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commentary

Multidimensional regulation of lipoprotein lipase: impact on biochemical and cardiovascular phenotypes[1]

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LPL contributes profoundly to physiologic lipoprotein metabolism and to tissue-specific substrate delivery and utilization (1). Perturbed LPL activity affects global energy balance, insulin action, body weight maintenance, and CVD risk; the latter alluded to by contemporary human genetic studies. LPL is the pivotal rate-limiting mediator of hydrolysis of core TGs from TG-rich lipoproteins, particularly chylomicrons and VLDL (2, 3). The products of LPL-mediated catalysis, such as fatty acids and monoacylglycerol, are handled differentially at local sites depending on the global hormonal and nutritional milieu, and local energy needs. For instance, in the fasted state, LPL activity in adipose tissue is suppressed, while it is increased in skeletal and cardiac muscle, shunting fatty acids away from storage and toward utilization in heavily oxidizing tissues. Conversely, after eating, LPL activity in adipose tissue is enhanced, while it is suppressed in skeletal and cardiac muscles, shunting fatty acids toward storage. Similar tissue-specific modulation of LPL activity is related to cold and exercise (1–4).

Recently, the complexity of the regulation of LPL secretion and activity has become more apparent; some insights have emerged from studying natural human genetic variants. LPL is regulated at transcriptional, posttranscriptional, and posttranslational levels (1–4); furthermore, depending on local milieu and needs, this regulation is tissue specific. At least 10 gene products govern the secretion and activity of LPL at different stages of its life cycle. LPL is primarily expressed in tissues that oxidize or store fatty acids in large quantities, such as the heart, skeletal muscle, and brown and white adipose tissue.

Although various factors influence LPL gene transcription (1–4), much of the physiological variation in LPL activity, i.e., related to feeding-fasting cycles and exercise, appears to be driven via posttranslational mechanisms by extracellular proteins. These proteins can be divided into two main groups: the liver-derived apolipoproteins, which are products of the *APOC1*, *APOC2*, *APOE*, *APOC3*, and *APOA5* genes, and the more broadly expressed angiopoietin-like (ANGPTL) proteins, specifically the products of the *ANGPTL3*, *ANGPTL4*, and *ANGPTL8* genes. But even prior to regulation by apolipoproteins and ANGPTL proteins, LPL secretion and delivery to the vascular space is in the hands of other intermediaries, including products of lipase maturation factor 1 (*LMF1*) and glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (*GPIHBP1*) genes. In addition to classical cellular and molecular biological studies of these various proteins, studies of naturally occurring human genetic variants have helped fill in some gaps in understanding of LPL regulation. Selected information regarding genes affecting LPL discussed below is summarized in **Table 1**.

CENTRAL ROLE OF LPL ITSELF

The central nonredundant role of basal LPL mass and activity in directing intravascular hydrolysis of TG-rich lipoproteins is underscored by the causative role of very rare homozygous loss-of-function variants in the *LPL* gene in patients with severe hypertriglyceridemia (essentially chylomicronemia) and increased risk of pancreatitis (5, 6). About 40% of patients diagnosed clinically with LPL deficiency have rare loss-of-function variants on both *LPL* alleles (5); among all patients with severe hypertriglyceridemia, ~10% have one or two probable pathogenic *LPL* variants. Rare instances of atherosclerosis observed in patients with complete LPL deficiency are exceptions that seem to prove the rule that severe chylomicronemia due to complete LPL deficiency is not associated with increased atherosclerosis risk (7). Heterozygotes for these

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TABLE 1. Selected gene products that interact with LPL and their genetic associations with plasma lipids and CHD risk

