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APOCIII: A MULTIFACETED PROTEIN IN CARDIOMETABOLIC DISEASE

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SUMMARY

ApoCIII has a well-recognized role in triglyceride-rich lipoproteins metabolism. A considerable amount of data has clearly highlighted that high levels of ApoCIII lead to hypertriglyceridemia and, thereby, may influence the risk of cardiovascular disease. However, recent findings indicate that ApoCIII might also act beyond lipid metabolism. Indeed, ApoCIII has been implicated in other physiological processes such as glucose homeostasis, monocyte adhesion, activation of inflammatory pathways, and modulation of the coagulation cascade. As the inhibition of ApoCIII is emerging as a new promising therapeutic strategy, the complete understanding of multifaceted pathophysiological role of this apoprotein may be relevant. Therefore, the purpose of this work is to review available evidences not only related to genetics and biochemistry of ApoCIII, but also highlighting the role of this apoprotein in triglyceride and glucose metabolism, in **the** inflammatory process and coagulation cascade as well as in cardiovascular disease.

Keywords: *APOC3* gene, ApoCIII protein, triglycerides, triglycerides (TG)-rich lipoproteins (TRL), *APOC3* gene expression, cardiovascular disease risk, therapeutic target.

Abbreviations: ApoB, apolipoprotein B; ApoCIII, Apolipoprotein CIII; APOE, apolipoprotein E; ASCVD, atherosclerotic cardiovascular disease; ASO, antisense oligonucleotide; CHD, coronary heart disease; ChREBP, Carbohydrate-responsive element-binding protein; CM, chylomicrons; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ERRa, estrogen-related receptor a; FCS, Familial Hyperchylomicronemia; FOXO1, forkhead box O1 protein; FVIIa-AT, Factor VIIa-antithrombin complex; G6PC, glucose-6-phosphatase; GalNAc3, N-acetyl galactosamine 3; GOF, gain of function; GPIHBP1, Glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1; GWAS, Genome Wide Association Studies; HDL-C, high density lipoprotein cholesterol; HDLs, high density lipoproteins; HFD, high-fat diet; HL, hepatic lipase; HNF4a, hepatic nuclear factor-4a; HSPGs, heparan sulfate proteoglycans receptor; HTG, hypertriglyceridemia; IGFBP1, insulin-like growth factor binding-protein 1; IHD, ischemic heart disease; IL-1, interleukin-1; IRS, insulin receptor substrate; IVD, ischemic vascular disease; LDLR, LDL receptor; LOF, loss-of-function; LPL, lipoprotein lipase; LRP1, LDL-C, low-density lipoprotein cholesterol; LDLR-related protein 1 receptor; LXRs, liver X receptors; MTTP, microsomal triglyceride transfer protein gene; MZ, monozygotic; NAFLD, non-alcoholic fatty liver disease; PPAR-a, Peroxisome proliferator-activated receptor alfa; PPAR- γ , Peroxisome proliferator-activated receptor gamma; PGC1b, Peroxisome proliferator-activated receptor gamma coactivator 1-beta; PUFAs, polyunsaturated fatty acids; lecithin-cholesterol acyl transferase, LCAT; cholesterol-ester transfer protein, CETP; RCT, reverse cholesterol transport; SDC1, syndecan-1; SNVs, single nucleotide variants; TF, tissue factor; TG, triglycerides; TNF-a, tumor necrosis factor α ; TRL, triglyceride-rich lipoproteins; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; VLDL, very low-density lipoprotein; WAT, white adipose tissue.

Introduction

Apolipoprotein CIII (ApoCIII) is one of the most abundant apolipoproteins in the blood, where it is present as an exchangeable component between high-density lipoprotein (HDL) and apolipoprotein B (ApoB)-containing lipoprotein particles (very-low and low density lipoproteins, VLDL and LDL). While in normolipidemic subjects, the majority of plasma ApoCIII is bound to HDL, in hypertriglyceridemic subjects it is bound to triglyceride (TG) rich lipoprotein (TRL), VLDL and chylomicrons (CM) (1,2).

ApoCIII was first isolated and characterized nearly 50 years ago by Brown *et al.* (3). Studies on protein structure and dynamics revealed that it is an 8.8 kDa glycoprotein synthesized mainly by the liver, and to a lesser extent, by the intestine (4–7). For several years, ApoCIII has been investigated mainly for its strong correlation with TG concentrations. Firstly, ApoCIII was recognized as an inhibitor of lipoprotein lipase (LPL) and hepatic lipase (HL) (8–10), key enzymes in the hydrolysis of TG contained in TRL (11). Secondly, in addition to its extracellular functions, ApoCIII was found to play an intracellular role by promoting VLDL-TG secretion by hepatocytes (12). Genome-wide association studies (GWAS) and Mendelian randomization studies have demonstrated a direct relationship between loss-of-function (LOF) mutations in ApoCIII-coding gene (*APOC3*) and reduced risk of atherosclerotic cardiovascular disease (ASCVD) (13). Further evidences have highlighted how ApoCIII levels directly associate with endothelial cells (EC) dysfunction, monocyte adhesion, thrombosis, type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD). All these data allowed the identification of ApoCIII as a therapeutic target to treat hypertriglyceridemia (HTG), T2DM and to reduce ASCVD risk.

