LIPOPROTEIN LIPASE: MOLECULAR STRUCTURE, REGULATION, AND UPDATES WITH CVD RISK

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Abstract

Because of recent efforts performed to create new medications for the treatment of elevated triglyceride blood levels, there is increasing interest in deeply understanding the lipoprotein lipase enzyme structure and mechanism of action. The purpose of this article is to summarize the recent studies of molecular structure and regulation of lipoprotein lipase enzyme (LPL), describing the correlation of this enzyme with the pathogenesis of cardiovascular diseases. **Highlights in this article:**

• X-ray crystallography of Lipoprotein lipase enzyme (LPL) revealed that the enzyme is not only active in a homodimer complex with GPIHBP1, but also in the monomeric form.

• Recent genetic association studies and annotation data-base suggested that tissue LPL is not only recognized as TG hydrolyzing key enzyme but also a modulator in many cellular mechanisms, including regulation of cell response to several macronutrients, mediating cellular events associated to immune cell response, controlling the proliferation of hematopoietic stem cells as well as regulation of cell response to insulin hormone.

• Several genetic studies identified more than 230 LPL gene polymorphisms have been identified to be associated with familial lipoprotein lipase deficiency, but NOT all are pathogenic.

Summary:

 Deep understanding of LPL molecular structure, regulation, and genetic polymorphic pattern could help in the early description of molecular risk factors for CVD, as well as the development of new therapeutic modalities for treating hypertriglyceridemia and hence, reducing the risk for cardiovascular diseases.

Abbreviations:

LMF1 : lipase maturation factor-1

HSPG: heparan sulfate proteoglycans

HS: heparan sulfate

GPIHBP1: glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1

ANGPTLS: angiopoietin-like proteins

TRL: triglyceride-rich lipoproteins

oxLDL: Oxidized low density lipoprotein

NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells

CSi: calculated insulin sensitivity index

HepG2: cell line exhibiting epithelial-like morphology that was isolated from a hepatocellular carcinoma

1. Introduction

The lipoprotein lipase is an extracellular water-soluble lipolytic enzyme, firstly discovered in 1943, referred to the lipase family and possesses many other names may be used in literatures, including clearing factor lipase, Diacylglycerol lipase, post-heparin lipase, and triacylglycerol protein acyl-hydrolase. [1, 2].

The significance role of LPL in lipid homeostasis attracts the researchers to study that molecule and its mode of regulation and action in both healthy and disease status, attempting to gather more information about its action, in addition enable researchers to develop potential therapeutic targets for hypertriglyceridemia individuals who are at risk for cardio-vascular diseases (CVD).

I- LPL: Molecular structure

LPL is expressed in TG-utilizing tissues and organs with a high oxidative metabolism as a monomeric glycosylated molecule in parenchyma cells, then translocated via glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) that mediated the translocation via extra cellular matrix to endothelial cells' capillary lumen surface, where it is bonded by ionic interactions with heparan sulphate glycosaminoglycan (HSPG) forming dimeric molecule with a molecular size of ~110 kDa.

Human LPL gene is located in the region 8p22 and composed of 10 exons, 9 introns, composing 30 kilobase (kb) pairs in length, and translated a protein of 448 amino acids with estimated molecular weight of ~55 kDa.[3, 4].

The globular structure of LPL is composed of two domains, the 1st domain involves the larger N-terminus domain which contains α helices structures around a central β sheet, containing an active site with an α/β hydrolase fold, a β5 loop (a.a. residues 54-64), a lid region (a.a. residues 216-239), as well as an oxyanion hole (a.a. Trp-55, Leu-133). The two β pleated sheet-layers are composed to formulate an elongated cylinder structure, forming the 2nd domain of the enzyme (smaller C-terminus domain). It mostly provides the substrate specificity of the LPL with high affinity for chylomicron, VLDL as well as for LDL- binding receptors. [5]

The monomers of the LPL non-covalent homodimer are arranged head-to-tail. The Ser/Asp/His triad is housed in a hydrophobic groove that is sealed off from the solvent by the lid. The C-terminal domain offers the lipid substrate to the lid area after binding to activator factor (ApoC-II) and lipid in the lipoprotein. The lipid interacts with both the lid region and the hydrophobic groove at the active site, causing the lid to migrate away from the active site and allowing access to it. The loops of the N-terminal domain folds back into the protein core, positioning one of the oxyanion hole's electrophiles for lipolysis. The lipid's glycerol backbone can then enter the active site and be hydrolyzed.[6]

For decades ago (till 2018), pure homogenous crystal structure of lipoprotein lipase enzyme was not clarified and LPL was assumed to be active in the form of non-covalent homodimer, which contains multiple functional domains required for normal hydrolytic activity including a catalytic domain, in addition to the sites involved in co-factor, heparin and lipid binding. [7, 8]. In 2018, X-ray crystallography and comparative modeling showed details about the structure homology of LPL and predicted the structure and functional regions of the LPL enzyme, revealing that both LPL and GPIHBP1-bound LPL are being active in a monomeric state.[9].