Protein	Gene Symbol/ Chromosomal Position	Effect on LPL Activity	Biochemical Phenotypes		CHD Association
			Rare Variants	Common Variants	
LPL	*LPL*/8p21.3	Reference	Hmz LOF: chylomicronemia Het LOF: increased risk of severe HTG	LOF: higher TG, lower HDL-C GOF: lower TG, higher HDL-C	Common LOF: increased CHD risk Common GOF: reduced CHD risk Rare LOF Het: likely increased CHD risk Rare LOF Hmz: none
LMF1	*LMF1*/16p13.3	Promotes	Hmz LOF: chylomicronemia Het LOF: increased risk of severe HTG	GWAS: none	Common or rare: none
GPIHBP1	*GPIHBP1*/8q24.3	Promotes	Hmz LOF: chylomicronemia Het LOF: increased risk of severe HTG	GWAS: none	Common or rare: none
apoC-I	*APOC1*/19q13.3	Inhibits	Het LOF: reduced TG	GWAS: none	Common or rare: none
apoC-II	*APOC2*/19q13.3	Promotes	Hmz LOF: chylomicronemia Het LOF: increased risk of severe HTG	GWAS: none	Common or rare: none
apoE	*APOE*/19q13.3	E2 isoform inhibits	Het rare LOF variants: dysbetalipoproteinemia	Hmz E2 plus 2° factors: dysbetalipoproteinemia	E2 Het or Hmz without dysbetalipoproteinemia: neutral or reduced CHD risk E2 Hmz in dysbetalipoproteinemia: increased CHD risk
apoC-III	*APOC3*/11q23.3	Inhibits	Het LOF: reduced TG, increased HDL-C	GOF from candidate gene studies: higher TG, lower HDL-C	Common GOF: increased risk MR rare Het LOF: reduced CHD risk
apoA-V	*APOA5*/11q23.3	Promotes	Hmz LOF: chylomicronemia Het LOF: increased risk of severe HTG	LOF from GWAS: higher TG; lower HDL-C	Common Het LOF: increased CHD risk MR rare Het LOF: increased CHD risk
ANGPTL3	*ANGPTL3*/1p31.3	Inhibits	Hmz LOF: familial combined hypolipidemia	GWAS: lower LDL-C, HDL-C, TG	Common or rare: none
ANGPTL4	*ANGPTL4*/19p13.2	Inhibits	Het LOF: reduced TG, increased HDL-C	GWAS: reduced TG, increased HDL-C	Common LOF: reduced CHD risk Rare Het LOF: reduced CHD risk
ANGPTL8	*ANGPTL8*/19p13.2	Inhibits	Het LOF: reduced TG, increased HDL-C	GWAS: none	Common or rare: none

Hmz, homozygous (can also refer here to compound heterozygous, or different mutations on two alleles); Het, simple heterozygous; LOF, loss-of-function; GOF, gain-of-function; HTG, hypertriglyceridemia; HDL-C, HDL cholesterol; TG, triglyceride; GWAS, genome-wide association study results; MR, Mendelian randomization study results.

loss-of-function variants are overrepresented in pools of patients with severe hypertriglyceridemia (formerly type 5 hyperlipoproteinemia) who do not have classical LPL deficiency (8).

In contrast, common small-effect *LPL* loss-of-function variants in the general population are associated with milder increases in plasma TGs, due in part to impaired clearance of VLDL (5). Furthermore, common LPL genetic variants that slightly raise TGs and lower HDL cholesterol are associated with increased coronary heart disease (CHD) risk, while a common LPL gain-of-function variant that modestly lowers TGs and raises HDL cholesterol has repeatedly been associated with protection from CHD (9, 10). Such genetic findings, in addition to consistently demonstrating the modestly altered biochemical phenotype, increasingly implicate partially altered LPL function as a determinant of CHD risk. A question that emerges is "do genetic variants in the interacting proteins impact on CHD risk in a manner consistent with their predicted effects on LPL?"

EARLY LIFE-CYCLE LPL HANDLING

Before its intravascular lipolytic activity undergoes in situ regulation, LPL needs to arrive safely at the endothelial surface of vasculature from its site of synthesis within various cell types, such as adipocytes or skeletal muscle. LMF1, encoded by the *LMF1* gene, is a membrane-bound chaperone protein located in the endoplasmic reticulum that is essential for the proper folding and assembly not only of LPL, but also of hepatic lipase and endothelial lipase (11). The involvement of LMF1 in the activity of multiple lipases is consistent with its implication initially as the causative gene for murine combined lipase deficiency (11, 12). As LMF1 participates exclusively in the maturation of homodimeric lipases, but not monomeric lipases, it probably mediates the assembly of inactive lipase subunits into active enzymes and may stabilize the active dimers (11, 12). Rare genetic variants in *LMF1* are associated with severe hypertriglyceridemia (chylomicronemia) in mice and humans, due to impaired lipid clearance from lipase

deficiency (12). Interestingly, common variants in *LMF1* have not been reported as being associated with either altered plasma lipoproteins or atherosclerosis in genome-wide association studies in populations.

