Published in final edited form as:
Heart Rhythm. 2014 March ; 11(3): 471–477. doi:10.1016/j.hrthm.2014.01.008.

Common Variation in Fatty Acid Metabolic Genes and Risk of Incident Sudden Cardiac Arrest:

Genetic Variation and Sudden Cardiac Arrest

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Disclosures. Dr Mozaffarian reports receiving research grants from GlaxoSmithKline, Sigma Tau, Pronova, and the National Institutes of Health for an investigator-initiated, not-for-profit clinical trial of fish oil and postsurgical complications; and small annual royalties from UpToDate for an online scientific chapter on fish oil. The other authors report no conflicts.

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Abstract

Background—There is limited information on genetic factors associated with sudden cardiac arrest (SCA).

Objective—To assess the association of common variation in genes in fatty acid pathways with SCA risk.

Methods—We selected 85 candidate genes and 1155 single nucleotide polymorphisms (SNPs) tagging common variation in each gene. We investigated the SNP associations with SCA in a population-based case-control study. Cases (n=2160) were from a repository of SCA in the greater Seattle area. Controls (n=2615), frequency-matched on age and sex, were from the same area. We used linear logistic regression to examine SNP associations with SCA. We performed p-min permutation tests to account for multiple comparisons within each gene. The SNP associations with corrected p-value < 0.05 were then examined in a meta-analysis of these SNP associations in nine replication studies totaling 2129 SCA cases and 23833 non-cases.

Results—Eight SNPs in or near 8 genes were associated with SCA risk in the discovery study, one of which was nominally significant in the replication phase (rs7737692, minor allele frequency 36%, near the *LPCAT1* gene). For each copy of the minor allele, rs7737692 was associated with 13% lower SCA risk (−21% to −5%) in the discovery phase and 9% lower risk (95% CI −16% to - 1%) in the replication phase.

Conclusions—While none of the associations reached significance with Bonferroni correction, a common genetic variant near *LPCAT1*, a gene involved in the remodeling of phospholipids, was nominally associated with incident SCA risk. Further study is needed to validate this observation.

Keywords

death; sudden; genetic epidemiology

INTRODUCTION

Sudden cardiac arrest (SCA) accounts for 10% of total mortality and 40% of mortality from coronary heart disease, the major cause of mortality in Western populations. [1] While a number of patient characteristics, including demographics, life style and clinical conditions are known risk factors for SCA, together, these known risk factors have low predictive value [2]. The possibility that genetic factors may also contribute to SCA risk was first suggested by familial syndromes with mutations in ion channel genes that predispose to SCA [3]. In addition, a parental history of SCA was found to be associated with higher SCA

risk in population-based studies, suggesting the existence of genetic risk factors for SCA in the community [4, 5].

Possible approaches to the search for genetic factors of SCA are genome-wide association studies (GWAS) and candidate gene studies [3]. While GWAS have uncovered numerous associations with metabolic endpoints, it has been more challenging to discover associations with complex diseases in spite of the formation of large consortia. An alternative to GWAS is the investigation of candidate genes based on knowledge of risk factors or the pathophysiology of the disease. We report here the result of a candidate gene approach based on the hypothesis that common variation in genes in pathways involved in fatty acid uptake and beta-oxidation, cell membrane fatty acid composition and metabolism of polyunsaturated fatty acids are associated with SCA risk.

We investigated the associations of common variants in 85 fatty acid metabolic genes with SCA risk among European Americans in a large population-based case-control study. Variants associated with risk were then investigated in a meta-analysis of these same associations in nine studies of sudden cardiac death participating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium [6].

METHODS

Design

We investigated genetic associations with sudden cardiac arrest (SCA) in two phases. In the discovery phase, we examined the associations of common genetic variation in 85 genes with SCA in a large population-based case-control study. Single nucleotide polymorphisms (SNPs) that met pre-specified criteria were then examined in a meta-analysis of *in silico* results of GWAS of SCA in nine studies in the CHARGE SCA Consortium. Below we describe the methods of the discovery study. Methods for the replication studies are summarized in the online supplement (Supplementary Tables 1 and 2).

