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Apolipoprotein C-II: New findings related to genetics, biochemistry, and role in triglyceride metabolism

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Abstract

Apolipoprotein C-II (apoC-II) is a small exchangeable apolipoprotein found on triglyceride-rich lipoproteins (TRL), such as chylomicrons (CM) and very low-density lipoproteins (VLDL), and on high-density lipoproteins (HDL), particularly during fasting. ApoC-II plays a critical role in TRL metabolism by acting as a cofactor of lipoprotein lipase (LPL), the main enzyme that hydrolyses plasma triglycerides (TG) on TRL. Here, we present an overview of the role of apoC-II in TG metabolism, emphasizing recent novel findings regarding its transcriptional regulation and biochemistry. We also review the 24 genetic mutations in the *APOC2* gene reported to date that cause hypertriglyceridemia (HTG). Finally, we describe the clinical presentation of apoC-II deficiency and assess the current therapeutic approaches, as well as potential novel emerging therapies.

Keywords

apoC-II; apoC-II deficiency; apoC-II mutations; triglyceride-rich lipoproteins; triglycerides; hypertriglyceridemia; lipoprotein lipase

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Conflict of interest

A.W., M. J. A., D. O. S., and A. T. R. are co-inventors on US patents for apoC-II mimetic peptides. A. W., D. O. S., and A. T. R. have a research cooperation agreement for apoC-II mimetic peptides with Corvidia Therapeutics, Inc., Waltham, MA, USA. R. L. D. has received grant support and modest honoraria from Ionis Pharmaceuticals and grant support from uniQure, Regeneron Pharmaceuticals, Zydus Pharmaceuticals, and Kowa Research Institute. L. F. and M. U. declare no conflict of interest.

1. Introduction

Numerous epidemiological and genetic studies demonstrate a strong positive correlation between elevated fasting and postprandial triglycerides (TG) with cardiovascular disease (CVD). Very severe hypertriglyceridemia (HTG), due to genetic and other causes, is also a well-known trigger for acute pancreatitis¹. As a consequence, the metabolism of triglyceride-rich lipoproteins (TRL) has been studied intensively for several decades² and numerous proteins involved in this pathway have been identified^{3–5}.

Lipoprotein lipase (LPL), a central enzyme in lipolysis of TRL, was first described in the mid-1950's⁶. An LPL activator, apolipoprotein (apo) C-II, was subsequently discovered in 1970 as a protein bound to very low-density lipoproteins (VLDL) and other lipoproteins, and was initially called glutamic acid lipoprotein or ApoLP-Glu based on its C-terminus amino acid (Glu)^{7,8}. The first apparent genetic cause of HTG was identified 8 years later due to apoC-II deficiency⁹, and now many other types of genetic mutations, including LPL itself, have been described¹⁰.

Given recent advances in apoC-II and growing interest in developing new therapies for HTG, this review will focus on apoC-II. We describe the clinical presentation of apoC-II deficiency, and the associated spectrum of *APOC2* mutations, as well as current and emerging clinical therapeutic options.

2. Overview of TRL metabolism

There are two main pathways whereby TRL deliver energy in the form of TG to peripheral tissues following lipolysis, and they differ depending on the source of TG, namely exogenous (dietary) (Fig. 1A) vs. endogenous (hepatic) sources (Fig. 1B).

As shown in Fig. 1A, the exogenous pathway involves the packaging of dietary lipids, primarily TG, into chylomicrons (CM) and their delivery to peripheral tissues either for energy production or storage in intracellular fat droplets, primarily in adipocytes. In contrast, VLDL mediate the transport of endogenous lipids to peripheral tissues (Fig. 1B). VLDL synthesis depends on TG availability, which are synthesized from free fatty acids (FFA) after their re-esterification in the liver¹¹. The liver receives FFA from three main extrahepatic sources: plasma FFA released by adipose tissue, uptake from CM remnants, and direct uptake of dietary FFA from the intestine *via* the portal vein¹². In the postprandial state, approximately 44% of TG on VLDL are synthesized from plasma FFA, 10% from diet-derived FFA, 15% from CM remnants, and about 8% from *de novo* lipogenesis. During fasting, 77% of TG of VLDL come from plasma FFA and only 4% from *de novo* lipogenesis¹³.

LPL, the main lipolytic enzyme for TRL, is first synthesized in the rough endoplasmic reticulum of parenchymal cells of adipose tissue, heart, and skeletal muscles as an inactive monomer^{14, 15}. Lipase maturation factor 1 (LMF1) dimerizes and activates LPL, as well as other related lipases, namely hepatic lipase¹⁶ and endothelial lipase¹⁷. A specific chaperone called Sel1L stabilizes the LPL-LMF1 complex¹⁸. Next, LPL is transported to the luminal surface of endothelial cells in conjunction with heparan sulfate proteoglycans (HSPG)^{19, 20}.

The glycosylphosphatidylinositol (GPI)-anchored high density lipoprotein-binding protein 1 (GPIHBP1) participates in this transport process^{21–23}, and also anchors LPL to the endothelial cell surface²⁴.

