Circulation

ORIGINAL RESEARCH ARTICLE

LPA Variants Are Associated With Residual Cardiovascular Risk in Patients Receiving Statins

Wei-Qi Wei, MD, PhD* et al

BACKGROUND: Coronary heart disease (CHD) is a leading cause of death globally. Although therapy with statins decreases circulating levels of low-density lipoprotein cholesterol and the incidence of CHD, additional events occur despite statin therapy in some individuals. The genetic determinants of this residual cardiovascular risk remain unknown.

METHODS: We performed a 2-stage genome-wide association study of CHD events during statin therapy. We first identified 3099 cases who experienced CHD events (defined as acute myocardial infarction or the need for coronary revascularization) during statin therapy and 7681 controls without CHD events during comparable intensity and duration of statin therapy from 4 sites in the Electronic Medical Records and Genomics Network. We then sought replication of candidate variants in another 160 cases and 1112 controls from a fifth Electronic Medical Records and Genomics site, which joined the network after the initial genome-wide association study. Finally, we performed a phenome-wide association study for other traits linked to the most significant locus.

RESULTS: The meta-analysis identified 7 single nucleotide polymorphisms at a genome-wide level of significance within the *LPA*/*PLG* locus associated with CHD events on statin treatment. The most significant association was for an intronic single nucleotide polymorphism within *LPA*/*PLG* (rs10455872; minor allele frequency, 0.069; odds ratio, 1.58; 95% confidence interval, 1.35–1.86; $P=2.6\times10^{-10}$). In the replication cohort, rs10455872 was also associated with CHD events (odds ratio, 1.71; 95% confidence interval, 1.14–2.57; $P=0.009$). The association of this single nucleotide polymorphism with CHD events was independent of statin-induced change in low-density lipoprotein cholesterol (odds ratio, 1.62; 95% confidence interval, 1.17–2.24; $P=0.004$) and persisted in individuals with low-density lipoprotein cholesterol ≤70 mg/dL (odds ratio, 2.43; 95% confidence interval, 1.18–4.75; $P=0.015$). A phenome-wide association study supported the effect of this region on coronary heart disease and did not identify noncardiovascular phenotypes.

CONCLUSIONS: Genetic variations at the *LPA* locus are associated with CHD events during statin therapy independently of the extent of low-density lipoprotein cholesterol lowering. This finding provides support for exploring strategies targeting circulating concentrations of lipoprotein(a) to reduce CHD events in patients receiving statins.

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Clinical Perspective

What Is New?

- A genome-wide association study identified variation at the *LPA* locus to be associated with coronary heart disease (CHD) events during statin therapy, independent of the extent of low-density lipoprotein cholesterol lowering.
- The association of the *LPA* locus with CHD events persisted in individuals with low-density lipoprotein cholesterol ≤70 mg/dL.
- This finding provides support for exploring strategies targeting circulating concentrations of lipoprotein(a) to reduce CHD events in patients receiving statins.

What Are the Clinical Implications?

- Genetic variants in *LPA* are associated with CHD events in individuals on statin therapy.
- The potential for lowering lipoprotein(a) with existing and emerging therapeutic agents may reduce CHD events in statin-treated patients, including those with low low-density lipoprotein cholesterol levels.

Coronary heart disease (CHD) affects >80 million Americans and remains the leading cause of mortality worldwide.[1] Statins reduce the incidence of CHD events. The major cardiovascular benefit of statin treatment is achieved through its ability to reduce circulating levels of low-density lipoprotein cholesterol (LDL-C). A meta-analysis of 26 randomized trials with 170 000 participants demonstrated that statin treatment significantly reduced the 5-year incidence of major CHD events by ≈20% for every 1-mmol/L (39-mg/dL) reduction in circulating levels of LDL-C.[2] However, clinical trials and retrospective observational cohort studies have reported considerable interindividual variability in LDL-C response to statins.[3–5] Recent findings from the Genomic Investigation of Statin Therapy (GIST) consortium have supported earlier evidence[6,7] that genetic factors contribute to this variation.[8] In their genome-wide association study (GWAS), GIST investigators identified single nucleotide polymorphisms (SNPs) at 4 loci significantly associated with the magnitude of statin-induced LDL-C reduction (*LPA, APOE, SLCO1B1,* and *SORT1/CELSR2/PSRC1).*

Although statin therapy decreases the incidence of CHD events,[2,9,10] events continue to occur despite lower LDL-C levels.[9,11–13] For example, a recent clinical trial suggested no additional benefit of LDL-C reduction with respect to major adverse cardiovascular events involving patients at lower cardiovascular risk.[12] The contribution of genetic variation to this residual CHD risk during statin therapy remains unknown, which impedes the development of an optimum approach to long-term reduction of CHD events.[7,14,15] We therefore conducted a multisite case-control GWAS to assess the genetic determinants of CHD events, defined as either acute myocardial infarction (AMI) or the need for revascularization, occurring during statin therapy, and the extent to which risk was dependent on change in LDL-C. Phenotypic information for cases and controls was ascertained across multiple sites of the Electronic Medical Records and Genomics (eMERGE) Network with a validated algorithm.[16]

METHODS

Availability of Data

Data from eMERGE network have been submitted to Database of Genotypes and Phenotypes (phs000360, phs000944.v1.p1). The authors declare that other genotyped and phenotypic data will be made available to other researchers through the Database of Genotypes and Phenotypes for purposes of reproducing the results.

