

Genetic association of long-chain acyl-CoA synthetase 1 variants with fasting glucose, diabetes, and subclinical atherosclerosis^S

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Abstract Long-chain acyl-CoA synthetase 1 (ACSL1) converts free fatty acids into acyl-CoAs. Mouse studies have revealed that ACSL1 channels acyl-CoAs to β -oxidation, thereby reducing glucose utilization, and is required for diabetes-accelerated atherosclerosis. The role of ACSL1 in humans is unknown. We therefore examined common variants in the human *ACSL1* locus by genetic association studies for fasting glucose, diabetes status, and preclinical atherosclerosis by using the MAGIC and DIAGRAM consortia; followed by analyses in participants from the Multi-Ethnic Study of Atherosclerosis, the Penn-T2D consortium, and a meta-analysis of subclinical atherosclerosis in African Americans; and finally, expression quantitative trait locus analysis and identification of DNase I hypersensitive sites (DHS). The results show that three SNPs in *ACSL1* (rs7681334, rs735949, and rs4862423) are associated with fasting glucose or diabetes status in these large (>200,000 subjects) data sets. Furthermore, rs4862423 is associated with subclinical atherosclerosis and coincides with a DHS highly accessible in human heart. SNP rs735949 is in strong linkage disequilibrium with rs745805, significantly associated with *ACSL1* levels in skin, suggesting tissue-specific regulatory mechanisms. This study provides evidence in humans of *ACSL1* SNPs associated with fasting glucose, diabetes, and subclinical atherosclerosis and suggests links among these traits and acyl-CoA synthesis.—Manichaikul, A., X-Q. Wang, W. Zhao, M. K. Wojczynski, K. Siebenthal, J. A. Stamatoyannopoulos, D. Saleheen, I. B. Borecki, M. P. Reilly, S. S. Rich, and K. E. Bornfeldt. Genetic association of long-chain acyl-CoA synthetase 1 variants with fasting glucose, diabetes, and subclinical atherosclerosis. *J. Lipid Res.* 2016. 57: 433–442.

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The enzyme long-chain acyl-CoA synthetase 1 (ACSL1), which catalyzes conversion of free long-chain fatty acids into their acyl-CoA derivatives, has emerged as a metabolic rheostat in mouse skeletal muscle, heart, and adipose tissue (1–3). Thus, mice deficient in ACSL1 in skeletal muscle exhibit a marked reduction in fatty acid utilization through β -oxidation and a concomitant increase in glucose utilization during fasting, and they are hypoglycemic during endurance training (4). In the heart, which relies heavily on fatty acids as an energy source, ACSL1 deficiency results in reduced fatty acid oxidation and increased glucose utilization (1, 2). Mice with adipose tissue-selective ACSL1 deficiency have reduced blood glucose levels during cold exposure (3). Furthermore, an inducible whole-body ACSL1-deficient mouse model exhibits lower blood glucose levels than matched controls (1). Thus, mouse studies have revealed a clear relationship between reduced blood glucose levels and reduced ACSL1 activity in several tissues due to metabolic flexibility in these tissues.

ACSL1 is ubiquitously expressed, with high levels in typical insulin target tissues, such as skeletal muscle, liver, and adipose tissue (5). ACSL1 is also expressed in myeloid cells,

Abbreviations: ACSL1, acyl-CoA synthetase 1; CAC, coronary artery calcification; CEU, northern Caucasian Europeans (from Utah); DHS, DNase I hypersensitive site; DIAGRAM, Diabetes Genetics Replication and Meta-Analysis; eQTL, expression quantitative trait locus; GWAS, genome-wide association study; MAGIC, Meta-Analyses of Glucose and Insulin-Related Traits Consortium; MAF, minor allele frequency; MESA, Multi-Ethnic Study of Atherosclerosis; MuTHER, Multiple Tissue Human Expression Resource; RREB1, ras responsive element binding protein 1; TLR, Toll-like receptor.

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particularly in myeloid cells stimulated with inflammatory molecules, such as Toll-like receptor (TLR) 4 ligands, Gram-negative bacteria, TLR1/2 ligands, TLR3 ligands, TNF- α , and IFN- γ (6–8). Both in mouse and human macrophages, plasma membrane-associated ACSL1 levels increase after TLR4 and IFN- γ stimulation (6, 9) and also increase in human macrophages exposed to increased metabolic activation by insulin, elevated glucose, and palmitate (10). When these cells are exposed to metabolic or inflammatory activation, ACSL1 is required for effective phospholipid

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replenishment, neutral lipid accumulation, and early atherosclerosis in the context of diabetes (6, 7, 11). Mice deficient in ACSL1 selectively in myeloid cells or endothelial cells do not exhibit reduced blood glucose levels (6, 12).