As shown in Fig. 2, LPL-mediated lipolysis of TRL are governed by several positive and negative regulators²⁵. Besides apoC-II, which will be discussed in more detail later, apoA-V also stimulates lipolysis, possibly by facilitating the binding of TRL to the endothelial cell surface *via* HSPG^{26, 27} and GPIHBP1²⁸. In contrast, both apoC-I and apoC-III have been shown by *in vitro* assays to inhibit LPL activity^{29, 30} and their overexpression in animal models raises plasma TG^{31, 32}. Larsson *et al.*³³ have shown that apoC-I and apoC-III compete with LPL for binding to lipid emulsion particles *in vitro* in a dose dependent manner. As a result, apoC-I and apoC-III inhibit lipolysis by decreasing LPL activity rather than inactivating the enzyme itself³³. In women, high levels of apoC-III were significantly and independently associated with impaired catabolism of VLDL, resulting in plasma HTG³⁴, but the main effect may be due to the ability of apoC-III to inhibit hepatic TRL or remnants uptake³⁵. The angiopoietin-like proteins (ANGPTL) 3, 4, and 8 have also recently been described to inhibit LPL³⁶. Although their overall effects on lipolysis are similar, they differ in their tissue specific expression, activity, and role, depending on nutritional and metabolic needs³⁷.

Finally, following TG lipolysis, the generated FFA may be taken up by cells *via* both receptor-mediated and receptor-independent pathways³⁸. Eventually, as TRL become depleted of TG, they dissociate from the endothelial cell surface and are removed by the liver. In addition to apoE and apoB-100, inactivated LPL bound to remnants can also serve as a ligand for removal by the liver³⁹. ApoC-II and the other apolipoproteins that modulate LPL eventually dissociate from remnant lipoproteins during lipolysis and then are transferred onto HDL⁴⁰, which serves as a reservoir for these proteins in the fasting state.

3. Transcriptional control of the human *APOC2* gene

3.1. Overview

The human *APOC2* gene resides within the human *APOE-APOC1-APOC4-APOC2* gene cluster on Chromosome 19 and is subject to complex transcriptional control (Fig. 3). ApoC-II is primarily expressed in the liver and secreted into plasma, but it is also produced by other tissues, including the intestine, macrophages, adipose tissue, brain, skin, lungs, retina, and retinal pigment epithelium^{41–46} where it may regulate lipolysis at the local level. Transcriptional regulation of the *APOC2* gene has been best characterized in liver and macrophages, and to a lesser extent, in the intestine. Each of these tissues regulates *APOC2* in a tissue-specific manner. For example, in liver, the farnesoid X receptor (known as FXR or NR1H4), which binds bile acids and regulates genes involved in bile acid synthesis and transport, plays a major role in *APOC2* gene transcription. In macrophages, however, key transcription factors for the *APOC2* gene include the liver X receptor (LXR), whose role is to correct for cellular cholesterol^{47, 48} and signal transducer and activator of transcription 1 (STAT1), which mediates cellular responses to interferons and certain cytokines and growth factors⁴⁹. In intestine, *ApoC2* gene expression increases with dietary lipids, as might be

expected, but recent work in mice has also demonstrated a unique regulatory mechanism involving CD36 sensing of dietary fat ⁵⁰.

In the following sections, we review the regulatory mechanisms of *APOC2* gene expression in the liver, macrophages, and intestine. The picture that emerges reveals distinctly different mechanisms of regulation, reflecting different physiological roles for apoC-II in these three different tissues.

3.2. Hepatic expression of ApoC-II

The main role of apoC-II secreted by the liver into the plasma is to enhance TG hydrolysis of VLDL and CM for energy delivery or storage. Consequently, the *APOC2* gene in the liver responds to metabolic cues by activation of transcription factors and nuclear hormone receptors.

Liver-specific expression of the *APOC2* gene, as well as *APOE*, *APOC1*, and *APOC4*, is coordinately controlled by two 319-bp liver-specific hepatic control regions, HCR.1 and HCR.2 (Fig. 3A) ^{51, 52}, located approximately 22 and 11 kb upstream of the *APOC2* gene transcriptional start site, respectively ⁵³. HCR.1 and HCR.2 share 85% nucleotide homology. Both contain FXR response elements that bind FXR/retinoid X receptor (RXR) heterodimers that mediate activation of the *APOC2* gene by bile acids ^{54, 55}, which are end products of cholesterol metabolism and potent signaling molecules. The FXR/RXR α binding site in HCR.1 (nt 214/226 of HCR.1) also binds hepatic nuclear factor-4 α (HNF4 α) and apoA-I regulatory protein-1 (ARP1) (Fig. 3A) ⁵⁶. HNF4 α is a master regulator of lipid metabolism in the liver ⁵⁷. It binds to and is activated by FFA ⁵⁸.

The -545/+18 *APOC2* gene proximal promoter contains five DNase-I-protected promoter segments: C-II-A, -B, -C, -D, and -E ⁵⁹. The C-II-B (-102—81) segment of the proximal promoter contains a hormone response element (HRE) that binds HNF-4 (Fig. 3A). Interestingly, a promoter mutation in a patient with chylomicronemia maps to position -86 of the *APOC2* promoter, within the CII-B element ⁶⁰. The C-II-C segment (-159—116) has a second HRE that binds the transcription factors ARP1 and eosinophil-associated, ribonuclease A-1 (EAR1) (Fig. 3A) ⁵⁹. The *APOC2* promoter is also stimulated by triiodothyronine (T3) via RXR α /thyroid hormone receptor β (known as T3T β or THR β) dimers, which likely bind the HRE at -140—155 of the *APOC2* promoter ⁶¹. In the presence of the appropriate ligands (bile acids, T3, retinoids, etc.), the two HREs in the apoC-II proximal promoter interact with the HCR.1 enhancer to promote liver-specific expression of the *APOC2* gene. This activation requires a long-range physical interaction between the HCR.1 FXR/RXR element, 11 kb upstream of the *APOC2* promoter, and the *APOC2* proximal promoter (Fig. 3A). Under different metabolic conditions, the same elements can recruit corepressors that inhibit *APOC2* gene expression ^{56, 59, 61}.

