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Wild, P. S., Zeller, T., Schillert, A., Kee, F., Evans, A., & Al, E. (2011). A Genome-wide association study identifies LIPA as a susceptibility gene for coronary artery disease. Circ Cardiovasc Genet, 4(4), 403-412. DOI: 10.1161/CIRCGENETICS.110.958728

Published in:
Circ Cardiovasc Genet

A Genome-Wide Association Study Identifies *LIPA* as a Susceptibility Gene for Coronary Artery Disease

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Background—eQTL analyses are important to improve the understanding of genetic association results. We performed a genome-wide association and global gene expression study to identify functionally relevant variants affecting the risk of coronary artery disease (CAD).

Methods and Results—In a genome-wide association analysis of 2078 CAD cases and 2953 control subjects, we identified 950 single-nucleotide polymorphisms (SNPs) that were associated with CAD at $P<10^{-3}$. Subsequent in silico and wet-laboratory replication stages and a final meta-analysis of 21 428 CAD cases and 38 361 control subjects revealed a novel association signal at chromosome 10q23.31 within the *LIPA* (lysosomal acid lipase A) gene ($P=3.7\times10^{-8}$; odds ratio, 1.1; 95% confidence interval, 1.07 to 1.14). The association of this locus with global gene expression was assessed by genome-wide expression analyses in the monocyte transcriptome of 1494 individuals. The results showed a strong association of this locus with expression of the *LIPA* transcript ($P=1.3\times10^{-96}$). An assessment of *LIPA* SNPs and transcript with cardiovascular phenotypes revealed an association of *LIPA* transcript levels with impaired endothelial function ($P=4.4\times10^{-3}$).

Conclusions—The use of data on genetic variants and the addition of data on global monocytic gene expression led to the identification of the novel functional CAD susceptibility locus *LIPA,* located on chromosome 10q23.31. The respective eSNPs associated with CAD strongly affect *LIPA* gene expression level, which was related to endothelial dysfunction, a precursor of CAD. **(*Circ Cardiovasc Genet*. 2011;4:403-412.)**

Key Words: coronary artery disease ■ genome-wide association studies ■ gene expression ■ genetic variation ■ genomics ■ eQTL ■ eSNP ■ LIPA

Received October 4, 2010; accepted April 26, 2011.

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The online-only Data Supplement is available at http://circgenetics.ahajournals.org/cgi/content/full/CIRCGENETICS.110.958728/DC1.

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Coronary artery disease (CAD) remains one of the major causes of death. Recent data indicate that classic risk factors and novel risk markers account for a large proportion of disease risk.[1,2] Despite these considerable advances, it remains apparent that the underlying causes of CAD are multifactorial and involve a complex interplay between acquired and inherited risk factors. The advent of genome-wide association (GWA) studies led to the identification of several genetic loci that associate with the risk of CAD.[3–7] The majority of these associations are located in genomic regions for which functional understanding is lacking.[8] Consequently, there exists a substantial gap in our understanding about how these single-nucleotide polymorphisms (SNPs) affect the pathophysiological mechanisms through which the loci contribute to disease. Variation in gene expression appears to be an important intermediate step underlying susceptibility of complex diseases.[9–15] The abundance of a gene transcript can be directly modified by polymorphisms; thus, transcript abundance mediated by genetic variation either alone or in combination with environmental factors might be considered as a quantitative trait that can be mapped.[15] When combined with GWA data, the analysis of the transcriptome can help to clarify and categorize effects of CAD-associated SNPs on gene expression (eSNPs).

Clinical Perspective on p 412

In the present study, a GWA case-control study in 5031 individuals followed by 2 stages of replication and a final meta-analysis of 59 789 cases and control subjects was performed. This approach led to the identification of a novel CAD susceptibility locus on chromosome 10q23.31, *LIPA*. Additionally, eQTL analysis, using a data set of global monocytic gene expression, revealed a strong effect of *LIPA* eSNPs on *LIPA* transcript levels, and *LIPA* transcript levels in turn showed association to prevalent cardiovascular risk factors and phenotypes of subclinical disease.

Methods

Study Design

A GWA study using the Genome-Wide Human SNP 6.0 Array (Affymetrix, Santa Clara, CA) was conducted to discover SNPs associated with CAD in the CADomics study (*C*oronary *A*rtery *D*isease and Gen*omics*), a case-control study of CAD (2078 CAD cases and 2953 control subjects). Replication of SNPs was performed in 2 steps. SNPs associated with CAD in the discovery stage at a threshold probability value of $<10^{-3}$, entered the first replication stage (in silico replication in 9487 cases and 30 171 control subjects of the following studies with European ancestry: CHARGE, GerMIFS I, GerMIFS II, MIGen, WTCCC-CAD, PennCATH, and MedStar). On the basis of a threshold probability value of $<10^{-4}$ in the pooled analysis of the discovery and the first replication stage, SNPs were selected for the second replication stage (wet laboratory replication in 9863 cases and 5237 control subjects of the following studies with European ancestry: ECTIM, AngioLueb, GoKard, LURIC, popgen, and MORGAM). A final meta-analysis was performed in 21 428 cases and 38 361 control subjects. SNPs passing a conservative threshold of statistical significance at $P<5\times10^{-8}$ in the final meta-analysis were further evaluated for their association to global gene expression in 1494 apparently healthy, population-based samples from the Gutenberg Heart Express (GHSExpress) study for identifying SNPs (eSNPs) that affect gene expression (eQTL transcripts). Finally, we explored eSNPs and respective eQTL transcripts for their association with cardiovascular risk factors and phenotypes of subclinical disease. The study design is depicted in Figure 1.