Therefore, ApoCIII should be considered as a multifunctional protein with major physiological relevance beyond TRL metabolism and with a key role in **cardiometabolic diseases**.

The genetics of ApoCIII

APOC3 gene is a member of the *APOA1/C3/A4/A5* gene cluster, an area in strong linkage with lipid metabolism. The gene encoding for ApoCIII is located on the long arm of chromosome 11 at position 23.3 (11q23.3).

Transcriptional control of gene expression

APOC3 gene expression is tightly regulated through several factors determining its tissue-specific expression. Scarce data exist regarding which factors regulate its expression in the intestine. By contrast, abundant research on mechanisms that control *APOC3* expression in the liver has been conducted (14–33). **Table 1** summarises the main factors regulating *APOC3* gene expression (18, 24–26, 30–32).

The history of APOC3 variants

Several *APOC3* single nucleotide variants (SNVs) have been described in humans. Both LOF(34–42) and gain-of-function (GOF) (43–45) variants have been discovered. Most of them were identified in isolated populations, but those within protein-coding sequences were subsequently tested to understand to what extent they contribute to TG variation in the general

population (34–45). **Supplementary Figure 1** summarizes LOF and GOF variants in the *APOC3* gene identified to date and their relative position in the protein sequence. **Table 2** shows all significant studies evaluating the effect of LOF and GOF *APOC3* variants on plasma ApoCIII concentrations and lipid profile in carriers vs. non-carriers. *In vitro* functional results, and kinetic studies were also reported (46–50).

Role of ApoCIII in TRL metabolism

All lipoproteins may contain ApoCIII, although **this apoprotein** is mainly represented in CM, VLDLs and their remnants and HDL. In its mature form, ApoCIII consists of 79 amino acids (from 99 amino acids of precursor protein), with 6 alpha amphipathic helices, capable of binding to lipids (5,7,51). **As well as the other** apolipoproteins, ApoCIII is a key player in plasma TG metabolism.

Kinetic studies have clearly shown that increased levels of plasma ApoCIII are the main determinant of plasma TG levels in the population **with** impaired TRL catabolism (52). In fact, ApoCIII concentration in normolipidemic subjects are about 8-10 mg/dl while up to 3-times higher levels can be found in patients with HTG (5). In normal conditions, most of the plasma ApoCIII is bound to HDL whereas, in HTG subjects, the majority is bound to TRL. Although ApoCIII is a relatively minor component of HDL protein, approximately 50% of total plasma ApoCIII may be **contained** in the HDL fraction (1,2,53). Therefore, while under normal conditions HDL particles work as the reservoir of ApoCIII, in HTG states there is an enrichment **of ApoCIII in TRL and a** concomitant shift within these lipoproteins from **predominantly** ApoE-containing particles (which are quickly removed from the circulation and have little tendency to form LDL) **to** ApoCIII-enriched particles (53–56).

As shown in **Figure 1**, ApoCIII influences TRL metabolism through different pathways: **a)** by promoting the assembly and production of VLDLs at the hepatic level **b)** by inhibiting the activity of LPL, **c)** by interfering with **ApoE**-mediated hepatic uptake of VLDL particles (57). Studies in genetically modified HTG mice overexpressing ApoCIII, displayed increased hepatic secretion of VLDL particles (58). By contrast, inhibition of ApoCIII synthesis in mice using antisense oligonucleotides (ASO) did not reduce VLDL secretion (59), so that the biological significance of ApoCIII as a regulator of hepatic VLDL assembly and release is still unclear, particularly in humans. Even though we can assume that ApoCIII raises plasma TG by inhibiting LPL (3,9,60,61), many *in vitro* and *in vivo* studies showed controversial results (56,62–64). Some of them have reported only minor effects of ApoCIII on LPL activity (62, 65). A clinical study by Gaudet *et al* (66) conducted in three patients with complete LPL deficiency showed that ASO-induced ApoCIII reduction was associated with a drastic reduction in plasma TG levels (66). This observation argues that ApoCIII may also act independently from LPL, suggesting the existence of other pathways of TG removal linked to ApoCIII.

Indeed, recent human kinetic studies showed that ApoCIII-containing TRL are cleared more slowly than ApoCIII-lacking TRL. This effect does not involve any changes in LPL activity, but it represents the consequence of the increase in the hepatic uptake of remnants through their interaction with specific hepatic receptors, namely heparan sulphate proteoglycans

receptor (HSPGs), syndecan-1 (SDC1), the LDL receptor (LDLR) and the LDLR-related protein 1 (LRP1) receptor (56,67–70).