Research Participants

We performed a 2-stage GWAS within the eMERGE Network.[17,18] The eMERGE Network is a consortium of US cohorts with DNA samples linked to electronic health record (EHR) data for conducting large-scale, high-throughput genetic research. The current phase of the eMERGE Network has 12 member sites. Dense genotypic data coupled to EHRs are in place at each eMERGE site for individuals selected for a range of initial phenotypes.[17] Each participating site obtained Institutional Review Board approval.

Discovery Cohorts

Our primary analysis was a meta-analysis of cases and controls identified at 4 eMERGE sites: Vanderbilt University Medical Center's BioVU resource,[19] the Geisinger Health System, the Mayo Clinic, and the Marshfield Clinic. We identified cases and controls with extant GWAS data at these 4 sites. At the same time, an additional large cohort was identified from Vanderbilt's BioVU resource and was genotyped at the Rikagaku Kenkyūjyo (RIKEN) Center for Genomic Medicine (BioVU-RIKEN) under an existing alliance with the Pharmacogenomics Research Network.[20]

Validation Cohort

To replicate the initial findings, we identified cases and controls from the Partners HealthCare Biobank, which joined the eMERGE network after the initial GWAS was underway. The Partners Biobank is a recontactable EHR-linked DNA biobank with 60 528 consented individuals. Among these individuals, 4930 had been genotyped at the time of this study. Our replication was limited to only genome-wide significant associations from the initial study.

Cohort for Phenome-Wide Association Study

After completing our GWAS, we conducted a phenome-wide association study (PheWAS) to investigate other potential associations with our found variant. PheWAS is a systematic approach to replicate and discover relationships between targeted genotypes and multiple phenotypes.[21,22] We used

11 566 individuals of European ancestry with genome-wide genotyping data available in BioVU, excluding those in the discovery cohort.

Phenotyping

Identification of CHD Events

We defined a CHD event as either AMI or the need for revascularization. Our algorithm used EHR data, including *International Classification of Diseases, Ninth Revision, Clinical Modification* (*ICD-9-CM*) codes, Current Procedural Terminology codes, and laboratory test results to identify a CHD event. We defined a CHD event while on statins as one that occurred at least 180 days after the earliest recorded date of statin use. The algorithm is published on https://PheKB.org and has been validated at 3 sites of the eMERGE network through manual chart validation.[16] Manual validation showed a 96% to 100% positive predictive value for the identification of CHD events during statin therapy (cases)[16] and 100% positive predictive value for controls. The BioVU-RIKEN cohort was limited to individuals identified as white in the EHR. We identified 1758 cases and matched them to 3516 controls for sex and age of statin initiation at a 1:2 ratio. We then added 726 controls on the basis of their availability in BioVU. For the other cohorts, we identified cases and controls from all individuals who had been genotyped.

We also collected information about type 2 diabetes mellitus, hypertension, smoking status, and CHD history for each individual. We used the previously validated algorithm for type 2 diabetes mellitus posted at https://PheKB.org.[23] We applied previously validated natural language processing and machine learning algorithms to determine ever/never smoking status.[24] We used presence or absence of *ICD-9-CM* codes to ascertain each individual's hypertension status (401.*) and whether the individual had a history of a CHD (410–414). These covariates were used to adjust analyses.

Extraction of LDL-C Response to Statin Treatment

We used the definition of statin response adopted by the GIST consortium[8] with modifications as follows. Statin medication exposure and dose and LDL-C measures were extracted from each individual's EHR by applying natural language processing algorithms that we have developed and validated.[4] To qualify, a participant was required to have at least 1 off-treatment LDL-C measurement and at least 1 on-treatment LDL-C measurement. We defined the off-treatment LDL-C as the median value of all LDL-C measures before the first mention of statin therapy in the EHR. We defined the on-treatment low-density lipoprotein as the median value of all LDL-C measures after the first mention of statin use. For cases, we used only the LDL-C results before the first CHD event during treatment. We calculated the magnitude of LDL-C–lowering effect of statin therapy and used it as a covariate.

PheWAS Approach

Following established protocols used in past PheWASs,[25,26] we grouped each individual's *ICD-9-CM* codes into 1837 disease phecodes. To be a case for each phecode, an individual needed to have relevant *ICD-9-CM* codes on ≥2 different days. Individuals who had only 1 relevant *ICD-9-CM* code for a phecode were neither cases nor controls. Controls were remaining individuals who also lacked related *ICD-9-CM* codes to the phecode (eg, an individual with ischemic heart disease does not serve as a control for an individual with an AMI). We analyzed all 1083 phecodes occurring in >20 patients.