Discovery Phase Study Population

Cases were selected from the Cardiac Arrest Blood Study Repository (CABS-R), a large population-based repository of data and specimens from adult out-of-hospital cardiac arrest patients who were attended by paramedics in Seattle and King county, Washington. SCA was defined as a sudden pulseless condition in apparently otherwise stable person in the absence of a non-cardiac cause of arrest. The records of 6003 persons identified by paramedics to be in cardiac arrest were reviewed and classified as definite, probable, possible, or non-SCA based on initial rhythm (e.g. ventricular fibrillation [VF] or asystole *vs.* pulseless electrical activity), circumstances (e.g. witnessed *vs.* un-witnessed) and possible contribution of comorbidities to the event. For the current analysis, we restricted our case population to those of European descent with a cardiac arrest classified as definite or probable SCA and with a presenting rhythm of ventricular fibrillation or asystole. We excluded nursing home residents to avoid misclassification as to the cause of death. We identified 2353 SCA cases between the years of 1988 and 2007 that met these criteria.

We identified population-based controls from the same geographic areas from three sources: 1. Controls (N = 809), previously identified as part of the Diet and Primary Cardiac Arrest Study, and individually matched to a subset of CABS-R cases without diagnosed heart disease prior to their SCA [7]. 2. Controls (N = 1774), randomly selected from controls in the Heart and Vascular Health Studies [8], a collection of case-control studies conducted at Group Health Cooperative, a large Health Maintenance Organization in Western Washington. 3. Because sources 1 and 2 did not include any subjects over the age of 80, we also recruited

446 controls specifically for this study from a random sample of Group Health enrollees aged 80 years and older. The combined controls were frequency-matched to cases on age and gender.

The Human Subject Review Committees of the University of Washington and Group Health Cooperative approved the study. All controls signed an informed consent form that included use of data and specimens for genetic studies. The use of repository data and samples from CABS-R for this study was authorized under a waiver of consent.

Blood collection

Paramedics obtained blood specimens from cases in the field after all emergency medical care had been provided and the patient was either clinically stable or deceased. Blood was collected in tubes containing EDTA and white blood cells were separated from plasma and red blood cells by centrifugation and stored at −80°C. DNA was extracted from thawed white blood cells using standard phenol extraction procedures. Blood samples for cases and controls were subjected to similar processing methods and identical DNA extraction methods.

Gene and SNP Selection

We included a total of 85 genes in fatty acid metabolic pathways (Table 1). For each gene, we identified SNPs that tagged common patterns of variation across the gene using information from the Genome Variation Server (GVS) (http://gvs.gs.washington.edu/GVS/index.jsp) and the International HapMap Project (http://hapmap.org). Data for common variants (minor allele frequency (MAF) ≥ 0.05) in European populations (GVS: PGA_CEPH; HapMap: CEU) for each gene and for 500kb on either side of the gene were downloaded. We used the Tagger pairwise algorithm for genes where data were obtained from HapMap [9] and the LDSelect algorithm for genes where data were obtained from GVS [10] to select tagSNPs ($r^2 \geq 0.80$). A list of genotyped SNPs with their regression results is shown in the online supplement (Supplementary Table 3).

Genotyping

Genotyping was performed at the University of California San Francisco in the laboratory of Dr Kwok (Department of Biopharmaceutical Sciences UCSF; San Francisco CA). Genotyping was done using BeadArray technology with a custom GoldenGate panel (Illumina, San Diego, CA). In addition, we supplemented the data from the Illumina panels with genotyping data obtained using Affymetrix Axiom panel. We genotyped 1608 SNPs in 85 genes involved in fatty acid metabolism. We also genotyped 93 SNPs identified as ancestry informative markers in the Multi-Ethnic Study of Atherosclerosis [11]. Samples from 4568 subjects were genotyped on the GoldenGate panels; of those, 4187 also had Axiom genotype data. An additional 816 samples had genotype data from the Axiom panel only. Exclusion criteria at the sample level were call rates <90%, sex mismatches or non-European by ancestry informative markers. Exclusion criteria at the SNP level were call rate <95%, out of Hardy-Weinberg equilibrium ($p<0.01$) or monomorphic. 1155 SNPs in 85 genes were included in this investigation. The investigation included up to 2160 cases with SCA and up to 2615 controls. Data on some of the genes, including *LPCAT1*, were available on 2005 cases and 2522 controls.

