale GWAS to be a promising candidate gene in the pathogenesis of T2D [6,8,24]. In a study with 14,358 French, Danish and Finnish individuals, rs2943641 located adjacent to the *IRS1* showed strong association with IR and T2D [8]. From data of common variants associated with serum lipids in > 100,000 individuals of European ancestry, the major allele of rs2972146 adjacent to *IRS1* was associated with higher TG and lower HDL-cholesterol concentrations [12]. A meta analysis of 36,626 individuals identified rs2943650 located near *IRS1* to be associated with body fat percentage [10]. Another study from the Wellcome Trust Case Control Consortium (WTCCC) study found that rs2943634 at chromosome 2q36.3, which is located near *IRS1*, had a genome-wide level significant association with CAD [6]. These SNPs at 2q36.3 are highly correlated, suggesting that they represent a single association signal.

We found one signal (rs10167219) associated with ABI in the regression model after accounting for multiple testing. rs10167219 is located in the same genomic region of interest, but its LD with the previously known SNPs is relatively weak ($r^2 = 0.12$). However, there was no significance in the association of rs10167219 with ABI stratified by the median HOMA-IR. Moreover, in the validation look-up in the CHARGE study, no SNP was associated with ABI. This result suggests that although IR and T2D may be mechanistically linked to CAD via subclinical atherosclerosis [27], an alternate mechanism for the IR-T2D-CAD genetic associations at 2q36.3-*IRS1* must be postulated.

In a recent paper investigating genome-wide association with carotid-IMT from the CHARGE consortium, three novel SNPs on chromosomes 8q24, 19q13, and 8q23.1 were found to be significantly associated with carotid-IMT but there were no significant associations with chromosome 2q36.3/*IRS1* signals [28]. Another study investigating common genetic variants associated with CAC and myocardial infarction found that genome-wide significant associations with CAC for SNPs on chromosomes 9p21 and 6p24 in 9961 participants of European ancestry but no chromosome 2q36.3/*IRS1* signal seen [29].

These data support our study finding of no genome-wide significant association of genetic variants at 2q36.3-*IRS1* with subclinical atherosclerosis traits, although IR and T2D appear to be mechanistically linked to CAD via subclinical atherosclerosis [27]. Of note, a variant at chromosome 9p21 has been found to be associated with abnormal glucose homeostasis as well as increased CAD risk and ABI variation [15], representing an intergenic region that does provide an example of an intergenic region associated with T2D, subclinical atherosclerosis, and CAD [30].

The proatherosclerotic milieu is characterized by various processes: change in adipocytokines, activation of low grade inflammation, and production of reactive oxygen species [31]. Thus, aside from genetic components, endothelial dysfunction, oxidative stress, and inflammatory processes contribute to the development of atherosclerosis by multiple interactive pathways. In addition to the *IRS1* locus, other genetic traits at different loci have been also associated with atherosclerosis including ABI [6,15,19]. These data suggest that SNPs associated with IR may not suffice to fully explain the development of atherosclerosis. Indeed, although HOMA-IR was associated with increased subclinical atherosclerosis, the association was not independent of the risk factors that comprise metabolic syndrome in the Multi-Ethnic Study of Atherosclerosis [32]. Taken together, these lines of evidence indicate that the pathophysiological processes involved in the initiation and progression of subclinical atherosclerosis are diverse.

There are several strengths to this study. First, we tested five traits of subclinical atherosclerosis in the current analysis, providing a comprehensive assessment of systemic vascular disease. Each of the traits reflects distinct pathophysiology of atherosclerosis at different vascular beds: for example, CAD by CAC, cerebrovascular disease by IMT, and peripheral arterial diseases by ABI. Second, the primary analysis in FHS was powered well enough (80% probability to detect SNPs explaining a reasonable degree of trait variability) to detect a common variant at 2q36.3-*IRS1* to be associated with subclinical atherosclerosis. Thus it can reasonably be concluded that common variants at this locus are not associated with subclinical atherosclerosis (especially ABI), and that 2q36.3-*IRS1* does not appear to provide a genetic link connecting IR, T2D and CAD via subclinical atherosclerosis.