Genotyping and Imputation

All genotyping was conducted with commercially available genome-wide SNP arrays with quality control criteria for variants before imputation listed in Table I in the online-only Data Supplement. The eMERGE phase 1 cohort, generated during the initial period of eMERGE, included data from Marshfield Clinic and partial data from the Geisinger Health System, the Mayo Clinic, and Vanderbilt BioVU for this study. The Geisinger, Mayo, and BioVU cohorts represented data collected subsequently. Genotyping for the Geisinger Health System, Mayo Clinic, and Marshfield Clinic and the other Vanderbilt samples was conducted within the eMERGE network. Genotyping for the BioVU-RIKEN set was conducted at RIKEN. Genotyping of Partners Biobank participants was conducted separately. All data sets are exclusive.

Genotype data were curated for quality control with PLINK.[27] For the BioVU-RIKEN cohort, results were filtered with a minor allele frequency ≥0.01. For other cohorts, we removed samples with (1) a per-individual call rate <95%; (2) per-individual autosome heterozygosity >5 SDs from the mean; (3) wrongly assigned sex; (4) one of each pair of individuals with a cryptic relationship closer than a third-degree relative (proportion identity by descent PI_HAT ≥0.125)[28] or both individuals from a duplicated pair (PI_HAT ≥0.95); (5) SNPs with a genotyping call rate <95%; and (6) SNPs with estimated allele frequencies in controls that were >10% different from the population estimate from the 1000 Genomes Project. We also aligned alleles to the genomic forward strand using 1000 Genomes Project allele frequency estimates. The remaining samples were assessed for population stratification with principal component analyses implemented in EIGENSOFT.[29,30]

To increase the power and coverage of the GWAS, we performed whole-genome imputation. We prephased haplotypes from post–quality control, strand-aligned genotype data using SHAPEIT2.[31] We then used IMPUTE2[32] to perform genotype imputation to the 1000 Genomes Project Phase 3 reference haplotypes (October 2014). Approximately 10 million direct or imputed SNPs passed the quality control filters and were evaluated for association.

Statistical Analysis

We assessed the relationship between genetic variation and the risk of developing a CHD event after statin exposure using the SNPTEST software package, version 2.2.0.[33] We assumed an additive effect of SNP alleles on risk and applied logistic regression with the frequentist test, adjusting for age, sex, type 2 diabetes mellitus, hypertension, smoking status, CHD history, and the top 10 principal components for ancestry. SNPs with an information score <0.4 were removed. The analysis was run on each discovery cohort individually, followed by a meta-analysis using METAL,[34] combining the results from all discovery cohorts and adjusting for multiple testing. We evaluated the SNPs only when

there were ≥2 cohorts with available information. The replication analysis was run separately. Regional association plots were generated with LocusZoom (http://locuszoom.sph.umich.edu/locuszoom/).

To evaluate the effect of changes in LDL-C on the top hits, we conducted further analyses adjusting for LDL-C change (defined as the difference between the median LDL-C before and after statin treatment) using individuals for whom this information was available from the largest study cohort (BioVU-RIKEN). We also conducted a survival analysis on the BioVU-RIKEN cohort (the only data set in which time-to-event data were known), with the end point defined as the first CHD event during statin treatment by computing Kaplan-Meier curves for the top hit. The survival analysis was done with the R statistical language 3.3.0. PheWAS was performed with the R PheWAS package[35] using an additive genetic model and adjusted for age, sex, and principal components. In addition, PheWAS was repeated with adjustment for statin use and the median LDL-C value.

RESULTS

Deploying validated algorithms across the discovery set identified 3099 cases with CHD events on statin and 7681 controls. The replication cohort from the Partners Biobank contributed another 160 cases and 1112 controls. The characteristics of these cohorts are listed in Table 1 and Table I in the online-only Data Supplement.

Primary Analysis

The meta-analysis identified 7 SNPs within the *LPA*/*PLG* locus that were associated with CHD events while on statin treatment (Figure 1 and Table 2) at genome-wide significance ($P<5\times10^{-8}$). The most significant association ($P=2.6\times10^{-10}$) was for rs10455872 at the *LPA*/*PLG* locus on chromosome 6 (Figure 1 and Table 2). The minor allele frequency of our cohort for rs10455872 is 7.8%, which is consistent with the 7% minor allele frequency in the European population according to the 1000 Genomes Project.[36] Carriers of the minor allele were more likely to have CHD events while on statin treatment than noncarriers (odds ratio [OR], 1.58; 95% confidence interval [CI], 1.35–1.86). An additional 6 variants were associated with case status within the *LPA*/*PLG* region: rs74617384, rs55730499, rs118039278, rs4252185, rs56393506, and rs2315065. All these variants were in strong linkage with the most strongly associated SNP, rs10455872, within *LPA* (Figure 2).