Statistical methods

Associations of genotype with SCA risk were assessed using logistic regression with robust or ‘sandwich’ standard errors to obtain odds ratios (OR) and their 95% confidence intervals. These regressions were adjusted for age category (≤40, 41–45, 46–50, 51–55, 56–60, 61–65,

66–70, 71–75, ≥76) and sex. A logistic linear model was used for all SNPs. The permutation-based p-min procedure and a Holm step-down procedure were used to adjust for multiple comparisons within a gene [12]. We performed sensitivity analyses adjusted for ancestry using eight principal components derived from ancestry informative markers to control for potential residual population stratification. Analyses were carried out using Stata 11.0 (StataCorp, College Station TX).

RESULTS

We examined the association of common SNPs in 85 candidate genes with the risk of incident SCA in a large population-based case control study among men and women of European ancestry. Mean age of the 2160 cases and 2615 controls was 67 years and 77% were men. Table 1 shows the list of genes that were examined.

After correction for multiple comparisons within each gene, we observed 8 SNPs, in 8 different genes, associated with SCA (Table 2). The genes included a transporter *SCL25A20*, the regulators of fatty acid oxidation *PRKAB1* and *MLYCD*, a gene involved in phospholipids biosynthesis, *LPCAT1*, a phospholipase A2 gene, *PLA2G4A*, two genes in leukotriene pathways, *ALOX5, ALOX5AP* and a receptor of prostaglandin E, *PTGER3*. The minor alleles at rs7737692 (in *LPCAT1*) and rs3780894 (in *ALOX5*) were associated with lower risk of SCA while the minor alleles at the other loci were associated with higher SCA risk (Table 2).

We investigated the association of the 8 SNPs with sudden cardiac death in nine other studies described in Supplemental Tables S1 and S2: The Atherosclerosis Risk in Communities Study (ARIC); the CARTAGENE Study (CARTAGENE); the Cardiovascular Health Study (CHS); the FinGesture Study (FinGesture), the Framingham Heart Study (FHS), the HARVARD Cohort SCD Study (HARVARD-SCD), The Helsinki Sudden Death Study (HSDS), Oregon Sudden Unexpected Death Study (Oregon-SUDS) and the Rotterdam Study (RS). In meta-analyses of the SNP associations in these studies, one SNP, rs7737692 (in *LPCAT1*) was nominally associated with sudden cardiac death (Table 3, Figure 1). The associations of the other 7 SNPs did not replicate (Table 3, Supplementary Figure 1). Figure 1 shows the association of rs7737692 in the individual studies. Overall, each copy of the minor allele was associated with an 8.9% lower risk of SCA (−17.1% to −0.7%; p-value=0.031) in the replication studies. The variant allele frequency in combined cases and non-cases ranged from 32.5% (HSDS) to 38.0% (CHS). In a combined meta-analysis of the 2129 cases and 23833 non-cases in both replication studies and the discovery study, each copy of the minor allele was associated with an overall 11.4% lower risk of SCA (−17.4% to −5.4%).

In a sensitivity analysis in the discovery study where heart rhythm shortly after the cardiac arrest event is available from paramedic incident reports, restriction of the SCA case group to cases with documented VF did not change the results for rs7737692; the log relative risk (standard error) for each copy of the minor allele was −0.155 (0.051) with restriction to cases in VF, and -0.143 (0.045) with all the cases.

DISCUSSION

In this large study of SCA in the community, we initially found an association of incident SCA with genetic variation in 8 genes in fatty acid metabolic pathways. One of these associations was nominally significant in a meta-analysis of results from nine GWAS of SCA: a common variant with 36% minor allele frequency in the discovery cohort,

rs7737692, located near the gene *LPCAT1*, was nominally associated with lower risk of incident SCA.