Whereas mouse studies indicate that ACSL1 might play a critical role in maintaining glucose homeostasis and atherosclerosis, the role of ACSL1 in humans is unknown. Because of the studies discussed above, we were particularly interested in the relationship between human *ACSL1* SNPs with variation in fasting glucose, diabetes, and risk of atherosclerosis. We interrogated published [Meta-Analyses of Glucose and Insulin-Related Traits Consortium (MAGIC) and Diabetes Genetics Replication and Meta-Analysis (DIAGRAM) consortium] genome-wide association study (GWAS) scans of fasting glucose (13, 14) and diabetes status (15) with respect to common [minor allele frequency (MAF) >5%] SNPs in the *ACSL1* locus (4q35.1). MAGIC contains >46,000 healthy nondiabetic individuals of European descent, while DIAGRAM has >56,000 individuals of primarily European descent (>12,000 with type 2 diabetes). For the common *ACSL1* SNPs identified through the MAGIC and DIAGRAM consortia, we conducted follow-up analyses of participants with and without diabetes from the Multi-Ethnic Study of Atherosclerosis (MESA) and in >200,000 subjects with and without type 2 diabetes included in the Penn-T2D consortium. The analyses in MESA allowed us to expand discoveries from the published GWAS effort to examine evidence of association *a*) across race/ethnic groups and *b*) with subclinical atherosclerosis traits, and the analysis in the Penn-T2D consortium allowed us to expand our analysis to a much larger sample size. The association between *ACSL1* SNPs and coronary artery calcification (CAC) was validated in African Americans (16). Furthermore, expression quantitative trait locus (eQTL) analysis was used to investigate if the identified *ACSL1* SNPs were associated with *ACSL1* levels in skin, lymphocytes, and adipose tissue, and finally, DNase I hypersensitive sites (DHSs) coinciding with these SNPs were analyzed in several fetal and adult human tissues.

Together, our results demonstrate that three SNPs in the *ACSL1* gene are associated with markers of fasting glucose or diabetes status and that one of these SNPs is also significantly associated with subclinical atherosclerosis. This represents the first indication of ACSL1 contributing to the regulation of fasting glucose and risk of diabetes and subclinical atherosclerosis in humans.

MATERIALS AND METHODS

Candidate association analyses from large-scale consortia

To perform association analysis of common variants within the *ACSL1* gene region, we examined published GWAS and custom-array results from the MAGIC and DIAGRAM consortia. We report the SNP demonstrating the strongest association with the analyzed traits for each study.

MAGIC. MAGIC published a GWAS of fasting glucose and fasting insulin in up to 46,196 nondiabetic participants of European

descent in 2010 (13). A subsequent effort from MAGIC performed a GWAS of the same traits with adjustment for BMI in an expanded sample of up to 51,750 nondiabetic participants of European descent (14).

DIAGRAM. The DIAGRAM consortium performed a GWAS of type 2 diabetes in 12,171 cases and 56,862 controls comprising the DIAGRAM v3 GWAS (15). The SNPs identified through GWAS contributed to the design of the Metachip, a custom genotyping array used for genetic association analysis of type 2 diabetes in an expanded sample of (primarily European descent) 34,840 cases and 114,981 controls.

Genetic association analysis of *ACSL1* variants in MAGIC and DIAGRAM. We focused on *ACSL1* variants within 50 kb of the *ACSL1* gene, using LocusZoom (17) to identify SNPs within the region from the published GWAS and custom genotyping array results. We then conducted candidate association analyses for each of the four selected published data sets, the original MAGIC GWAS (13), the MAGIC BMI-adjusted GWAS (14), the DIAGRAM v3 GWAS (15), and the DIAGRAM Metachip (15). For each lookup, we applied a Bonferroni correction for the number of SNPs identified within the target region. Here, we report those SNPs reaching the specified Bonferroni threshold for at least one of the published data sets.

Analysis of *ACSL1* SNPs in MESA

MESA. The MESA is a longitudinal cohort study of subclinical cardiovascular disease and risk factors in 6,814 men and women 45 to 84 years of age, initially free of overt disease, that predict progression to disease or progression of subclinical disease (18). The MESA Family Study (MESAFS) recruited 1,595 African American and Hispanic family members 45 to 84 years of age specifically for genetic analysis, and the MESA Air Pollution Study recruited an additional 257 participants (19). MESA participants had detailed medical histories and examinations for anthropometry, blood pressure, and vascular imaging [to obtain measures of subclinical atherosclerosis of CAC and common (cIMT) and internal carotid intima-media thickness (iIMT)] (20). Glucose concentrations were determined from fasting samples, and diabetes status was defined using the 2003 American Diabetes Association fasting criteria (fasting glucose ≥ 126 mg/dl) (21) and/or diabetes treatment. Characterization of MESA subjects included in the present study is included in the supplementary Materials and supplementary Tables 1 and 2. MESA participants were genotyped using the Affymetrix Human SNP array 6.0 (22). Imputation was conducted with IMPUTE v2 using Phase 1 v3 of the cosmopolitan 1,000 genomes reference set (23), followed by robust quality control (24).

Genetic association analysis in MESA. MESA participants were stratified by race/ethnic group using an unrelated subset of individuals from MESA and from MESAFS, by selecting, at most, one individual from each pedigree, based on inferred relationships in KING (25). Linear regression of quantitative phenotypes or logistic regression of dichotomous phenotypes was performed under an additive 1 d.f. genetic dosage model in R. For phenotypes with a substantial familial component, we performed analysis using linear mixed-effects models for quantitative traits, or generalized estimating equations for dichotomous traits, to account for familial relationships as implemented in the package R/GWAF (26). A basic model including age, gender, study site, and principal components of ancestry was used. Fixed effect meta-analysis was performed to combine race/ethnic specific results using METAL (27).