It has been also proposed that ApoCIII prevents the elimination of lipoprotein particles displacing ApoE from TRLs (67,68). However, this mechanism is questionable as anti-*APOC3* ASO treatment did not affect ApoE levels on TRL (56). However, the results by Ramms *et al* (71), suggested a new metabolic model in which ApoE determines the effects of ApoCIII in mediating either the inhibition of hepatic uptake of TRLs or the activity of LPL in adipose tissue.

Role of ApoCIII in HDL function and metabolism

In addition to LPL, it has been demonstrated that ApoCIII influences the activity of other enzymes, particularly LCAT and CETP, which are crucial in HDL metabolism and function (72).

Experimental studies have shown that ApoCIII inhibits LCAT activity while stimulates CETP activity (54). It has also been reported that ApoCIII in HDL can bind to scavenger-receptor BI (SR-BI) (73), thus inhibiting the transfer of cholesterol from HDL and LDL to hepatocytes (73). Furthermore, biochemical studies revealed the presence of two types of HDL particles, those with ApoCIII and those without ApoCIII (74). **Nevertheless**, the percentage of ApoCIII in HDL particles has been reported to be significantly correlated with the risk of coronary artery disease (CAD) (74). In vitro experiments indicate that the deterioration of anti-apoptotic effects of HDL observed in CAD patients was associated with the remodeling of HDL proteome, **and this was** mainly characterized by the increase in ApoCIII content (75).

Less is known about a potential direct effect of ApoCIII on HDL-mediated cholesterol efflux. Recently, the work by Luo *et al* (76) highlighted that the presence of ApoCIII in HDL impairs the HDL-mediated cholesterol efflux capacity. Since both ApoCIII_{HDL} and ApoCIII_{HDL} ratio (apoCIII_{HDL} over plasma ApoCIII) inversely correlate with cholesterol efflux capacity, the authors hypothesized that elevated ApoCIII in HDL could competitively bind to SR-BI and interrupt, or impair, HDL-mediated **reverse cholesterol transport** (RCT). In addition, **when** evaluating the interaction effect of ApoCIII and ApoE on HDL metabolism, Morton *et al* (77) found that ApoE and ApoCIII cooperate in affecting HDL metabolism. Moreover, subspecies of HDL **containing** ApoE but not ApoCIII, uniquely participate in classical pathways of RCT. As ApoE promotes metabolic steps in RCT, when ApoCIII coexists with ApoE on HDL, abolishes these benefits (77). Overall, these observations strongly suggest that the increase of ApoCIII in HDL may deteriorate the atheroprotective functions of these particles, thus indicating another mechanism by which ApoCIII **might** influence ASCVD risk.

The role ApoCIII on vascular endothelial cells dysfunction, monocyte adhesion and inflammation

Basic science studies have highlighted that ApoCIII is also involved in several other crucial checkpoints promoting atherogenesis, such as monocyte adhesion to EC and smooth muscle cell (SMC) proliferation (78,79). It is known that the activation of nuclear factor κ B (NF- κ B) is responsible for endothelial dysfunction and monocyte adhesion (80). Kawakamy *et al* (80) have found that ApoCIII activates PKC β and NF- κ B thus increasing VCAM-1 and ICAM-1 expression in endothelial cells and promoting monocyte adhesion. Moreover, NF- κ B also drives the enhancement of β 1-integrin expression in

monocytes, **thus** further favouring the adhesion of these cells to EC (81). Consistently, it has been observed that ApoCIII-rich VLDL directly activates EC and monocytes recruitment (82). In an elegant study aimed at elucidating the atherogenic role of ApoCIII, Yingchun *et al* (82) fed ApoCIIItg/LDLR^{-/-} and LDLR^{-/-} littermates with an atherogenic diet for 12 weeks. Interestingly, authors found that ApoCIIItg mice had increased inflammation and endoplasmic reticulum (ER) stress in the aorta, with increased infiltration of macrophages and VCAM-1 expression, as compared to littermates. In fact, TRLs+ApoCIII can induce MCP1 expression in human umbilical vein EC, **it** promotes the expression of IL6 and MCP1 in peritoneal macrophages and, stimulated by LPS, can increase the expression of VCAM-1. These results suggested that TRL enriched in ApoCIII could induce a greater level of inflammation than TRL alone (82). In a subsequent study, the same authors examined whether statin treatment was able to suppress ApoCIII-induced EC activation in vitro and in vivo (83). Despite a limited direct effect on ApoCIII levels, they found that statins were able to hinder ApoCIII's stimulation of vascular adhesiveness by downregulating VCAM-1. Additionally, Botteri *et al* (84) showed that the exposure of myotubes and isolated skeletal muscle to VLDL increases the levels of ER stress and inflammatory markers and attenuates the insulin signalling pathway (84). Moreover, their experiment highlighted that ApoCIII simulates the effects of VLDL confirming the crucial role of this apolipoprotein in mediating VLDL actions (84).

Finally, with the aim to investigate the role of ApoCIII in restenosis and clarify how smooth muscle cells respond to TRLs with or without ApoCIII, Li *et al* (79) studied various ApoCIII transgenic and knockout mice undergoing endothelial denudation to model restenosis. They showed that ApoCIII-enriched TRL, via Akt signalling pathway, promoted SMC proliferation, VCAM-1 expression, and reactive oxygen species (ROS) production, thus worsening atherogenesis and coronary stenosis in mice with severe HTG (79).

