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

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2010 and 2015 at the Broad Institute as previously described.[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 nonduplicate reads were locally realigned and base qualities were recalibrated using Genome Analysis Toolkit software.[21] Variants were jointly called using Genome Analysis Toolkit HaplotypeCaller software. The sensitivity of the selected variant quality score recalibration threshold was 99.6% for single-nucleotide polymorphisms and 95% for insertion or 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 were extracted from exome sequences generated at the Regeneron Genetics Center between 2014 and 2015 as previously described.[16]

Damaging *LPL* Variant Ascertainment

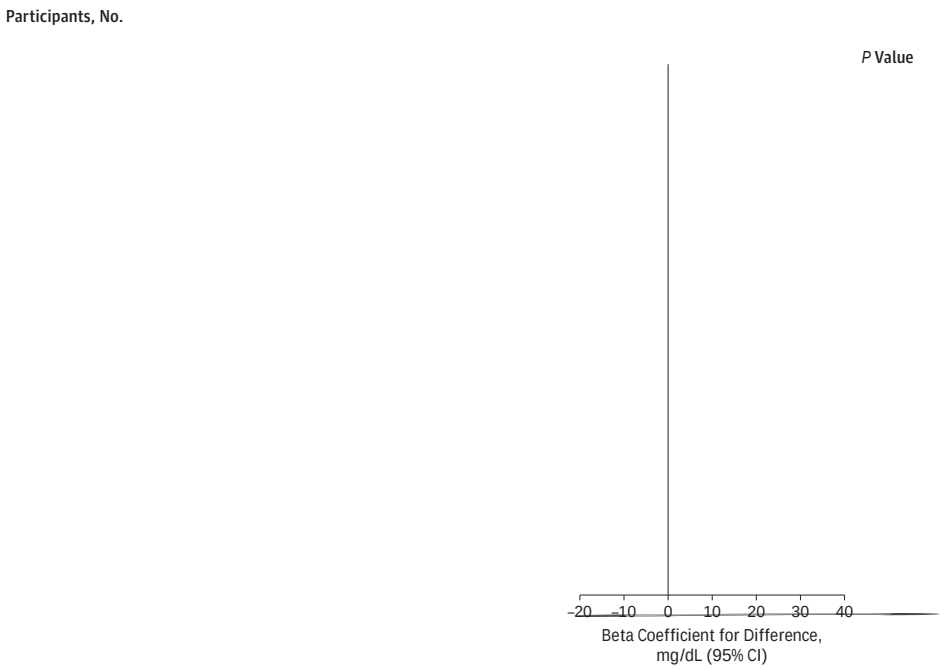
The positions of genetic variants were based on the complementary DNA reference sequence for *LPL* (RefSeq NM_000237.2). Rare *LPL* variants (minor allele frequency <1%) were annotated with respect to the following 3 classes in a sequential fashion: (1) loss-of-function variants, ie, single base changes 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–messenger RNA 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]; and (3) missense variants predicted to be damaging or possibly damaging by *each* of 5 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 its associated Loss-of-Function Transcript Effect Estimator (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 squared, sex, study cohort, and the first 5 principal components of ancestry. Principal components of ancestry were based on observed genotypic differences across subpopulations (eg, 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 using Cochran-Mantel-Haenszel statistics for stratified 2-by-2 tables without continuity correction as implemented previously.[9,18,28]

Common variants (allele frequency >1%) at the *LPL* locus independently associated with circulating triglyceride levels were ascertained via analysis of the Global L.3(ace9(minimiz)5(es)-286.9(conf50y)-281N58n)-293(53)-32d(293(53)-3)14.9(tld 4.3(5

Figure 1. Association of Damaging Lipoprotein Lipase Gene (*LPL*) Mutations With Circulating Lipid Concentrations



Discussion

The protein-coding exons of *LPL* 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 0.40% of individuals carried a rare damaging mutation in *LPL*. These carriers had increased circulating triglyceride levels (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.