For each of the three SNPs identified in consortium studies (Table 1), primary analyses in MESA focused on the phenotype analyzed in discovery of each SNP, including glucose (log-transformed) and diabetes status. Secondary analysis examined subclinical atherosclerosis (cIMT, iIMT, CAC presence/absence, and Agatston score for those with CAC >0).

Penn-T2D consortium. The Penn-T2D consortium used publicly available DIAGRAM meta-analyses data, updated by adding additional T2D GWAS/Metachip studies that have not previously contributed data to the DIAGRAM consortium (findings from these analyses are currently submitted for publication). The Penn-T2D consortium consists of data from 56,241 type 2 diabetes cases and 187,815 controls.

Replication and validation in MESA and Penn-T2D for SNPs identified in consortia studies

For each of the three *ACSL1* SNPs identified in MAGIC and DIAGRAM associated with fasting glucose and diabetes (Table 1), analyses were focused on replicating and validating association with the corresponding trait analyzed in discovery of each SNP in MESA and the Penn-T2D consortium. Statistical significance for replication of the three SNPs was determined at a Bonferroni-corrected threshold of $\alpha = 0.05/3 = 0.017$, whereas $\alpha = 0.05$ was considered as nominal evidence of replication. For secondary analysis of subclinical atherosclerosis traits in MESA, we report findings as significant if they reach a Bonferroni-corrected threshold of $\alpha = 0.05/12 = 0.0042$, corresponding to three SNPs and four traits, or 12 tests.

Meta-analysis of CAC in African Americans

This study amassed data on 5,823 African Americans from eight US studies in CAC, examining various definitions of CAC: $\ln[\text{CAC}+1]$; CAC present/absent; $\ln(\text{CAC})$ where CAC >0; and BLOM transformed CAC, defined by Gomez et al. (28). Results from the eight studies were meta-analyzed in METAL (16). For this validation study, we used the results from subjects with CAC >0 on 2,520 subjects. This cohort included African Americans from MESA (29% of subjects). Statistical significance for replication of the three SNPs was determined at a threshold of $\alpha = 0.05/3 = 0.017$ after Bonferroni correction.

CARDIoGRAMplusC4D consortium

In order to investigate association of the three identified *ACSL1* SNPs with cardiovascular events (myocardial infarction, symptomatic coronary events, or coronary stenosis), we took advantage of the CARDIoGRAMplusC4D consortium. CARDIoGRAM GWAS is a meta-analysis of 22 GWASs in participants of European descent involving 22,233 cases and 64,762 controls, and C4D GWAS is a meta-analysis of GWASs in participants of European and South Asian descent involving 15,420 coronary heart disease cases and 15,062 controls. GWAS data from both of these consortia contain nonoverlapping participants (29). We used this publicly available data and conducted a fixed-effects meta-analysis yielding a sample size of 37,653 coronary heart disease cases and 80,182 controls.

Multiple Tissue Human Expression Resource analysis

The Multiple Tissue Human Expression Resource (MuTHER) consortium data (<http://www.muth.ac.uk>) were used to investigate if the three identified *ACSL1* SNPs were associated with *ACSL1* levels, representing an eQTL analysis. MuTHER contains genome-wide expression (Illumina HT-12v3 array) profiles on lymphocytes, subcutaneous fat, and skin biopsies from ~856 twins from the TwinsUK BioResource. MuTHER was interrogated for changes in probe ILMN_1684585 (*ACSL1*).

TABLE 1. Summary of SNPs identified in the *ACSL1* gene region from MAGIC and DIAGRAM

Data	Trait	Number of <i>ACSL1</i> Region SNPs ^e	SNP ID	HG 19 Position	Effect/ Other Allele	Consortia Results				MESA Results ^b			
						EAF	Beta	SE	P	EAF ^c	Beta	SE	P
MAGIC GWAS	Fasting glucose in nondiabetics	173	rs7681334	185,710,859	A/G	0.455	0.016	0.004	8.4E-6	0.496/0.452/ 0.644/0.928	0.003	0.002	0.177
MAGIC BMI-adjusted GWAS	Fasting glucose in nondiabetics	183	rs4862423	185,726,548	T/C	0.350	0.017	0.003	4.6E-7	0.407/0.268/ 0.502/0.661	0.004	0.002	0.037
Data	Trait	Number of SNPs ^e	SNP ID	HG 19 Position	Effect/ Other Allele	EAF ^d	Odds Ratio	95% CI	P	EAF ^c	Odds Ratio	95% CI	P
DIAGRAM v3 GWAS	Diabetes status	173	rs735949	185,716,232	T/C	0.825	1.11	(1.05, 1.17)	7.8E-5	0.853/0.948/ 0.909/0.9993	1.04	(0.870, 1.25)	0.716
DIAGRAM MetaboChip	Diabetes status	9					1.09	(1.05, 1.12)	3.7E-6				

CEU, northern Caucasian Europeans (from Utah); CI, confidence interval; EAF, effect allele frequency; HG19, human genome version 19.

