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Association of Rare and Common Variation in the Lipoprotein Lipase Gene with Coronary Artery Disease

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Drs. Khera and Kathiresan had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Abstract

Importance—The activity of lipoprotein lipase (LPL) is the rate-determining step in clearing triglyceride-rich lipoproteins from the circulation. Mutations that damage the *LPL* gene lead to lifelong deficiency in enzymatic activity and can provide insight into the relationship of LPL to human disease.

Objective—Determine if rare and/or common variants in the *LPL* gene are associated with early-onset coronary artery disease (CAD).

Design, Setting, and Participants—Cross-sectional study. The *LPL* gene was sequenced in 10 CAD case-control cohorts of the multinational Myocardial Infarction Genetics Consortium and a nested CAD case-control cohort of the Geisinger Health System DiscovEHR cohort between 2010 and 2015. Common variants were genotyped in up to 305,699 individuals of the Global Lipids Genetics Consortium and up to 120,600 individuals of the CARDIoGRAM Exome Consortium between 2012 and 2014. Study-specific estimates were pooled via meta-analysis.

Exposure—Rare damaging mutations in *LPL* included loss-of-function variants and missense variants annotated as pathogenic in a human genetics database or predicted to be damaging by computer prediction algorithms trained to identify mutations that impair protein function. Common variants in the *LPL* gene region included those independently associated with circulating triglyceride levels.

Main Outcomes and Measures—Circulating lipid levels and CAD.

Results—Among 46,891 individuals with *LPL* gene sequencing data available, mean age was 50 years (SD 12.6) and 51% were female. 188 participants (0.40%; 95%CI 0.35–0.46) carried a damaging mutation in the *LPL* gene – 105 of 32,646 control participants (0.32%) and 83 of 14,245 (0.58%) early-onset CAD cases. Compared to 46,703 non-carriers, the 188 heterozygous carriers of a *LPL* damaging mutation displayed higher plasma triglycerides (Beta coefficient= +19.6 mg/dL; 95%CI 4.6–34.6) and higher odds of CAD (odds ratio 1.84; 95%CI 1.35–2.51; P<0.001). An analysis of 6 common *LPL* variants noted an odds ratio for CAD of 1.51 (95%CI 1.39–1.64; $P=1.1\times10^{-22}$) per standard deviation increase in triglycerides.

Conclusions and Relevance—The presence of rare damaging mutations in the *LPL* gene was significantly associated with higher triglyceride levels and presence of CAD. However, further research is needed to assess causal mechanisms by which heterozygous LPL deficiency could lead to CAD.

Introduction

The enzymatic activity of lipoprotein lipase (LPL) serves as the rate-determining step in the postprandial clearance of circulating triglyceride-rich lipoproteins.[1] Homozygous LPL deficiency, known as familial chylomicronemia syndrome, is associated with marked elevations in chylomicrons, severe hypertriglyceridemia, and recurrent pancreatitis.[2] However, an increased risk of coronary artery disease (CAD) in this condition has not been

observed, potentially because the large circulating chylomicrons are unable to penetrate the arterial wall.[3,4] By contrast, in heterozygous LPL deficiency, the attenuated capacity for lipolysis leads to a buildup of circulating chylomicron remnants and intermediate-density lipoproteins which are rich in both triglycerides and cholesterol. A study of nine such individuals suggested an increased risk of CAD,[5] but this association has not been confirmed.

In this study, the *LPL* gene was sequenced to test the hypothesis that rare, damaging mutations leading to heterozygous LPL deficiency are associated with differences in circulating lipid levels as well as higher odds of early-onset CAD. In addition, to provide complementary evidence, independent common (allele frequency > 1%) variants in the *LPL* gene region were also tested for association with CAD.

Methods

Study Populations

The *LPL* gene was sequenced in participants of 10 previously described CAD case-control cohorts (eTable 1). Studies included the Italian Atherosclerosis Thrombosis and Vascular Biology (ATVB) study,[6] the Exome Sequencing Project Early-Onset Myocardial Infarction (ESP-EOMI) study,[7] a nested case-control of the Jackson Heart Study (JHS),[8] the South German Myocardial Infarction study,[9] the Ottawa Heart Study (OHS),[10] the Precocious Coronary Artery Disease Study (PROCARDIS),[11] the Pakistan Risk of Myocardial Infarction Study (PROMIS),[12] the Registre Gironi del COR (Gerona Heart Registry or REGICOR) study,[13] the Leicester Myocardial Infarction study,[14] and the North German Myocardial Infarction study.[15] Clinical data was assessed in each study. The majority of CAD cases in this analysis (97.5%) were ascertained with onset at an early age (defined as ≤50 years in men and ≤60 years in women). Informed consent was obtained from all participants of contributing studies, each of which received ethical approval from respective institutional review boards.