The experimental results using X-ray crystallography in 2019 showed that the LPL- GPIHBP-1

complex includes a structure with a novel inhibitor bound in the LPL active site. The LPL lid and lipid-binding areas are included in the inhibitor-bound structure, making it the first full crystal structure of LPL and providing insight into how these sections of LPL contribute to tri-glyceride-rich lipoprotein (TRL) substrate recognition.[10, 11]. It also revealed that the lipoprotein lipase-GPIHBP1 complex has overall similarity in structure with pancreatic lipase (PL) and consists of two distinct portions attached to each other by peptide linkage, involving different actions including acyl-glycerol hydrolase activity, lipid binding, catalysis, heparin, and enzyme-cofactor interaction.[5].

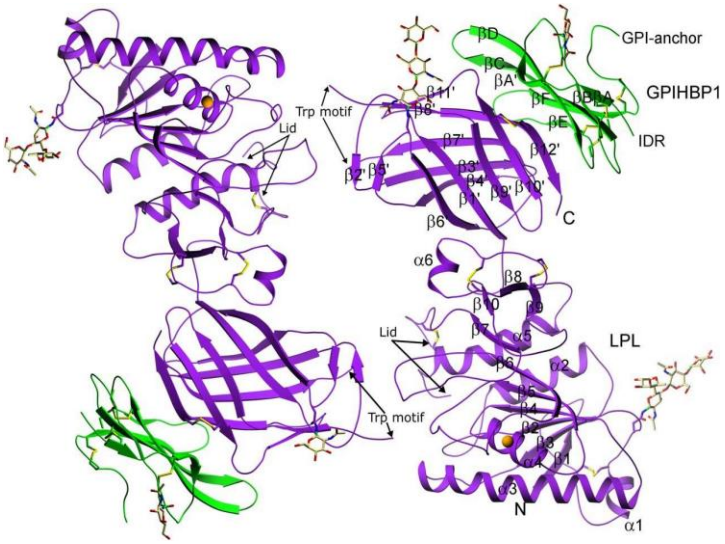

Figure (1): *Ribbon representations of the LPL-GPIHBP1 complex in the asymmetric crystallographic unit show the structure of the LPL-GPIHBP1 complex.[5]*

A single calcium ion (orange sphere) is coordinated by A194, R197, S199, D201, and D202, while LPL (purple) has five disulfide linkages (C54-C67, C243-C266, C291-C302, C305-C310, and C445-C465). It also has two N-linked glycans (at N70 and N386). There is one N-linked glycan in GPIHBP1 (green) (at N78). LPL has two domains that are joined by a hinge region: an N-terminal /-hydrolase domain (N) with six helices and ten strands, and a C-terminal flattened -barrel domain (C) with twelve strands. The GPIHBP1 -strands are numbered in accordance with the nomenclature suggested for LU domain proteins. Inherently disordered region, or IDR.

LPL - Molecular functions and mediatory roles

Several molecular functions of LPL have been identified based on genetic association studies and genetic annotation data-base, describing the functions and regulatory effect of LPL enzyme in a wide range of molecular functions including Lipoprotein particle binding, Protein-membrane adaptor activity, Signaling receptor binding, Phosphatidylserine 1-acylhydrolase activity, Triglyceride binding, and 1-acyl-2-lyso-phosphatidylserine acyl-hydrolase activity**. [12].**

Using bio-informatics “gene ontology”-, LPL has been identified to play regulatory roles in different cellular processes and responses including cholesterol homeostasis, chylomicron remodeling, fatty acid biosynthesis, mediating cellular response to different fatty acids (lipoic acid, arachidonic acid, oleic acid, linoleic acid, palmitoleic acid, linoleic acid, and palmitoleic acid).[12-15].

A strong association has been identified between DNA methylation patterns and the insulin sensitivity index CSi, which is determined by an intravenous glucose challenge. Two CpGs in

the genes CTNND2 (cg04615668) and LPL (cg07263235) correlate to the two most important signals of connection.[16]

Table (1): Major identified cellular roles of LPL enzyme.