^aThe *ACSL1* gene region was defined as the genomic region containing the *ACSL1* gene itself ± 50 kb.

^bResults for MESA are presented based on combined meta-analysis across race/ethnic groups.

^cEAF for MESA shown separately for Caucasian/African American/Hispanic/Chinese.

^dEAF reported for HapMap CEU because this information is not included in the DIAGRAM summary files.

Identification of chromatin accessibility and transcription factor binding motifs

Chromatin accessibility data were obtained from public repositories for human cell lines [ENCODE Consortium (30)] as well as fetal and adult tissues and primary cell lines [Roadmap Epigenomics Consortium (31) and unpublished observations] and intersected with the three SNPs examined by this work. Transcription factor binding motif locations were determined as described by Vierstra et al. (32).

Human study approvals

Each study obtained approval from their respective institutional review board and the ethics committee of each participating institution, including the University of Alabama at Birmingham, Washington University, University of Mississippi Medical Center, University of Minnesota, Northwestern University, Kaiser Permanente (Oakland, CA), University of Washington, Columbia University, Johns Hopkins School of Medicine, University of California Los Angeles School of Medicine, Wake Forest University School of Medicine, University of Michigan Health Sciences and Behavioral Sciences, the Cedars-Sinai Medical Center, the University of Virginia, and the University of Pennsylvania, and informed consent from participants. All methodology was compliant with the principles set forth in the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Protection of Human Subjects.

RESULTS

ACSL1 association analysis in published results from MAGIC

After Bonferroni correction (significance threshold $\alpha = 0.05/173 = 2.9 \times 10^{-4}$) for 173 SNPs within *ACSL1* in the MAGIC GWAS, we identified rs7681334 as significantly associated with fasting glucose (MAF = 0.40, $P = 8.4 \times 10^{-6}$; Fig. 1A and Table 1). After Bonferroni correction (significance threshold of $\alpha = 0.05/183 = 2.7 \times 10^{-4}$) for 183 SNPs within *ACSL1* in the MAGIC BMI-adjusted GWAS, we identified rs4862423 in addition to rs7681334 as significantly associated with fasting glucose (MAF = 0.43, $P = 4.6 \times 10^{-7}$; Fig. 1B and Table 1).

ACSL1 association analysis in published results from DIAGRAM

We identified rs735949 as significantly associated with diabetes status (MAF = 0.06, $P = 7.8 \times 10^{-5}$) after Bonferroni correction for 173 *ACSL1* SNPs in the DIAGRAM v3 GWAS. We also identified rs735949 as significantly associated with diabetes status (MAF = 0.06, $P = 3.7 \times 10^{-6}$) after correction for 9 *ACSL1* SNPs in the DIAGRAM MetaboChip data (Fig. 1C, D and Table 1).

Replication of *ACSL1* results identified from consortia studies in MESA

In combined meta-analysis across race/ethnic groups for nondiabetic participants from MESA, we identified nominal (but not Bonferroni-corrected) evidence of replication for the association of *ACSL1* rs4862423 with BMI-adjusted fasting glucose; further, the observed direction of effect was consistent with that seen in MAGIC (Table 1;