An unexpected new twist in FXR regulation of the *APOC2* promoter was the recent discovery of a long noncoding RNA, liver-specific TG regulator (lncLSTR), which inhibits *APOC2* gene expression ⁶². Depletion of lncLSTR leads to increased LPL activity and plasma TG clearance. lncLSTR does not directly interact with the *APOC2* gene but instead binds to transactive response DNA-binding protein 43 kDa (TDP-43), a transcriptional

repressor of the sterol 12 α -hydroxylase (*CYP8B1*) gene (Fig. 3B). This interaction counteracts the repression of the *CYP8B1* gene by TDP-43. Depleting lncLSTR causes more TDP-43 to bind to the *CYP8B1* promoter and inhibits *CYP8B1* expression. This alters the bile acid pool by increasing the muricholic acid (MCA)/cholic acid (CA) ratio, which increases *APOC2* gene expression through the FXR pathway, and decreases plasma TG levels (Fig. 3B) ⁶².

New insights have also recently been gained into regulation of the *APOC2* gene by cyclic adenosine monophosphate (cAMP). Streicher *et al.* ⁶³ previously reported a cAMP response element located between -170 and -36 of the *APOC2* gene promoter (Fig. 3A), with a 6-fold up-regulation of *APOC2* mRNA levels in HepG2 cells after incubation with the cAMP activator forskolin. Recently, cAMP-responsive-element-binding protein H (CREB-H), a transcription factor highly expressed in the liver and small intestine induced by fasting, was found to increase the expression of apoC-II and other LPL coactivators. Multiple nonsynonymous mutations in the cAMP responsive element binding protein 3 like 3 (*CREB3L3*) gene, which encodes CREB-H, have been found in individuals with extreme HTG ⁶⁴. Based on these findings, CREB-H, as well as the lncLSTR pathway, appear to be promising new targets for therapeutic intervention.

Although *Apoc2* mRNA levels are relatively invariant in adult rat livers, it can rapidly change in newborn rats in response to suckling. It rapidly increases immediately after birth, and then decreases slightly, reaching adult liver levels around the suckling/weaning transition period (day 20 of age) when they switch to a carbohydrate-rich diet ⁶⁵. Hepatic *Lpl* and *Apoc2* gene expression levels change in parallel, thus enabling hydrolysis of the high levels of milk fat ingested during suckling ⁶⁵. In pregnant rats fed a protein-restricted diet, placental *Apoc2* mRNA also increases 17.6-fold, along with changes in other genes involved in cholesterol and lipoprotein transport ⁶⁶.

Several known drugs, namely fibrates, statins, and ezetimibe influence hepatic *APOC2* gene expression in humans ⁶⁷. Fibrates decrease *APOC2* gene expression through peroxisome proliferator-activated receptor (PPAR) α ⁶⁵, whereas PPAR γ coactivator 1 α (PGC-1 α) partners with HNF4 α to increase hepatic *APOC2* mRNA levels under fasting conditions ⁶⁸. PGC-1 α also regulates a number of genes in response to fasting ⁶⁸. The promoter elements involved in modulating hepatic *APOC2* gene expression by these factors have not yet been defined. Finally, not only *APOC2* but also *APOC3* and *APOC1* gene expression in the liver are influenced by certain drugs and metabolic disorders, as well as age, sex and diet ^{65, 67}. When assessing potential effects of drugs to treat HTG on gene expression, measuring hepatic *APOC2* gene expression is, clearly, of great importance, but expression of other proteins affecting lipolysis must be taken into consideration as well.

Nonetheless, studies of hepatic *APOC2* gene expression has provided a wealth of information into the metabolic signals that influence apoC-II levels in plasma. This information may be useful in future drug development.

3.3. Macrophage specific expression of ApoC-II

ApoC-II is also expressed in macrophages and has been localized in macrophages within murine arterial lesions⁵⁵. Presumably, it facilitates the delivery of TG to macrophages, which are metabolically very active cells, but this may be pro-atherogenic based on the findings that decreased expression of LPL in macrophages decreases atherosclerosis in mice⁶⁹.

Macrophage-specific expression of the *APOE-APOC1-APOC4-APOC2* gene cluster depends on two 620-bp macrophage-specific multi-enhancer elements termed ME.1 and ME.2, located 32.85 and 20.25 kp upstream of the *APOC2* promoter (Fig. 3A)⁷⁰. Both ME.1 and ME.2 are required for *APOE* gene expression in macrophages, adipose tissue, and brain^{71, 72}. It was demonstrated that two LXR response elements (LXRE) within ME.1 and ME.2 were essential for stimulation of *APOC2* promoter activity by LXR and RXR in macrophages⁵⁵. As LPL is also stimulated by LXR, Mak *et al.*⁵⁵ hypothesize that secretion of apoC-II and LPL together from LXR-activated macrophages will result in increased local LPL activity. Moreover, ATP-binding cassette transporter A1 (ABCA1) is also upregulated by LXR in macrophages, and since apoC-II, as well as apoE, apoC-I, and apoC-IV are all able to act as acceptors for cholesterol efflux from macrophages⁷³, the coordinate upregulation of *ABCA1* and this gene cluster may enhance cholesterol efflux from macrophages, including foam cells in the arterial wall⁵⁵. Finally, LXR is has been shown to have an anti-inflammatory role in macrophages⁴⁷.