There were also several limitations in this study. First, despite 80% power to exclude a false negative result of reasonable effect size, our sample size may have been too small to exclude a much smaller association of 2q36.3-*IRS1* variants with subclinical atherosclerosis. Second, the current study population, the Framingham Heart Study, is included in the CHARGE validation data, representing 6.6% of the total CHARGE-GWAS sample size. So the testing of rs10167219 in CHARGE was not, strictly speaking, a replication. However, this would only be an issue if rs10167219 did “replicate”. SNPs rs10167219 since rs10167219 are located in repeat elements of the genome, increase the chance of genotyping errors although all quality control measures (call rate, HWE *P*-value) indicated that the genotypes were of good quality. Finally, we only examined common variant associations at the locus. Our analysis does not exclude the possibility that a rare variant at 2q36.3-*IRS1* could account for the shared genetic association of IR, T2D, CAD and subclinical atherosclerosis.

5. Conclusion

In this study, we identified one SNP in the chromosome 2q36.3 region that was associated with ABI in the primary analysis in FHS population. However, this association was not validated in the larger CHARGE ABI meta-analysis. We did not find any association between four known SNPs on chromosome 2 and five distinct subclinical atherosclerosis traits, which are clinical measures previously shown to have a considerable effect on the incidence of clinical cardiovascular diseases. This is a negative study but the current finding provides an important implication that common variant genetic information at chromosome 2q36.3-*IRS1* does not appear to contribute to phenotypic associations among IR-T2D-CAD. This finding suggests that genetic predisposition around *IRS1* does not fully explain the relationship between IR and subclinical atherosclerosis. For better understanding of genetics in the development of diabetes-related atherosclerosis, further investigation to find alternate mechanisms determining IR-T2D-CAD associations is required.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations and acronyms

IRS1	insulin receptor substrate 1
FHS	Framingham Heart Study
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
SNP	single nucleotide polymorphism
CT	computed tomography
CAC	coronary artery calcium
AAC	abdominal aortic calcium
CCA	common carotid artery
ICA	internal carotid artery
IMT	intima-media thickness
ABI	ankle-brachial index
IR	insulin resistance
T2D	type 2 diabetes
CAD	coronary artery disease

Fig. 1.

A regional association plot for four SNPs known to be associated with insulin resistance (rs2972146, rs2943641, rs2943650), type 2 diabetes (rs2943641) or CAD (rs2943634, at the center of the plot), and a SNP (rs10167219) at 2q36.3 associated with ABI (corrected P value of rs10167219 = 0.0045). Linkage disequilibrium (LD) is shown in relation to rs10167219; four SNPs (rs10498199, rs950503, rs11675231, rs964818) in high LD with rs10167219 are displayed in red; the four known SNPs (rs2943634, rs2943641, rs2972146, rs2943650) in relatively low LD with rs10167219 are displayed in white. The LD map is from HapMap 2, B36, CEU population. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Anthropometric and clinical characteristics and subclinical atherosclerosis traits in Framingham Heart Study subjects at examination 6.

	Mean $\pm$ SD or %
Age (yr)	57.8 $\pm$ 9.6
Women (%)	54.9%
BMI (kg/m ²)	27.8 $\pm$ 5.1
Waist circumference (cm)	97.1 $\pm$ 13.5
Systolic blood pressure (mmHg)	127.5 $\pm$ 18.5
Fasting glucose (mg/dL)	102.3 $\pm$ 25.6
Triglycerides (mg/dL)	139.0 $\pm$ 135.1
HDL-cholesterol (mg/dL)	51.8 $\pm$ 16.0
Log-transformed TG/HDL-cholesterol	0.45 $\pm$ 0.02
LDL-cholesterol (mg/dL)	127.7 $\pm$ 33.3
<i>Comorbidity status</i>	
Antidiabetic medication	4.0%
Antihypertensive medication	24.0%
Lipid-lowering medication	9.8%
<i>Subclinical atherosclerosis traits</i>	
Mean $\pm$ SD (range) or %	
AAC (Agatston unit) ^a	1580.0 $\pm$ 2556.7 (0–23630)
CAC (Agatston unit) ^a	232.6 $\pm$ 553.9 (0–5016)
CCA-IMT (mm)	0.71 $\pm$ 1.21 (0.42–3.41)
ICA-IMT (mm)	0.66 $\pm$ 1.58 (0.28–4.75)
Ankle-brachial index (ABI)	1.10 $\pm$ 0.10 (0.5–1.39)
AAC ^b 4776.2 (Agatston unit)	10.0%
CAC 300 (Agatston unit)	18.2%
CCA-IMT 1.0 (mm)	4.9%
ICA-IMT 1.0 (mm)	17.5%
ABI < 0.9	3.1%