Replication of the observed associations with regard to lipid levels and CAD was performed via analysis of the previously described DiscovEHR study.[16] DiscovEHR participants were recruited as part of the MyCode Community Health Initiative of the Geisinger Health System and Regeneron Genetics Center. The present analysis was restricted to early-onset CAD cases and CAD-free controls (age <55 years for men or <65 years for women for both cases and controls). Median values for serially measured laboratory and anthropometric traits were calculated for all individuals with two or more measurements in the electronic health records (EHR) following removal of likely spurious values that were >3 standard deviations from the intra-individual median value. Participants were considered to have CAD if they had a history of coronary revascularization in the EHR, or history of acute coronary syndrome, ischemic heart disease, or exertional angina (ICD-9 codes 410*, 411*, 412*, 413*, 414*) with angiographic evidence of obstructive coronary atherosclerosis (>50% stenosis in at least one major epicardial vessel from catheterization report). CAD-free controls were defined as individuals without any case criteria or any single encounter or problem list diagnosis code indicating CAD.

Across all studies, the effect of lipid-lowering therapy in those reporting use at the time of lipid measurement was taken into account by dividing the measured total cholesterol and LDL cholesterol by 0.8 and 0.7 respectively.[16–19] Because remnant cholesterol was not measured in study cohorts, values were estimated according to the following formula: Remnant cholesterol = Total cholesterol – HDL cholesterol – LDL cholesterol.[20]

In order to extend the analysis to common variants in the *LPL* gene, summary statistics of two large genome-wide association studies were analyzed. The effect of common *LPL* variants on circulating triglyceride levels was used as a proxy for influence on LPL activity. The relationship of common *LPL* variants with triglyceride levels was assessed in an analysis of up to 305,699 individuals from 73 cohorts of the Global Lipids Genetics Consortium genotyped using the HumanExome BeadChip (Illumina, San Diego, CA) between 2012 and 2014. These same variants were subsequently linked to CAD in up to 120,600 individuals also genotyped between 2012 and 2014 in the previously reported CARDIoGRAM Exome Consortium study.[15]

Gene Sequencing

Whole exome sequencing of the Myocardial Infarction Genetics Consortium was performed at the Broad Institute (Cambridge, MA, USA) as previously described between 2010 and 2015.[7] In brief, sequence data of all participants were aligned to a human reference genome build GRCh37.p13 using the Burrows–Wheeler Aligner algorithm. Aligned non-duplicate reads were locally realigned and base qualities were recalibrated using the Genome Analysis ToolKit (GATK) software.[21] Variants were jointly called using the GATK HaplotypeCaller software. The sensitivity of the selected variant quality score recalibration threshold was 99.6% for single nucleotide polymorphisms and 95% for insertion/deletion variants as empirically assessed using HapMap controls with known genotypes included in the genotyping call set. *LPL* sequence data from the Geisinger Health System DiscovEHR participants was extracted from exome sequences generated at the Regeneron Genetics Center between 2014 and 2015 as previously described.[16]

Damaging *LPL* Variant Ascertainment

The positions of genetic variants were based on the complementary DNA reference sequence for *LPL* (GenBank accession number: NM_000237.2; http://www.ncbi.nlm.nih.gov/nuccore/NM_000237.2). Rare (minor allele frequency <1%) *LPL* variants were annotated with respect to the following three classes in a sequential fashion: 1) loss of function variants: single base changes that introduce a stop codon leading to premature truncation of a protein (nonsense), insertions or deletions (indels) of DNA that disrupt the translated protein's amino acid sequence beyond the variant site (frameshift), or point mutations at sites of pre-mRNA splicing that alter the splicing process (splice-site); 2) variants annotated as "pathogenic" in ClinVar, a publicly available archive of genetic variations associated with clinical phenotypes;[22] 3) missense variants predicted to be damaging or possibly damaging by *each* of five computer prediction algorithms (LRT score, MutationTaster, PolyPhen-2 HumDiv, PolyPhen-2 HumVar, and SIFT) as performed previously.[7,23] Software used to annotate observed variants included Variant Effect Predictor (version 81) and associated LOFTEE plugin,[24,25] and the dbNSFP database (version 3.0b1).[26]

