LIPA Variants in Genome-Wide Association Studies of Coronary Artery Diseases
Loss-of-Function or Gain-of-Function?

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Genome-wide association studies (GWASs) have identified multiple coronary artery disease (CAD) risk loci, yet moving from association to mechanistic insights and therapeutic translation remains a major challenge. Several GWASs have identified LIPA as a novel locus for CAD. LIPA encodes lysosomal acid lipase (LAL), the major lysosomal enzyme hydrolizing cholesteryl esters (CEs) and triglycerides derived from lipoproteins taken up by cells. Before its GWAS discovery for CAD, loss-of-function (LOF) mutations in LIPA were identified as the cause of rare Mendelian disorders, including Wolman disease, an infantile-onset disorder due to complete LOF mutations, as well as cholesteryl ester storage disease (CESD), a later-onset disorder with residual LAL activity resulting in hepatosplenomegaly, hyperlipidemia, liver failure, and premature atherosclerosis. Although rare LIPA LOF alleles in CESD are linked to accelerated atherosclerosis and hyperlipidemia, surprisingly the common LIPA CAD risk alleles are not associated with altered plasma lipids, liver traits, or reduced expression of LIPA in liver. Indeed, the CAD risk alleles have no expression quantitative trait locus (eQTL) in liver tissue yet do, however, have eQTLs for higher LIPA mRNA in monocytes and macrophages.

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These paradoxical data raise important questions for the field, particularly as LAL enzyme replacement therapy is approved for clinical use in Wolman disease and CESD, but whether this will ameliorate premature atherosclerosis and affect cardiovascular outcomes remains to be determined. First, what is the directionality of CAD causal variant(s) at the LIPA GWAS locus—are they LOF as might be anticipated based on the effects of rare Mendelian variants that cause CESD and atherosclerosis, or are these unexpectedly gain of function variants that increase LIPA expression and function in monocytes/macrophages, as suggested by the eQTL studies? If gain-of-function or LOF variants in monocytes/macrophages contribute to CAD in the general population, what is the underlying biological mechanism? Second, is the CAD-associated actions of the LIPA GWAS locus cell specific, restricted to actions in monocytes/macrophages and not active in hepatocytes, as one might surmise based on cell-specific eQTL data? Third, what is the genetic mechanism of the CAD locus in the disease-relevant cells—and is there one or many functional variants that contribute to the GWAS CAD signal?

In this issue, Morris et al. have begun to tackle these intriguing questions in their study of the potential causal variant for CAD at the GWAS LIPA locus and the directionality of its actions (Figure). They report that rs1051338 (NM_000235.3:c.46A>C, p.Thr16Pro), a coding variant in high linkage disequilibrium with the GWAS lead single-nucleotide polymorphisms (SNPs), may serve as the potential causal variant at the LIPA locus for CAD. By using in silico prediction, overexpression of LAL in COS7 cell lines, and comparing LAL expression and activity in primary macrophages from risk allele and nonrisk allele carriers, the authors propose that the risk allele (C) at rs1051338, which encodes a nonsynonymous threonine to proline change (Thr16Pro) within the signal peptide of LAL, may impair LAL protein translocation from the endoplasmic reticulum resulting in proteosomal degradation and reduced LAL protein and activity in macrophages. The results also showed that lysosomal LAL activity in the risk allele carriers was lower than that in the nonrisk allele carriers and that this was associated with a trend toward reduced efflux after [3H]-cholesterol–labeled acetylated low-density lipoprotein loading. Thus, they propose that the GWAS risk locus for CAD is indeed LOF and mediated by the rs1051338 LIPA variant.

Although these data are highly suggestive, they are not yet definitive for rs1051338 being “the” causal variant at the LIPA GWAS locus. The effects of the variant on LAL protein degradation were determined by exogenous LAL overexpression in COS7 cell line, a line that is not a disease-relevant cell type and the results mainly relied on the use of pharmacological inhibitors. Some experiments were repeated using human monocyte–derived macrophages but only in 4 homozygous risk allele and nonrisk allele carriers, which is a small sample size for detection of the modest effects expected of a common variant for a complex trait identified by GWAS. Indeed, this is revealed by the lack of difference in LIPA expression in the monocytes and macrophages in the presented data despite the published eQTL for rs1051338 and other linked GWAS lead SNPs at the locus. Furthermore, the functional impact of the variant examined in this study focused only on the efflux capacity of [3H]-cholesterol–labeled acetylated low-density lipoprotein.
and failed to show a statistically significant difference by allele groups. Other phenotypes relevant to the LOF of \textit{LIPA}, such as lysosomal CE hydrolysis, autophagy, and macrophage alternative activation were not studied.

In addition to rs1051338, there are other linked SNPs at the \textit{LIPA} locus, including those of similar allele frequency yet showing stronger association with increased risk of CAD and also with higher mRNA expression in eQTLs. Some of these SNPs overlie open chromatin marks and other epigenetic features suggesting regulatory actions that might increase \textit{LIPA} expression and contribute to CAD. This study does not exclude this alternative hypothesis, one that is supported indirectly by data showing that increasing free cholesterol levels in lysosomes inhibit lysosome acidification and function and subsequent hydrolysis of lipoprotein CE, and that extracellular lysosomal synapse can degrade aggregated low-density lipoprotein and contribute to foam cell formation. The functional effects, or lack thereof, of the linked SNPs in the region on \textit{LIPA} transcription and mRNA expression in both monocytes and macrophages remain to be studied. This is particularly important, as this study did not address the published and surprising eQTL data of higher \textit{LIPA} mRNA levels if the rs1051338 coding variant is indeed causal for CAD and encodes LOF of LAL activity.

Studying cell lines with endogenous \textit{LIPA} expression on an isogenic background when the only genetic difference is each individual SNP will provide more reliable data than studying endogenous cells where the effects of individual variants in high linkage disequilibrium at the locus cannot be separated—as is the case for the current studies by Morris et al. Gene editing of human-induced pluripotent stem cells to introduce separately each risk allele or to correct each risk allele, ideally differentiating the human-induced pluripotent stem cell lines to macrophages for functional studies, is ultimately required to provide definitive data in support of causal effects of any individual or combination of SNP variants.

In summary, Morris et al present important data suggesting that the rs1051338 Thr16Pro variant, in the LAL signal peptide, may be a causal LOF variant at the \textit{LIPA} GWAS locus. Yet these studies do not address fully the \textit{LIPA} paradox. Ultimately, gene editing of isogenic human-induced pluripotent stem cell with differentiation to macrophages (and other \textit{LIPA} and CAD-relevant cells) coupled to study the primary macrophages of much larger numbers of risk and nonrisk allele carriers is warranted to parse the individual effects of each of the linked variants at the \textit{LIPA} GWAS locus. Targeted mouse models with knockin of human \textit{LIPA} CAD alleles will also help to reveal the in vivo effects of specific variants at this GWAS locus.
locus on atherosclerosis. Further mechanistic study of macrophage LIPA in CAD risk will shed light on the potential for benefit and risk in therapeutic targeting of LIPA in CAD, particularly in the context of the availability of LAL replacement therapy currently approved for use in patients with CESD.

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None.

References


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