Role of ApoCIII in coagulation cascade and thrombosis

Several lines of evidence suggest a direct effect of ApoCIII on thrombosis (85). In a prospective study including 663 subjects with angiographically proven ASCVD, it was observed an independent positive association between elevated ApoCIII and enhanced thrombin generation (86). The same authors (87) found that elevated circulating levels of ApoCIII (but not other lipids or apolipoproteins) were involved in a progressive increase in factor II (FII) coagulant activity in the plasma of patients with or without CAD. In addition, ApoCIII was found to be strongly associated with the activated FVII-anti-thrombin (FVIIa-AT) complex. FVIIa-AT is an indirect marker of intravascular exposure of tissue factor (TF), thus indirectly suggesting an activation of the extrinsic coagulation pathway. In a recent study, including 127 patients with venous thromboembolism and 299 controls, levels of apolipoproteins CI, CII, CIII and E were associated with the plasma concentration of several coagulation factors such as vitamin K-dependent factors, factor XI, antithrombin, clot lysis time as well as factor VIII and von Willebrand factor levels (88). These results may suggest that increased concentration of ApoCIII **might** be potentially used as a biomarker for the identification of subjects with pro-thrombotic diathesis and increased risk of thrombosis (89).

The demonstration that the administration of volanesorsen, an anti-ApoCIII ASO, is able to cause a significant drop in platelets count (90) raised the possibility that the ApoCIII inhibition might unfavourably interfere with platelets production or activity. By analysing a population of 42,503 participants in the UK Biobank, Khetarpal *et al* (91) tested whether heterozygous carriers of LOF mutations in *APOC3* were at increased risk of thrombocytopenia (91,92). They did not find any differences between carriers and non-carriers thus concluding that this hematologic abnormality might be a **drug** or class-specific side effect and not **directly** related to the inhibition of ApoCIII. **Further** studies are needed to clarify the role of ApoCIII in influencing platelet function and count.

Other potential atherogenic effects of ApoCIII

ApoCIII has been implicated in other potential proatherogenic effects. It has been shown that this apolipoprotein increases the affinity of LDL particles to proteoglycans in the artery wall (93,94), thus influencing the accumulation of atherogenic lipoproteins in the vessel wall. Since ApoCIII does not directly bind proteoglycans, the enhanced binding may result from a conformational change associated with increased ApoCIII content by which ApoB and/or ApoE become more accessible to proteoglycans (93,94). This hypothesis has been confirmed in the work by Hiukka *et al* (95) showing that LDL particles with a high ApoCIII/ApoB ratio have reduced content of unesterified cholesterol, sphingomyelin and ceramide. These changes in lipid composition lead to higher membrane fluidity, thus allowing ApoB to acquire a conformation that is more favorable for proteoglycan binding (96). In the artery wall, LDL is exposed to several enzymes, including sphingomyelinases (SMase) that promote aggregation and fusion of retained lipoproteins (97). ApoCIII is an activator of SMase and ApoCIII-enriched LDL displays increased susceptibility to hydrolysis and aggregation by SMase (95).

The role of ApoCIII in the risk of clinical complications of ASCVD

In addition to the above-mentioned experimental evidence linking ApoCIII to atherogenesis, a bulk of studies have convincingly demonstrated that ApoCIII contributes to clinical manifestations of ASCVD in humans (13, 36, 39, 40, 74, 98–104). To this regard, of special interest are the genetic studies testing the impact of LOF *APOC3* variants with large TG-lowering effect on CVD risk. It has been reported that the R19X LOF mutation (36) is associated with 60% decrease in the risk of coronary artery calcification. In the study by Crosby *et al* (39), the risk of coronary heart disease (CHD) among 498 carriers of any rare LOF *APOC3* mutation was 40% lower than the risk among 110,472 non-carriers (39). Moreover, LOF mutations were also associated with a 46% reduction in levels of ApoCIII **and 41% and 36%** risk reduction of ASCVD and ischemic heart disease (IHD), **respectively** (40). A still open question is whether the reduced ASCVD risk found in *APOC3* LOF carriers is driven by lowering plasma TG levels or by other lipid-related effect, such reduction of LDL-C, cholesterol remnants or increased HDL-C (98). Recently, Wulff AB *et al* (13) performed a mediation and meta-analysis study, showing that the low risk of ASCVD observed in *APOC3* LOF mutation carriers was accompanied by a reduction of remnant cholesterol (mean 43%) without any changes in LDL-C (mean 4%). Although not significant, the percentage of non-carriers taking lipid lowering therapies (9%) was higher than that of *APOC3* LOF mutation carriers (6%). **Moreover**, ApoB, a marker

of the total number of atherogenic lipoprotein particles, was 13% lower in LOF heterozygotes compared to non-carriers (13,99). Ference *et al* (105), evaluating 63 cohort or case-control studies conducted in North America or Europe between 1948 and 2017, found similar lower risk of CHD per unit difference in ApoB, after comparing patients having LPL-triglyceride-lowering and LDL-C-lowering variants (105). Therefore, the clinical benefit of lowering TG and LDL-C levels may be proportional to the absolute change in ApoB (105).