After LPL is secreted from parenchymal cells, it needs to reach the endothelial cell surface. GPIHBP1, encoded by the *GPIHBP1* gene, seizes LPL in the interstitial space (13, 14) and shuttles it across endothelial cells to the lumenal surface; it also tethers LPL to the capillary endothelium (15). The reader is referred to the beautiful work from Young (15) in defining the structure and function of this fascinating protein. The essential role of GPIHBP1 in handling LPL is emphasized by the consequences of rare homozygous genetic variants that compromise GPIHBP1 function, including impairing its ability to bind LPL, resulting in mislocalization of LPL in the interstitial space and consequent severe hypertriglyceridemia (chylomicronemia). As with *LMF1*, there is no evidence that milder common heterozygous variants within *GPIHBP1* are associated with either altered plasma lipoproteins or atherosclerosis in genome-wide association studies in populations.

REGULATING LPL IN ITS WHEELHOUSE: APOLIPOPROTEINS

Several apolipoproteins exert direct and indirect effects on LPL once it settles upon its site of action. apoC-I is the product of the *APOC1* gene on chromosome 19. The human *APOC1* transgenic mouse has combined hyperlipidemia (16). apoC-I has been reported to inhibit LPL activity by preventing binding of LPL to the lipid-water interface of TG-rich lipoproteins and also by rendering LPL more susceptible to interference by ANGPTL4 (17). Recently, a rare variant (allele frequency 0.82%) in a noncoding region of *APOC1* was associated with a 13.6% reduction in plasma TG, but with no change in CHD risk (18). Tangentially, a relatively common *APOC1* missense variant (p.T45S) seen only in North American indigenous people was associated with lower body mass index (19).

apoC-II is a constituent of chylomicrons, VLDL, LDL, and HDL particles. apoC-II contains three amphipathic α-helices, an N-terminal lipid binding domain, and a C-terminal LPL binding domain (20). It is an essential cofactor for LPL activity; homozygous rare loss-of-function variants in *APOC2* leading to apoC-II deficiency are associated with a chylomicronemia phenotype that resembles LPL deficiency (21). Perhaps paradoxically, apoC-II concentrations are elevated in hypertriglyceridemia (22); overexpression of apoC-II in animal models also leads to severe hypertriglyceridemia (23), although an analogous situation in humans has not been observed. Rare heterozygous *APOC2* variants are among those that are overrepresented in pools of patients with severe hypertriglyceridemia (24), but not with increased CVD risk. There is no evidence that common *APOC2* variants are associated with either altered plasma lipoproteins or atherosclerosis in genome-wide association studies.

apoE, encoded by the *APOE* gene, is a component of TG-rich lipoproteins, their remnants, and HDL (25). Common polymorphisms at two sites in the *APOE* coding sequence underlie the classical apoE protein isoform system: E4 (arginine at both residues 112 and 158, without counting the prepeptide sequence; otherwise these are residues 130 and 176), E3 (cysteine at residue 112, arginine at residue 158), and E2 (cysteine at both residues 112 and 158). Homozygosity for the E2 isoform, in the context of additional genetic or nongenetic factors, can be associated with hyperlipidemia, characterized by increased plasma levels of chylomicron and VLDL remnants, associated with xanthomatosis and early atherosclerosis (26). In this pathological state, E2-containing particles accumulate in plasma: increased plasma cholesterol in these patients results from impaired clearance of E2-bearing particles, while increased TG is caused by interference with LPL by E2 (27). The hypertriglyceridemia in E2 transgenic mice can be corrected by directly activating LPL by overexpressing apoA-V, but not by deleting apoC-III (28). Also, there are a few ultra-rare apoE protein coding variants that can have dominant effects on the lipid phenotype, also with an associated increased CHD risk (29).