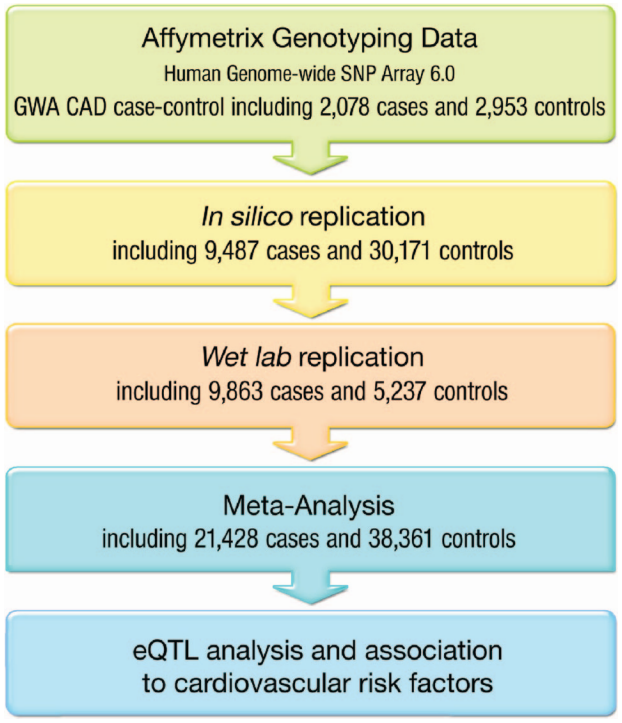

Figure 1. Study design of the CADomics Study. The study consisted of a discovery GWA stage, followed by 2 stages of replication (in silico and wet laboratory) in independent study samples and a final meta-analysis. SNPs with genome-wide significance ($P<5\times10^{-8}$) were further explored for their association with global gene expression (eSNPs, eQTLs) in monocytes and cardiovascular risk factors. Statistical evidence for association was combined across several stages, using a final meta-analysis.

Description of Study Samples

CADomics is a case-control study including the hospital-based catheter laboratory Athero*Gene* Registry[16] and the population-based Gutenberg-Heart Study (GHS). For the present analysis, individuals with angiographically proven CAD (stenosis >50% in 1 major coronary artery), nearly 60% presenting with acute myocardial infarction, were included as cases, and individuals without a history of myocardial infarction and/or history of CAD were taken from the population-based cohort as control subjects. The GHSExpress study is a subsample of GHS participants—who served as control subjects in the CADomics study—from which RNA was directly extracted from monocytes isolated from fresh blood samples. Characteristics of the CADomics and the GHSExpress study samples are provided in Table 1 and Online-only Data Supplement Table I. Further detailed description of the studies is provided in the Online-only Data Supplement Materials. Descriptions of the studies used for replication stages are provided in the Online-only Data Supplement Materials and Online-only Data Supplement Table II.

Genotyping

For CADomics, genomic DNA was isolated from buffy coats of EDTA plasma samples as described elsewhere.[17] Genotyping was conducted on the Affymetrix Genome-Wide Human SNP 6.0 Array; quality control on sample and SNP level was performed according to standardized criteria.[18] Genotyping was performed in individuals of European descent only. A detailed description of genotyping methods and quality control is provided in the Online-only Data Supplement Materials. In total, 5031 samples and 608 247 SNPs were included in the analyses. Online-only Data Supplement Table III

Table 1. Characteristics of the CADomics Study

	CADomics	
	Cases (n=2078)	Control Subjects (n=2953)
Study design		
Ascertainment scheme	Hospital-based	Population-based
Ethnicity	Caucasian	Caucasian
Country of origin	Germany	Germany
Age range, y	26–84	35–74
Age, y	60.8±10.1	55.3±10.8
Female sex, n (%)	456 (21.9)	1491 (50.5)
Myocardial infarction n (%)	1212 (58.3)	0
Cardiovascular risk factors		
Diabetes mellitus, n (%)	436 (21.0)	180 (6.1)
Dyslipidemia, n (%)	1353 (65.1)	792 (26.8)
Family history of myocardial infarction, n (%)	773 (37.2)	513 (17.4)
Hypertension, n (%)	1491 (71.8)	1506 (51.0)
Obesity, n (%)	528 (25.4)	661 (22.4)
Smoking		
Never, n (%)	752 (36.2)	1392 (47.2)
Ex-smoker, n (%)	722 (34.8)	1008 (34.2)
Smoker, n (%)	603 (29.0)	550 (18.6)
Body mass index, kg/m^2	27.8±4.0	27.0±4.7
Total cholesterol, mg/dL	209±47	226±41
LDL-cholesterol, mg/dL	133±41	144±35
HDL-cholesterol, mg/dL	48±14	58±16
Triglycerides, mg/dl	161±100	123±71
RR systolic, mm Hg	132±24	134±18
RR diastolic, mm Hg	73±13	84±10

Data presented are the absolute and relative frequency of patients for categorical and mean±SD for continuous traits.

provides information on genotyping platforms and methods used for all replication studies.

Global Gene Expression

Isolation of total RNA and analysis of gene expression were performed as recently described.[15] In brief, total RNA was isolated from monocytes of 1606 participants of the GHSExpress Study and hybridized to Illumina HT-12 v3 BeadChips (Illumina Inc, San Diego, CA). Arrays were quantile-normalized and transformed using the arcsinh function. After quality control, 14 027 expressed RefSeq transcripts in 1494 samples were used for eQTL analyses. Detailed description of the methods is given in the Online-only Data Supplement Materials.

Cardiovascular Risk Factors and Phenotypes of Subclinical Disease

eQTL transcripts and eSNPs were investigated for associations with prevalent cardiovascular risk factors (LDL- and HDL-cholesterol, triglycerides, diabetes mellitus, HbA_{1c}, and systolic and diastolic blood pressure) and phenotypes of subclinical disease (flow-mediated vasodilation and carotid macroangiopathy). Methods of risk factor measurements and descriptions of phenotype assessment are described in the Online-only Data Supplement Materials.