The association between ApoCIII or ApoCIII-containing lipoproteins and ASCVD risk has been studied in several retrospective and prospective study. Pechlaner *et al* (100) directly measured apolipoproteins by mass spectrometry in the Brunech Study and compared their associations with incident ASCVD. They found that the apolipoproteins most significantly associated with incident cardiovascular disease were ApoCII, ApoE and ApoCIII (HR=1.38; 95% CI 1.17-1.63). These associations were independent of HDL and non-HDL-C and were extended to the risk of stroke and myocardial infarction. Plasma ApoCIII levels were measured in all baseline samples included in a prospective nested case-control study among participants of the European Prospective Investigation of Cancer (EPIC)-Norfolk cohort (101). Despite ApoCIII levels were significantly associated with the risk of CAD (HR=1.91; 95% CI 1.48-2.48), results from the mediation analysis found that the elevated levels of remnant lipoproteins, small dense LDL, and low-grade inflammation may explain this association. In a recent prospective study, it was reported that the risk for both fatal and nonfatal myocardial infarction was significantly increased in patients with an increased fraction of VLDL or LDL containing ApoCIII (102). As reported above, in two prospective studies, HDL subgroups (containing ApoCIII and without ApoCIII) were differentially associated with CVD, **showing that an** increase in HDL enriched in ApoCIII **was associated** with a higher CVD risk (74). Similarly, the presence of ApoCIII on LDL correlated with the development of CVD (103). These findings suggest that the presence or absence of ApoCIII on lipoprotein particles may turn HDL into an atherogenic lipoprotein, and the dose of ApoCIII on LDL and VLDL may determine the degree of their atherogenicity (103).

Furthermore, the impact of ApoCIII levels on ASCVD risk has been recently confirmed in a meta-analysis including a total of 3163 cases of cardiovascular events from 5 retrospectives and 7 prospective studies (104). Authors have shown that while 5-mg/dL increase in total plasma ApoCIII translates into a relative risk estimate of 1.33 (95% CI 1.07–1.66) for cardiovascular events, a comparable increase in the non-HDL ApoCIII fraction translates into a 2.48-fold increased risk (95% CI 1.48–4.32) (104).

These results suggest that the robust association between ApoCIII and ASCVD might be explained by the ApoCIII content in atherogenic lipoproteins **so that** ApoCIII might be **considered as a** potent marker of the atherogenicity of lipoprotein particles.

ApoCIII, glucose metabolism and T2DM

Emerging evidence suggests that ApoCIII may also be involved in the pathophysiology of T2DM through pancreatic beta cell apoptosis and glucose homeostasis (14).

As previously mentioned, the expression of ApoCIII is increased by insulin deficiency, and insulin resistance and hyperglycemia induce ApoCIII transcription (18,24). In addition, mice overexpressing ApoCIII are more susceptible to high-fat diet (HFD)-induced diabetes (106). In humans, elevated levels of circulating ApoCIII are associated with insulin resistance (25) and, consequently, T2DM. In fact, many observational studies have reported a positive correlation between circulating ApoCIII concentrations and the incidence of both type 1 and T2DM (107–110). However, despite many of these associations are apparently independent from plasma TG levels and other confounding factors, the cross-sectional nature of these investigations does not permit to give definite conclusions and it is still not clear if ApoCIII is *itself* the driver of hyperglycemia and diabetes onset (111). Nevertheless, findings from a study involving a multi-ethnic population of 4579 individuals showed that the association might probably be determined primarily by TG-related mechanisms (112). In fact, increased total ApoCIII concentrations were associated with a higher rate of diabetes whereas ApoCIII-defined HDL subspecies had an opposite effect. These results were also supported by *in vivo* study in which overexpression of the *APOC3* gene resulted in elevated circulating triacylglycerol levels and increased susceptibility to hepatic insulin resistance and fatty liver disease (113).

These results have been also confirmed in a randomized, double blind, placebo-controlled trial performed on 15 adult patients with T2DM (HbA1c >7.5% [58 mmol/mol]) and HTG (TG >200 and <500 mg/dL) that have been treated with volanesorsen (114). In this study, the action of volanesorsen was not only associated with the reduction of plasma ApoCIII and TG and increased HDL-C levels but also with the improvement of glucose disposal and insulin sensitivity (114).

Recently, Bozzetto *et al* (115) evaluated if ApoCIII concentrations associate with differences in body mass index (BMI) or glycemic response during oral glucose tolerance test in young and healthy monozygotic (MZ) twins. Despite they did not find any relation between ApoCIII and acquired differences in BMI, they showed that ApoCIII levels were associated with early dysregulation of glucose metabolism independently of obesity and genetics.