The protein coded by *LPCAT1* (lpcat1) is an enzyme that transfers a fatty acid in the form of acyl-CoA to lyso-phosphatidyl choline in order to reconstitute phosphatidyl choline (PC). The process of de-acylation of PC by phospholipase 2 followed by re-acylation by a lyso-PC acyl transferase, known as the Land's cycle [13], effectively replaces one fatty acid with another. It has been proposed that lpcat1 participates in the Land's cycle in erythrocytes where it can replace unsaturated fatty acyl chains damaged by free radicals [14], and in alveolar cells where it appears needed for surfactant production [15]. In addition to these specialized roles, lpcat1 was recently found in the surface layer of organelles called "lipid droplets" where lpcat1 biosynthesizes PC via the Land's cycle [16]. Lipid droplets are ubiquitous cellular organelles that store lipids such as triacylglycerol [17]. These lipid droplets are present in the heart where they appear to protect the heart from oxidative damage. Hearts from mutant mice specifically devoid of heart lipid droplets oxidize fatty acids more actively, produce more reactive oxygen species (ROS) and show greater decline in contractile function with age than wild type mice [18]. The acyl-CoA synthetase *ACSL3* is suggested to provide the fatty acid needed for PC synthesis in the lipid droplets [19].

Interestingly, we recently reported an association of *ACSL3* with a greater likelihood of survival following SCA [20]. Further studies are needed to explore how lpcat1-mediated PC synthesis and remodeling in lipid droplets might influence the risk of incident SCA.

We broadly selected genes in fatty acid metabolic pathways for this investigation of genetic association with incident SCA. The gene selection was guided in part from the observation of associations of membrane and circulating fatty acids, long-chain n3 fatty acids [7, 21] as well as fatty acid biomarkers of de novo lipogenesis [22], with risk of incident SCA. These observations prompted us to select genes in several pathways, including the metabolic conversion of essential fatty acids into longer chain polyunsaturated fatty acids, fatty acid synthesis and control of fatty acid synthesis, and genes potentially involved in the remodeling of membrane fatty acids (the Land's cycle mentioned above). Additionally, polyunsaturated fatty acids are a source of eicosanoids, and cellular and animal experiments suggest eicosanoids may influence fibrillation [23–25], ion channel function [26, 27], vessel wall inflammation [28] and subclinical disease [29], processes that may influence the risk of SCA. For these reasons, we investigated genes involved in prostaglandin and leukotriene synthesis. Finally, fatty acids are a major source of energy for the heart [30]. A shift in substrate preference occurs in diseases that increase the risk of SCA, including cardiac hypertrophy [31] and uncontrolled diabetes [32]; and accumulation of intermediates of fatty acid oxidation influence arrhythmogenesis in isolated cardiac tissues and cardiac myocytes [33, 34]. For these reasons, we examined genes that may be involved in the uptake and transport of fatty acids in the heart, fatty acid beta-oxidation in mitochondria and the control of beta-oxidation. None of the genes we investigated were shown to be associated with SCA after Bonferroni correction for multiple comparisons. Whereas demonstrated associations might point to the involvement of a gene product or pathway, lack of strong evidence for genetic association is admittedly less informative. Other approaches, such as case-control comparison of gene expression or directly measured metabolites, will be needed to further explore the potential role of these pathways in the pathophysiology of SCA.

We used tag SNPs to cover common genetic variation in each gene and the nominal association we observed with SCA risk may be due to another SNP in linkage disequilibrium with the associated SNP. We cannot comment on association with less common or rare SNPs (1–5% and <1% minor allele frequency, respectively). The nominal significance of one out of 8 associations we tested could be a chance finding. Whether

LPCAT1 genetic variation affects lpcat1 biological activity is not known; however in a meta-analysis of eQTLs (expression quantitative trait loci) in 5311 blood samples [35], rs7737692 was associated with expression levels of *LPCAT1* ($p = 7\times10^{-6}$). The study was restricted to study subjects of European descent and results may not be generalizable to other ethnicities. Study strengths include the population-based design, the hypothesis-directed investigation, the large number of SCA cases, and replication of the findings in a consortium of nine SCA studies.

