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Gain-of-function variants in lipid genes enhance biological insight and point toward therapeutic opportunities

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Dyslipidemia is associated with atherosclerotic cardiovascular disease (ASCVD), the leading cause of death worldwide¹. Plasma levels of low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides are all highly correlated with ASCVD risk and are demonstrated heritable traits². The study of individuals with extreme lipid phenotypes has led to the identification of rare variants in multiple genes that have a major influence on lipoprotein metabolism. In several cases, these discoveries have contributed to the validation of new therapeutic targets and ultimately to effective and approved therapeutics. For example, loss-of-function (LoF) mutations in *MTTP* and *APOB* that cause abetalipoproteinemia and hypobetalipoproteinemia, respectively, are associated with very low LDL-C and led to the development of lomitapide, a small molecule MTP inhibitor, and mipomersen, an antisense oligonucleotide (ASO) to *APOB*, both approved for homozygous familial hypercholesterolemia (HoFH)³. In a family with familial combined hypolipidemia (low LDL-C, HDL-C, and triglycerides), LoF mutations in *ANGPTL3* provided validation of this secreted protein as a therapeutic target, and evinacumab is an inhibitory antibody approved for HoFH and being studied for treatment of hypertriglyceridemia⁴. An especially interesting example is that of *PCSK9*; initial studies in families with very high LDL-C identified rare missense variants in *PCSK9* that are now known to be gain-of-function (GoF) variants, whereas some individuals with very low LDL-C levels were found to have classic LoF variants. *PCSK9* is now a target of two approved antibodies and an approved siRNA therapeutic (inclisiran)⁵. The example of *PCSK9* demonstrates the immense biological insights derived from allelic series that include GoF variants, and the lipid field has been interested in the identification of GoF variants in other genes known to affect lipoprotein metabolism. In this issue of *Circulation*, Dijk and colleagues report the first GoF variant in the *LIPC* gene which encodes the enzyme hepatic lipase (HL).

The investigators focused on DNA sequencing of individuals with extreme lipid phenotypes using a custom panel of known lipid-related genes. In one proband with very low levels of LDL-C and HDL-C they identified a private variant in *LIPC*: p.Glu97Gly (E97G). Within the extended family, carriers of the *LIPC*-E97G variant exhibited low LDL-C and

HDL-C levels. The authors used homology-based modeling to hypothesize that the amino acid substitution modifies a conserved motif affecting substrate access to HL's catalytic site. Experimental studies confirmed that the E97G mutation altered substrate specificity, leading to *increased* HL phospholipase activity with no effect on triglyceride lipase activity *in vitro*. Mice overexpressing *LIPC-E97G* recapitulated the combined hypocholesterolemic phenotype and provided key insight into the variant's effect on lipoprotein kinetics: this novel GoF variant was shown to promote the clearance of cholesterol-containing lipoprotein remnants from blood. Surprisingly, much of this accelerated clearance in mice was by extra-hepatic tissues (namely, adipose tissue depots and oxidative tissues). The discovery of this private GoF variant *LIPC-E97G* demonstrates that HL phospholipase activity is specifically critical in apoB-lipoprotein and HDL-C metabolism.

LIPC-E97G represents the first GoF variant in *LIPC/HL*, and is shown to lead to hypocholesterolemia. Importantly, LoF variants in *LIPC/HL* were first identified by Hegele in 1991 as a rare cause of *hypercholesterolemia*⁶. Thus, *LIPC* joins *PCSK9* as another lipid gene in which both LoF and GoF variants have been identified and result in plasma lipid levels at opposite ends of the spectrum, creating a full allelic series. Interestingly, the best-known GoF variant in the lipid field is a variant in lipoprotein lipase (*LPL*), a close relative of HL. Rare biallelic LoF mutations in *LPL* cause familial chylomicronemia syndrome (FCS) characterized by severe hypertriglyceridemia and risk of pancreatitis. In contrast, the relatively common *LPL* variant S447X is a surprising GoF variant which truncates the protein at the C-terminal by only two amino acids. This loss of terminal amino acids enhances LPL activity, thereby decreasing plasma triglycerides, increasing HDL-C, and modestly protecting against cardiovascular disease⁷. This fascinating experiment of nature proved that therapeutic intervention to increase LPL activity would not only reduce triglycerides but would also reduce risk of ASCVD and has fueled intense interest in developing other approaches to increasing LPL activity. It also led to the use of this GoF variant in a gene therapy approach for LPL deficiency in humans (alipogene tiparvovec) that was approved in Europe⁸.