apoC-III, encoded by the *APOC3* gene, is a constituent of several apoB-containing lipoproteins, including chylomicrons, VLDL and LDL, and also HDL particles (30). Like apoC-I, apoC-III may inhibit LPL activity by preventing binding of LPL to the lipid-water interface of TG-rich lipoproteins and also by rendering LPL more susceptible to interference by ANGPTL4 (17). There is also substantial evidence that apoC-III plays a role in hepatic TG-rich lipoprotein production (31). Transgenic overexpression of human *APOC3* in mice is associated with severe hypertriglyceridemia (32), while targeted deletion of murine *Apoc3* is associated with low TG and protection from postprandial hypertriglyceridemia (33). Rare loss-of-function variants of *APOC3* are associated with moderately reduced TG, increased HDL cholesterol, and reduced CHD risk (34–36). Common variants in *APOC3*, particularly those in the promoter associated with failure to downregulate in the presence of insulin (37), have been associated with elevated TG and reduced HDL cholesterol (38). Thus, the human genetic data support a functional role for apoC-III in modulating both plasma lipoproteins and CHD risk in a manner that reflects the association of LPL genetic variation with these traits. Although the predominant opinion had been that apoC-III raised plasma TGs primarily by inhibiting LPL activity (39), the recent demonstration that antisense-mediated suppression of apoC-III plasma levels in patients with familial chylomicronemia syndrome, who had no measurable LPL activity, nevertheless dramatically lowered their elevated plasma TG levels indicates that apoC-III lowers TGs in a non-LPL-dependent manner (40). This now appears to be inhibition by apoC-III of receptor-mediated hepatic clearance of TG-rich lipoproteins (41). Whether interfering with apoC-III production or activity could prevent CHD in individuals with milder TG elevations remains to be proven.

apoA-V, encoded by the *APOA5* gene, is an exchangeable apolipoprotein discovered bioinformatically (42), that has a central role in metabolism of TG-rich lipoproteins (43). Some liver-derived apoA-V is secreted into plasma and facilitates LPL-mediated TG hydrolysis, while another portion is recoverable intracellularly, in association with cytosolic lipid droplets (43). Loss of apoA-V function is positively correlated with elevated plasma TG and increased CHD risk (44, 45). Homozygous rare loss-of-function variants in *APOA5* cause severe hypertriglyceridemia, essentially chylomicronemia (6). Heterozygous rare loss-of-function variants in *APOA5* were associated with modestly increased TG (in absolute terms), along with modestly decreased HDL cholesterol and increased CHD risk (44). Common variation at the *APOA1-C3-A4-A5* locus, likely by modulating apoA-V expression, was associated with increased TG, decreased HDL cholesterol, and increased CHD risk (45), although the locus is genetically complex and linkage disequilibrium with functional variants affecting apoC-III are possible. The common functional *APOA5* p.S19W coding variant is associated with a 3- to 6-fold increased risk of clinically ascertained hypertriglyceridemia (26). Another common functional *APOA5* variant, namely the −1131C>T promoter variant, was associated with increased TG, reduced HDL cholesterol, and increased CHD risk in a meta-analysis of 101 studies (46). Thus, the human genetic data support a functional role for apoA-V in modulating both plasma lipoproteins and CHD risk in a manner that parallels the association of these traits with LPL genetic variation.

ANGPTL PROTEINS AND LPL: LAYERS OF REGULATION

ANGPTL3, 4, and 8, (encoded by *ANGPTL3*, *ANGPTL4*, and *ANGPTL8* genes, respectively) each inhibit LPL activity (47). ANGPTL3 has N-terminal coiled-coil domains; cleavage by protein convertases frees the N-terminal domain to specifically inhibit LPL activity, possibly by interfering with its dimerization. ANGPTL3 was first identified as the causative gene explaining low plasma TG levels in KK/San mice (48). Common variants in *ANGPTL3* are associated with mild variations in plasma TG levels in normolipidemic populations (45). Ultra-rare large-effect loss-of-function variants in *ANGPTL3* are associated with markedly reduced levels of TGs and LDL cholesterol adhering to additive or autosomal codominant inheritance, while HDL cholesterol levels are also reduced following a recessive model; the phenotype had been referred to as "familial combined hypolipidemia" or "familial hypobetalipoproteinemia type 2" (18, 49). A recent whole-genome sequencing study confirmed the associations of rare variants in *ANGPTL3* (allele frequencies <0.5%) with reduced TGs (by 16–20%) and with reduced levels of non-HDL cholesterol (18). No study has shown a clear relationship between genetic variation in *ANGPTL3* and CHD risk. Here, the human genetic findings differentiate the possible modulatory role of ANGPTL3 on LPL function from the effects of apoC-III, apoA-V, and even ANGPTL4, because genetic variation at these other loci has minimal effect on LDL cholesterol with divergent effects on HDL cholesterol levels and CHD risk.