AAC, abdominal aorta calcification; CAC, coronary artery calcification; CCA, common carotid artery; ICA, internal carotid artery; IMT, intima-media thickness.

^aData from Framingham Heart Study examination 7.

^bUpper 10% value was determined arbitrarily.

Table 2

Association of four IR-T2D-CAD SNPs at chromosome 2q36.3-*IRS1* with five traits of subclinical atherosclerosis.

SNP	Model 1		Model 2	
	Beta	SE	Beta	SE
CCA-IMT				
rs2943634	0.0051	0.0049	0.30	0.0067
rs2943641	0.0042	0.0049	0.39	0.0065
rs2972146	0.0039	0.0049	0.42	0.0062
rs2943650	0.0042	0.0049	0.39	0.0066
ICA-IMT				
rs2943634	0.0191	0.0130	0.14	0.0241
rs2943641	0.0157	0.0128	0.22	0.0210
rs2972146	0.0155	0.0128	0.22	0.0208
rs2943650	0.0151	0.0128	0.24	0.0205
CAC ^a				
rs2943634	-0.0265	0.0978	0.79	-0.0217
rs2943641	-0.0162	0.0959	0.87	-0.0066
rs2972146	-0.0392	0.0958	0.68	-0.0291
rs2943650	-0.0157	0.0960	0.87	-0.0056
AAC ^a				
rs2943634	0.0496	0.1109	0.66	0.0829
rs2943641	0.0396	0.1087	0.72	0.0819
rs2972146	0.0183	0.1086	0.87	0.0603
rs2943650	0.0397	0.1088	0.72	0.0825
ABI				
rs2943634	0.0029	0.0026	0.25	0.0024
rs2943641	0.0041	0.0025	0.11	0.0035
rs2972146	0.0038	0.0025	0.13	0.0032
rs2943650	0.0041	0.0025	0.10	0.0036

CCA and ICA, common and internal carotid artery; IMT, intima-media thickness; CAC, coronary artery calcium; AAC, abdominal aortic calcium; ABI, ankle-brachial index.

1) Model 1: age and sex were adjusted, 2) Model 2: age, sex, BMI, smoking status, SBP, antihypertensive medication, fasting glucose, antidiabetic medication, LDL-cholesterol, lipid-lowering medication, and log-transformed TG/HDL-cholesterol ratio were adjusted.

SBP was excluded from adjusting variables.

α log-transformed (1 + CAC) and log-transformed (1 + AAC) were used.

Table 3Association of candidate SNP rs10167219 at 2q36.3-*IRS1* with ABI.^a

	Effect	SE	P-value	Corrected P ^b
<i>Primary analysis</i>				
All subjects (n = 2863)	-0.0137	0.0034	0.00005	0.0045
<i>Sex</i>				
Men (n = 1282)	-0.0166	0.0054	0.002	0.18
Women (n = 1581)	-0.0126	0.0042	0.003	0.27
<i>HOMA-IR</i>				
Highest quartile (n = 620)	-0.0210	0.0082	0.011	0.96
Lower three quartile (n = 1921)	-0.0102	0.0038	0.008	0.72
<i>Validation in the CHARGE ABI GWAS data</i>				
N = 35,404	-0.0005	0.0014	0.70	—

^a age, sex, BMI, smoking status, antihypertensive medication, fasting glucose, antidiabetic medication, LDL-cholesterol, lipid-lowering medication, and log-transformed TG/HDL-cholesterol ratio were adjusted. HOMA-IR; homoeostasis model assessment of insulin resistance.

^b calculated by dividing the nominal P-value by the number of effective loci for 2q36.3 region (n = 18) and traits of subclinical atherosclerosis (n = 5).