In summary, we report a genetic variant near *LPCAT1* nominally associated with incident SCA risk. Further studies are needed to explore possible effect of this genetic variation on lpcat1-mediated PC synthesis and remodeling of lipid droplets in the heart.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding sources: The Sudden Cardiac Blood Repository Study was financed by the National Lung, Heart, and Blood Institute, grants RO1-HL092144, RO1-HL092111, and RO1-HL088456. Funding sources for the other contributing studies are listed in the Supplementary Information.

Abbreviations

SCA	sudden cardiac arrest
GWAS	genome-wide association studies
SNPs	Single nucleotide polymorphisms
CABS-R	Cardiac Arrest Blood Study Repository
OR	odds ratio
PC	phosphatidyl choline

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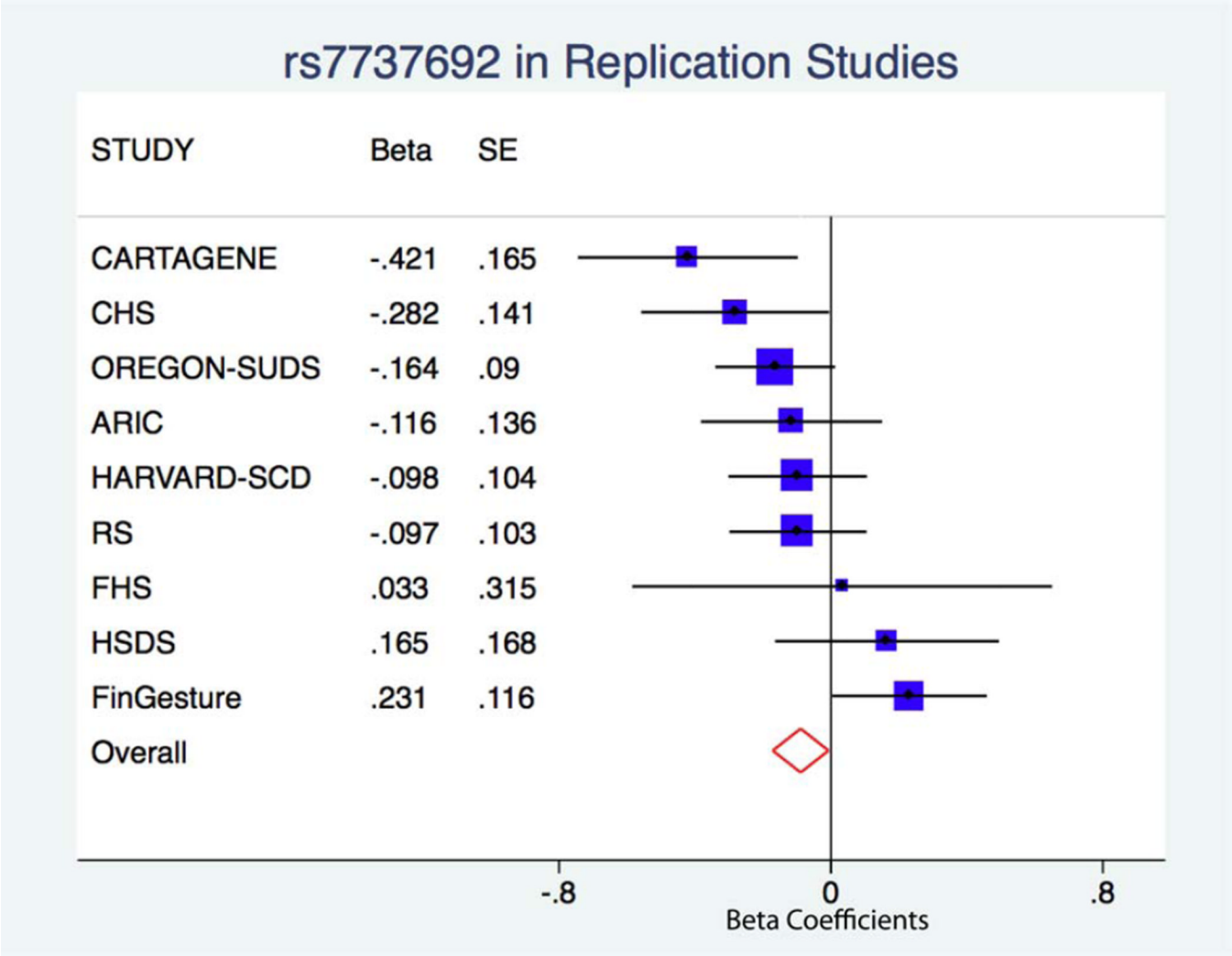

Figure 1.