ANGPTL4 was initially identified as a peroxisome proliferator-activated receptor-dependent fasting-induced ANGPTL family member that inhibits LPL activity in an adipocyte-specific manner (47, 50, 51). Similar to ANGPTL3, ANGPTL4 also has N-terminal coiled-coil domains; cleavage by protein convertases frees the N-terminal domain to inhibit LPL activity, possibly by interfering with dimerization. Fasting, cold exposure, and exercise all affect ANGPTL4-mediated inhibition of LPL activity, which results in decreased storage of fatty acids in white adipose tissue and increased oxidation in muscle (47). ANGPTL4 appears to interact with LPL in at least three compartments: intracellularly, in the subendothelial spaces, and intravascularly. This multi-dimensional regulation allows for swift adaptation of LPL levels during different physiological conditions, such as fasting, exercise, and cold. It is difficult to speculate on the relative importance of these three levels for LPL inhibition, as the relative importance of each level may be determined by the global physiological state and may further depend on the specific target tissue.

In the current issue of the *Journal of Lipid Research*, Dijk et al. (52) report several complementary lines of investigation of the functional role of ANGPTL4 affecting intracellular LPL. In cotransfected CHO cells, primary adipocytes, and adipose tissue from wild-type and *Angptl4*$^{-/-}$ mice, they showed that ANGPTL4 reduced intracellular LPL protein levels, indicating an effect on LPL processing in addition to catalytic function. Absence of ANGPTL4 caused the mature-glycosylated form of LPL to accumulate and resulted in increased secretion of LPL. When they blocked endoplasmic reticulum-Golgi transport, they abolished differences in LPL abundance between wild-type and *Angptl4*$^{-/-}$ adipocytes, indicating that ANGPTL4 acts relatively late, after LPL is processed in the endoplasmic reticulum. Also, physiological changes in adipose tissue ANGPTL4 expression during fasting and cold were associated with inverse changes in the amount of mature-glycosylated LPL in wild-type mice, but not in *Angptl4*$^{-/-}$ mice. These experiments indicate that ANGPTL4 induces loss of intracellular LPL, probably by stimulating degradation after LPL processing in the endoplasmic reticulum. They provide experimental ballast to the idea that the intracellular interaction of ANGPTL4 with LPL can lead to measurable downstream phenotypic consequences.

Furthermore, ANGPTL4 has become extremely topical lately due to recent reports showing that carriers of either rare inactivating mutations or common loss-of-function variants (10, 53) had an improved plasma lipid profile, with reduced TGs and higher HDL cholesterol, together with reduced CHD risk. In particular, the *ANGPTL4* p.E40K variant, which is present in ~3% of Caucasians and has virtually no inhibitory activity due to attenuated

extracellular accumulation of both the full-length protein and N-terminal fragments, was previously shown to be associated with reduced TG and increased HDL cholesterol (54). Although earlier studies could not definitively tie this variant to cardioprotection (55), recent genetic findings clearly make this link (10, 53). Whole-genome sequencing of Icelanders also confirms an association between the ANGPTL4 p.E40K variant and reductions in TGs, non-HDL cholesterol, and CHD risk by 11.9%, 0.16 mmol/l, and 20%, respectively (18). Thus, the studies by Dijk et al. (52) provide mechanistic information about multiple actions of ANGPTL4 to inhibit adipose-derived LPL, helping to explain the improved lipid profile and protection from CHD among carriers of loss-of-function variants.