Statistical Analysis

The association of rare, damaging *LPL* mutations with lipid phenotypes in the Myocardial Infarction Genetics Consortium and the DiscovEHR studies was estimated using linear regression with adjustment for age, age^2, sex, study cohort, and the first five principal components of ancestry. Principal components of ancestry were based on observed genotypic differences across subpopulations (e.g. race or ethnicity) in the overall study. Inclusion of principal components as covariates in linear regression analyses increases statistical power for true relationships and minimizes confounding by ancestry.[27] The association of *LPL* mutations with odds of CAD was determined via meta-analysis utilizing Cochran–Mantel–Haenszel statistics for stratified 2-by-2 tables without continuity correction as implemented previously.[9,18,28]

Common (allele frequency > 1%) variants at the *LPL* locus independently associated with circulating triglyceride levels were ascertained via analysis of the Global Lipid Genetics Consortium cohorts. The association of variants with inverse normal transformed residuals of natural log of triglyceride levels was determined in a model adjusted for age, age^2, sex and up to four principal components of ancestry. For any given genetic locus, such as *LPL*, multiple variants may be associated with circulating triglyceride levels in an independent fashion. Sequential forward selection provides a statistical framework to identify such independent variants.[29,30] The relationship of all genetic variants in the *LPL* locus with triglycerides was first determined. This analysis was then repeated using regression conditional upon the most strongly associated variant, continuing the process until the top result was no longer significant at a pre-specified P-value threshold of $< 5 \times 10^{-8}$ (to represent genome-wide significance). To aid in interpretability, the beta coefficients derived from this analysis were converted into units of mg/dL using data from the National Health and Nutrition Examination Survey from 2005–2012, in which a similar transformation was employed (substituting self-reported race for principal components of ancestry) to yield a conversion factor of 60.7 mg/dL change in triglyceride level per one unit change in inverse normal transformed values.

These same common *LPL* variants were linked to CAD using summary-level test statistics in the previously reported CARDIoGRAM Exome Consortium study.[15] The cumulative association of these variants with odds of CAD was determined, standardized per genetic standard deviation increase in triglyceride levels. Explicitly, if × is the association of each variant with the outcome of interest, and y the association of each variant with triglyceride levels, then the estimated association of a one-standard deviation increase in triglycerides mediated by *LPL* locus variants is calculated as a fixed effects meta-analysis of x/y for all variants. This method is mathematically equivalent to a previously reported approach.[31]

Analyses were performed using R version 3.2.2 software (The R Project for Statistical Computing, Vienna, Austria). All reported P-values were two-tailed, with a value < 0.05 used as a threshold for statistical significance unless otherwise specified.

Results

Gene sequencing of *LPL* was performed in 22,533 participants of the Myocardial Infarction Genetics Consortium, including 12,395 controls and 10,138 cases with CAD (Table 1). 123 loss of function or missense variants in the *LPL* gene with minor allele frequency <1% were identified. Of these 123, 52 variants were classified as damaging (Table 2). Eight of these 123 variants led to loss of function, including 5 premature stop ("nonsense") codons, 2 splice acceptor/donor variants, and 1 frameshift mutation. Only about 25% of missense variants in any given gene have strongly damaging impact on protein function;[32] additional annotation algorithms were thus needed for the 115 missense variants. Six were previously deemed pathogenic based on ClinVar annotation. In addition, 38 of the 109 remaining missense variants were predicted to be damaging by each of 5 computer prediction algorithms. Because any individual damaging mutation was rare (eTable 2), the 52 damaging variants were aggregated for subsequent analyses of phenotypic consequences.

A total of 97 individuals in the Myocardial Infarction Genetics Consortium cohorts carried one of the 52 damaging *LPL* mutations, including 60 cases (0.59%; 95%CI 0.46 – 0.77%) and 37 controls (0.30%; 95%CI 0.21 – 0.42%) as noted in eTable 3. Circulating lipid levels were available in 16,200 (72%) of the participants, including 72 of 97 (74%) of mutation carriers. Median triglyceride levels were 183 versus 147 mg/dL in *LPL* mutation carriers and non-carriers respectively. In an adjusted linear regression model, circulating triglyceride levels were 25.6 mg/dL (95%CI −2.5 – 53.5) higher in mutation carriers as compared to non-carriers although there was no significant association (P = 0.07) (Figure 1 & eTable 4). Furthermore, mutation carriers were at increased odds of having clinical hypertriglyceridemia (triglyceride levels ≥ 150 mg/dL) – odds ratio 1.88; 95%CI 1.13 – 3.20; P = 0.02.