Forest plot depicting the log relative risk (beta coefficient) of rs773762 per allele (95% confidence interval) on risk of sudden cardiac arrest across the individual replication studies and overall using inverse variance modeling.

CARTAGENE = Cardiac arrest and gene study; CHS = Cardiovascular Health Study; OREGON-SUDS= Oregon Sudden Unexpected Death Study; ARIC = Atherosclerosis Risk in Communities Study; HARVARD-SCD = Harvard Cohort Sudden Cardiac Death Study; RS = Rotterdam Study; FHS = Framingham Offspring Study and Framingham Third Generation Study; HSDS = Helsinki Sudden Death Study; FinGesture = FinGesture Study.

Table 1

Genes examined for their association with SCA

Fatty acid synthesis
ACSL1 (Acyl-CoA synthetase, long chain)
ACSL3 (Acyl-CoA synthetase, long chain)
ACSL4 (Acyl-CoA synthetase, long chain)
ACSL5 (Acyl-CoA synthetase, long chain)
ACSS1 (Acyl-CoA synthetase, short chain)
ACSS2 (Acyl-CoA synthetase, short chain)
FASN (Fatty acid synthase)
FADS3 (Delta-9 desaturase)
SCD5 (Delta-9 desaturase)
SREBF1 (Sterol regulatory element binding protein 1)
SREBF2 (Sterol regulatory element binding protein 2)
SCAP (SREBP cleavage activating protein)
INSIG1 (Insulin induced gene 1)
FADS1 (Delta-5 desaturase)
FADS2 (Delta-6 desaturase)
ELOVL5 (Long-chain fatty-acyl elongase, member 5)
ELOVL6 (Long-chain fatty-acyl elongase, member 6)
ELOV2 (Elongation of very long chain fatty acids)
ELOV4 (Elongation of very long chain fatty acids)
Fatty acid uptake
CD36 (Fatty acid translocase)
SLC27A1 (Long-chain FA transporter)
SLC27A3 (Long-chain FA transporter)
SLC27A4 (Long-chain FA transporter)
SLC27A6 (Long-chain FA transporter)
FABP3 (Fatty acid binding protein)
Acylation-reacylation of cell membranes
LPGAT1 (Lysophosphatidylglycerol acyltransferase)
AGPAT3 (Lysophosphatidylglycerol acyltransferase)
AGPAT4 (Lysophosphatidylglycerol acyltransferase)
LPCAT1 (Lysophosphaditylcholine acyltransferase)
PNPLA8 (Calcium-independent phospholipase A2 γ)
PLA2G4C (Cytosolic phospholipase A2 γ)
Fatty acid oxidation and control of oxidation
LPL (Lipoprotein lipase)
MLYCD (Malonyl-CoA decarboxylase)
ACACA (Acetyl-CoA carboxylase, subunit A)
ACACB (Acetyl-CoA carboxylase, subunit B)
ACADVL (Acetyl-CoA decarboxylase, very long chain)

CPT1B (Carnitine palmitoyltransferase 1)

CPT2 (Carnitine palmitoyltransferase 2)

SLC25A20 (Carnitine acylcarnitine translocase)

ACADM (Acyl-CoA dehydrogenase, medium chain)

HADHA (Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha)

HADHB (Hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, beta)

HADH (Hydroxyacyl-CoA dehydrogenase)

ECHS1 (Enoyl CoA hydratase, short chain 1)

ACAT1 (Acetyl-CoA acetyl transferase)

DCI (Dodecenoyl-CoA delta isomerase)

DECR1 (Dienoyl-CoA reductase 1)

PRKAA2 (AMP-activated protein kinase α2 subunit)

PRKAG3 (AMP-activated protein kinase γ3 subunit)

PRKAB1 (AMP-activated protein kinase ß1 subunit)