Apolipoprotein C-III (gene *APOC3*) is an endogenous inhibitor of LPL that is transported on triglyceride-rich lipoproteins. LoF variants in *APOC3* are proven to reduce triglyceride levels (through increased LPL activity) and provide protection against ASCVD. One missense variant in *APOC3* (A43T) behaves like a LoF variant due to reduced lipid binding and rapid clearance of poorly lipid-bound protein, providing insight into ApoC-III structure-function⁹. *APOC3-Q38K* is an interesting potential GoF variant that may provide further insight. Like *LIPC-E97G*, this mutation was initially identified in a single family with increased plasma triglycerides¹⁰. *APOC3-Q38K* has been reported to increase very low-density lipoprotein (VLDL) assembly and secretion via *de novo* lipogenesis¹¹. Recent work also demonstrates that the substitution increases the LPL inhibitory capacity of the protein and enhances ApoC-III's affinity for ApoB-containing lipoproteins while reducing its affinity for HDL particles¹². Other GoF variants in *APOC3* are likely to be identified. In contrast to *APOC3*, Apolipoprotein A-V (gene *APOA5*) is an enhancer of LPL. LoF variants in *APOA5* have been reported in association with hypertriglyceridemia and increased risk of pancreatitis and ASCVD¹³. To date, no GoF variants in *APOA5* have been characterized, but their discovery will be of great interest in helping define the structure-function properties

of ApoA-V and as a potential tool for *APOA5*-based therapeutics. Leverage of GoF variants in a therapeutic setting has influenced the development of gene therapy approaches as seen with Factor IX Padua in hemophilia¹⁴.

Continued identification and mechanistic study of other naturally-occurring GoF variants in lipid genes will provide expansion of the allelic series, insight into structure-function, and potentially alternative approaches to lipid-lowering therapeutic strategies (Figure). *LIPC-E97G* alters HL's phospholipid substrate preference to resemble more closely that of its relative *LIPG* (endothelial lipase, EL). *ANGPTL3* is an inhibitor of EL and the LDL-lowering effect of *ANGPTL3* inhibition is apparently due to the increased activity of EL¹⁵. One can imagine how a GoF variant in EL (not yet described) might result in a hypocholesterolemic phenotype and further elucidate the role of phospholipase activity in lipoprotein catabolism. Detailed variant-driven structure-function assessment of other key players in the lipase pathways (e.g., apolipoproteins, chaperones, inhibitors) will add to our understanding of the biology of these proteins and enhance our therapeutic arsenal.

These interesting findings by Dijk, et al. arose from the discovery of a private variant identified in a single family with an extreme lipid phenotype. Careful mechanistic study of this variant revealed *LIPC-E97G* as the first GoF variant in HL and demonstrates the power of 'N of 1' discoveries in humans with extreme phenotypes. Structure-function analysis of HL and other proteins and their variants of interest continues to uncover novel and relevant biology with significant therapeutic implications. The insight gained from *LIPC-E97G* makes a strong case for detailed mechanistic investigations of other proteins and their naturally occurring mutations, leveraging human phenotypic and genotypic data to understand protein structure-function and provide insights into new therapeutic approaches.