The presence of a rare, damaging *LPL* mutation was associated with an odds ratio for CAD of 1.96 (95%CI 1.30 – 2.96; P = 0.001) in a combined analysis of the Myocardial Infarction Genetics Consortium studies (Figure 2). This association was most pronounced in those with a loss of function mutation in *LPL* (Table 2). Within the subgroup of 2,592 CAD cases and 5,341 controls free of CAD with an observed LDL cholesterol (<130 mg/dL), an increased odds of CAD among carriers of a damaging *LPL* mutation remained apparent (odds ratio = 2.15; 95%CI 1.14 – 4.06; P = 0.02).

Independent replication of the increased circulating triglyceride levels and CAD was performed in 24,358 individuals from the Geisinger Health System DiscovEHR cohort (Table 1). This cohort included 4,107 individuals with early-onset (age <55 years in men or <65 years in women) CAD as ascertained based on medical records and 20,251 CAD-free controls. Ninety-one individuals were heterozygous carriers of a damaging *LPL* mutation, including 23 (0.56%; 95%CI 0.36 – 0.85%) CAD cases and 68 (0.34%; 95%CI 0.26 – 0.43%) CAD-free controls. Circulating triglycerides were 17.2 mg/dL (95%CI −0.5 – 34.9; P = 0.06) higher in mutation carriers as compared to non-carriers (Figure 1 & Table 2). These individuals were at increased odds of early-onset CAD (odds ratio 1.67; 95%CI 1.04 – 2.69; P = 0.03).

In a combined analysis of the Myocardial Infarction Genetics Consortium and DiscovEHR cohorts, a damaging *LPL* mutation was present in 188 of 46,891 individuals (0.40%; 95%CI 0.35 – 0.46%). A meta-analysis of the association with lipid levels demonstrated that these mutations were associated with a 19.6 mg/dL increase in circulating triglycerides (95%CI 4.6 – 34.6), a 3.6 mg/dL decrease in HDL cholesterol (95%CI −5.7 – −1.5), and a 5.6 mg/dL increase in remnant cholesterol (95%CI 2.3 – 9.0) as displayed in Figure 1. These mutations were additionally associated with increased odds of early-onset CAD (odds ratio 1.84; 95%CI 1.35 – 2.51; P < 0.001) as demonstrated in Figure 2.

Beyond rare, damaging mutations, common variants at the *LPL* locus were analyzed to assess for a similar link to triglyceride levels and CAD. In an analysis of up to 305,699 individuals, six common variants (minor allele frequency ranging from 1–29%) were robustly ($P < 5\times10^{-8}$) and independently associated with plasma triglycerides. The minor (less common) allele of four of these variants were associated with decreased triglyceride levels, suggesting gain of lipoprotein lipase activity, and two were linked to increased triglyceride levels, consistent with decreased activity. In an analysis of up to 120,600 individuals of CAD case-control studies, we confirmed that each of these variants was associated with odds of CAD (P < 0.002 for each) with the expected directionality. A roughly linear relationship was noted in this dataset between association with triglyceride levels and odds of CAD (eFigure 1). A weighted analysis that combined these six variants demonstrated an odds ratio for CAD of 1.51 (95%CI 1.39 – 1.64; $P= 1.1 \times 10^{-22}$) per standard deviation increase in triglycerides mediated by *LPL* locus variants.

Discussion

The protein-coding exons of the *LPL* gene were sequenced in 46,891 individuals from an international collaboration of CAD case-control cohorts and patients of a large health care organization. In this study, approximately 1 in 249 (0.40%) individuals carried a rare, damaging mutation in *LPL*. These carriers have increased circulating triglyceride levels (Beta coefficient = +19.6 mg/dL) and an odds ratio of 1.84 for early-onset CAD. An analysis using common variants in *LPL* similarly demonstrated a significant association with CAD.