	LPL molecular function	**References**
1	**Apolipoprotein binding**	[17]
2	**Calcium ion binding**	[5, 18]
3	**Heparan sulfate proteoglycan binding**	[19]
4	**Heparin binding**	[19, 20]
5	**Lipase activity**	[21]
6	**Lipoprotein lipase activity**	[5, 22, 23]
7	**Phospholipase A1 activity**	[24, 25]
8	**Phospholipase activity**	[25] [26]
9	**Protein homodimerization activity**	[27]
10	**Triglyceride lipase activity**	[24]

II- LPL: Molecular regulation and CVD risk

Because of the intensively reported positive correlation between elevated blood triglycerides level (TAG) -in both fasting and postprandial status- with coronary heart disease (CHD), many research studies have been reported regarding the pathways of TAG-rich lipoproteins (TRL) and the possible positive and negative mediators of lipolysis. The significance role of LPL in lipid delivery attracts many researchers to study the different possible mechanisms of regulation of that molecule, attempting to gather the information that enables researchers to use the enzyme in solving related clinical problems.[28]

The activity of LPL enzyme is regulated at different physiological and pathological status such as during fasting, exercise, and in diabetics via post-translational mechanisms driven by different extracellular proteins in a tissue specific mechanism, controlling the delivery and distribution of the free fatty acids among tissues based on variations in metabolic status.[29]

Several mediators governed LPL-mediated lipolysis of TRL, including hormonal regulation (Insulin, glucagon, growth hormone, and adrenaline) and non-hormonal regulation which includes positive mediators; non-esterified fatty acids, apolipoproteins APOA5, and apolipoprotein C-II, while both liver-derived apolipoprotein C-III and TNF-a are classified as negative mediators.[30-32].

Positive mediators:

Apo-C-II

ApoC-II is an apolipoprotein LPL-activator cofactor discovered in 1970 as a surface protein bound to different lipoprotein molecules including very low-density lipoproteins (VLDL), chylomicrons, and HDL.[33] It contains 79 amino acids and a MW of 8916 Dalton and composed of just three helices as described using Nuclear magnetic resonance and secondary structure prediction studies.[34]. It is expressed by liver tissue to enhance TG hydrolysis, playing a crucial role in triglyceride-rich lipoproteins metabolism.

Despite the fact that the ApoC-II binding site is still unknown, it is believed that residues from both the N- and C-terminal domains are necessary for this interaction to occur. Both the N- and C-terminal domains of LPL have heparin binding sites that are separated from the lipid binding sites. As a result, LPL serves as a link between lipoproteins and the cell surface. It's important to note that LPL's catalytic activity is unrelated to its capacity to bind to receptors or cell surfaces.[35].

For each LPL homodimer molecule, two molecules of the Apo-C-II -could attach for starting hydrolysis in a simultaneous manner for single lipoprotein molecule. This reaction is regulated by the number of free fatty acids released, however, the APOC2 gene itself is regulated in response to the metabolic status through many transcriptional factors.[36, 37].

The deficiency of ApoC-II was reported early as a genetic cause for elevated TG. A research team in 2016 succeeded to create ApoC-II mutant mice as a therapy for patients with apoC-II deficiency.[38, 39].

Low cardiovascular mortality is associated with high levels of circulating apoC-II. These results are in line with the treatment approach of increasing apoC-II to lower cardiovascular risk.[40].

More than 24 genetic mutations in the *APOC2* gene has associated with hypertriglyceridemia and reported to impair the activity of LPL.[38]

Liver-derived apolipoprotein, APOA5

The liver-derived apolipoprotein APOA5 appeared to play an important role in LPL activity. The APOA5 variants are genetically discovered and identified -through genetic association studies among severe and moderate hypertriglyceridemia-to play not only as a stimulator of lipolysis through facilitating the binding of TRL to the endothelial cell surface *via* heparan sulfate proteoglycans (HSPG), but also as a regulator of all lipoprotein subclasses distribution which may shift lipoproteins towards atherogenic changes for high risk patients.

The apolipoprotein A5 genetic variant c.553G > T (rs2075291) was positively correlated with elevation of triglycerides levels in coronary artery disease (CAD) patients receiving atorvastatin and lipid lowering drug.[41-43]. other polymorphism; rs662799 of the APOA5 gene C-allele carriers demonstrated significant rates of low HDL and hypertriglyceridemia, as well as variations in triglyceride, HDL cholesterol, and adiponectin levels among white obese individuals.[44].