Statistical Methods

In the discovery GWA analysis, association of CAD with SNPs was tested with the use of an additive genetic model in a logistic regression. In both replication steps (in silico and wet laboratory replication), fixed-effects meta-analysis using inverse-variance weighting was performed with the R package MetABEL.[19]

Associations between SNPs and transcripts were investigated with the use of the median test,[20] with a significance level of probability $<10^{-8}$, corresponding to a probability value of $<10^{-12}$ in an ANOVA[20] for the samples that passed quality control for both genotype and expression data. SNPs located within 500 kb of either the 5′ or 3′ end of the associated gene were considered as *cis*-acting SNPs; otherwise, they were called to act in *trans*. Only associations of transcripts without SNPs in probe sequences are reported.[21]

Associations of eSNPs and eQTL transcripts with cardiovascular risk factors were analyzed using logistic and linear regression for qualitative and quantitative traits, respectively. Triglycerides and HbA_{1c} were log-transformed before analysis.

Probability values were corrected for multiple testing with the use of false discovery rate[22] and a significance level of 0.05.

All analyses were performed using R, version 2.10.1 (http://www.r-project.org).

Results

Discovery GWA Study, Replication, and Final Meta-Analysis

The discovery GWAS revealed 950 SNPs that were associated with CAD at a level of $P<10^{-3}$ in the 2078 CAD cases and 2953 population-based control subjects of the CADomics study. The strongest association was observed for the previously described region at 9p21.3 (lead SNP rs1333049: $P=4.28\times10^{-7}$; odds ratio [OR], 1.22; 95% confidence interval [CI], 1.12 to 1.32). Detailed results of all associated SNPs are provided in Online-only Data Supplement Table IV.

All 950 SNPs were selected for in silico replication in 7 independent case-control studies (9487 cases and 30 171 control subjects). Only SNPs with $P<10^{-4}$ in the pooled analysis of CADomics and the in silico replication studies were selected for wet laboratory replication (Online-only Data Supplement Table IV). For loci with several CAD-associated SNPs, tagSNPs were selected for replication. A total of 20 SNPs was genotyped in 6 additional replication studies including 9863 cases and 5237 control subjects. Results of the discovery GWA study, both replication stages and the subsequent meta-analysis finally including 21 428 cases and 38 361 control subjects, are presented in Table 2.

As expected, the chromosome 9p21.3 locus revealed the strongest association with CAD in the meta-analysis of all 14 studies included (lead SNP rs1333049: $P=7.12\times10^{-58}$; OR, 1.27; 95% CI, 1.23 to 1.31; Online-only Data Supplement Figure I). A locus on chromosome 10q23.31, so far not known to be associated with CAD, also reached genome-wide significance in the meta-analysis (Figure 2A; rs1412444: $P=3.71\times10^{-8}$; OR, 1.1; 95% CI, 1.07 to 1.14; rs2246833: $P=4.35\times10^{-8}$; OR, 1.1; 95% CI, 1.06 to 1.14).

Identification of eSNPs and eQTL Transcripts

All SNPs that reached genome-wide significance (Table 2) were further tested for association with monocytic transcripts in *cis* (SNPs located within 500 kb of either the 5′ or 3′ end of the associated gene) and *trans*. SNPs rs1412444 and rs2246833, located on chromosome 10q23.31 in intronic regions of the *LIPA* (lysosomal acid lipase A) gene, showed a strong association with expression of the *LIPA* transcript itself ($P=1.3\times10^{-96}$ and $P=4.0\times10^{-96}$, respectively; Figure