TG-interacting factor 1 (TGIF1), a transcriptional repressor, was found to bind to ME site(s) in the *APOE/C1/C4/C2* locus (Fig. 3A) and repress *APOC2*, as well as *APOA4*, gene expression in the liver⁷⁴. While the mechanism of repression is not entirely clear, it is interesting that the ME.1 and ME.2, in addition to enhancing macrophage-specific *APOC2* gene expression through LXR, also participate in repression of liver-specific *APOC2* expression in the liver, through TGIF1.

APOC2 mRNA levels were found to increase during 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced differentiation of promonocytic U937 cells into monocytes and macrophages⁷⁵. Interestingly, differentiation of monocytes into macrophages markedly increases STAT1 protein synthesis⁷⁶. Trusca *et al.*⁷⁶ found two STAT1 binding sites involved in *APOC2* gene regulation: one in the proximal promoter (nt -500—493) and the second in ME.2, 20 kb upstream of the *APOC2* promoter (Fig. 3A). The ME.2 STAT1 site was previously shown to be important for macrophage-specific *APOE* gene expression. For *APOC2* expression in macrophages, not only the ME.2 STAT1 site but also the STAT1 binding site in the proximal promoter confer macrophage-specific promoter activity. STAT1 on both the proximal and the distal binding sites were found to interact with RXR α on the RXR α /T3R β binding site (nt -140—-155 of the proximal *APOC2* promoter). This complex of transcription factors on the *APOC2* promoter, including both short- and long-range interactions, confers macrophage-specific gene expression.

The markedly different regulation of the *APOC2* gene in macrophages vs. hepatocytes necessarily reflects a need to respond to different physiological signals in these two tissues. As macrophages play a major role in atherosclerosis, and macrophage apoC-II is predicted

to have a role in local lipid metabolism, generating mice with macrophage-specific *ApoC2* gene knockouts could potentially uncover a specific role for macrophage apoC-II in atherosclerosis.

3.4. Intestinal regulation of apoC-II

Early on, apoC-II was noted to be synthesized by mucosal epithelial cells in rat small intestine ⁷⁷. In humans, it is expressed in jejunal enterocytes at relatively high levels ^{78, 79}, but the overall contribution of the intestine vs. the liver in maintaining plasma levels of apoC-II is not known. In mice, it was recently shown that the *Apoc2* gene is induced in the intestine more than 3-fold after an oil gavage ⁵⁰, suggesting a role for apoC-II in the postprandial metabolism of CM. The mechanism involves lipid-mediated downregulation of CD36, which is involved in dietary fat sensing in the enterocyte, and is impaired in the metabolic syndrome ⁵⁰. More detailed studies are needed, however, to better understand the exact mechanism for *Apoc2* gene regulation in the intestine.

4. ApoC-II biochemistry

After cleavage of a 22-amino acid signal peptide, the mature full-length apoC-II protein contains 79 amino acids and has a MW of 8916 Da ⁸⁰ (Fig. 4A). Retention of the signal peptide in transgenic mice due to a specific mutation has recently been shown to cause the selective binding of apoC-II to HDL, leading to HTG ⁸¹. ApoC-II contains no N-linked glycosylation sites and does not appear to undergo significant O-linked glycosylation.

Plasma levels of apoC-II are approximately 4.5 $\mu\text{mol/L}$ (40 mg/L) ⁸² in normolipidemic individuals but can be considerably higher when there is an accumulation of TRL in plasma. There are several factors, in addition to drugs described earlier, such as age, sex, diet, diabetes mellitus (DM) type 2, or chronic kidney disease that can affect plasma apoC-II concentration. ApoC-II levels increase until the age of 60 years in women, and until age 40 years in men ⁸³. Moreover, overweight-obese women have higher plasma apoC-II concentrations than normal-weight women ³⁴. A high-carbohydrate diet has been associated with increased apoC-II concentrations relative to apoC-III in VLDL ⁷⁹. The increased apoC-II levels in DM type 2 ⁸⁴ and chronic renal failure ⁸⁵ are associated with elevated TG in plasma. Because the plasma levels of TRL even in the postprandial state are usually far less than 0.5 $\mu\text{mol/L}$, there is enough apoC-II to exist in multiple copies per TRL particle. The plasma concentrations of apoC-III, which inhibits lipolysis and clearance of TRL, are twice as high as apoC-II ⁸², and it may be the ratio of these two proteins that regulates TRL catabolism. However, it has been recently shown that in women the apoC-III:apoC-II ratio was not associated with either apoB-100 levels or plasma TG concentration ³⁴.

Based on secondary structure prediction and nuclear magnetic resonance analysis ^{86, 87}, apoC-II contains only 3 helices (Fig. 4A). The first helix (Fig. 4A) at the N-terminus, residue 16 to 36 (historical nomenclature, based on residue 1 occurring after signal peptide cleavage) is relatively long and is primarily responsible for lipoprotein binding. It forms a classic Type A amphipathic helix, with a large hydrophobic face, two positively charged residues (Lys) close to the water/lipid interface and three negatively charged residues (Glu) on the hydrophilic side of a helix. This amino acid configuration allows apoC-II to bind

tightly to a lipid surface⁸⁸. The second helix is relatively short and only encompasses residues 50–56, and from nuclear magnetic resonance analysis this region appears to be mostly in a random coil configuration⁸⁶. The third helix (Fig. 4A) on the C-terminus (residues 63–76, in historical nomenclature) forms a G-type helix similar to what is commonly found in globular proteins, and does not show a strong polarization of hydrophobic and hydrophilic amino acids, and hence does not bind to lipids avidly⁸⁹. Based on natural mutations^{90, 91}, site-directed mutagenesis,⁹² and synthetic peptide studies⁹³, the C-terminal helix (Fig. 4A, B) is responsible for LPL activation⁹². Residues Tyr-63, Ile-66, Asp-69, and Gln-70 (HGVS-recommended nomenclature: Tyr-85, Ile-88, Asp-91, and Gln-92), all within helix 3, are thought to form a binding site for LPL⁹⁴ and have been proposed to lead to its activation by helping guide the TG substrate into the active site of LPL⁸⁷.