Finally, ANGPTL8, previously known under numerous aliases, including Gm6484, RIFL, lipasin, and β-trophin, is expressed in liver and both brown and white adipose tissue (51). Like ANGPTL3, its expression is stimulated by feeding and reduced by fasting (51); a direct effect on LPL activity has not been demonstrated. Instead, one model suggests that ANGPTL8 exerts its effects by activating ANGPTL3 in an endocrine manner to inhibit LPL activity in the heart and skeletal muscle (51). Mice lacking ANGPTL8 had lower TG levels, associated with both reduced VLDL secretion and increased LPL activity, indicating that ANGPTL8 plays a key role in the metabolic transition between fasting and refeeding; it might be required to direct fatty acids to adipose tissue for storage in the fed state (56). Although the ANGPTL8 locus did not yield a positive signal when common variants were studied with respect to plasma lipids and atherosclerosis end points, a low frequency ANGPTL8 stop codon variant (rs145464906, p.Q121X, allele frequency 0.1%) was associated with reduced TG and increased HDL cholesterol, but not with protection from CHD (57).

CAN GENETICS HELP SOLVE THE RIDDLE, MYSTERY, AND ENIGMA OF LPL REGULATION?

Do any patterns emerge after assembling genetic data related to common and rare variants in genes that impact upon LPL? To narrow the focus through the lens of human genetics; what does the amalgam of data on the role of genetic variation affecting candidates in the LPL pathway in Table 1 tell us not only about TG metabolism, but also about its potential role in atherosclerosis risk? Assuming that the fulcrum is the effect of LPL variation on plasma lipoproteins and atherosclerosis risk, then factors that interact with LPL at its multiple sites and roles may show comparable or analogous effects on these phenotypic readouts. Differences between the associations seen with LPL variation and variation of one of its interacting factors may also be hypothesis-generating.

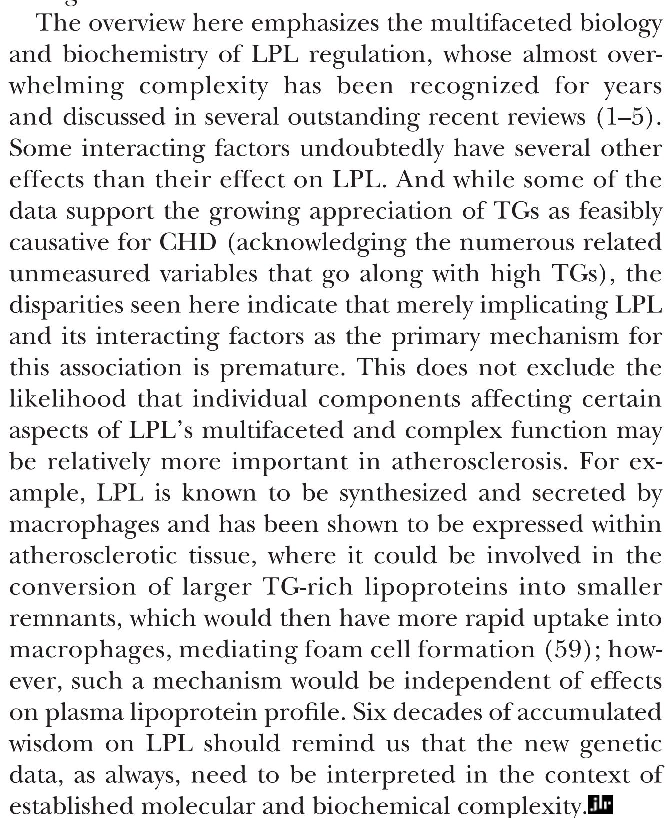
First, for reference, rare homozygous loss-of-function variants in *LPL* result in severe hypertriglyceridemia (familial chylomicronemia). This phenotype is also seen in patients with rare homozygous loss-of-function variants in *LMF1*, *GPIHBP1*, *APOC2*, and *APOA5* genes, consistent with the predictions of the effects of these gene products that promote LPL activity. In contrast, rare heterozygous loss-of-function variants in genes that encode inhibitors of LPL, such as *APOC3*, *ANGPTL3*, *ANGPTL4*, and *ANGPTL8*, are associated with reduced TG levels. However, some differences are seen regarding other components of the lipid profiles, such as differential effects on LDL and HDL cholesterol seen with variants in *ANGPTL3*, but not with variants in the other genes. The divergent associations seen with ANGPTL3 and ANGPTL4 variants may be due, in part, to the unique functions of ANGPTL4, as characterized by Dijk et al. (52). Finally, heterozygous ultra-rare *APOE* variants are associated with the specific form of hypertriglyceridemia seen in dysbetalipoproteinemia. Thus, rare large-effect genetic variants across this particular portfolio of genes are generally associated with marked perturbations in the lipid profile in a direction consistent with the known effect of the respective gene product on LPL activity.