The *LPA* locus was the only 1 of the 4 loci identified by the GIST consortium as being associated with LDL-C response to statin therapy to also be associated with the risk of CHD events at genome-wide significance ($<10^{-8}$) in this study (data for the other 3 GIST loci are presented in Table II in the online-only Data Supplement).

Replication

We tested the top associations at the *LPA*/*PLG* locus in the cohort from the Partners Biobank (Table 3). The top SNP from the primary analysis, rs10455872, was associated with CHD events on statins (OR, 1.71; 95% CI, 1.14–2.57; $P=0.009$). Two other top SNPs, rs74617384 and rs55730499, were also replicated. The effect size and direction were similar to those in the discovery set.

Table 1. Demographic Characteristics of the Discovery and Replication Sets

		Number	Sex (M/F ratio)	White, %	Age, y	T2DM, %	Hypertension, %	Smoker, %	LDL, mg/dL
Discovery cohort									
BioVU-RIKEN*	Cases	1758	2.26	100	73.59±11.47	33	87	22	87.19±31.86
	Controls	4242	1.65	100	68.94±11.19	17	36	41	102.09±31.60
eMERGE phase 1	Cases	528	1.89	98	82.91±10.92	37	95	46	100.77±27.75
	Controls	1199	0.85	96	75.30±11.15	25	56	40	112.74±25.80
BioVU	Cases	321	2.32	100	73.40±12.26	34	92	46	86.66±26.22
	Controls	134	0.71	100	69.47±12.59	19	68	22	109.59±31.87
Geisinger	Cases	424	3.46	100	77.51±9.52	38	93	86	94.04±24.15
	Controls	1264	0.81	99	68.49±13.50	40	77	62	101.80±24.62
Mayo	Cases	68	2.17	99	74.18±10.58	15	98	60	95.67±19.86
	Controls	842	1.05	96	81.81±10.33	8	42	57	112.83±25.23
Replication cohort									
Partners	Cases	160	2.4	87	72.85±9.98	41	98	60	92.11±29.14
	Controls	1112	0.8	89	65.41±10.97	19	67	42	112.47±28.91

LDL values displayed as mean ± standard deviation. eMERGE indicates Electronic Medical Records and Genomics; F, female; LDL, low-density lipoprotein; M, male; and T2DM, type 2 diabetes mellitus.*For the RIKEN cohort, we identified 1758 cases and matched them to 3516 controls for sex and age of statin initiation at a 1:2 ratio. We then added 726 controls based on their availability in BioVU.

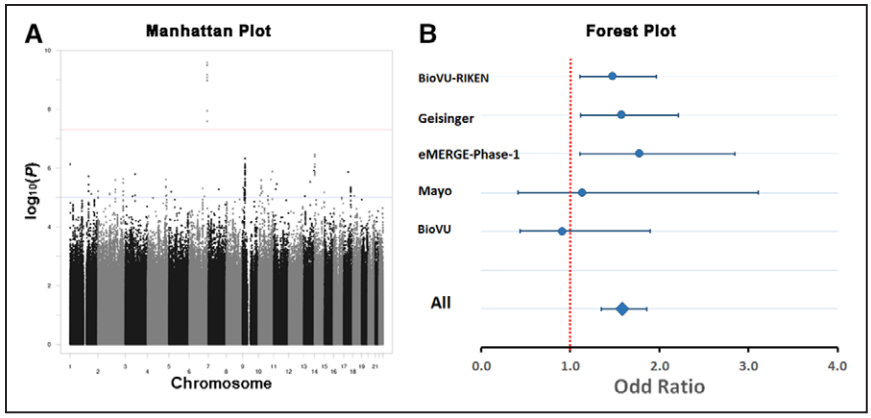

Figure 1. Results of the genome-wide association study meta-analysis.
A, Manhattan plot presenting the –log10 *P* values from the meta-analysis (n=10 780; 3099 cases and 7681 controls) on the association with coronary heart disease (CHD) events on statin. *P* values were generated with logistic regression analysis. **B**, Forest plot of the association of rs10455872 with CHD from each discovery cohort. eMERGE indicates Electronic Medical Records and Genomics.

Effects of LDL-C Changes on the Associations of *LPA* SNPs With CHD

To account for the effect of low-density lipoprotein lowering on the association of top findings in the *LPA* locus with CHD events, we extracted LDL-C changes for 474 cases and 832 controls from the BioVU-RIKEN cohort and repeated the analysis adjusting for statin-induced change in LDL-C. As shown in Table 3, the *LPA* locus was associated with CHD events while on statin treatment independently of LDL-C changes. The effect size was unchanged after adjustment for LDL-C change (rs10455872; OR before adjustment, 1.58; OR after adjustment, 1.62; Table 3).

LDL-C–Stratified Analysis

We then collected data on individuals from both discovery and validation cohorts and performed a stratified analysis for individuals with various LDL-C levels. On the basis of their available LDL-C results, we classified individuals into 2 distinct groups, ie, a group with mean LDL-C ≤70 mg/dL (n=480) and a group with mean LDL-C >70 mg/dL (n=4069). As shown in Table 4, rs10455872 was significantly associated with CHD in individuals with LDL-C ≤70 mg/dL (OR, 2.43; *P*=0.015) and was similar regardless of adjustment for age, sex, and race.