The exact binding site of apoC-II on LPL is not known, but it involves both the N- and C-terminal domains of LPL, with two apoC-II molecules bound per dimer of LPL^{95, 96}. By crosslinking studies, the C-terminus of apoC-II has been shown to be linked to specific amino acid residues near the lid region of LPL, and thus may cause displacement of the lid and facilitate TG entry into the active site⁹⁷. It depends somewhat on the nature of the lipid substrate, but when TRL, a natural substrate, is used, apoC-II increases the apparent $V_{\max}$ of LPL and only has a limited effect on the K_m ⁹⁵. Based on oil-drop tensiometer studies, it was recently established that the initial binding of apoC-II and LPL to lipoproteins depends on the surface pressure of TRL⁹⁸. Both proteins readily bind to newly secreted TRL, which have relatively low surface pressure, but as the surface pressure gradually increases with lipolysis due to retention of FFA, diglycerides, and monoglycerides on the surface of TRL, LPL dissociates unless it is retained to the TRL surface by apoC-II. Eventually, the surface pressure on the remnant particles becomes too high and both apoC-II and LPL are displaced, thus stopping any further lipolysis⁹⁸.

5. Clinical presentation of apolipoprotein C-II deficiency

Clinically, HTG is an extremely heterogeneous phenotype, with both monogenic and polygenic etiologies; therefore, the diagnosis of apoC-II deficiency requires a step-wise process⁹⁹. First, polygenic HTG is relatively common with contributions of both rare and common genetic variants¹⁰⁰. In addition, secondary causes of HTG should always be excluded when first evaluating a patient. These include uncontrolled or inadequately-managed DM type 1 or type 2, hypothyroidism, chronic renal insufficiency, nephrotic syndrome, alcohol overuse, excessive intake of long-chain TG and free sugars, and certain medications (e.g. estrogen, beta-blockers, diuretics, isotretinoin, glucocorticoids, serotonin reuptake inhibitors, and atypical antipsychotic drugs, such as clozapine and olanzapine)^{101, 102}.

Polygenic chylomicronemia, perhaps better known as type V hyperlipidemia, is fairly common with a prevalence of about one in 600¹⁰³, but in this condition, both CMs and VLDLs are elevated. On the other hand, familial chylomicronemia syndrome (FCS), formerly known as type I hyperlipoproteinemia, is a group of extremely rare monogenic conditions with a reported prevalence of about one in 1 million, equally distributed in both

genders, and higher frequencies are found in some populations due to a founder effect^{90, 104}. Among FCS, the most common cause is LPL deficiency (OMIM 238600) and apoC-II deficiency (OMIM 207750) is less frequent⁹⁰. In addition, other proteins associated with the TRL metabolism, such as deficiencies or defects of apoA-V (OMIM 144650), LMF1 (OMIM 246650), and GPIHBP1 (OMIM 615947) are all rare causes of FCS⁹⁰. Auto-antibodies against LPL¹⁰⁵, apoC-II¹⁰⁶, and GPIHBP1¹⁰⁷ also cause HTG and when severe, non-familial chylomicronemia syndrome.

Due to the autosomal recessive inheritance pattern of FCS, there may be no previous family history. In addition, since the first clinical manifestation of LPL or apoC-II deficiency often occurs during childhood or adolescence, HTG is unlikely to be the first clinical finding. Usually, patients first come to medical attention when they present with varying degrees of abdominal pain from vague queasiness to incapacitating pain due to acute pancreatitis. The presence of excess CMs is thought to be the inciting cause of pancreatitis. Frequently, when the pain is mild, it is often dismissed as common childhood illnesses. The pain may also be mistaken for an acute abdomen, presaging unnecessary exploratory procedures.

Other notable FCS-associated clinical features include eruptive xanthomas, lipemia retinalis, and hepatosplenomegaly. Eruptive xanthomas are transient cutaneous rash-like yellowish papules, 3 to 5 mm in diameter, on the trunk, elbows, or buttocks. They typically occur when TG levels peak in FCS patients due to the uptake of TRL by macrophages. They are normally nonpruritic and painless unless they are present on sensitive areas, such as the soles of feet. Lipemia retinalis is engorgement of the retinal vessels by milky and viscous lipemic plasma, visible only by fundoscopic examination. Hepatosplenomegaly is the result of macrophage uptake of TGs in liver due to persistent HTG.

When one or more FCS-associated clinical features are present in children or adolescents, the workup should include plasma TG, as well as total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, and apoB. In FCS, fasting TG levels are usually over 11.3 mmol/L (1,000 mg/dL) and often over 22.6 mmol/L (2,000 mg/dL). Typically, untreated plasma TG levels greatly exceed TC, and the TG/TC ratio of $>2.2 \text{ (mmol/L)/(mmol/L)}$ or $>5 \text{ (mg/dL)/(mg/dL)}$ ^{92, 99} is a useful indication for FCS. Importantly, low levels of apoB ($<0.75 \text{ g/L}$ or $<75 \text{ mg/dL}$) alone and especially a high TG/apoB ratio $>10 \text{ (mmol/L)/(g/L)}$ or $>8.8 \text{ (mg/dL)/(mg/dL)}$ help to differentiate FCS from type V hyperlipidemia¹⁰⁸.