PRKAG1 (AMP-activated protein kinase γ1 subunit)

PRKAB2 (AMP-activated protein kinase ß2 subunit)

Polyunsaturated fatty acid release and eicosanoid synthesis

PLA2G4A (Phospholipase A2, IVA)

PLA2G2A (Phospholipase A2, IIA)

PLA2G12A (Phospholipase A2, XIIA)

PLA2G12B (Phospholipase A2, XIIB)

PLA2G5 (Phospholipase A2, V)

PLA2G7 (Lipoprotein-associated phospholipase A2)

PTGS1 (Cyclooxygenase 1)

PTGS2 (Cyclooxygenase 2)

CBR1 (PGE 9-reductase)

DHRS4 (Dehydrogenase/reductase, SDR family)

PTGER3 (Prostaglandin E receptor 3)

PTGDS (Prostaglandin D synthase)

PTGES (Prostaglandin E synthase)

PTGES2 (Prostaglandin E synthase 2)

PTGIS (Prostaglandin I synthase)

TBXAS1 (Thromboxane synthase I)

AKR1C3 (Prostaglandin F synthase)

PTGFRN (Prostaglandin F2 receptor negative regulator)

HPGD (Hydroxy prostaglandin dehydrogenase)

ALOX5 (Arachidonate 5-lipoxygenase)

ALOX5AP (Arachidonate 5-lipoxygenase activating protein)

GPX3 (Glutathione peroxidase 3)

GPX7 (Glutathione peroxidase 7)

LTA4H (Leukotriene A4 hydrolase)

EPHX2 (Epoxide hydrolase 2)

LTC4S (Leukotriene-C4 synthase)

GGT1 (Gamma-glutamyl transferase 1)

GGT7 (Gamma-glutamyl transferase-like 3)

GGT5 (Gamma-glutamyl transferase-like activity 1)

ALOX12 (Arachidonate 12-lipoxygenase)

ALOX15 (Arachidonate 15-lipoxygenase)

CYP2J2 (Arachidonic acid epoxygenase)

Table 2

SNPs associated with risk of incident SCA[†]

SNP ID	Gene (# of SNPs)	Allele/ Alternate	MAF	Beta Coefficient	SE	p-value
rs7623023	*SLC25A20* (3)	G/A	0.34	0.1198	0.0439	0.0064
rs4213	*PRKAB1* (2)	G/T	0.31	0.1089	0.0463	0.0188
rs8060065	*MLYCD* (6)	C/G	0.05	0.2259	0.0919	0.0139
rs7737692	*LPCAT1* (28)	G/A	0.36	−0.1425	0.0448	0.0015
rs4402086	*PLA2G4A* (51)	G/A	0.26	0.1550	0.0458	0.0007
rs3780894	*ALOX5* (28)	G/A	0.16	−0.1980	0.0607	0.0011
rs4769058	*ALOX5AP* (16)	C/T	0.04	0.2914	0.1026	0.0045
rs6685546	*PTGER3* (37)	C/T	0.14	0.2221	0.0608	0.0003

[†]Results for 8 SNPs that met the threshold for within-gene multiple comparison, from analyses in 2003 CABS-R cases and 2518 controls

Table 3

Associations of 8 SNPs selected for replication with incident SCA in the replication studies [†]

SNP ID	Gene	Allele/ alternate	MAF	Beta coefficient	SE	p-value
rs7623023	*SLC25A20*	G/A	0.35	0.0439	0.0406	0.28
rs4213	*PRKAB1*	G/T	0.31	−0.0337	0.0419	0.42
rs8060065	*MLYCD*	C/G	0.06	−0.1243	0.0921	0.18
rs7737692	*LPCAT1*	G/A	0.36	−0.0905	0.0420	0.03
rs4402086	*PLA2G4A*	G/A	0.28	−0.0325	0.0438	0.46
rs3780894	*ALOX5*	G/A	0.16	0.0735	0.0523	0.16
rs4769058	*ALOX5AP*	C/T	0.04	−0.092	0.1060	0.38
rs6685546	*PTGER3*	C/T	0.18	−0.0148	0.0531	0.78

[†]Meta-analysis results