Lipase maturation factor 1 (LMF1)

Lipase maturation factor 1 (LMF1) is another LPL bound protein that is essential for the folding and assembly of LPL (chaperone protein). Several LMF1 variants are newly identified to be

associated with severe hypertriglyceridemia, however, a recent molecular research in 2020 have studied the genetic spectrum of the LMF1 gene variants (19 studied SNPs) among the patients with severe elevation of serum triglycerides (SHTG) revealed non-significant correlation.[45, 46]

LPL Negative mediators

Angiopoietin-like proteins (ANGPTLs)

Endogenous LPL antagonists ANGPTL3, 4, and 8 proteins contribute to the tissue-specific control of LPL in response to various physiological stimuli.[47]. Whereas ANGPTL3 is only expressed in the liver, ANGPTL4 and ANGPTL8 are widely distributed in both adipose tissue and the liver. A linker region connects an N-terminal coiled-coil and a C-terminal fibrinogen-like domain in the molecular structure of all ANGPTL proteins, with the exception of ANGPTL8. Proprotein convertases (proteases involved in protein modulation) cleave both ANGPTL3 and 4 in the space that connects two functional domains.[27, 48] For LPL binding and inhibition, a conserved area, LAXGLLXLGXGL, known as specific epitope 1 (SE1) is necessary and shared by the N-terminal domains of ANGPTL3 and ANGPTL4.[49] The coiled-coil domains of ANGPTL3 and 4 share structural similarities with ANGPTL8, including the conserved motif for LPL binding. As a result, ANGPTL8 is an unusual ANGPTL family member.[50].

Humans with low plasma lipid levels have been found to have loss-of-function variations of ANGPTL3, 4, and 8.[51-53], indicating them as prospective targets for the treatment of metabolic and cardiovascular illnesses.[47].

Angiopoietin Like 3 protein (ANGPTL3):

ANGPTL3 is an angiogenesis like protein, expressed mainly in liver and perform a stimulatory effect on human plasma lipid metabolism, achieved by suppressing plasma TG clearance via inhibition of LPL activity. The mechanism of *ANGPTL3* in LPL activity may involve unfolding of the homodimer LPL- *GPIHBP1* complex. Molecular research in 2017 has reported that patients with harmful variants (impair the function) of *ANGPTL3* -using whole exome sequencing (WES)- have improved plasma lipoprotein patterns, including lowering of both TG and cholesterol levels.[54-56].

Angiopoietin Like 4 protein (ANGPTL4):

ANGPTL4 is a LPL-regulator mediates inactivation of the lipoprotein lipase LPL as it promotes intracellular cleavage of LPL in adipocytes, and thereby plays a role in the regulation of triglyceride clearance from the blood stream and in lipid metabolism. *It* was found catalyzes the unfolding of LPL's amino-terminal hydrolase domain.[57].

Next Generation Sequence analysis showed that mutations in both angiogenesis-like proteins, *ANGPTL3/ANGPTL4* were associated with lowering in circulatory TG and decreased the risk for CVD development. *It was* found that *TNF*-α increases the expression of *ANGPTL4* protein in addition to the increase of mRNA.[51, 58-60].

Angiopoietin Like 8 protein (ANGPTL8):

ANGPTL8 is a novel regulator of lipid metabolism -discovered 2012-, expressed in both liver and adipose tissue and regulated under different nutritional stress conditions, including fasting and nutrient excess involving RAS/RAF/MAPK signaling.[61]

It has been demonstrated that the activation of ANGPTL8 protein is a result of binding with ANGPTL3, and this binding increases the efficacy of ANGPTL3 towards LPL. White adipose tissue may be better able to store energy if the ANGPTL3-8 complex forms during feeding and directs dietary fatty acids away from the heart and muscle.[62].

Plasma levels of ANGPTL8 proteins are known to be increased in atherosclerotic individuals, where, ANGPTL8 inhibits plasma LPL and leads to an accumulation of circulating lipoproteins that promotes inflammation and the pathogenesis of atherosclerosis. Hence, even while intracellular ANGPTL8 may have an anti-inflammatory effect by preventing NF-kB activation, extracellular activities of circulating ANGPTL8 may result in inflammation.[63].

Apolipoprotein-C-III (APOC3):

APOC3 is a small protein encodes for 79 amino acids and expressed mainly by liver cells and, to a lesser extent in epithelial cells of small intestine and present on chylomicrons, VLDL, HDL, and LDL particles. It composed two alpha helices, and the amino acid tryptophan residues in the carboxyl-terminal of apoC-III is important for the ability of apoC-III to interact with tri-glyceride-rich lipoproteins.[35, 62, 64-66].

Two genetic studies suggested that people with the APOC3 null mutation R19X have 50% lower plasma levels of apoC-III, 35% lower plasma triglycerides, noticeably lower postprandial triglycerides, and significantly lower coronary artery calcification (CAC) scores -which is a known risk factor for cardiovascular events and a marker for coronary artery disease- than people without the mutation.[62, 67].