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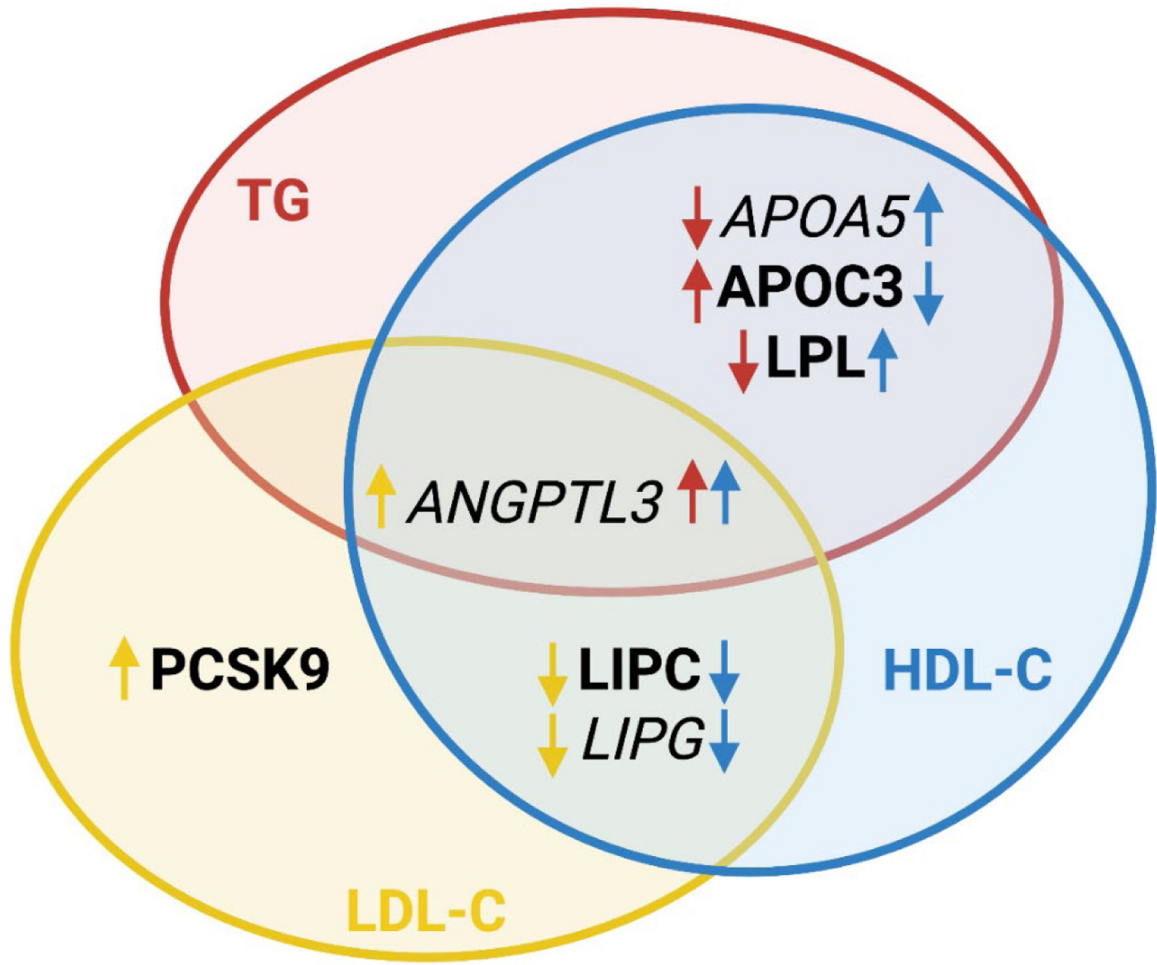


Figure. Observed or predicted effects on plasma lipids by gain-of-function (GoF) variants in selected lipid genes.

The diagram depicts the observed (**bold**) and predicted (*italic*) effects of GoF variants in selected genes on triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). Color of the arrows refers to the lipid parameter affected by GoF variants in indicated genes: red - TG, blue - HDL-C, and yellow - LDL-C. Direction of the arrows indicates the direction of effect: ↑ increase or ↓ decrease of indicated lipid parameters. Gene symbols: APOA5, apolipoprotein A5; APOC3, apolipoprotein C3; LPL, lipoprotein lipase; ANGPTL3, angiopoietin like 3; PCSK9, proprotein convertase subtilisin/kexin type 9; LIPC, lipase C, hepatic type; LIPG, lipase G, endothelial type.