Table 2. Results of Discovery GWA, Replication Stages, and Final Meta-Analysis

SNP	Chr	Position, bp	Gene	Risk Allele	Non–Risk Allele	Risk Frequency	GWA		Replication Step 1		Replication Step 2		Meta-Analysis	
							P Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)
rs1333049*	9	22115503	Intergenic	C	G	0.4858	$4.28*10^{-7}$	1.22 (1.12–1.32)	$1.80*10^{-44}$	1.30 (1.25–1.35)	$8.12*10^{-16}$	1.22 (1.17–1.29)	$7.12*10^{-58}$	1.27 (1.23–1.31)
rs7865618*	9	22021005	MTAP	A	G	0.5854	$6.25*10^{-5}$	1.18 (1.09–1.28)	$3.94*10^{-25}$	1.22 (1.17–1.26)	$1.69*10^{-5}$	1.11 (1.06–1.17)	$1.72*10^{-27}$	1.18 (1.14–1.21)
rs7044859*	9	22008781	MTAP	A	T	0.4586	$2.03*10^{-5}$	1.17 (1.08–1.27)	$7.02*10^{-24}$	1.21 (1.16–1.25)	$1.99*10^{-5}$	1.13 (1.07–1.20)	$3.93*10^{-27}$	1.18 (1.15–1.22)
rs1412444*	10	90992907	LIPA	T	C	0.3245	$6.29*10^{-4}$	1.13 (1.04–1.23)	$4.12*10^{-5}$	1.11 (1.05–1.16)	$2.39*10^{-4}$	1.10 (1.05–1.16)	$3.71*10^{-08}$	1.10 (1.07–1.14)
rs2246833*	10	90995834	LIPA	T	C	0.3270	$6.78*10^{-4}$	1.13 (1.04–1.23)	$2.24*10^{-5}$	1.10 (1.05–1.15)	$5.26*10^{-4}$	1.10 (1.04–1.15)	$4.35*10^{-08}$	1.10 (1.06–1.14)
rs365302	6	159566321	FNDC1	C	T	0.2393	$3.72*10^{-4}$	1.17 (1.07–1.29)	$3.24*10^{-5}$	1.11 (1.06–1.16)	$8.11*10^{-3}$	1.11 (1.03–1.20)	$8.37*10^{-7}$	1.11 (1.06–1.15)
rs16893526	6	82572034	Intergenic	G	A	0.9123	$8.71*10^{-4}$	1.26 (1.09–1.46)	$9.21*10^{-5}$	1.14 (1.07–1.22)	$1.60*10^{-2}$	1.12 (1.02–1.22)	$4.69*10^{-6}$	1.13 (1.07–1.21)
rs294917	6	159547065	FNDC1	T	C	0.2359	$7.56*10^{-4}$	1.17 (1.07–1.29)	$4.58*10^{-5}$	1.11 (1.05–1.16)	$5.06*10^{-2}$	1.06 (1.00–1.12)	$1.21*10^{-5}$	1.09 (1.05–1.13)
rs7848524	9	21691432	AL355679.20. RP11–47303.1	T	C	0.4793	$7.25*10^{-5}$	1.18 (1.09–1.28)	$6.97*10^{-5}$	1.08 (1.04–1.12)	$8.64*10^{-2}$	1.05 (0.99–1.10)	$2.36*10^{-5}$	1.07 (1.04–1.10)
rs2782552	6	159563684	FNDC1	A	C	0.2393	$2.25*10^{-4}$	1.18 (1.07–1.29)	$3.25*10^{-5}$	1.11 (1.06–1.16)	$1.68*10^{-1}$	1.04 (0.98–1.10)	$4.63*10^{-5}$	1.08 (1.04–1.12)
rs6682490	1	88597878	Intergenic	A	T	0.1630	$6.25*10^{-4}$	1.21 (1.09–1.35)	$7.20*10^{-5}$	1.17 (1.08–1.26)	$1.20*10^{-1}$	1.05 (0.99–1.12)	$1.68*10^{-4}$	1.10 (1.05–1.15)
rs16893523	6	82560898	Intergenic	G	A	0.9113	$2.99*10^{-4}$	1.31 (1.13–1.51)	$1.54*10^{-5}$	1.15 (1.08–1.23)	$5.55*10^{-1}$	0.97 (0.87–1.08)	$7.28*10^{-4}$	1.10 (1.04–1.16)
rs17412370	11	80404012	Intergenic	T	G	0.7834	$1.18*10^{-4}$	1.22 (1.10–1.34)	$9.36*10^{-5}$	1.11 (1.05–1.17)	$5.13*10^{-1}$	0.98 (0.93–1.04)	$1.13*10^{-2}$	1.05 (1.01–1.09)
rs4849561	2	117990472	Intergenic	C	T	0.8517	$4.35*10^{-4}$	1.22 (1.09–1.37)	$8.27*10^{-5}$	1.13 (1.06–1.20)	$4.04*10^{-1}$	0.97 (0.90–1.04)	$1.58*10^{-2}$	1.06 (1.01–1.11)
rs13197670	6	82626603	Intergenic	G	C	0.9223	$1.98*10^{-4}$	1.31 (1.12–1.52)	$1.54*10^{-6}$	1.19 (1.11–1.27)	$3.05*10^{-3}$	1.16 (1.05–1.27)	$3.48*10^{-2}$	1.06 (1.00–1.12)
rs1421521	18	60236486	Intergenic	G	A	0.6498	$4.29*10^{-4}$	1.17 (1.07–1.27)	$9.03*10^{-5}$	1.08 (1.04–1.13)	$6.53*10^{-2}$	0.95 (0.91–1.00)	$5.58*10^{-2}$	1.03 (1.00–1.06)
rs1348330	4	171789432	Intergenic	C	T	0.3376	$2.79*10^{-4}$	1.17 (1.08–1.27)	$6.44*10^{-6}$	1.09 (1.05–1.13)	$9.22*10^{-4}$	0.92 (0.87–0.96)	$8.78*10^{-2}$	1.03 (1.00–1.06)
rs11143677	9	75525136	Intergenic	A	G	0.5443	$1.80*10^{-4}$	1.16 (1.07–1.26)	$4.74*10^{-6}$	1.14 (1.08–1.20)	$4.11*10^{-2}$	0.94 (0.90–1.00)	$1.07*10^{-1}$	1.03 (0.99–1.07)
rs368771	4	171750312	HSP90AA6P	C	A	0.3343	$5.07*10^{-4}$	1.16 (1.07–1.27)	$9.11*10^{-6}$	1.09 (1.05–1.13)	$4.76*10^{-4}$	0.91 (0.86–0.96)	$1.37*10^{-1}$	1.02 (0.99–1.06)
rs4692845	4	171771856	Intergenic	A	G	0.3363	$5.15*10^{-4}$	1.16 (1.07–1.27)	$6.57*10^{-6}$	1.09 (1.05–1.13)	$1.11*10^{-4}$	0.90 (0.85–0.95))	$1.41*10^{-1}$	1.02 (0.99–1.06)

Discovery GWA was performed in 2078 CAD cases and 2953 control subjects. In silico replication was performed in 9487 cases and 30 171 control subjects of the following studies: CHARGE, GerMIFS I, GerMIFS II, MIGen, WTCCC-CAD, PennCATH, and MedStar. Wet lab replication was performed in 9863 cases and 5237 control subjects of the following studies: ECTIM, AngioLueb, GoKard, LURIC, popgen, and MORGAM. Final meta-analysis included 21 428 CAD cases and 38 361 control subjects.

*Indicates results with genome-wide significance.