Furthermore, from the cohort of 6000 BioVU-RIKEN study individuals with detailed longitudinal EHR data, we identified 67 cases and 69 controls with a mean LDL-C ≤70 mg/dL before the CHD event; all were white men. The *P* values for association with CHD events were 0.008 (without adjustment) and 0.0078 (adjusted for age) in these individuals with LDL-C ≤70 mg/dL before their CHD events.

Survival Analysis

We conducted a survival analysis of rs10455872 in the BioVU-RIKEN cohort (Figure 3). The group with 2 copies of the G allele (n=27) developed CHD earlier than the group with 1 copy (n=593) and no copy (n=4721). In the multivariate Cox regression analysis, age ($P<2\times10^{-16}$), sex ($P=1.90\times10^{-4}$), smoking status ($P<2\times10^{-16}$), and rs10455872 ($P=2.05\times10^{-6}$) were significantly associated with CHD events during statin treatment. We also found similar results for those individuals with mean LDL-C ≤70 mg/dL ($n_{g=0}$=3086; $n_{g=1}$=519; $n_{g=2}$=20; P=0.01).

PheWAS Analysis

We performed a PheWAS for rs10455872 in 11 566 individuals of European ancestry from 3 Illumina genome-wide SNP arrays available in BioVU (HumanCore-

Table 2. Genome-Wide Significant Associations in Discovery Meta-Analysis

Chromosome	Position	SNP	Gene	Minor Allele	Frequency	OR (95% CI)	Direction	*P* Value
6	161010118	rs10455872	*LPA*	G	0.078	1.58 (1.35–1.86)	+++-+	2.6×10^{-10}
6	160997118	rs74617384	*LPA*	T	0.078	1.58 (1.35–1.86)	+++-+	3.2×10^{-10}
6	161005610	rs55730499	*LPA*	T	0.080	1.56 (1.33–1.83)	+++-+	6.7×10^{-10}
6	160985526	rs118039278	*LPA*	A	0.078	1.56 (1.33–1.83)	+++-+	8.5×10^{-10}
6	161123451	rs4252185	*PLG*	C	0.078	1.69 (1.43–2.01)	++–+	1.1×10^{-9}
6	161089307	rs56393506	*LPA*	T	0.181	1.31 (1.17–1.47)	+++++	1.1×10^{-8}
6	161108144	rs2315065	*LPA/PLG*	A	0.088	1.53 (1.31–1.78)	++-++	2.6×10^{-8}

The direction column indicates the direction of effect in each of the 5 cohorts analyzed (in the order of Electronic Medical Records and Genomics phase 1, Geisinger, Mayo, BioVU, and BioVU-RIKEN). CI indicates confidence interval; OR, odds ratio; and SNP, single nucleotide polymorphism.

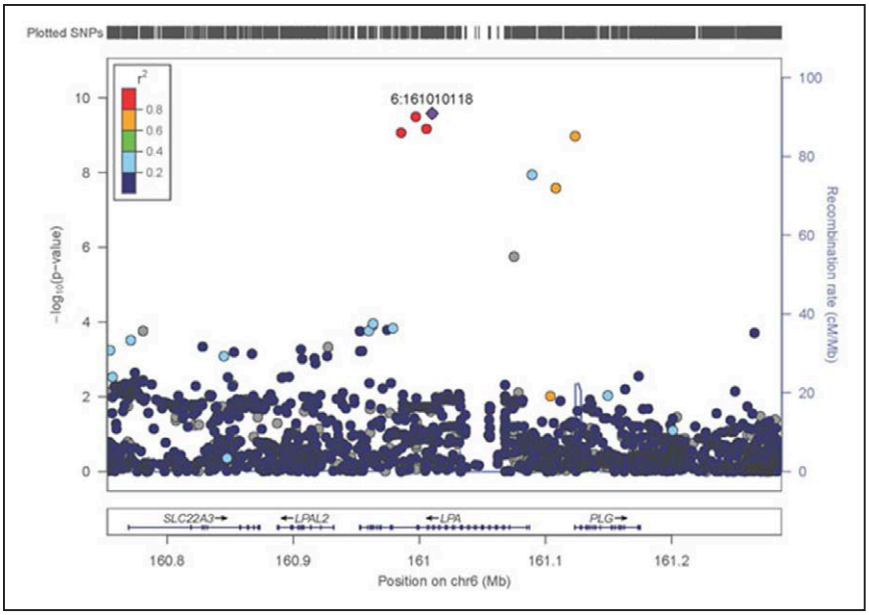

Figure 2. Regional association plots of the *LPA* locus on association with coronary heart disease events during statin treatment.
The color of the single nucleotide polymorphisms (SNPs) is based on the linkage disequilibrium with the lead SNP (purple). Reference sequence genes in the region are shown on the bottom. *P* values were generated with logistic regression analysis. cM/Mb indicates centimorgan/mega base pair.