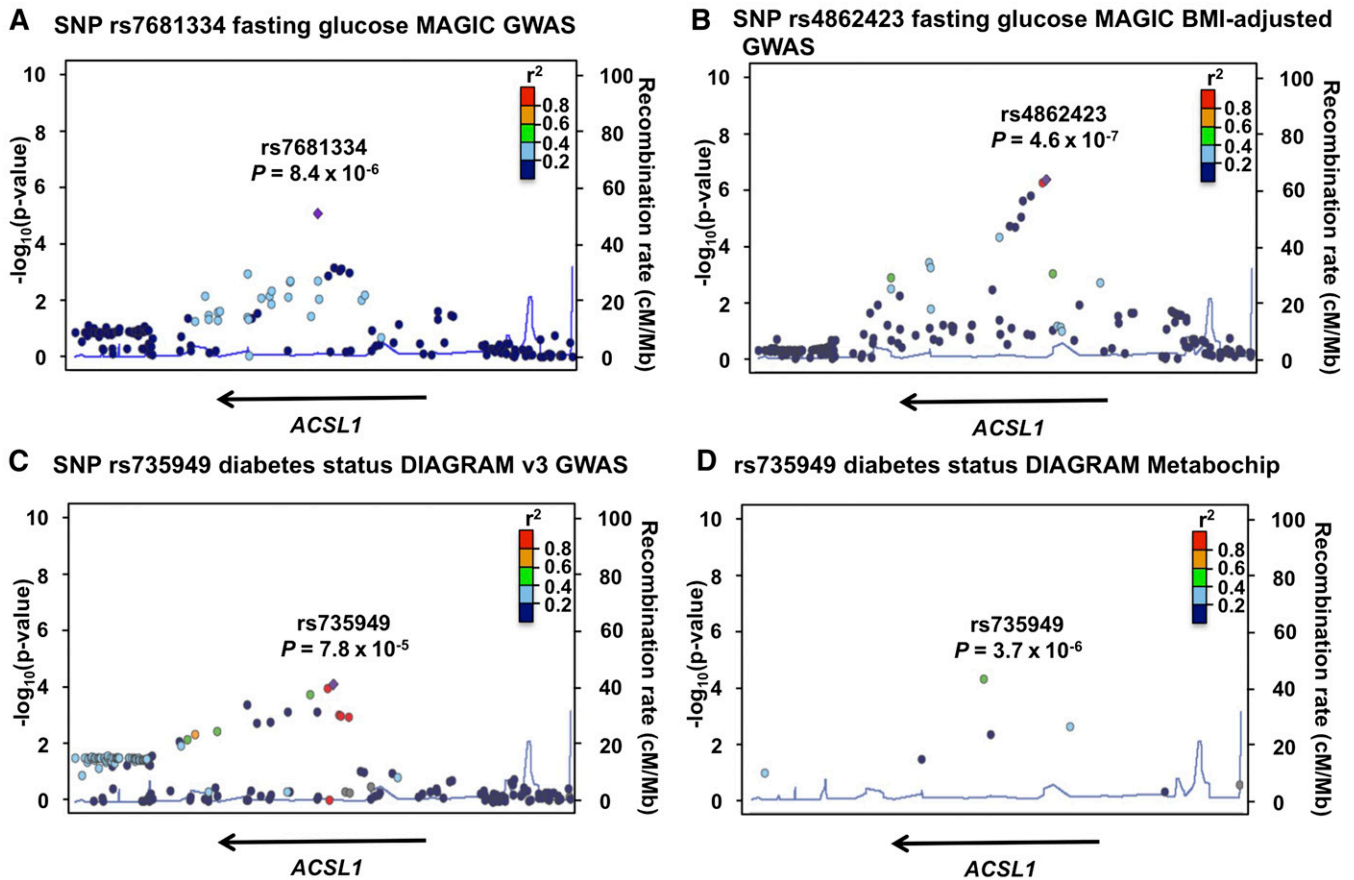


Fig. 1. Regional association plots for statistically significant SNPs identified through candidate association studies in *ACSL1* in published results from genetic consortia. SNP rs7681334 association with fasting glucose in the original MAGIC GWAS (A), SNP rs4862423 association with fasting glucose in the MAGIC BMI-adjusted GWAS (B), SNP rs735949 association with diabetes status in DIAGRAM v3 GWAS (C), and SNP rs735949 association with diabetes status in DIAGRAM MetaboChip (D). The plots are generated using LocusZoom with 1000 Genomes CEU as the reference for calculating linkage disequilibrium.

$P = 0.037$). The effect allele (T/C) frequency was 0.350 in MAGIC European Caucasians and 0.407 in MESA Caucasian participants. The observed effect size and strength of association for rs4862423 was most notable in MESA African Americans without diabetes compared with other race/ethnic groups ($P = 0.033$; supplementary Table 3). We did not observe additional race/ethnic specific associations for fasting glucose-related SNPs (supplementary Tables 3 and 4). We did not observe statistically significant evidence of replication for rs7681334 with fasting glucose or for rs735949 with diabetes status (Table 1 and supplementary Table 5). Thus, these results demonstrate that the T allele in rs4862423 (T/C) associates significantly with increased fasting blood glucose levels in MAGIC, with suggestive evidence of replication in MESA.

Replication and validation of *ACSL1* SNP associations in Penn-T2D meta-analyses

In a second study [Penn-T2D meta-analyses (Penn-T2D Meta) data], which is larger than either of the two consortia used for discovery analysis and the MESA replication study, we were able to confirm the association of *ACSL1* rs735949 with diabetes status. Furthermore, rs7681334, which was associated with fasting blood glucose in MAGIC (Table 1) was also significantly associated with diabetes

status in the Penn-T2D Meta (Table 2). The observed direction of effect was consistent with that seen in MAGIC and DIAGRAM. *ACSL1* rs4862423, which was associated with fasting glucose in the BMI-adjusted MAGIC with suggestive evidence of replication in MESA (Table 1), showed a suggestive association with diabetes status ($P = 0.065$) in the Penn-T2D Meta for which the direction of effect was consistent with that seen with fasting glucose in MAGIC and MESA. Thus, the association of *ACSL1* rs735949 and rs7681334 with fasting glucose and diabetes status is replicated in large cohorts. The association of rs4862423 with fasting glucose was significant in the BMI-adjusted MAGIC in nondiabetic subjects, yet failed to reach significant association with diabetes status in the Penn-T2D Meta.