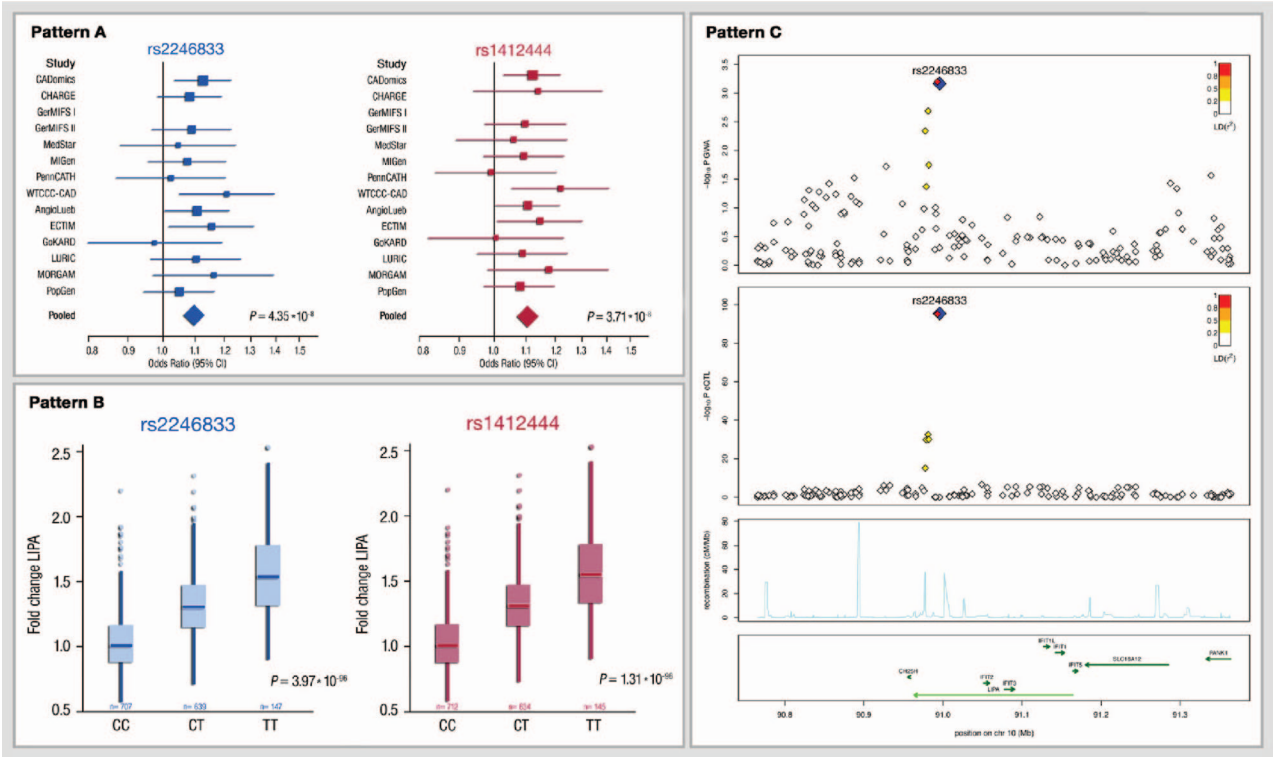

Figure 2. Identification of the CAD-related locus *LIPA* on chromosome 10q23.31. **A**, Forest plots for rs2246833 and rs1412444. Meta-analysis of the association of rs2246833 and rs1412444 with CAD was performed in a case-control design including 14 independent cohorts of European ancestry with n=59 789. Individual studies are plotted against the individual ORs. Horizontal lines are the CIs corresponding to the probability value threshold of 5×10^{-8}. Vertical line indicates that the value is consistent with no association. If an SNP was not available in a study, there is no data point for that study. Diamond represents the meta-analytic effect size. For reasons of quality control, after imputation no data are available for GerMIFS I. **B**, Association of the eSNPs rs2246833 and rs1412444 with *LIPA* gene expression. Box plots are shown for the fold change of *LIPA* expression in relation to the genotype. Fold change of *LIPA* expression was calculated relative to median expression of the nonrisk allele genotype (**C**). **C**, Locus-specific regional association plots for discovery GWA and eQTL analysis results on chromosome 10q23.31 (*LIPA*). The figure shows from top to bottom: (i) log10(P) of the association between SNPs and case and control status (primary GWA); (ii) log10(P) of the association between SNPs and *LIPA* expression (eQTL transcript); and (iii) recombination fraction based on HapMap and positions of genes. SNP rs2246833, with the smallest eQTL P is represented by a blue diamond. Other SNPs are color-coded according to pairwise LD (r^2) with this SNP. (see legend in figure). Note that SNP rs1412444 is colored in red (r^2=0.985).

2B and Table 3). Both *LIPA* SNPs were in strong linkage disequilibrium (r^2=0.985), and for both SNPs, the CAD risk allele (T) was associated with higher *LIPA* expression. Figure 2C displays regional plots for the association of *LIPA* eSNPs and eQTL transcripts in relation to CAD. A "platform validation" was conducted in 119 monocytic samples, using quantitative reverse-transcriptase–polymerase chain reaction analyses, and the association of *LIPA* SNPs with *LIPA* transcripts was successfully replicated (rs1412444: $P=3.87\times10^{-8}$, rs2246833: $P=1.52\times10^{-8}$; see also Online-only Data Supplement Figure II).

The CAD-associated SNPs in the 9p21.3 region, rs1333049, rs7865618, and rs7044859, showed no association to global monocytic gene expression.