Although clinical features are usually indistinguishable among different genetic etiologies in FCS, there may be subtle differences. ApoC-II deficiency is usually less severe with a later onset than LPL deficiency^{104, 109}. In addition, there seems to be a stronger association between apoC-II deficiency and insulin-dependent DM; however, it is unclear whether beta-cell destruction due to recurrent pancreatitis is the sole cause of DM.

To confirm the diagnosis of apoC-II deficiency, special biochemical and/or molecular analyses are necessary. An informative diagnostic algorithm of FCS published recently may be helpful⁹⁹. In theory LPL enzyme activity should facilitate the diagnosis of FCS. In practice, it is only available as a research assay, limiting utility for clinical use. Moreover, results may vary greatly between laboratories. LPL enzyme activity is determined by

collecting two sets of plasma, before and after an intravenous heparin administration (60 U/kg body weight). Heparin facilitates the release of LPL tethered to the endothelium into the blood stream for an *in vitro* activity assay⁶. Absent or greatly reduced LPL enzyme activity in the post-heparin plasma is diagnostic for LPL or apoC-II deficiency after excluding hepatic lipase deficiency¹¹⁰. Then, apoC-II deficiency is apparent when LPL activity is restored on adding apoC-II protein or “normal” plasma *in vitro*¹¹¹. Alternatively, LPL activity can be analyzed in adipose tissues¹¹². There also exist several ELISAs for measuring apoC-II mass, but they are not routinely used by most clinical laboratories and may be misleading, as patients with missense mutations that inactivate apoC-II may nonetheless have normal levels of apoC-II mass in plasma. The post-heparin LPL activity test, however, is only available at specialized centers and the use of heparin can trigger thrombocytopenia.

In recent years, the definitive diagnosis of FCS has increasingly relied on molecular diagnosis employing multi-gene panels targeting FCS-associated genes^{90, 113}. However, when novel *APOC2* variants are identified, additional studies may be necessary to prove their pathogenicity, including familial segregation analysis if a family history is present¹¹¹. In addition to providing a definitive diagnosis, the knowledge of molecular etiology can facilitate family screening to implement pre-symptomatic management, since TG levels can greatly fluctuate based on dietary intake of fat.

6. Genetics of apolipoprotein C-II deficiency

Due to the rarity of apoC-II deficiency, less than 30 *APOC2* mutations have been published in the literature (Table 1). Only 13 of these mutations are reported in Online Mendelian Inheritance in Man (OMIM: <https://www.omim.org/entry/608083>). Most are missense mutations, but nonsense, frameshift, splice-site due to deletion/insertion, loss of translational initiation due to methionine or promoter mutations have all been described (Fig. 4B; Table 1). Several *APOC2* mutations are in helix 1 and presumably impair LPL activation due to poor lipoprotein binding of its cofactor apoC-II (Fig. 4A, B). Mutations present in the third helix (Fig. 4A and B) likely interfere with LPL activation¹¹⁴. There are also cases of apoC-II deficient patients with clinical features but unknown genetic mutations (Table 2).

Four mutations, namely K55Q, K19T, E38K and R-19* (HGVS-recommended nomenclature: K77Q, K41T, E60K, and R4*) (Fig. 4B; Table 1) account for more than half of all *APOC2* mutations found in the ExAc exome consortium database of over 60,000 individuals (<http://exac.broadinstitute.org/gene/ENSG00000234906>). Based on the frequencies of these 4 alleles alone, approximately 1.5 individual per 100,000 in the general population would be expected to have bi-allelic apoC-II deficiency. This is much higher than an estimated prevalence derived from published case reports and reported DNA sequencing results of patients with HTG¹¹³. It is plausible that apoC-II deficiency, as with many other rare diseases, is under-diagnosed. There may also be an ascertainment bias leading to a falsely elevated frequency in the ExAc exome database, which collected the data from several studies on CVD and dyslipidemia. Furthermore, the K55Q and E38K (HGVS: K77Q and E60K) mutations are much more common in individuals from African descent, which

suggests a greater frequency of apoC-II deficiency in this population and less in the general population.

7. Clinical therapy for apolipoprotein C-II deficiency

The main goal of therapy in FCS, including apoC-II deficiency, is to prevent acute pancreatitis, the most serious morbidity and mortality in this condition. Because conventional TG-lowering medications are largely ineffective in FCS, medical nutrition therapy is absolutely essential. A fat-restricted dietary regimen with fat less than 15% of total daily calories or <20 g/day is usually recommended¹¹⁵. Suppressing TG to <11.3 mmol/L (<1,000 mg/dL) lowers pancreatitis risk considerably, and guidelines typically advise suppression <5.65 mmol/L (<500 mg/dL) when feasible¹¹⁶. Medium-chain TG may be used for cooking to improve palatability, since they are not incorporated into CMs.

Fibrates and/or niacin are regularly added as adjunct therapy, since they may result in some additional TG lowering¹¹⁷, although their effectiveness is unreliable in this disorder. Fish oil supplements should be used with caution; though they have been used successfully in some individuals¹¹⁸, they might also contribute to CM levels¹¹⁹.

Since it is very difficult for patients to maintain a long-term fat-restricted diet, novel therapies are urgently needed in FCS. Taking advantage of the multiple regulatory mechanisms of LPL function, specifically, inhibitors of LPL, apoC-III and ANGPTL3, have all emerged as potential targets to enhance the catabolism of TRL. Antisense-inhibitors of *APOC3*^{120, 121}, and *ANGPTL3*^{122, 123}, as well as a monoclonal antibody to ANGPTL3 have been extensively investigated in recent years. Even though an antisense oligonucleotide therapy has already been shown to be effective in LPL deficiency¹²¹, its efficacy in apoC-II deficiency has not been documented.