Other lines of evidence suggest that ApoCIII may influence β -cell function. In rats, increased ApoCIII concentrations in pancreatic islets promoted a local inflammatory milieu, with consequent β -cell failure (117). Moreover, *in vitro* and animal studies suggested a role of ApoCIII in inducing islet insulin resistance (118). Insulin resistance and β -cell failure are the major defects in T2DM. Preventing insulin resistance at the islet level is thus crucial to preserve β -cell mass, especially during the late phase of T2DM, where β cells are progressively lost. Overall, these studies support the notion that the regulation of *APOC3* expression pathway may be involved in the crosstalk between glucose and lipid metabolism (119). Therefore, studying ApoCIII helps in understanding TG swings in chronic inflammatory states (such as T2DM) but whether its inhibition translates into a potential benefit still remains to be addressed.

The role of ApoCIII in hepatic steatosis

ApoCIII was also suggested as a risk factor for hepatic steatosis. However, the physiological function of ApoCIII in lipid metabolism of the hepatocyte is still unclear and matter of debate.

Duivenvoorden *et al.* (120) showed that *APOC3* knockout mice are more susceptible to develop diet-induced obesity and NAFLD. Moreover, in order to gain mechanistic insight about the pivotal role of ApoCIII protein in liver, Cheng *et al.* (121) fed *APOC3*-transgenic and wild-type mice with a high fructose or high fat diet. Then, these authors tried to determine the effect of *APOC3* on hepatic TG synthesis and secretion, inflammation and the progression of NAFLD. They provide compelling evidence that while *APOC3* is clearly culpable for HTG, it does not play a pivotal role in the pathogenesis of NAFLD in high fat-fed *APOC3*-tg mice (121). Apparently, also studies in humans do not support a role of ApoCIII in favoring NAFLD (122–127).

Although Petersen *et al.* (122) reported that subjects with the common genetic variants within the *APOC3* promoter (-455T > C rs2854116 and -482C > T rs2854117) are at increased risk of developing NAFLD, Kozlitina *et al.* (123) found no association between these two *APOC3* variants and NAFLD in humans. This latter observation was supported by three other independent clinical studies showing that carriers of neither one nor both *APOC3* alleles are associated with NAFLD (124–126). Finally, results of a large meta-analysis (127) involving seven clinical trials have clearly disproved the association between polymorphisms in *APOC3* and the risk of NAFLD.

Moreover, given the role of ApoCIII in facilitating the intracellular VLDL-TG assembly and secretion (4), this protein is probably an ill target for therapeutic intervention of NAFLD. In a study on chow- and WD-fed C67BL/6 and apoC3^{-/-} mice treated with the ASO against ApoCIII, authors found that the secretion of TG by the liver was apparently unaffected by the treatment. In this study, authors observed a non-statistically significant increase of liver TG levels in this mouse model (59).

Considering all these data, more studies are warranted to definitively establish if ApoCIII is a predisposing factor for exacerbating the development of NAFLD in humans and if its inhibition is associated with adverse outcomes.

ApoCIII as a therapeutic target

Previously summarised evidence allowed identifying ApoCIII as a therapeutic target to treat HTG and, potentially, T2DM, and to control ASCVD risk. At present, clinical trials evaluating the benefit of reducing ApoCIII synthesis in HTG are under way.

We have recently summarized the available evidence on the efficacy and safety of a recently developed, second-generation ASO directed towards *APOC3* mRNA synthesis, named volanesorsen, that has been used to treat HTG (128). Pre-clinical and clinical studies have highlighted an unprecedented efficacy of this silencing technology in reducing TG even in the more resistant form of HTG as familial chylomicronemia syndrome (FCS) (59,90). The administration of volanesorsen subcutaneously has led to a significant reduction of TG levels, with 77% of FCS subjects achieving TG below 750 mg/dl as compared with only the 10% in the placebo group (90). Nevertheless, in a recent published meta-analysis on 3 randomized controlled clinical studies including a total of 156 patients (with diabetes, FCS or with moderately high levels of TG) (129), authors showed that the benefit of volanesorsen was also extended to HDL-C (+ 40%), VLDL-C (- 73%), ApoB-48 (- 65%), ApoCIII (- 75%), and VLDL ApoCIII (- 84%). This dose-dependent decrease in ApoCIII obtained with volanesorsen might be particularly interesting if considering the role of ApoCIII as risk factor for ASCVD (130).

However, despite these promising data, Phase III trials have raised some concerns especially with regards to the severe thrombocytopenia side effect (90). As previously mentioned, the decrease in platelet count was an unexpected result and an interesting debate is still ongoing to establish the possible causes of this side effect (91,92,128).

Considering the potential severity of side effects, the benefit/risk ratio of volanesorsen has been considered positive only by the European Medical Agency (EMA) so far, leading to the authorization of volanesorsen for the treatment of FCS patients with high risk of acute pancreatitis for whom an alternative drug does not currently exist (128,131,132).