Association of LIPA eSNPs and eQTL Transcripts With Cardiovascular Risk Factors and Phenotypes of Subclinical Atherosclerosis

To explore potential mechanisms mediating the genetic risk, the relationship of *LIPA* mRNA transcript and the respective *LIPA* eSNPs rs1412444 and rs2246833 to cardiovascular risk factors (LDL- and HDL-cholesterol, triglycerides, diabetes mellitus, HbA_{1c}, and systolic and diastolic blood pressure) and subclinical atherosclerotic disease (endothelial function measured and carotid macroangiopathy) was investigated. Detailed results are provided in Table 4. Elevated *LIPA* expression was significantly associated with lower HDL-cholesterol levels ($P=2.5\times10^{-3}$) and impaired endothelial function measured by flow-mediated vasodilation ($P=4.04\times10^{-3}$), whereas associations with higher levels of LDL-cholesterol and triglycerides did not reach statistical significance. In contrast, no significant association between *LIPA* eSNPs and any cardiovascular risk factor was observed.

eSNPs Located in Known CAD Loci

In addition to SNPs identified in our analysis, we performed an eQTL analysis for SNPs previously reported to be associated with CAD and/or myocardial infarction,[3–5,7,23] but not found in our analysis. Of 26 SNPs investigated (Online-only Data Supplement Table V), only 3 SNPs in 2 loci were associated with eQTL transcripts (Table 3). In our data, the locus on chromosome 1p13 (represented by SNPs rs599839 and rs629301) revealed a strong association with *PSRC1* transcripts with the risk allele for both SNPs associated with

Table 3. eSNPs Associated With CAD and Gene Expression

	Location SNP			eQTL Transcript			
SNP	Chr	bp	Gene	Probe ID	Transcript	*P* Value	SNP Effect
SNPs associated with CAD and with gene expression							
rs1412444	10	90992907	LIPA	ILMN_1718063	LIPA	$1.31*10^{-96}$	In gene
rs2246833	10	90995834	LIPA	ILMN_1718063	LIPA	$3.97*10^{-96}$	In gene
CAD-SNPs from literature associated with gene expression							
rs629301*	1	109619829	CELSR2	ILMN_1671843	PSRC1	$8.74*10^{-38}$	*cis*
				ILMN_2315964	PSRC1	$1.22*10^{-10}$	*cis*
rs599839	1	109623689	PSRC1	ILMN_1671843	PSRC1	$1.71*10^{-36}$	*cis*
				ILMN_2315964	PSRC1	$2.31*10^{-10}$	*cis*
rs6725887	2	203454130	WDR12	ILMN_1739942	FAM117B	$8.07*10^{-21}$	*cis*

Results from eQTL analysis of CAD-associated SNPs ($P<5\times10^{-8}$), based on results of the present CADomics study and SNPs of previously published loci for CAD. SNPs located within 500 kb of either the 5′ or 3′ end of the associated gene were considered as *cis*-acting; otherwise, they were called to act in *trans*.

*The corresponding published tagSNP for rs629301 is rs646776.

decreased transcript levels of *PSRC1*. For the second locus, the risk allele of SNP rs6725887, located within the *WDR12* gene on chromosome 2q33, was associated with decreased *FAM117B* transcript levels (located close to *WDR12*).

The association of these eSNPs and eQTL transcripts with cardiovascular risk factors and phenotypes of subclinical disease was further analyzed (Table 4). Significant associations between increased *PSRC1* transcript levels and lower LDL-cholesterol levels ($P=8.2\times10^{-3}$), higher HDL-cholesterol levels ($P=3.0\times10^{-3}$), lower systolic and diastolic blood pressure ($P=9.9\times10^{-5}$ and $P=3.5\times10^{-4}$, respectively), and an improved endothelial function ($P=2.2\times10^{-4}$) were observed. As previously reported[3,4,24] the risk alleles of eSNPs rs599839 and rs629301 were robustly associated with increasing LDL cholesterol levels ($P=3.96\times10^{-4}$ and $P=3.93\times10^{-4}$). In addition, the risk alleles were associated with the extent of atherosclerotic plaques ($P=1.44\times10^{-3}$ and $P=1.23\times10^{-3}$). No significant association was found for *FAM117B* transcript levels and respective eSNPs with cardiovascular risk factors and phenotypes of subclinical disease.

Discussion

A GWA study for CAD was performed, and loci identified were further evaluated to explore their potential functional relevance by (1) testing functionality of genetic variants in relation to gene expression and (2) correlating expression levels with CAD risk factors and disease precursors such as endothelial function and carotid atherosclerosis.

In addition to the previously known locus on chromosome 9p21, our study identified the *LIPA* gene on chromosome 10q23 as a novel CAD susceptibility locus ($P=3.71\times10^{-8}$ and $P=4.35\times10^{-8}$ for SNPs rs1412444 and rs2246833). In the subsequent eQTL analysis, *LIPA* genotypes displayed a strong association with *LIPA* transcripts ($P=1.31\times10^{-96}$ and $P=3.97\times10^{-96}$, respectively), with the CAD risk allele being associated with higher *LIPA* expression. Further, elevated *LIPA* expression itself was related to lower HDL-cholesterol levels and impaired endothelial function, a precursor of CAD.

In humans, the *LIPA* gene encodes lysosomal acid lipase (LAL).[25,26] LAL hydrolyzes cholesteryl esters and triglycerides delivered to the lysosome. If LAL is missing and/or not active, trigylcerides and cholesteryl esters accumulate in the cell, resulting in foam cell formation and as a consequence in atherosclerotic plaque.[27] Mutations in the *LIPA* gene are the cause of the cholesteryl ester storage disease and Wolman disease.[28,29] Patients with these diseases also have premature cardiovascular disease.[29] Residual LAL activity determines the severity of clinical symptoms, with Wolman patients having the lowest residual activity.[30]

Our data demonstrate that the *LIPA* CAD risk allele is associated with increased *LIPA* expression. Increased intrinsic *LIPA* expression might enhance intracellular release of fatty acids and cholesterol through the lysosomal route,[27] possibly explaining the association of the risk allele with impaired endothelial function, a precursor of atherosclerosis.[31] Furthermore, increased *LIPA* expression is expected to be associated with increased LAL activity. Unesterified cholesterol is a hallmark of atherosclerotic lesions.[32] In fact, cholesteryl ester hydrolysis has been shown to be a critical step in the enzymatic modification of LDL particles in the intima, conferring the ability to activate complement to LDL and rendering them proatherogenic.[33,34] Thus, the risk allele could increase the generation of enzymatically modified LDL and free cholesterol in the arterial intima, thereby promoting foam cell formation, complement activation, and an inflammatory process.