Exome, MEGA, and OncoArray). The analysis result showed significant associations between rs10455872 and coronary disease, including the phenotypes for coronary atherosclerosis, chronic ischemic heart disease, unstable angina, and myocardial infarction (Figure 4). The signals also remained significant after adjustment by median LDL-C value and statin use.

DISCUSSION

To identify genetic variants associated with CHD events during statin treatment, we conducted a multisite case-control GWAS (>10 700 statin users) in the context of routine clinical care. We identified variants in the *LPA/PLG* locus associated with risk of CHD events while on statin therapy. Each copy of the risk allele G at the lead variant, rs10455872, was associated with a 58% increased risk of CHD events. The minor allele frequency for rs10455872 in European populations is 7%. The association was independent of the LDL-C–lowering effect of statin treatment and was present in individuals with low LDL-C (≤70 mg/dL).

The *LPA* gene encodes apolipoprotein(a), a liver-derived protein with homology to plasminogen. Apolipoprotein(a) is covalently bound to apolipoprotein B on a low-density lipoprotein particle, forming a particle designated lipoprotein(a) (Lp[a]). Circulating Lp(a) levels vary widely across individuals and ethnic groups, and >70% of the variation can be attributed to variants at the *LPA* locus, including Kringle IV repeats.[37–39] In a previous study of 1822 individuals, the minor allele G of rs10455872 was associated with an increase in circulating Lp(a) levels of ≈25%.[40]

Plasma Lp(a) level is an independent predictor for CHD. The Copenhagen City Heart Study reported a stepwise increase of AMI risk associated with elevated Lp(a) concentration.[41] Bennet and colleagues[42] conducted a meta-analysis of 9870 individuals with CHD. They

Table 3. Replication Results

		Discovery Cohorts		Validation Cohort
		Without Adjustment for LDL-C Change: OR (95% CI)	With Adjustment for LDL-C Change: OR (95% CI)	OR (95% CI, *P* Value)
rs10455872	*LPA*	1.58 (1.35–1.86)	1.62 (1.17–2.24)	1.71 (1.14–2.57, 0.0093)
rs74617384	*LPA*	1.58 (1.35–1.86)	1.62 (1.17–2.24)	1.71 (1.14–2.57, 0.0093)
rs55730499	*LPA*	1.56 (1.33–1.83)	1.57 (1.14–2.17)	1.67 (1.11–2.50, 0.0134)
rs118039278	*LPA*	1.56 (1.33–1.83)	1.60 (1.16–2.21)	1.55 (1.01–2.39, 0.0456)
rs4252185	*PLG*	1.69 (1.43–2.01)	1.73 (1.23–2.43)	1.34 (0.87–2.07, 0.1903)
rs56393506	*LPA*	1.31 (1.17–1.47)	1.28 (1.02–1.60)	1.17 (0.86–1.61, 0.3198)
rs2315065	*LPA/PLG*	1.53 (1.31–1.78)	1.58 (1.16–2.16)	1.44 (0.98–2.14, 0.0660)

Effect of adjusting for LDL-C change with statin treatment on coronary heart disease risk associated with top single nucleotide polymorphisms in discovery cohorts and replication results of 160 cases and 1112 controls from the Partners Biobank adjusted by age, sex, and race. CI indicates confidence interval; LDL-C, low-density lipoprotein cholesterol; and OR, odds ratio.

Table 4. Subanalyses of Individuals With Different LDL-C Values

LDL-C, mg/dL	No. (Cases/Controls)	No Adjustment		Adjusted by Age, Sex, and Race	
		P Value	OR (95% CI)	*P* Value	OR (95% CI)
≤70	480 (187/293)	0.016	2.34 (1.18–4.75)	0.015	2.43 (1.19–5.07)
>70	4069 (947/3122)	<0.001	1.42 (1.16–1.73)	<0.001	1.48 (1.20–1.82)

Subanalyses of individuals with a mean LDL-C ≤70 mg/dL and mean LDL-C >70 mg/dL on statin therapy before the coronary heart disease event. CI indicates confidence interval; LDL-C, low-density lipoprotein cholesterol; and OR, odds ratio.

found that individuals in the top tertile of Lp(a) levels were 1.45 times more likely to develop CHD than those in the bottom tertile (OR, 1.45; 95% CI, 1.32–1.58). The association changed slightly after adjustment for smoking, lipids, blood pressure, diabetes mellitus, and body mass index.[43,44] Notably, a mendelian randomization study of *LPA* variants associated with both Lp(a) levels and CHD risk provided further evidence for a causal role of Lp(a) in the pathogenesis of CHD.[40]

We observed that the minor allele of rs10455872 was associated with a 58% increase in CHD risk in statin-treated patients, which is similar to the 47% increase in CHD in carriers of this allele reported for non–statin-treated patients.[40] Our findings are also consistent with the finding of an association of this SNP with CHD in statin-treated patients in an *LPA* candidate gene study (OR, 1.41; 95% CI, 1.17–1.68).[14] Although this variant has been previously associated with less LDL-C reduction in response to statin therapy,[8] the change in LDL-C levels could not explain all the increased CHD risk. Donnelly et al[14] reported that the minor allele of rs10455872 was associated with a 0.10-mmol/L low-density lipoprotein reduction, corresponding to only an ≈2% increase in CHD risk.[2,45,46] Our follow-up analysis adjusting for LDL-C change further supports the independent role of rs10455872 in predicting on-treatment risk of a CHD event.