There are also several other targets which help to lower HTG, some of which are under investigation. Microsomal triglyceride transfer protein (MTP) inhibitor, lomitapide, has a limited approval for use in homozygous familial hypercholesterolemia. In theory, it could be re-purposed to decrease VLDL production⁹⁰. However, this would be well outside the label and not without significant risk; therefore, we think it is inadvisable outside extraordinary circumstances. To reduce TG synthesis, inhibitors of diacylglycerol O-acyltransferase 1 (DGAT1) may be potential treatment options for FCS⁹⁰. Recently, Millar *et al.*¹²⁴ have shown that anacetrapib treatment alone and in conjunction with atorvastatin increased apoC-II but also apoC-III levels and had no effect on direct clearance of VLDL.

Particularly for apoC-II deficiency, therapeutic plasma exchange with donor plasma that contains apoC-II is the most direct therapy and can be life-saving during severe pancreatitis episodes. This can also be implemented as a prophylactic therapy to rapidly lower TG if needed¹²⁵. The recent development of an apoC-II mimetic peptide, which incorporates the third helix of apoC-II, has shown some promise as potential future therapy. Intravenous injection of this peptide normalized TG in a mouse model of apoC-II deficiency⁸¹. Furthermore, the apoC-II mimetic peptide potentiated LPL activity in other non-apoC-II deficient HTG conditions, suggesting a potential broader utility⁹³.

8. Conclusions

ApoC-II is an important cofactor for the full activation and enzymatic activity of LPL in the hydrolysis of TG on TRL. ApoC-II mutations can promote a prodigious rise in plasma TG levels due to chylomicronemia and consequently can provoke acute pancreatitis. In addition to apoC-II, there are several other important regulators of TRL metabolism, including apoC-III, apoA-V, ANGPTL 3, 4 and 8. Therefore, TRL metabolism is a complex and multifaceted process that involves numerous proteins, as well as complex gene regulation.

Although great strides have been made in understanding the biology and biological role of apoC-II in TRL metabolism, there is undoubtedly much more to be investigated. Not only it is important in identifying novel targets for the treatment of HTG, but also in understanding the relationship between HTG and CVD risks. Currently, the most important therapy for apoC-II deficiency remains to be medical nutrition therapy with a strict low-fat diet. The inhibition of several LPL regulatory protein targets currently being investigated may soon provide better treatment options for patients with apoC-II deficiency and other forms of FCS. Finally, investigating TRL metabolism may not only uncover additional targets for FCS and the more common forms of HTG, but also may better reveal the relationship between TG metabolism and CVD risk.

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Fig. 1. Pathways for TRL metabolism

(A) The exogenous pathway. Dietary lipids are absorbed in the small intestine, packaged into CM and secreted into lymph just 1 hour after dietary fat consumption. CM contain apoB-48 as the main structural protein and acquire apoC-II and apoE (shown) as well as additional apolipo-proteins such as apoC-I, apoC-III, and apoA-V (not shown) in the perimesenteric lymphatics. Once in circulation, CM are hydrolysed by LPL bound to endothelial cells to FFA and mono-glycerides (MG) and are converted to CM remnants. CM remnants are removed from circulation by the liver *via* binding to the low-density lipoprotein receptor (LDLR) or the LDL receptor-related protein (LRP). Free fatty acids (FFA) and glycerol generated during lipolysis are utilized by parenchymal tissues.

(B) The endogenous pathway. Hepatically derived VLDL are present in the plasma in the both the fasting and postprandial states. VLDL, which contain apoB-100 as their main structural protein, are secreted into the systemic circulation, and like CM acquire many of the same apolipo-proteins in plasma. The lipolysis of VLDL bound to LPL on endothelial cells generates smaller particles deplete of TG, which are also called remnants or intermediate density lipoproteins (IDL). A fraction of VLDL particles undergoes further lipolysis and is converted to LDL particles without apoE that are taken up by the liver or peripheral tissues *via* the LDLR. Excess LDL can be deposited into the vessel wall, where it can cause atherosclerosis.

Fig. 2. Lipolytic complex

LPL action is dependent on multiple regulators, both positive (green) and negative (red). LMF1 is responsible for proper folding and assembly of LPL, whereas Sel1L stabilizes the LPL-LMF1 complex. LPL is transported from parenchymal cells to the endothelial cell surface of the capillary lumen, where it binds to GPIHBP1. ApoC-II is an essential cofactor for LPL activation, whereas apoC-I and apoC-III may inhibit lipolysis. ApoA-V stabilizes the LPL-apoC-II complex by helping TRL to bind to the endothelial cell surface *via* HSPG. ANGPTL 3, 4, and 8 all inhibit LPL but in different tissue beds. Generated FFA and MG during TG hydrolysis are taken up by cells for energy metabolism or storage.