The significant association of the *LIPA* eSNPs rs1412444 and rs2246833 with CAD, their strong association with expression and the relation between transcript levels, and subclinical disease in apparently healthy individuals strongly supports a causal role for the *LIPA* gene in atherosclerosis.

We also studied the relationship of previously published loci to gene expression, cardiovascular risk factors, and

Table 4. Effect of eQTL Transcripts (A) and eSNPs (B) on Cardiovascular Risk Factors and Phenotypes of Subclinical Atherosclerotic Disease

eQTL Transcripts

Transcript	Probe ID	LDL-Cholesterol, mg/dL	HDL-Cholesterol, mg/dL	Log Triglycerides, mg/dL	Diabetes Mellitus, %	Log HbA_{1c}, %	Systolic Blood Pressure, mm Hg	Diastolic Blood Pressure, mm Hg	Carotid Macroangiopathy, Yes/No	Flow-Mediated Vasodilation, %
		Strength of association (β estimates for continuous traits or OR for dichotomous traits with 95% CI, *P* Value)								
LIPA	ILMN_1718063	4.58 (−0.58; 9.74)	−3.51 (−5.78; −1.23)	0.06 (−0.01; 0.13)	0.84 (0.49; 1.44)	0.01 (−0.01; 0.03)	−2.71 (−5.25; −0.17)	0.003 (−1.39; 1.40)	0.94 (0.60;1.46)	−1.06 (−1.79; −0.34)
		0.082	$2.5*10^{-3}$*	0.0836	0.533	0.148	$3.9*10^{-2}$	0.997	0.782	$4.04*10^{-3}$*
PSRC1	ILMN_1671843	−12.02 (−20.94; −3.11)	6.00 (2.041; 9.95)	−0.14 (−0.26; −0.02)	0.61 (0.24; 1.56)	−0.01 (−0.04; 0.02)	−8.76 (−13.17; −4.36)	−4.43 (−6.85; −2.00)	0.35 (0.16; 0.76)	2.39 (1.12–3.65)
		$8.2*10^{-3}$*	$3.0*10^{-3}$*	$2.8*10^{-2}$	0.303	0.524	$9.96*10^{-5}$*	$3.5*10^{-4}$*	$8.05*10^{-3}$*	$2.2*10^{-4}$*
PSRC1	ILMN_2315964	−1.95 (−5.01; 1.12)	0.27 (−1.09; 1.63)	−0.01 (−0.05; 0.03)	1.10 (0.8; 1.57)	−0.01 (−0.02; 0.01)	−1.65 (−3.17; −0.12)	−0.75 (−1.59; 0.09)	0.89 (0.702; 1.167)	0.36 (−0.08; 0.79)
		0.213	0.701	0.591	0.574	0.370	$3.4*10^{-2}$	0.080	0.398	0.105
FAM117B	ILMN_1739942	7.70 (0.97; 14.44)	0.67 (−2.3; 3.64)	0.07 (−0.02; 0.16)	1.28 (0.63; 2.66)	−0.007 (−0.03; 0.02)	1.13 (−2.18; 4.45)	0.27 (−1.55; 2.09)	1.35 (0.76; 2.44)	0.02 (−0.93; 0.98)
		$2.5*10^{-2}$	0.658	0.136	0.506	0.538	0.503	0.771	0.322	0.962

eSNPs

SNP	Chr	Gene	Risk/ Non–Risk Allele	LDL-Cholesterol, mg/dL	HDL-Cholesterol, mg/dL	Log Trigylcerides, mg/dL	Diabetes Mellitus, %	Log HbA_{1c}, %	Systolic Blood Pressure, mm Hg	Diastolic Blood Pressure, mm Hg	Carotid Macroangiopathy, Yes/No	Flow-Mediated Vasodilation, %
				Strength of association (β estimates with 95% CI, *P* Value)								
rs1412444	10	LIPA	T/C	1.11 (−0.8; 3.02)	−0.68 (−1.55; 0.18)	0.01 (−0.01; 0.04)	0.88 (0.71; 1.1)	0 (−0.01; 0.01)	0.16 (−0.8; 1.11)	−0.1 (−0.62; 0.41)	1.06 (0.89; 1.25)	0 (−0.27; 0.27)
				0.25	0.12	0.37	0.28	0.94	0.75	0.70	0.51	0.98
rs2246833	10	LIPA	T/C	1.25 (−0.65; 3.14)	−0.76 (−1.62; 0.1)	0.02 (−0.01; 0.04)	0.9 (0.73; 1.12)	0 (−0.01; 0.01)	0.17 (−0.78; 1.11)	−0.17 (−0.68; 0.35)	1.06 (0.89; 1.25)	−0.02 (−0.29; 0.25)
				0.20	0.084	0.25	0.35	0.99	0.73	0.52	0.52	0.88
rs629301	1	CELSR2	T/G	3.93 (1.83; 6.04)	−0.05 (−1.01; 0.9)	0 (−0.03; 0.03)	1.07 (0.84; 1.36)	0 (0–0.01)	0.37 (−0.68; 1.43)	−0.28 (−0.85; 0.29)	1.43 (1.16; 1.76)	0.12 (−0.18; 0.42)
				$2.0*10^{-4}$*	0.91	0.96	0.59	0.35	0.49	0.34	$1.0*10^{-3}$*	0.44
rs599839	1	PSRC1	A/G	3.96 (1.87; 6.06)	0.06 (−0.89; 1.01)	0 (−0.03; 0.03)	1.1 (0.86; 1.4)	0 (0–0.01)	0.39 (−0.66; 1.44)	−0.18 (−0.75; 0.38)	1.44 (1.17; 1.76)	0.07 (−0.23; 0.36)
				$2.0*10^{-4}$*	0.90	0.92	0.45	0.36	0.47	0.53	$1.0*10^{-3}$*	0.66
rs6725887	2	WDR12	C/T	−1.76 (−4.38; 0.86)	0.13 (−1.07; 1.32)	−0.04 (−0.07; 0)	0.97 (0.72; 1.3)	0 (−0.01; 0.01)	−0.33 (−1.65; 0.98)	−0.58 (−1.3; 0.13)	0.77 (0.6; 0.99)	0.4 (0.02; 0.77)
				0.20	0.84	0.042	0.82	0.87	0.62	0.11	$4.2*10^{-2}$	$3.7*10^{-2}$