These results permit several conclusions. First, heterozygous LPL deficiency was associated with the presence of early-onset CAD. By identifying 188 carriers of a rare, damaging mutation, an association with higher triglycerides and remnant cholesterol and lower HDL cholesterol was established along with an odds ratio for early-onset CAD of 1.84. This susceptibility to CAD may be due to impaired lipolysis of triglyceride-rich lipoproteins. Triglyceride-rich lipoproteins are known to penetrate directly into the arterial wall and are selectively retained in the intima, thus promoting the development of cholesterol-rich foam cells and an inflammatory response that accelerate atherosclerosis.[33]

Second, a complementary common variant analysis involving six independent *LPL* variants confirmed the association of genetic variation in *LPL* with CAD. In an analysis in over 300,000 individuals, each common variant's association with triglyceride levels was used as a proxy for influence on lipoprotein lipase activity. Association of these same variants with CAD in over 120,000 individuals demonstrated an odds ratio for CAD of 1.51 per standard

deviation increase in triglycerides associated with common *LPL* locus variants. These findings confirm and extend previous common variant studies that have suggested similar trends.[15,34,35]

Third, these data add to considerable recent genetic evidence that beyond LDL cholesterol, lipoprotein lipase and its endogenous regulation – via facilitator (APOA5) and inhibitor proteins (APOC3, ANGPTL4) – represent an important determinant of human atherosclerosis. Similar approaches have been used to demonstrate that damaging mutations in *APOA5* are associated with a significant increase in odds of CAD.[7,20] By contrast, rare inactivating mutations in *APOC3* and *ANGPTL4* confer substantial vascular protection.[9,15,16,36] Ongoing research will seek to clarify the mechanistic interactions between these proteins. However, in each case, CAD risk is likely to be affected by lifelong alterations in lipoprotein lipase activity. Whether therapy to alter this pathway will decrease risk of CAD remains unknown.

A key strength of the present analysis is that the *LPL* gene was sequenced in a large number of individuals to analyze the entire spectrum of damaging mutations, each of which was rare in the population. Second, concordant results were demonstrated between CAD case-control studies of the Myocardial Infarction Genetics Consortium and the DiscovEHR study participants from the Geisinger Health System, in whom CAD status was ascertained based on electronic health records. This reinforces the potential utility of ongoing efforts, such as the UK Biobank and the All of Us Research Program (a cohort study within the Precision Medicine Initiative), which will facilitate large-scale interrogations of genetic variants as they relate to human disease.

Several limitations should be acknowledged. The approach to annotating rare missense variants in the *LPL* gene using prediction algorithms and the ClinVar database has been previously validated and is fully reproducible.[7,23] However, because functional validation of each variant was not performed, this method may have led to misclassification in some cases. Second, because the effect of LPL activity on regulation of circulating triglyceride levels is most pronounced following a meal,[37] the degree of triglyceride elevation among mutation carriers would likely have been greater if post-prandial triglyceride levels were available. Third, this study assessed the association of *LPL* mutations with susceptibility to early-onset CAD; effect estimates might differ among individuals with later onset of disease. Fourth, both triglycerides and calculated remnant cholesterol, the primary lipid components of triglyceride-rich lipoproteins, were increased in those harboring a *LPL* mutation. Because remnant cholesterol was estimated and not directly measured in the present analysis, additional research is needed to determine the relative contributions of these components to human CAD.

Conclusions

The presence of rare damaging mutations in the *LPL* gene was significantly associated with higher triglyceride levels and presence of CAD. However, further research is needed to assess causal mechanisms by which heterozygous LPL deficiency could lead to CAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

Are heterozygous carriers of a damaging mutation in the gene encoding lipoprotein lipase at increased odds of coronary artery disease?

Findings

Gene sequencing identified a damaging mutation in the lipoprotein lipase gene in 188 of 46,891 (0.40%) individuals from coronary artery disease case-control studies; these mutations were associated with a 19.6 mg/dL increase in plasma triglycerides and an odds ratio of 1.84 for presence of coronary artery disease.

Meaning

The presence of rare damaging mutations in the lipoprotein lipase gene was significantly associated with higher triglyceride levels and presence of coronary artery disease. However, further research is needed to assess causal mechanisms by which heterozygous lipoprotein lipase deficiency could lead to coronary artery disease.