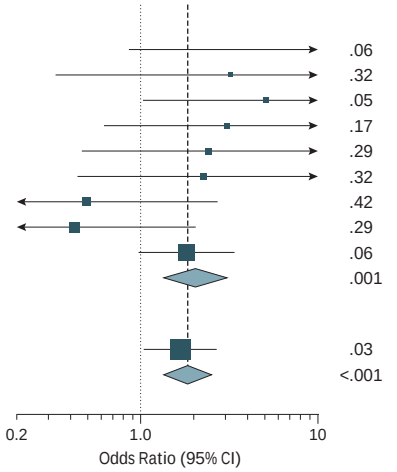
These results permit several conclusions. First, heterozygous LPL deficiency was associated with the presence of early-onset CAD. By identifying 188 carriers of a rare damaging mutation, an association with higher levels of triglycerides and remnant cholesterol and lower levels of high-density lipoprotein 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 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 6 independent *LPL* variants confirmed the association of genetic variation in *LPL* with CAD. In an analysis in more than 300 000 individuals, each common variant's association with triglyceride levels was used as a proxy for influence on LPL activity. Association of these same variants with CAD in more than 120 000 individuals demonstrated an odds ratio for CAD of 1.51 per 1-SD increase in triglyceride levels 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-C, LPL and its endogenous regulation—via facilitator (apolipoprotein A5 [APOA5]) and inhibitor (apolipoprotein C3 [APOC3], angiopoietin-like 4 [ANGPTL4]) proteins—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 LPL activity. Whether therapy to alter this pathway will decrease risk of CAD remains unknown.

Figure 2. Association of Damaging Lipoprotein Lipase Gene (*LPL*) Mutations With Coronary Artery Disease (CAD) Among 46 891 Individuals in 11 Studies

	LPL Mutation Carriers/Total, No.			
	CAD Cases	CAD-Free Controls	Odds Ratio for CAD (95% CI)	*P* Value
Myocardial Infarction Genetics Consortium				
JHS[8]	0/18	0/693	NA	
PROCARDIS[11]	7/914	0/910	15.05 (0.86-263.89)	.06
REGICOR[13]	3/369	1/391	3.20 (0.33-30.87)	.32
OHS[10]	6/572	2/968	5.12 (1.03-25.45)	.05
North German[15]	6/858	2/878	3.08 (0.62-15.32)	.17
ATVB[6]	5/1791	2/1719	2.40 (0.47-12.40)	.29
Leicester[14]	5/1201	2/1090	2.27 (0.44-11.75)	.32
South German[9]	2/400	4/398	0.49 (0.09-2.72)	.42
ESP-EOMI[7]	2/989	7/1471	0.42 (0.09-2.04)	.29
PROMIS[12]	24/3026	17/3877	1.82 (0.97-3.39)	.06
Combined Heterogeneity: I^2=21%, P=.26	60/10138	37/12395	1.96 (1.30-2.96)	.001
Geisinger Health System				
DiscovEHR[16]	23/4107	68/20251	1.67 (1.04-2.69)	.03
Overall Heterogeneity: I^2=12%, P=.33	83/14245	105/32646	1.84 (1.35-2.51)	<.001



In each study, the relationship of rare damaging mutations in *LPL* with risk of CAD was determined. *P* values for association tests and confidence intervals were determined using exact methods. A meta-analysis across studies was performed using 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 0. An odds ratio in the Jackson Heart Study (JHR3clSQq6(when)-191uho Study Studyls

A key strength of the present analysis is that *LPL* 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 EHRs. 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 *LPL* 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 level elevation among mutation carriers would likely have been greater if postprandial 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, levels of both triglycerides and calculated remnant cholesterol, the primary lipid components of triglyceride-rich lipoproteins, were increased in individuals harboring an *LPL* mutation. Because the level of 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 *LPL* was significantly associated with higher triglyceride levels and presence of CAD. However, further research is needed to assess whether there are causal mechanisms by which heterozygous LPL deficiency could lead to CAD.

ARTICLE INFORMATION

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