Table shows the strength of association by β estimates or ORs with the corresponding 95% CI and uncorrected *P* values.
**P* values that remain significant after correction for false discovery rate.

phenotypes. The association of the risk alleles on the 1p13 locus with decreased *PSRC1* transcript and increased LDL-cholesterol levels had been reported previously.[24] Further, our data showed significant association for 1p13 eSNPs and *PSCR1* transcript levels with blood pressure and endothelial function, indicating that this genetic risk locus might act through these CAD risk factors. In human liver, the 1p13 locus affects transcript levels of *CELSR2*, *PSRC1,* and *SORT1,* with the strongest regulatory effect for *SORT1*.[3,24] Further, in a recent study by Musunuru et al,[35] liver-specific transcriptional regulation of the *SORT1* gene by C/EBP transcription factors was shown, and *SORT1* has been nominated as the causal gene at the 1p13 locus for LDL-cholesterol and myocardial infarction. However, as previously reported by our group,[15] *SORT1* was not *cis*-regulated in our data set of global monocytic gene expression, suggesting a different mechanism of transcript regulation of the 1p13 locus in monocytes, and does not exclude *PSRC1* as an important contributor to lipid levels and coronary artery disease.

Some limitations merit consideration. Cases comprise individuals with severe coronary atherosclerosis documented by angiography and myocardial infarction. Gene expression studies were performed in monocytes. Hence, other cell types might yield different results. Finally, we did not test expression profiles in cases. However, because patients are receiving CAD treatment, medication probably would severely modify expression patterns.

Overall, the use of genome-wide SNP data and the monocyte transcriptome (GHSExpress, http://genecanvas.ecgene.net/uploads; for review, see Reference 15)[15] led to the identification of a novel locus potentially relevant for the development of CAD. The respective eSNPs strongly affected *LIPA* gene expression, and the *LIPA* expression level itself was related to subclinical disease as assessed by vascular endothelial function. The consistency of our results between genetic variants, *LIPA* expression level, and disease precursor identifies *LIPA* as an attractive research candidate for follow-up functional studies, also emphasized by the association between LAL deficiency and the rare cholesteryl ester storage disease and Wolman disease.

Appendix

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Acknowledgments

We appreciate the contribution of participants of the Gutenberg Heart Study and the AtheroGene Registry. We gratefully acknowledge the excellent medical and technical assistance of all technicians, study nurses, and coworkers involved in the Gutenberg Heart Study. We thank Andreas Weith, Detlev Mennerich, and Werner Rust for help during technical performance of GWA and global gene expression experiments.

Sources of Funding

The Gutenberg Heart Study is funded through the government of Rheinland-Pfalz ("Stiftung Rheinland Pfalz für Innovation," contract No. AZ 961–386261/733), the research programs "Wissen schafft Zukunft" and "Schwerpunkt Vaskuläre Prävention" of the Johannes

Gutenberg-University of Mainz, and its contract with Boehringer Ingelheim and PHILIPS Medical Systems, including an unrestricted grant for the Gutenberg Heart Study. Specifically, the research reported in this article was supported by the National Genome Network "NGFNplus" (contract No. project A3 01GS0833 and 01GS0831) by the Federal Ministry of Education and Research, Germany.

Disclosures

Drs Reilly and Rader were supported by GlaxoSmithKline through an Alternate Drug Discovery Initiative research alliance award.

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CLINICAL PERSPECTIVE

In relation to polygenic coronary artery disease, recent genome-wide association studies have revealed interesting novel loci whose pathophysiological significance is incompletely understood at present. Variation in gene expression may be an important intermediate link between common genetic variants and phenotypes. In our study, combining information from genome-wide association studies and global gene expression in peripheral blood monocytes, a cell type central to the atherosclerotic process, we identified interesting single-nucleotide polymorphisms in the *LIPA* (lysosomal acid lipase A) gene on chromosome 10q23 in relation to coronary artery disease. *LIPA* gene expression was also associated with endothelial function, an intermediate phenotype of coronary artery disease. Consistent associations at the genetic, gene expression, subclinical disease, and disease levels support a causal relationship and add a pathophysiologically plausible candidate for future investigation in cardiovascular risk assessment as well as a potential therapeutic target at all stages of the disease process. The approach of combining genome-wide association studies information and global gene expression shows a successful way to further exploit genome-wide data in relation to coronary artery disease. If further confirmed, biomarkers of the lysosomal acid lipase A pathway may be candidate markers for risk prediction in primary and secondary prevention and may potentially serve as targets for interventional strategies if proven to be causal.