Second, common variants in *LPL* encoding a gain-of-function are associated with relatively mildly reduced TGs, while those encoding a loss-of-function are associated with relatively mildly increased TGs. These mild phenotypes are also seen in carriers of common small effect variants in *APOC3*, *APOA5*, *ANGPTL3*, and *ANGPTL4* in genome-wide association study populations. Furthermore, the direction of the effect on plasma lipids is essentially consistent with the effect of the respective gene product on LPL activity. Interestingly, for several canonical genes in which rare variants underlie severe hypertriglyceridemia, such as *LMF1*, *GPIHBP1*, and *APOC2*, there are no accompanying common variants that produce association signals for mild perturbations in TGs in genome-wide association studies. Finally, homozygosity for the E2 isoform encoded by *APOE* is associated with the specific form of hypertriglyceridemia seen in dysbetalipoproteinemia, while the *APOC1* and *ANGPTL8* genes also have no common variants associated with the plasma lipid profile in genome-wide association study populations.

Third, homozygous rare loss-of-function variants in *LPL* that cause chylomicronemia are not obviously associated with CHD risk, while rare heterozygous loss-of-function variants in *LPL* that contribute to polygenic severe hypertriglyceridemia seem to be associated with increased CHD risk. In contrast, common variants in LPL encoding gain-of-function that are associated with relatively mildly reduced TGs (and increased HDL cholesterol) are associated with reduced CHD risk, while those encoding loss-of-function that are associated with relatively mildly increased TGs (and reduced HDL cholesterol) are associated with increased CHD risk. Rare variants in *APOC3*, *APOA5*, and *ANGPTL4* are associated with altered CHD risk in a direction that is consistent with their effects on TGs (and HDL cholesterol). Heterozygosity for *APOE* rare variants or homozygosity for the common *APOE* E2 isoform in the presence of secondary factors underlies dysbetalipoproteinemia, which has been associated with early CHD in observational studies. Common or rare variants in *LMF1*,

GPIHBP1, *APOC2*, and *ANGPTL8* are not associated with CHD risk, irrespective of their effects on lipids. Similarly, there is no demonstrated association between *ANGPTL3* or *APOC1* variants and CHD risk.

The less consistent associations between CHD and variants in these LPL-interacting gene products could have several explanations. First, it may be that plasma TGs are not the direct causative agent and may merely serve as an indirect marker for causal factors, such as the cholesterol content of atherogenic remnants, that are not measured clinically or epidemiologically (58); different gene products with the same impact on TG levels may have different effects on such unmeasured analytes. Also, the various gene products may have additional and variable effects on other unmeasured causative variables outside plasma lipoprotein metabolism. Overall, the findings demonstrate how variation in the genes encoding the interacting factors with LPL does not automatically translate on a one-to-one basis with associations with severe hypertriglyceridemia, mild deviations in plasma lipids, or CHD risk, based simply on the predicted gain or loss of function of the interacting factor.



The overview here emphasizes the multifaceted biology and biochemistry of LPL regulation, whose almost overwhelming complexity has been recognized for years and discussed in several outstanding recent reviews (1–5). Some interacting factors undoubtedly have several other effects than their effect on LPL. And while some of the data support the growing appreciation of TGs as feasibly causative for CHD (acknowledging the numerous related unmeasured variables that go along with high TGs), the disparities seen here indicate that merely implicating LPL and its interacting factors as the primary mechanism for this association is premature. This does not exclude the likelihood that individual components affecting certain aspects of LPL's multifaceted and complex function may be relatively more important in atherosclerosis. For example, LPL is known to be synthesized and secreted by macrophages and has been shown to be expressed within atherosclerotic tissue, where it could be involved in the conversion of larger TG-rich lipoproteins into smaller remnants, which would then have more rapid uptake into macrophages, mediating foam cell formation (59); however, such a mechanism would be independent of effects on plasma lipoprotein profile. Six decades of accumulated wisdom on LPL should remind us that the new genetic data, as always, need to be interpreted in the context of established molecular and biochemical complexity.

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