ACSL1 SNP association with subclinical atherosclerosis in MESA

For subclinical atherosclerosis traits, we observed association of *ACSL1* rs7681334 with CAC Agatston score (among participants with CAC >0) in combined meta-analysis across race/ethnic groups (Table 3; $P = 0.003$). The association was stronger in participants without diabetes (Table 3; $P = 0.001$). We further observed association of rs4862423 with CAC in participants without diabetes (Table 3; $P = 0.002$). Race/ethnic stratification provides consistent

TABLE 2. Summary of association with type 2 diabetes status of the three identified SNPs in the *ACSL1* gene region from Penn-T2D Meta

SNP ID	Effect Allele	Noneffect Allele	Beta	SE	<i>P</i>	N Case	N Control
rs7681334	A	G	0.031	0.009	6.56E-04	56,241	187,815
rs735949	T	C	0.069	0.014	1.02E-06	56,241	187,815
rs4862423	T	C	0.024	0.013	6.51E-02	20,516	66,774

directions of effect for both of these *ACSL1* SNPs for Caucasian, African American, and Hispanic participants, but not Chinese participants (supplementary Tables 6 and 7). In addition, both SNPs showed stronger effects on CAC in nondiabetics compared with those with diabetes (Cochran's Q test for heterogeneity, rs7681334, $P = 0.12$; rs4862423, $P = 0.04$).

CAC results in meta-analysis of African Americans

The association of rs4862423 with CAC observed in MESA African Americans among those with CAC scores >0 was significant in the expanded sample (Table 4). However, the association of rs7681334 with CAC in MESA was not observed in the African American sample. Furthermore, an association between rs735949 and CAC was observed in the African American sample, but not in MESA (Tables 3 and 4). Thus, the association of rs4862423 with subclinical atherosclerosis was consistent between the two studies. The association of rs735949 and rs7681334 with CAC appeared to be influenced by other factors.

Analysis of association of *ACSL1* SNPs in the CARDIoGRAMplusC4D consortium

In addition to testing *ACSL1* SNPs for association with diabetes and subclinical atherosclerosis, we determined whether the *ACSL1* SNPs modified risk for clinical end points (coronary heart disease). In the CARDIoGRAMplusC4D consortium data, there were no significant associations of *ACSL1* SNPs rs7681334, rs735949, or rs4862423 with coronary heart disease (supplementary Table 8).

Chromatin accessibility at the *ACSL1* locus

The three trait-associated *ACSL1* SNPs are located within the first (rs4862423) and second (rs7681334, rs735949) introns of the *ACSL1* gene. Noncoding GWAS SNPs are concentrated in regulatory DNA elements, where they may modulate transcription factor binding (33). Thus, we asked whether these *ACSL1* polymorphisms coincided with DHSs, which generically indicate transcription factor

binding at active regulatory elements (30). *ACSL1* utilizes alternative promoters, which appear to have different levels of tissue selectivity (Fig. 2A). SNP rs4862423 is located in intron 1 of *ACSL1*, a region that is highly accessible to DNase I in fetal heart tissue, and to a lesser extent in other fetal tissues (Fig. 2B) as well as adult heart, mucosal (gastric and small bowel), pancreatic, and skeletal muscle tissue (data not shown). This site does not appear to be active in the hepatocyte cell line HepG2 but is accessible in multiple cultured epithelial and skeletal muscle cell lines (data not shown). Furthermore, rs4862423 coincides with a recognition site for RREB1 (ras responsive element binding protein 1; Fig. 2C). Although rs735949 coincides with a DHS in primary skin melanocytes (data not shown), we did not observe regulatory element activity coinciding with rs7681334 in any of the cell lines and tissues for which DHS data are available.

eQTL analysis

ACSL1 SNPs were analyzed for evidence of eQTLs using the MuTHER consortium data on three tissues: subcutaneous fat, lymphocytes, and skin. There were no significant associations for any of the three SNPs with probe ILMN_1684585 (*ACSL1*). *P* values for rs7681334 were 0.45 in fat, 0.59 in lymphocytes, and 0.76 in skin. The corresponding *P* values for rs735949 were 0.13, 0.56, and 0.054, and for rs4862423, they were 0.35, 0.73, and 0.68 (supplementary Table 9). The most significant SNP associated with probe ILMN_1684585 in skin biopsies was rs745805 ($P = 0.0014$), which is in high linkage disequilibrium with rs735949 ($r^2 = 0.832$ in 1000G pilot 1 CEU; supplementary Table 9). These results suggest that rs735949 might be associated with *ACSL1* expression levels in some tissues.

DISCUSSION

The role of *ACSL1* in humans is unknown, although mouse studies have shown that this enzyme plays an important

TABLE 3. Summary of statistically significant genetic association results for Agatston calcium score in MESA

Group	SNP ID	HG 19 Position	Effect/Other Allele	EAFA	N	Beta	SE	<i>P</i>
All participants	rs7681334	185,710,859	A/G	0.497/0.450/ 0.647/0.944	3,852	-0.126	0.043	0.003
Participants without diabetes	rs7681334	185,710,859	A/G	0.496/0.452/ 0.644/0.928	3,172	-0.157	0.046	0.001
Participants without diabetes	rs4862423	185,726,548	T/C	0.407/0.270/ 0.499/0.658	3,172	-0.150	0.048	0.002

Results are presented based on the basic model of genetic association including adjustment for age, gender, study site, and principal components of ancestry.

^aEAFA for MESA shown separately for Caucasian/African American/Hispanic/Chinese participants.