Noteworthy, new targeting approaches of ASOs, using a triantennary N-acetyl galactosamine (GalNAc3) modified ASOs that targets the asialoglycoprotein receptor (ASGPR) in hepatocytes, has been recently developed. This new ASO, AKCEA-APOCIII-LRx, was recently tested and results have shown similar efficacy than volanesorsen, with 20–30-fold lower dosing, thus minimizing systemic exposure (133). As volanesorsen, the benefit of the administration of AKCEA-APOCIII-LRx was also associated with significant reduction in total cholesterol, apoB, non-HDL-C, VLDL, and increases in HDL-C (133). Nevertheless, AKCEA-APOCIII-LRx was also well tolerated with one injection site reaction of mild erythema, and no flu-like reactions, liver or renal safety signals and, **more importantly**, no thrombocytopenia events (133).

Further studies are needed to clarify the efficacy and safety of pharmacological inhibition of ApoCIII in treating not only hypertriglyceridemia but also ASCVD. In this context, the ongoing trial with AKCEA-APOCIII-LRx might probably help answering to this question (134).

Conclusions

The “ApoCIII saga” is a clear example of how the translational research approach in metabolic disease has the potential to influence quality of life and health-span beyond the boundaries of traditional disease-based research. From the analysis of this big plethora of data, it emerges how the role of ApoCIII in humans is more complex than initially thought. Investigations using anti-ApoCIII ASO in LPL deficient states have been crucial in illustrating that ApoCIII regulates TRL catabolism beyond the modulation of LPL function. In addition, **novel evidences have highlighted how** ApoCIII may act in other pathways involved in the network between lipids, glucose metabolism, endothelial cells dysfunction, inflammation and coagulation cascade. Even though other investigations are needed to better clarify this complex interplay, the available evidence may clearly expand the role of ApoCIII in cardiometabolic diseases. In this context, the development of new drugs aimed at inhibiting ApoCIII-synthesis is emerging as a very promising strategy in controlling severe HTG states. It remains to be established if this approach may be useful also in controlling the residual risk associated with atherosclerosis and other metabolic disorders.

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FIGURE LEGEND

Figure 1. Apolipoprotein CIII (ApoCIII) and Triglyceride (TG)-Rich Lipoproteins (TRL) Metabolism.

Plasma concentration of TG is a function of the relative equilibrium between production and clearance of TRL particles (16). These processes are directly related to the balance between de novo synthesis of TRL by the liver (VLDL), intestinal absorption of fat from the diet (CM), lipolysis in peripheral tissues (LPL) and hepatic clearance, i.e., impaired clearance, or increased production, or both (CM remnants, VLDL and VLDL remnants). Fluctuations in either production or clearance rates may lead to accumulation of TG ultimately leading to HTG. During LPL-driven hydrolysis of TGs contained in VLDL and CM, there is a transfer of ApoCIII from VLDL to HDL particles and then back to other neo-secreted VLDLs (54-55). ApoCIII influences TRL metabolism through different pathways: **A**) by promoting the assembly and production of VLDLs at hepatic level, **B**) by inhibiting the activity of LPL and **C**) by interfering with ApoE-mediated hepatic uptake of VLDL particles.

Positive (→) and negative (—|) effects were reported.

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Figure 1. Apolipoprotein CIII (ApoCIII) and Triglyceride (TG)-Rich Lipoproteins (TRLs) Metabolism.