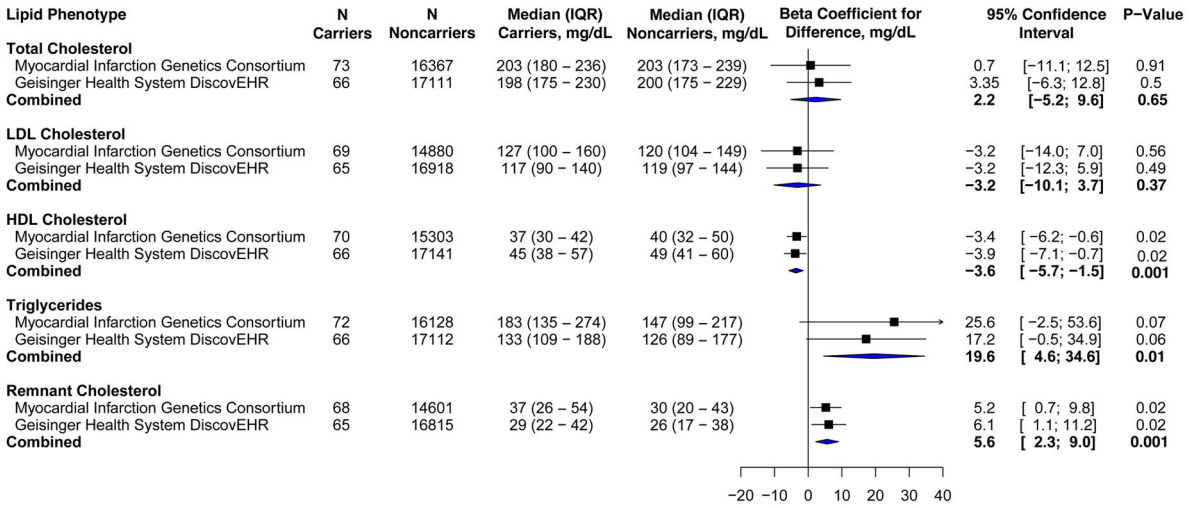

Figure 1.

Association of Damaging *LPL* Mutations with Circulating Lipid Concentrations

Beta coefficients reflective of the difference in lipid concentrations between carriers of a damaging *LPL* mutation, as compared to noncarriers, were derived from linear regression analysis that included adjustment for age, age^2, gender, cohort, and the first five principal components of ancestry. Principal components of ancestry are based on observed genotypic differences across subpopulations (e.g. race or ethnicity) in the overall study. Inclusion of principal components as covariates in linear regression analyses increases statistical power for true relationships and minimizes confounding by ancestry.[27] Fixed-effects meta-analysis was used to combine results across cohorts (P for heterogeneity > 0.50 for each lipid phenotype). The number of participants from each study cohort with lipid fraction values available is displayed.