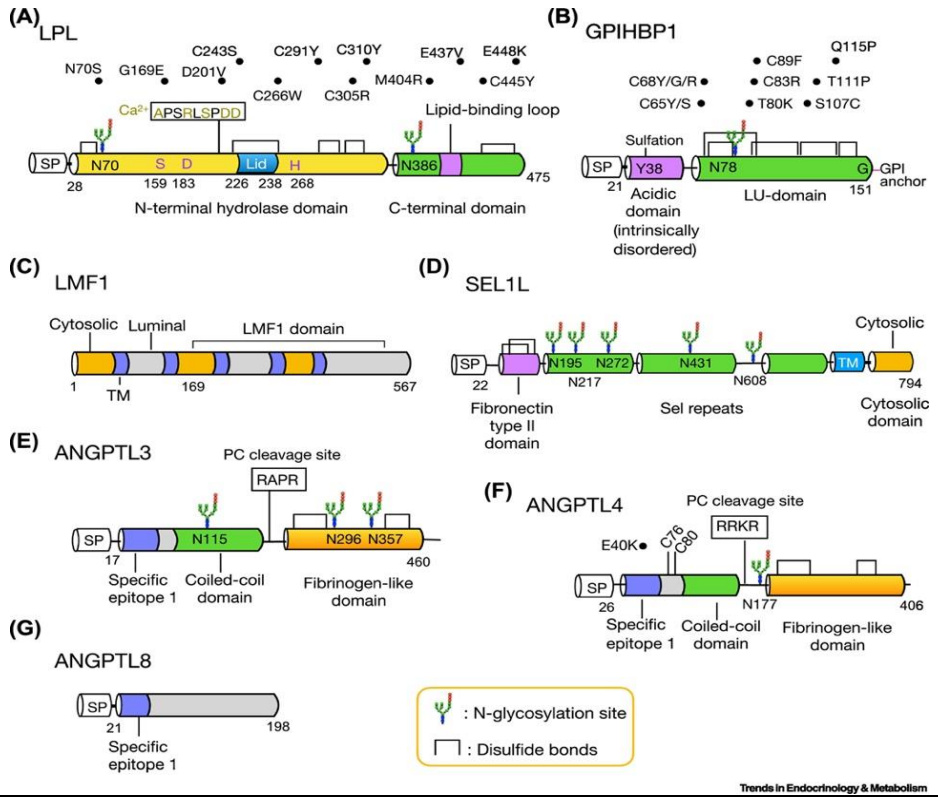

Figure (2)

shows the amino acid domains composition of:

LPL (a), GPIHBP1 (b), LMF1 (c), SEL1L (d), ANGPTL3 (e), ANGPTL4 (f), and ANGPTL8 (g). [68]

Colors of the LMF1's cytosolic, transmembrane, and luminal domains are, respectively, orange, purple, and gray (c). Its oligomerization depends on ANGPTL4's two cysteine residues, C76 and C80 (f). The structure above lists a number of human mutations for LPL (a), GPIHBP1 (b), and ANGPTL4 (f). Signal peptide (SP); transmembrane domain (TM).

LPL – Updates: Dyslipidemia regulation and CVD risk

The focus of medication development for reducing atherosclerotic CVD has been focused on lowering of total cholesterol and low-density lipoprotein (LDL). The subpopulation of high-risk patients still faces cardiovascular risks, despite the significant drops in LDL cholesterol levels.[69] Both hypertriglyceridemia and atherosclerotic CVD are amenable to treatment with the novel technologies that boost LPL activity. The therapeutic modalities based on enhancement of LPL activity are newly approaches in treating both hypertriglyceridemia and atherosclerotic CVD.[70]

In early onset atherosclerosis and CVD, such as coronary artery disease (CAD) and myocardial infarction (MI), The LPL deficiencies cause hypertriglyceridemia and hyperlipidemia and is particularly significant in the development of MI. [71, 72]

Current molecular research on LPL have revealed its "odd" functions in mediating cellular events associated to immune cell response, controlling the proliferation of hematopoietic stem cells, and regulating the levels of circulating cells and phenotypes.[70] These results were enforced by that resulted in 2021 -using cytokine arrays- that in various breast cancer subtypes, the LPL lipolytic effect on lipoprotein particles produces lipoprotein hydrolysis products that induce many pro-tumorigenic cytokine expression profiles.[73] These inflammatory cytokines and chemokines were found to cause oxidation of LDL in addition to apo-lipoprotein modification of Apo-B, as it transitions to the highly oxidized condition.[74]

The significance of systemic LPL activity in lowering the risk of CVD has once again been proven by a number of methods to deactivate the inhibitors that deactivate LPL action. On the other hand, mounting evidence points to a correlation between aortic macrophage numbers and inflammatory processes and focal arterial expression of LPL.[70]