TABLE 4. Summary of results on genetic association results for Agatston calcium score in meta-analysis of CAC among African Americans

SNP ID	HG 19 Position	Effect/Other Allele	EAF	N	Beta	SE	P
rs4862423	185,726,548	T/C	0.2448	2,523	-0.1757	0.0611	0.004
rs735949	185,716,232	T/C	0.954	2,523	-0.343	0.1322	0.009
rs7681334	185,710,859	A/G	0.4555	2,523	-0.0066	0.0538	0.912

role as a metabolic rheostat mechanism in several tissues, and hence, that loss of ACSL1 results in reduced blood glucose levels due to reduced fatty acid oxidation under conditions in which excess energy is needed. We demonstrate here that specific SNPs in the *ACSL1* gene are significantly associated with fasting glucose (rs7681334 and rs4862423) or with diabetes status (rs735949) in the MAGIC and DIAGRAM consortia. We also report evidence of replication of the association of rs4862423 with fasting glucose in nondiabetic participants from MESA, and association of both rs7681334 and rs735949 with diabetes status in Penn-T2D Meta. Although the Penn-based consortium includes the DIAGRAM data, it contains twice the number of cases included in DIAGRAM. Together, these four studies make a strong case for significant association of the three *ACSL1* SNPs with fasting glucose and diabetes. Furthermore, whereas MAGIC and DIAGRAM included subjects primarily of European descent, MESA includes subjects of different race/ethnic groups (mostly Caucasian, African American, and Hispanic participants and a smaller fraction of Chinese participants). The smaller sample size in MESA (7,847 participants) compared with the MAGIC and DIAGRAM consortia and Penn-T2D Meta probably contributed to the lower level of significance and statistical power. It is unlikely that the association of the *ACSL1* SNPs with glucose levels is significantly confounded by BMI or adiposity because no nominally statistically

significant association (defined as $P < 0.05$) was observed for any of the three SNPs in a large-scale GWAS meta-analysis of BMI (34).

The present study also demonstrates a significant association of rs4862423 with subclinical atherosclerosis, measured as CAC scores >0 in MESA and in a follow-up meta-analysis study in African Americans. The directionality of the association was different for fasting glucose and CAC. Thus, rs4862423 allele T was associated with increased fasting glucose and decreased CAC. The reason for the difference in directionality is unclear. Analysis of cardiovascular events in CARDIoGRAMplusC4D did not show association with the three *ACSL1* SNPs. It is possible that the association occurs only with early atherosclerosis or with calcification, rather than with more advanced lesions and clinical events. In this context, it is interesting that ACSL1 in mice is required for early lesions of atherosclerosis, with a less obvious effect on advanced lesions (6).

All three *ACSL1* SNPs are located in intronic regions of *ACSL1* (introns 1 and 2), and our studies demonstrate that the two SNPs most clearly associated with fasting glucose and diabetes (rs7681334 and rs735949) do not associate with identified DNase I accessible regions in several cells, fetal or adult tissues most likely to be relevant for these phenotypes. However, we show that rs735949 is associated with a DNase accessible region in primary skin melanocytes. A previous study identified association of rs735949