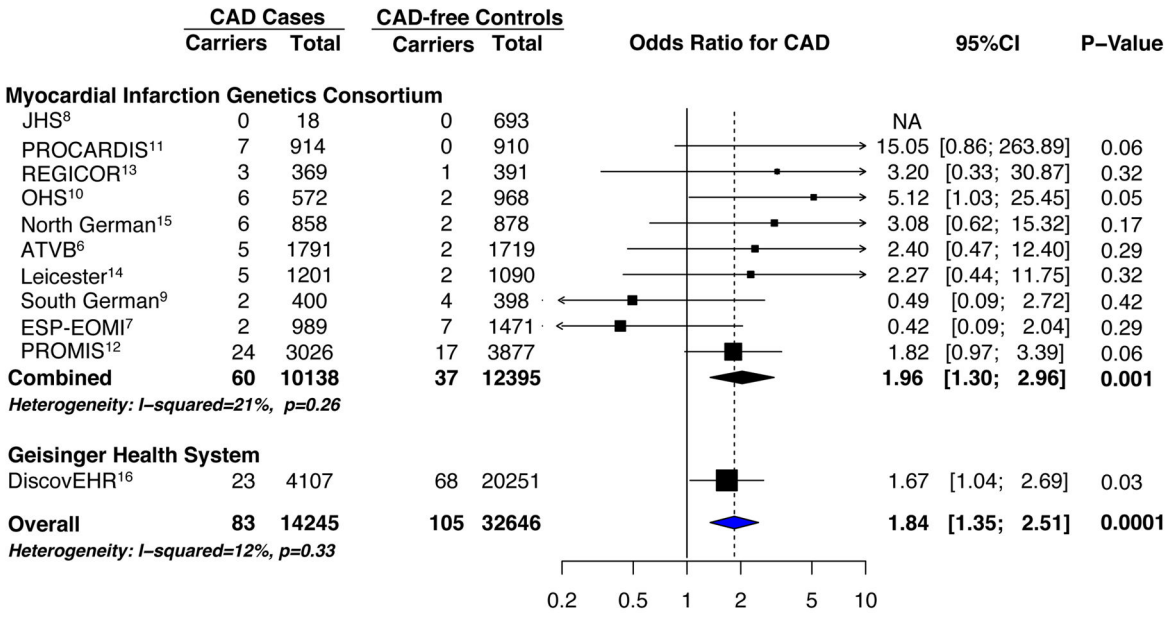

Figure 2.

Association of Damaging *LPL* Mutations with Coronary Artery Disease among 46,891 Individuals in 11 Studies.

In each study, the relationship of rare, damaging mutations in *LPL* with risk of coronary artery disease (CAD) was determined. P-values for association tests and confidence intervals were determined using exact methods. A meta-analysis across studies was performed with the use of the Cochran–Mantel–Haenszel statistics for stratified 2-by-2 tables. This method combines score statistics and is particularly useful when some observed odds ratios are zero. NA – Not Available; an odds ratio in the JHS cohort was not available due to absence of identified carriers of a damaging *LPL* mutation. The full study names are as follows: Jackson Heart Study (JHS), the Precocious Coronary Artery Disease Study (PROCARDIS), the Registre Gironi del COR (Gerona Heart Registry or REGICOR) study, the Ottawa Heart Study (OHS), the North German Myocardial Infarction study, the Italian Atherosclerosis Thrombosis and Vascular Biology (ATVB) study, the Leicester study, the South German Myocardial Infarction study, the Exome Sequencing Project Early-Onset Myocardial Infarction (ESP-EOMI) study, the Pakistan Risk of Myocardial Infarction Study (PROMIS), and the DiscovEHR project of the Regeneron Genetics Center and the Geisinger Health System.

Table 1

Baseline Characteristics of the Myocardial Infarction Genetics Consortium and Early-Onset CAD DiscovEHR Study Participants.

	Myocardial Infarction Genetics Consortium		Geisinger Health System DiscovEHR Cohort[c]	
	CAD Cases (n = 10,138)	**CAD-free Controls (n = 12,395)**	**CAD Cases (n = 4,107)**	**CAD-free Controls (n = 20,251)**
Age, years, median (IQR)	45 (41–50)	60 (48–68)	52 (47–57)	46 (35–55)
Female Sex, n (%)	1,294 (28%)	4,276 (19%)	2,169 (53%)	16,334 (81%)
BMI, kg/m^2, median (IQR)	26 (24–29)	27 (25–31)	32 (28–38)	31 (26–37)
Current Smoker, n (%)	4,322 (47%)	2,406 (21%)	986 (24%)	4,110 (20%)
Medical History				
Type 2 Diabetes, n (%)	2,190 (25%)	1,942 (19%)	1,520 (37%)	2,661 (13%)
Hypertension, n (%)	2,918 (47%)	3,741 (42%)	3,373 (82%)	6,848 (34%)
Lipid-lowering Medication [a]	2,739 (31%)	473 (5%)	2,494 (61%)	3,711 (18%)
Lipid Phenotypes, mg/dL				
Total Cholesterol, median (IQR)[b]	216 (181–252)	197 (168–228)	209 (184–240)	198 (173–227)
LDL Cholesterol, median (IQR) [b]	138 (107–171)	120 (96–147)	124 (101–151)	117 (96–142)
HDL Cholesterol, median (IQR)	37 (31–45)	42 (33–53)	44 (37–53)	50 (42–61)
Triglycerides, median (IQR)	166 (116–246)	133 (90–198)	154 (112–215)	120 (85–167)
Remnant Cholesterol, median (IQR)	33 (23–48)	28 (19–40)	33 (22–50)	24 (16–35)

Percentages indicative of participants with non-missing values.

[a] At the time of lipid measurement.

[b] Total and LDL cholesterol values were divided by 0.8 and 0.7 respectively in those on lipid-lowering medication to estimate untreated values.