In cultured macrophages, systemic LPL deficit lowers the quantity of induced peritoneal macrophages and the lipid content of adipose macrophages, regulating both pro- and anti-inflammatory gene expression. While, no effect was observed of macrophages gene expression in the presence or absence of LPL does within regressing atherosclerotic plaques.[75]

It was discovered that adding of LPL in experimental study increases the affinity of the binding as well as the number of binding sites for low density lipoproteins (LDL) and very low density lipoproteins (VLDL) to HepG2 cells and fibroblasts by 20–30 times.[76]

Since both chylomicron and VLDL are too large to be trancytosed through endothelial cells and into the artery intima, severe elevation of TG characterized by the accumulation of chylomicrons and large VLDLs is typically not considered pro-atherogenic but both remnants particles; IDL and LDL do transcytosed and considered to be atherogenic.[77]

Research studies on hyperlipidemic rabbits concluded that the remaining lipoprotein particles are allegedly more atherogenic than LDL itself, and showed that VLDL and IDL particles enter and are kept in the artery intima to the same amount as LDL particles.[78] Two observations serve to support this claim. First off, macrophages in the artery intima can pick up leftover particles without first oxidizing them.[79] Second, compared to LDL particles, they contain almost 40 times more cholesterol per residual particle. VLDL remnants may have an even greater atherogenic potential than oxLDL and chylomicron remnants, which were previously thought to be the major atherogenic lipoproteins.[79, 80]

The mechanism exploring this finding conclude that the marginated TG rich-lipoproteins are hydrolyzed intravascularly, and the leftover lipoproteins (remnant particles) and some still-attached inactive LPL molecules are detached from the endothelial cells. The hepatic absorption of leftover particles is facilitated by the inactive LPL acting as a ligand for many internalization receptors on hepatocytes.[81, 82] LDL receptor, VLDL receptor, LRP1, and SCD1 are some of these receptors.[83].

III-LPL Genetic polymorphisms and CVD risk

Recent genetic variant investigations included more than 300,000 individuals revealed that high plasma lipoprotein triglyceride levels are substantially associated with an increased risk of atherosclerotic cardiovascular disease (CVD).[84].

Several studies -based on genome wide association and genotyping- has discovered numerous of genetic variants that has been identified to be associated with dyslipidemia among CVD patients, recording more than 230 LPL-gene polymorphisms identified to be associated with familial lipoprotein lipase deficiency as recorded by US National library of Medicine in 2021. However, not all LPL gene identified-variants were pathogenic.

The LPL Genetic variants – according to ClinVar data-base 2021 -has been recorded about 56 LPL genetic polymorphisms identified as pathogenic and result especially with Hyperlipoproteinemia-I. Two LPL mutations, G118E and P207L have been found In Quebec, Canada to cause complete loss of LPL activity in homozygotes and 50% loss in heterozygotes.[55, 70, 85].

Two variants; c.347G > C and c.472 T > G has been discovered in the LPL gene mutation spectrum of neonates in 2021 with a recurrent infection during the hyperlipidemia stage, implying a link between LPL mutations and immunological weakness as well as lipid metabolic abnormalities.*[68]*.

Genetic association study and meta-analyses studies has been recorded significant associations for common polymorphisms in LPL; Asn291Ser, HindIII, and Ser447Ter polymorphisms, in regulating Alzheimer's disease (AD) risk.[86]. Meta-analysis study in 2021 has described both *LPL* HindIII and S447X polymorphisms as protective factors for Asians against myocardial infarction (MI), and the *LPL* HindIII polymorphism only for Caucasians.[87].

The protective property observed against MI was explained by that the two C-terminal amino acids are lost from the LPL protein due to the LPL S447X (rs328) polymorphism which already had been demonstrated to be linked to higher plasma post-heparin activity and LPL protein production, as well as lower TG levels.[88] These findings were in concordance with the results demonstrated the same protection against MI on Chinese according to studies conducted in 2008.[87] and on Egyptians for X allele.[89] However, neither the Swedish nor the Costa Rican populations demonstrated this effect.[90] No significant association also was observed between the genetic polymorphism LPL HindIII (rs320) and coronary artery disease (CAD) in Saudi population.[91]. While 584C/T polymorphism was significantly associated with CAD risk, and lipid profile in Egyptian post-myocardial infarction subjects.[89, 92].

Moreover, LPL HindIII was examined for association with MI-diabetic patients vs MI-nondiabetics -in addition to both (APOC3 SstI and LPL Ser447-Ter)- for dyslipidemia to measure the relative risk for MI development, suggesting a positive association of H2H2 or S2S2 genotypes with dyslipidemia and increased risk of myocardial infarction in Egyptian population.[89].