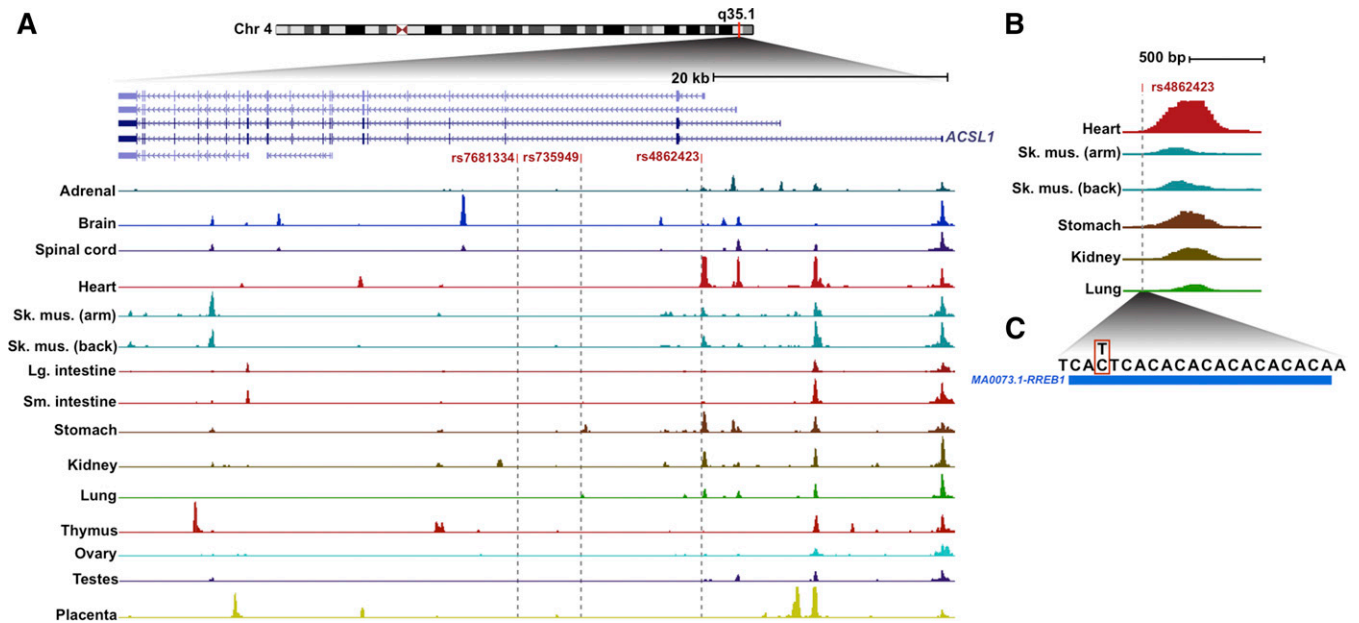


Fig. 2. Chromatin accessibility at the *ACSL1* locus. A: Normalized densities of DNase I cleavages are shown for 14 human fetal tissues, with the positions of three GWAS SNPs indicated by gray vertical lines. B: Localization of rs4862423 within a DNase I accessible region with the highest activity in fetal heart. C: Position of SNP (red box) within a binding motif of RREB1 (blue box).

with islet transcription factors (35). It is possible that rs7681334 and rs735949 coincide with regulatory elements active in other tissues, ages, or disease states relevant to fasting glucose and diabetes traits, or that these SNPs are in linkage disequilibrium with other regulatory variants at this locus. Indeed, rs735949 was in strong linkage disequilibrium with another SNP in the same *ACSL1* intron 2, rs745805, which is significantly associated with *ACSL1* expression in skin, suggesting links between rs735949 and *ACSL1* expression in a tissue-selective manner. However, rs745805 is not located within a region of appreciable DNase I hypersensitivity, nor does it coincide with a transcription factor recognition sequence (data not shown). Thus, the *ACSL1* SNPs investigated in regions that do not coincide with DHSs may be in linkage disequilibrium with transcription factor binding-altering SNPs within regulatory elements nearby. Furthermore, eQTL analysis did not reveal significant associations with the SNPs investigated and *ACSL1* levels in fat, skin, or lymphocytes. *ACSL1* expression is known to be regulated differently in different tissues (7), and the *ACSL1* gene contains three alternative exon 1s controlled by different promoters (36). It can therefore not be ruled out that one or several of the SNPs are associated with *ACSL1* expression levels in other tissues or in disease states. Furthermore, a long noncoding RNA, *SLED1*, is located in the vicinity of rs7681334 and rs735949. The putative role of *SLED1* is unknown, but it is plausible that these SNPs could affect *SLED1*.

ACSL1 rs4862423, which is associated with fasting glucose and CAC, coincides with an *ACSL1* DHS active in multiple fetal and adult tissues (including heart and pancreas) and is localized within a binding motif for RREB1. RREB1 has recently been implicated by GWAS as a regulatory factor associated with glucose levels (37). Mass spectrometry-based analysis of the human proteome (Proteomics DB) has demonstrated that heart, pancreas, lymphocytes, and colon express RREB1 (38). The role of RREB1 in regulation of blood glucose and atherosclerosis is unknown, but interestingly, RREB1 was recently demonstrated to modify histones by aiding in removal of histone H3 lysine 27 trimethylation (H3K27me3) marks, which often characterize silenced regions, thereby promoting *Ucp1* and *Cidea* expression in brown adipocytes (39). This mechanism of action of RREB1 was shown to be due to recruitment of Jmjd3, an H3K27me3 demethylase. Thus, RREB1 binding to the motif associated with rs4862423 might alter chromatin modification and increase *ACSL1* transcription in tissues that express RREB1. This hypothesis remains speculative until tissues expressing RREB1 from subjects with rs4862423 polymorphism have been examined by electrophoretic mobility shift assays for differences in RREB1 binding and *ACSL1* expression. SNP rs4862423 is located in intron 1 of human *ACSL1*. Previous studies have identified other SNPs located in this intron as being associated with metabolic parameters in humans (40, 41): rs6552828 has been associated with gains in maximal O₂ uptake after exposure to an exercise program in sedentary adults participating in the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study (40), and rs9997745 has been

associated with increased metabolic syndrome risk (41). A recent study suggested that rs6552828 (located near rs4862423) might result in differences in *ACSL1* expression (42). It is therefore possible that rs4862423 and other polymorphisms in intron 1 of the *ACSL1* gene result in altered expression of *ACSL1* in specific tissues.

In summary, our study is the largest to date examining *ACSL1* SNPs in humans, and the first to examine the association between *ACSL1* SNPs, fasting glucose, diabetes, and subclinical atherosclerosis. Our results demonstrate that three SNPs in the *ACSL1* gene are associated with markers of fasting glucose or diabetes status and that one of these SNPs is also significantly associated with subclinical atherosclerosis. This represents the first evidence of *ACSL1* association with regulation of fasting glucose and risk of diabetes and subclinical atherosclerosis in humans and suggests possible links between these traits and acyl-CoA synthesis. **Fig**

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