Given the known association of rs10455872 with circulating Lp(a) levels, these data suggest a causal role for Lp(a) in residual CHD risk for individuals on statins. Previously, a meta-analysis of 9 clinical trials reported an association of atorvastatin treatment with a decrease in Lp(a) levels.[47] A similar effect was also observed in a small study of both atorvastatin and rosuvastatin.[44] However, the JUPITER trial reported zero median change in Lp(a) with rosuvastatin and placebo.[43] Further study is needed to clarify the degree to which the association seen in our work was the result of statin-mediated change in Lp(a) levels; current data suggest that the effect is small and may vary by statin.

Despite substantial evidence that Lp(a) promotes the progression of atherosclerosis and increases the risk of thrombosis in individuals with high plaque burden,[48] a recent report from the dal-Outcomes trial showed no association between Lp(a) level and risk of ischemic cardiovascular events after acute coronary syndrome.[49] The disparate findings may be the result of differences in study cohorts and outcome definitions. Subjects in dal-Outcomes had a recent acute coronary syndrome, whereas our study included all statin users regardless

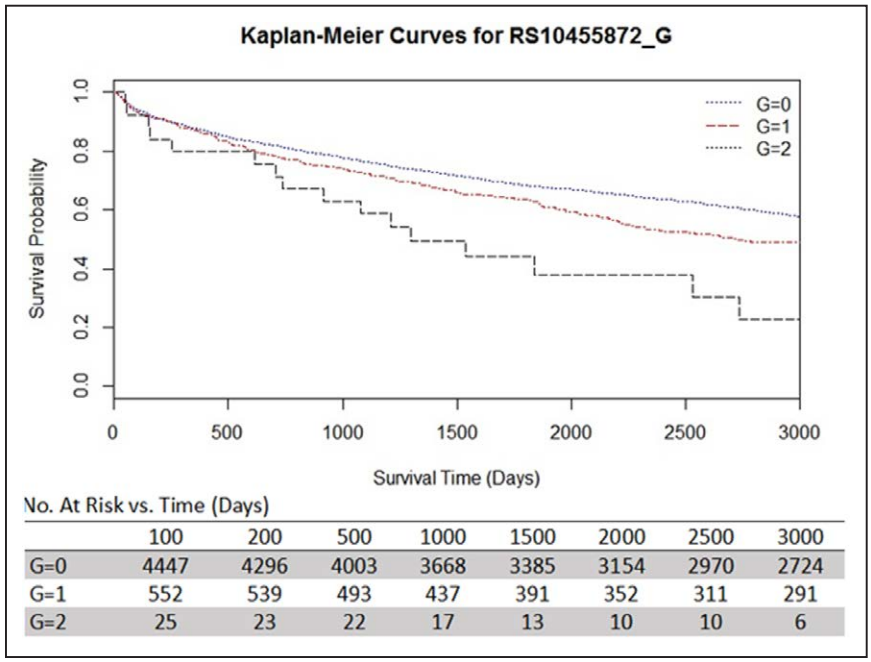

Figure 3. Kaplan-Meier curves by rs10455872 with coronary heart disease events.
The table shows survival probabilities across up to 3000 days. The *P* value from the log-rank test is 1.92×10^{-6}. G indicates the minor allele for rs10455872.