Fig. 3. Transcriptional control of the human *APOC2* gene

(A) Relative gene position and location of major regulatory elements that control *APOC2* gene and nearby genes in *E/C-I/C-IV/C-II* gene cluster on Chromosome 19. The liver-specific elements HCR.1 and HCR.2 are 319 bp elements approximately 22 kb and 11 kb, respectively, upstream of the *APOC2* promoter. Both contain FXR elements required for bile-acid – dependent activation of the *APOC2* gene. The HCR.1 FXRE (nt 214/226 of HCR.1) also binds HNF-4 and ARP1. The proximal *APOC2* gene promoter is 545 bp long and contains binding sites for HNF-4 (–102 – –81), RXR α /T3R β (–140 – –155), cAMP (between –36 and –170) and STAT1 (–500 – –493). Long-range interactions between FXR/RXR α in HCR.1 and RXR α at the RXR/T3R β binding site have been shown to mediate hepatocyte-specific bile-acid regulation of the *APOC2* gene (shown by upper bracket and green + at top of Fig. 3A). The RXR α /T3R β binding site also binds ARP1 and EAR1, which may affect transcriptional activation by RXR. The ME.1 and ME.2 are 620-bp multienhancer elements that contain LXREs, both located at nt 442 – 466 of each ME. The LXRE in ME.2 regulates the entire gene cluster. In macrophages, two STAT1 binding elements, one in ME.2 (between nt 174 – 182 of the 620 bp ME.2 element) and one between nucleotides –500 and –493 of the proximal *APOC2* promoter, upregulate *APOC2* gene expression. Long-range interactions between STAT1 bound to ME.2 and RXR α at the RXR/T3R β binding site have been shown to mediate macrophage-specific STAT1 regulation of the *APOC2* gene (shown by lower bracket and purple + at top of Fig. 3A). Fibrates, statins and ezetimibe decrease *APOC2* gene expression but the promoter elements involved have not been defined. Figure is not to scale.

(B) Regulation of *APOC2* gene by long noncoding RNA. lncLSTR indirectly inhibits *APOC2* gene expression by a bile-acid-dependent mechanism, involving CYP8B1 and its transcriptional repressor TDP-43. Figure is not to scale.

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Fig. 4. ApoC-II structure

(A) Sequence and the helical wheel plot of apoC-II helices. Charged residues are colored light blue, hydrophobic residues are green, neutral polar residues orange and Q and N are colored red. Position of hydrophobic moment and score is shown in center of helix.

(B) Human *APOC2* gene mutations. Position of known mutations are listed below exon-intron diagram of *APOC2* gene with position 1 after the signal peptide cleavage site. * or X: STOP codon. The four mutations shown in bold account for more than half of all *APOC2* mutations.

Table 1

ApoC-II mutations associated with HTG.

	ApoC-II Variant	Mutation	Position ^{a, b}	Type	Allele count per 100,000	Clinical features (TG mmol/L)	Source
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. 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):179 13-6
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):117 4-83
10	APOLIPOPROTEIN C-II (BARI)	Introduction stop codon (Tyr ₃₇)/ Termination	Y37* (Y59X)	Nonsense	<1	HTG (56.5), P, X	BBRC 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

	ApoC-II Variant	Mutation	Position ^{a, b}	Type	Allele count per 100,000	Clinical features (TG mmol/L)	Source
12	APOLIPOPROTEIN C-II (SAN FRANCISCO)	p.Glu ₃₈ Lys	E38K (<i>E60K</i>)	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 (<i>R72T</i>)	Missense	<1	HTG (33.9), P, G	J Clin Endocrinol Metab. 2017 Feb 13
14	APOLIPOPROTEIN C-II (AFRICAN)	p.Lys ₅₅ Gln	K55Q (<i>K77Q</i>)	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* (<i>Y85X</i>)	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 (<i>fsT90</i>)	Frameshift	<1	HTG (107.0)	NEJM (1978) 299:1421-1424, PNAS USA (1987) 84:270-273, J Medical Genetics (1988) 25:649-652
17	APOLIPOPROTEIN C-II (ONTARIO)	p.Gln ₇₀ Ter	Q70* (<i>Q92X</i>)	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 (<i>fsQ92</i>)	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 (<i>L94P</i>)	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 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

HTG, hypertriglyceridemia; X, xanthomas; C, chylomicronemia; P, pancreatitis; H/S, hepatosplenomegaly; LR, lipemia retinalis;

G, hyperglycemia, glucose intolerance or diabetes mellitus; O, other features (coronary, cerebral and/or iliofemoral atherosclerosis, hypertension, rupture of aortic aneurysm, macrocephaly, neurologically abnormalities, developmental delay, vomiting) ; A, atherosclerosis; O, triglycerides levels reported in mmol/L.

^a#1 is first amino acid of mature peptide after signal peptide cleavage

^b#1 is initiator methionine; first amino acid of protein before signal peptide cleavage (HGVS-recommended nomenclature)

* or X: STOP codon.

Table 2

ApoC-II deficiencies with unknown genetic mutations

	ApoC-II Variant	Mutation	Clinical features (TG mmol/L)	Source
1	APOLIPOPROTEIN C-II (BETHESDA)	Unknown	HTG (13.6)	J Lipid Res. 1988 Mar;29(3):273-8
2	APOLIPOPROTEIN C-II (TOKYO #2)	Unknown	HTG (36.5), P	J Atheroscler Thromb. 2013;20(5):481-93
3	APOLIPOPROTEIN C-II (TOKYO #3)	Unknown	HTG (172.6), C, X, LR	Eur J Pediatr 1989 Apr;148(6):550-2
4	APOLIPOPROTEIN C-II (TOSHIGI)	Unknown	HTG (6.1), G, CAD	Clin Exp Med 2002 May;2(1):29-31

HTG, hypertriglyceridemia; P, pancreatitis; C, chylomicronemia; X, xanthomas; LR, lipemia retinalis; G, Hyperglycemia, glucose intolerance or diabetes mellitus; CAD, coronary artery disease; (),triglycerides levels reported in mmol/L.