Homozygous *GPIHBP1* mutation (c.182-1G > T) has been identified to be positively associated to familial chylomicronemia syndrome with severe hypertriglyceridemia, altering the post transitional modification mechanism, affecting almost 50% of the cysteine-rich Lys (c.182-1G > T).[93]**.**

Several genetic researchers have examined the influence of *APOC3* genetic polymorphisms on lipid plasma levels and increased risk for CVD, and recorded that the null mutation of R19X is associated with lowering in plasma apoC-III levels by 50%, and lowering in TG plasma level by 35%, and significantly lowering in coronary artery calcification (CAC) scores.[62, 67]. This was enforced in 2020 by a research concluded that lifelong apoC-III deficiency is cardioprotective.[94] Several genetic studies examined the polymorphisms in ApoC-II gene and identified many SNPs to be associated with the elevation in triglyceride blood level. [38]

Table (2): Genetic variants of ApoC-II identified to be associated with hypertriglyceridemia

	ApoC-II Variant	**Mutation**	**Position**[a, b]	**Type**	**Allele count per 100,000**	**Clinical features (TG mmol/L)**	**Reference**
1	APOLIPOPROTEIN C-II (-190T→A)	T→A substitution in apo C-II promoter, 190 bp up-stream of major transcription start site. 2-fold reduction in activity (after subtracting background)	Promoter (−190T→A)	Substitution	<1	HTG (50.0), X	BBRC 2007 Mar 2;354(1):62-5
2	APOLIPOPROTEIN C-II (−86A→G)	A→G substitution in apoC-II promoter, 86 bp upstream of major transcriptional start site. No protein expression	Promoter (−86A→G)	Substitution	<1	HTG (18.9), C, P	J Lipid Res. 1996 37:2599-2607
3	APOLIPOPROTEIN C-II (PARIS-1)	$Met_{(-22)}$→Val/No initiation codon/Loss of signal peptide and first 8 aa	M-22V (*M1V*)	Absent	<1	HTG (10.7), P	JBC 1989 Dec 15;264(35): 20 839-42
4	APOLIPOPROTEIN C-II ($PARIS_2$, BARCELONA)	Introduction stop codon ($Arg_{(-19)}$)/Termination	R-19* (*R4X*)	Nonsense	4	HTG (20.3)	J Lipid Res. 1992 Mar;33(3): 361-7, J Lipid Res.

	ApoC-II Variant	Mutation	Position[a, b]	Type	Allele count per 100,000	Clinical features (TG mmol/L)	Reference
							1992 33:1823-1832
5	APOLIPOPROTEIN C-II (JAPAN, VENEZUELA)	1 bp deletion at Gln_2; frameshift resulting in 17 aa truncated protein	fsQ2 (*fsQ24*)	Frameshift	<1	HTG (12.4; 12.3)	Atherosclerosis 1979 Sep;34(1)53-65, Am J Hum Genet (1991) 48:383-389
6	APOLIPOPROTEIN C-II (SHANGAI)	1 bp deletion/2 bp insertion/$Asp_{29}Glu_{30}$→$Ala_{29}Ter_{30}$	fsD7 (*fsD29*)	Frameshift	<1	HTG (17.42), C, P	Lipids Health Disease 2016 15:12
7	APOLIPOPROTEIN C-II (NIJMEGEN)	1 bp deletion and frameshift at Val_{18}/introduction stop codon	fsV18* (*fsV40X*)	Frameshift	<1	HTG (4.0–15.0), H/S, LR, P	JBC 1988 Dec 5;263(34):17913-6
8	APOLIPOPROTEIN C-II VARIANT (ApoC-II-v)	p.Lys_{19}Thr	K19T (*K41T*)	Missense	87	HTG (7.3;3.7), G	J. Lipid Res. 1990 31:385–396, Dis Markers 1991 Mar–Apr 9(2):73–80, But see also Clin Gen (1994) 45:292-7
9	APOLIPOPROTEIN C-II (WAKAYAMA)	Trp_{26}→Arg	W26R (*W48R*)	Missense	<1	HTG (10.3), P	BBRC 1993 Jun 30;193(3):1174-83
1	APOLIPOPROTEIN C-II	Introduction stop codon	Y37* (*Y59X*	Nonsens	<1	HTG (56.5),	BBRC