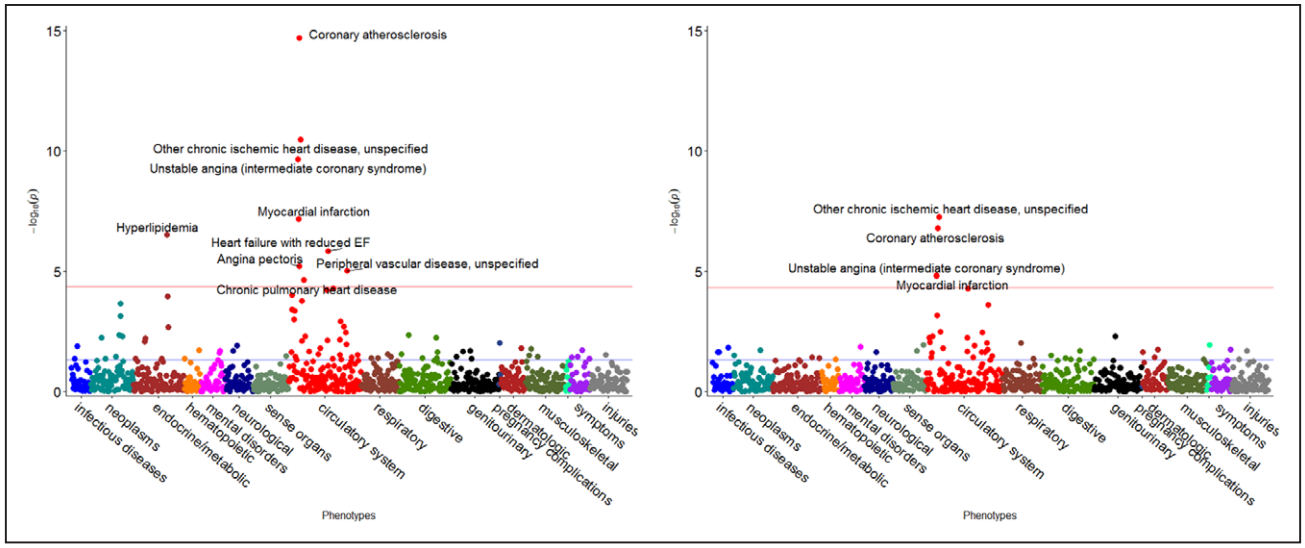

Figure 4. Phenome-wide association study results of rs10455872 on 11 566 additional individuals of European ancestry.
Coronary atherosclerosis, other chronic ischemic heart disease, and unstable angina were top hits associated with the single nucleotide polymorphism adjusted by sex and age (**left**). Other chronic ischemic heart disease, coronary atherosclerosis, unstable angina, and myocardial infarction remained significant after adjustment by sex, age, median low-density lipoprotein cholesterol, and statin use (**right**). EF indicates ejection fraction.

of CHD history. In addition, our definition of an event contains both AMI and the need for revascularization. Nevertheless, a limitation of our study was that we were not capable of measuring Lp(a) in subjects. In the future, a mendelian randomization study of *LPA* in a mix of statin and nonstatin users could further elucidate this effect.

The variant rs10455872 may influence circulating levels of Lp(a) by altering *LPA* expression. Lu et al[50] reported that the carriers of rs10455872 have a higher level of *LPA* mRNA than noncarriers. However, we cannot rule out other roles of this variant in regulating circulating Lp(a) levels. By querying the Genotype-Tissue Expression[51,52] databases, we found that rs10455872 is an expression quantitative trait locus for *SLC22A3,* a gene located upstream of *LPA*. *SLC22A3* is a polyspecific organic cation transporter that is expressed in the liver, kidney, and intestine. Further work is needed to determine its involvement in lipid metabolism and CHD.

The PheWAS also supported the association between rs10455872 and CHD. This association was independent of statin use and median LDL-C value, further supporting the primary findings from the GWAS. We did not observe other significant signals in the PheWAS, which suggests that drugs mediating Lp(a) may not have other significant effects (positive or negative), making Lp(a) a desirable target for further drug development.

The finding in this GWAS adds to the evidence for an important role of Lp(a) in contributing to cardiovascular risk in patients on statin therapy. The potential for lowering Lp(a) with existing and emerging therapeutic agents thus holds promise for further reducing CHD events in statin-treated patients.[53]

Limitations

Our analysis combined data for all statins and statin doses because of differing practice patterns across study sites. Although most individuals were receiving atorvastatin or simvastatin, many received ≥2 different statins or doses (Table III in the online-only Data Supplement). In addition, although individuals were taking the medication on the basis of their refill records in the EHR, we were not able to definitively ascertain compliance. Most of our data were based on populations of European ancestry. Further study is needed to determine whether rs10455872 is associated with similar residual CHD risk in other ethnic populations because Lp(a) levels vary widely across ethnic groups. Our validation analysis focused on the top signal, rs10455872. Although rs10455872 explains ≈25% of the variance in circulating Lp(a) levels, a future study using variations within the *LPA* gene will be of interest. Furthermore, we cannot rule out a possible role of *PLG* in the risk of CHD events while on statin. *PLG* encodes plasminogen, which is critical for both intravascular and extravascular fibrinolysis,[54] and patients with plasminogen deficiency have an increased risk of thrombosis.[55–57] A follow-up study is needed to evaluate the relationship between *PLG* and statin treatment. Although we imputed the genotype data, we cannot rule out the possibility that important rare or low-frequency genetic variants were missed. We did not examine aspirin therapy in this study because of its high use as a secondary prevention strategy in our cohorts. For example, in the Vanderbilt cohort, 99% of cases had aspirin documented in their EHRs. Given the very small number of patients not using aspirin in this cohort, we lack the statistical power to rigorously

quantify the relative contribution of aspirin as a covariate. Finally, our definition of CHD events included both AMI and the need for revascularization. We were not able to conduct an analysis using AMI alone because of limited statistical power.

CONCLUSIONS

Our GWAS demonstrates that genetic variants in *LPA* are associated with CHD events while on statin therapy, highlighting *LPA* as an important contributor to residual CHD risk.

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None.

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