[c] Participants were considered to have early-onset (men <55 years, women <65 years) coronary artery disease if they had a history of coronary revascularization in the electronic health records, or history of acute coronary syndrome, ischemic heart disease, or exertional angina (ICD-9 codes 410*, 411*, 412*, 413*, 414*) with angiographic evidence of obstructive coronary atherosclerosis (>50% stenosis in at least one major epicardial vessel from catheterization report). Participants were considered to have diabetes if they had at least 2 out of (i) a history of type 2 diabetes in the electronic health record, (ii) antidiabetic medication use, or (iii) fasting glucose greater than 126 mg/dL or hemoglobin A1c greater than 6.5 percent Participants were considered to have hypertension if they had a history of hypertension in the electronic health records, antihypertensive medication use, or systolic blood pressure greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg.

CAD: coronary artery disease; IQR: interquartile range; SI conversion factor: To convert cholesterol to mmol/L, multiply values by 0.0259. To convert triglyceride levels to mmol/L, multiple values by 0.01129.

Table 2

Association of Damaging *LPL* Mutations with Coronary Artery Disease by Rare Variant Class in the Myocardial Infarction Genetics Consortium Studies and Early-Onset CAD DiscovEHR Study

Variant Class	Loss of Function	ClinVar Pathogenic	Predicted Damaging Missense	Combined
Myocardial Infarction Genetics Consortium				
Number of Variants	8	6	38	52
N (%) Carriers of 10,138 CAD Cases	7 (0.07%)	15 (0.15%)	38 (0.37%)	60 (0.59%)
N (%) Carriers of 12,395 Controls	2 (0.02%)	5 (0.04%)	30 (0.24%)	37 (0.30%)
Beta Coefficient for Association with Triglycerides, mg/dL (95% CI)	+35.6 (−4.8 – 119.4) P = 0.41	+ 18.2 (−50.3 – 86.7) P = 0.60	+ 25.6 (−7.3 – 58.5) P = 0.13	+ 25.6 (−2.5 – 53.5) P = 0.07
Odds Ratio for CAD (95% CI)	4.33 (0.85 – 21.96) P = 0.08	3.47 (1.25 – 9.58) P = 0.02	1.55 (0.96 – 2.50) P = 0.07	1.96 (1.30 – 2.96) P = 0.001
Geisinger Health System DiscovEHR Cohort				
Number of Variants	3	7	15	25
N (%) Carriers of 4,107 CAD Cases	1 (0.02%)	6 (0.15%)	16 (0.39%)	23 (0.56%)
N (%) Carriers of 20,251 Controls	2 (0.01%)	28 (0.14%)	38 (0.19%)	68 (0.34%)
Beta Coefficient for Association with Triglycerides, mg/dL (95% CI)	+194.6 (92.7 – 296.4) P < 0.001	+29.3 (−0.8 – 59.3) P=0.06	+2.4 (−20.1 – 24.9) P=0.83	+ 17.2 (−0.5 – 34.9) P = 0.07
Odds Ratio for CAD (95% CI)	2.47 (0.22 – 27.2) P = 0.46	1.06 (0.44 – 2.55) P=0.90	2.08 (1.16 – .2.69) P=0.01	1.67 (1.04 – 2.69) P = 0.03

Rare variants refer to those with minor allele frequency <1% in the sequenced population. Loss of function variants were defined as single base changes that introduce a stop codon leading to premature truncation of a protein (nonsense), insertions or deletions (indels) of DNA that disrupt the translated protein's amino acid sequence beyond the variant site (frameshift), or point mutations at sites of pre-mRNA splicing that alter the splicing process (splice-site). Predicted damaging variants refer to those predicted to be deleterious or possibly deleterious by *each* of five *in silico* prediction algorithms (LRT score, MutationTaster, PolyPhen-2 HumDiv, PolyPhen-2 HumVar and SIFT). Beta coefficients reflective of the difference in triglyceride concentrations between carriers of a damaging *LPL* mutation, as compared to noncarriers, were derived from linear regression analysis that included adjustment for age, age^2, gender, cohort, and the first five principal components of ancestry. Principal components of ancestry were based on observed genotypic differences across subpopulations (e.g. race or ethnicity) in the overall study. Inclusion of principal components as covariates in linear regression analyses increases statistical power for true relationships and minimizes confounding by ancestry.[27] The association of *LPL* mutations with risk of CAD was determined via meta-analysis implementing Cochran–Mantel–Haenszel statistics for stratified 2-by-2 tables.

CAD: coronary artery disease; CI: confidence interval.