	ApoC-II Variant	Mutation	Position[a, b]	Type	Allele count per 100,000	Clinical features (TG mmol/L)	Reference
0	(BARI)	(Tyr_{37})/Termination	)	e		P, X	1990 May 16; 168:1118-1127
11	APOLIPOPROTEIN C-II (PADOVA)	Introduction stop codon (Tyr_{37})/Termination	Y37[*] (*Y59X*)	Nonsense	<1	HTG (56.5), P, X	J Clin Invest 1986 Feb;77(2)520-7, J Clin Invest 1989 Oct;84(4)1215-9
12	APOLIPOPROTEIN C-II (SAN FRANCISCO)	p.Glu_{38}Lys	E38K (*E60K*)	Missense	59	HTG (3.2; 2.3; 11.3)	Hum Mol Genet. 1993 Jan;2(1):69-74
13	APOLIPOPROTEIN C-II (PHILADELPHIA)	Arg_{72}→Thr	R50T (*R72T*)	Missense	<1	HTG (33.9), P, G	J Clin Endocrinol Metab. 2017 Feb 13
14	APOLIPOPROTEIN C-II (AFRICAN)	p.Lys_{55}Gln	K55Q (*K77Q*)	Missense	238	HTG (63.1; 17.5; 8.8; 14.9), X, P, G, O	J Clin Invest. 1986 Feb;77(2):595-601
15	APOLIPOPROTEIN C-II (AUCKLAND)	Tyr_{63}Ter/Termination	Y63[*] (*Y85X*)	Nonsense	<1	HTG (326.0; 189.8), H/S, O	Ann Neurol. 2003 Jun;53(6):807-10
16	APOLIPOPROTEIN C-II (TORONTO)	Deletion 1 bp Thr_{68}→ Frameshift, alteration of 6 aa and termination at aa 74	fsT68 (*fsT90*)	Frameshift	<1	HTG (107.0)	NEJM (1978) 299:1421-1424,

	ApoC-II Variant	Mutation	Position[a, b]	Type	Allele count per 100,000	Clinical features (TG mmol/L)	Reference
							PNAS USA (1987) 84:270-273, J Medical Genetics (1988) 25:649-652
17	APOLIPOPROTEIN C-II (ONTARIO)	p.Gln_{70}Ter	Q70[*] (*Q92X*)	Nonsense	<1	HTG (>3.37)	Cir Cardiovasc Genet (2012) 5:66-72
19	APOLIPOPROTEIN C-II (ST. MICHAEL)	Insertion 1 bp, Gln_{70}→Pro/Frameshift 97X	fsQ70 (*fsQ92*)	Frameshift	1	HTG (15.0), P, A	J Clin Invest (1987) 80:1597-1606, Clin Biochem (1992) 25:309-312
21	APOLIPOPROTEIN C-II (HONGKONG)	Leu_{72}→Pro (C-terminal helix)	L72P (*L94P*)	Missense	<1	HTG (82.0), X	Clin Chim Acta. 2006 Feb;364(1–2):256-9
22	APOLIPOPROTEIN C-II (NIJMEGEN, ApoC-II-C-IV)	Deletion of promoter and Exon 1	Del Promoter, Ex 1	Deletion	<1	HTG (21.0), C, P	BBRC 2000 Jul 14;273(3):108 4-7
23	APOLIPOPROTEIN C-II (HAMBURG, TOKYO)	Intron 2 + 1 Gly→Cys/Splice defect	Splice NT 2 G+1 to C	Splice	<1	HTG (20.9), P	J Clin Invest. 1988 Nov;82(5):148 9-94, Atherosclerosis 1997

	ApoC-II Variant	Mutation	Position[a, b]	Type	Allele count per 100,000	Clinical features (TG mmol/L)	Reference
							Apr;130(1–2):153-60
24	APOLIPOPROTEIN C-II (TUZLA)	Homozygous deletion of exons 2, 3 and 4	Del Ex 2, 3, 4	Deletion	<1	C (52.6), O	Clin Chim Acta. 2015 Jan 1;438:148-53
25	*APOC2* gene c.133_134delTC	homozygous frameshift mutation on exon 3	exon 3	frameshift mutation		c.133_134delTC	Appl Clin Genet. 2020 Mar 26;13:63-69
26	homozygous missense variant R72T in APOC2	homozygous missense variant					J Clin Endocrinol Metab. 2017 May 1;102(5):1454-1457.

Conclusion

- Computational algorithms and bio-informatics are recent tools used to predict the 3-D-structure of Lipoprotein lipase enzyme, providing more information about the molecular structure, functions, and mechanisms of regulation.
- Activation/inhibition of LPL is regulated at the post-translational phase via many extracellular proteins that has been involved in the CVD risk, and could be targeted for future therapy.
- Better understanding of LPL molecular structure and regulation, in addition to genetic polymorphisms identification could lead to early description of CVD molecular risk factors as well as the development of novel strategies for intervention.

Recommendations

Familial segregation analysis may be required to demonstrate the pathogenicity of all identified

genetic variants associated with dyslipidemia as TG levels can significantly change depending on dietary consumption of fat. Deep knowledge of the molecular etiology might facilitate family screening to apply pre-symptomatic therapy in addition to establishing a conclusive diagnosis.

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