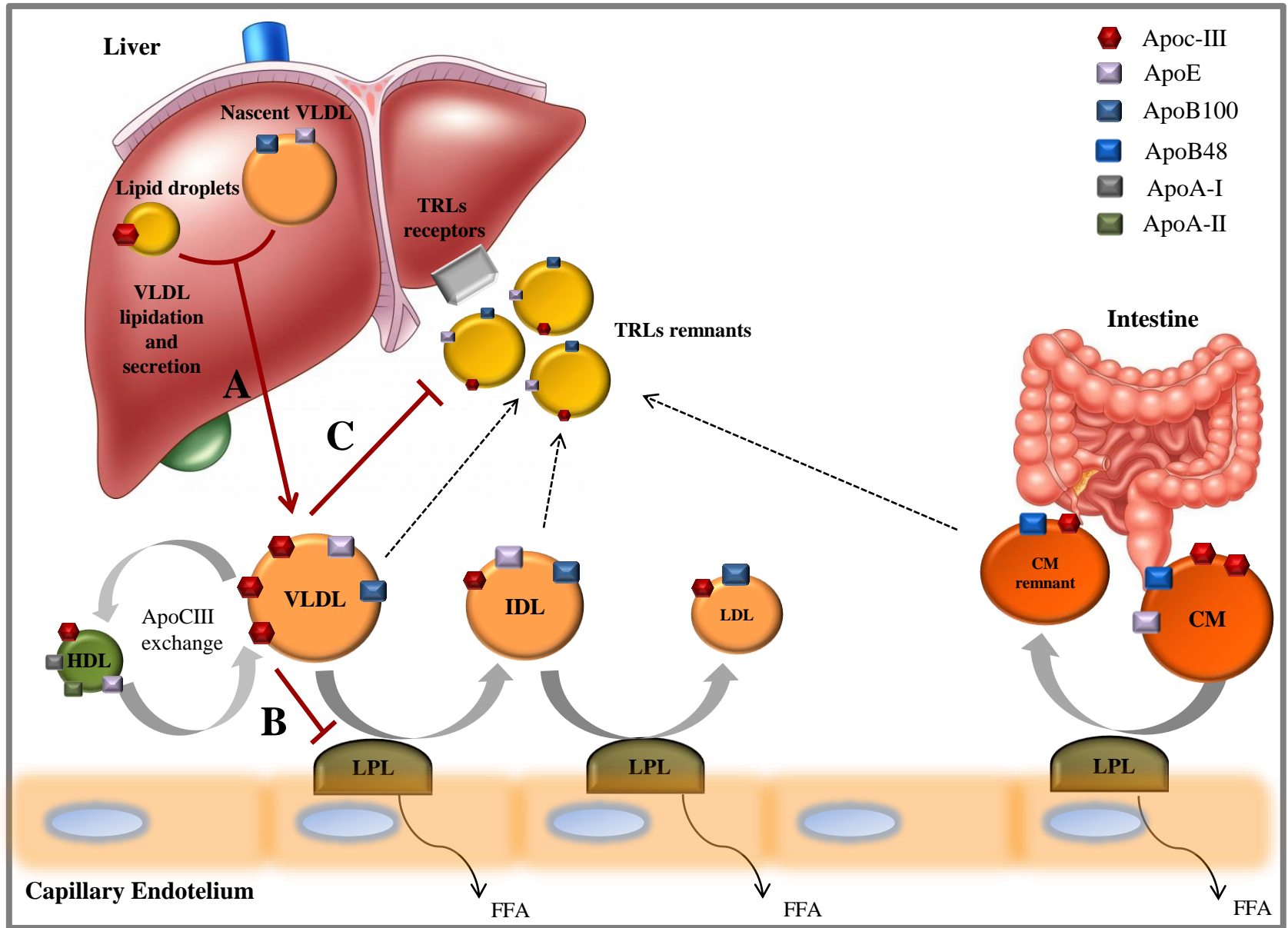


Table 1. Modulators of *APOC3* gene expression

| Effect on <i>APOC3</i> expression | Factors | Mechanism of action | References |
|-----------------------------------|-----------------------------|--|--|
| ▼ | Insulin | Phosphorylation and nuclear exclusion of FOXO1 | J Lipid Res 1994 Nov;35(11):1918-24 (18) J Clin Invest. 2004; 114: 1493–1503 (25) |
| ▼ | Glucose | Activation of by ChREBP and HNF-4 α | Arterioscler Thromb Vasc Biol 2011; 31:513–519 (24) Biochim Biophys Acta. 2011 Aug;1812(8):995-1006 (26) |
| ▲ | Saturated fatty acids | Induced expression of the PGC1- β | Arterioscler Thromb Vasc Biol 2008; 28:1660–1665 (32) |
| ▼ | Polyunsaturated fatty acids | Activation of PPAR α , and subsequent deactivation of FOXO1 | J Lipid Res 1996; 37:907-925 (19) J Clin Lipidol 2016; 10: 1442.e4–1451.e4 (30) J Biol Chem. 1995;270(22):13470–13475 (31) |

Table 1 summarizes the main factors that up - (▲) or down - (▼) regulate *APOC3* gene expression.

ChREBP, Carbohydrate-responsive element-binding protein; HNF4 α , hepatic nuclear factor-4 alpha; FOXO1, forkhead box O1 protein, PGC1- β , Peroxisome proliferator-activated receptor gamma coactivator 1-beta; PPAR-a, Peroxisome proliferator-activated receptor alfa.

Table 2. APOC3 LOF and GOF gene variants and their effect on lipid metabolism.

| Coordinates of SNVs | Type of SNVs | Effects on lipid profile (carriers vs. non-carriers) | Ethnic Origin | N° Carriers | References |
|--|--------------|--|--|---------------------------------|---|
| c.280A>G; p. Thr74Ala (rs121918381) | - | high amounts of plasma non-glycosylated ApoCIII ₀ no changes in lipid profile | Japan | 1 | Maeda et al. (34) |
| c.232A>G; p. Lys58Glu (rs121918382) | LOF | lower plasma ApoCIII (- 30 % to - 40 %) HDL cholesterol above the 95th percentile positive charge at position 58 is crucial for ApoCIII binding to lipids and for promoting TAG secretion | Deutsch - | 2 - | von Eckardstein et al. (35) Qin et al. (49) |
| Ala23Thr | LOF | lower plasma ApoCIII lower TG (- 25 % to - 48 %) lower Total Cholesterol (- 56 % to - 78 %) less efficient lipid binding of ApoCIII-Thr23 might lead to a faster catabolism of free ApoCIII and poorer competition with apoE Ala23Thr protein might prevent effective fusion between lipid droplets and VLDL precursors | Mayan - | 3 - | Liu et al. (47) Sundaram et al. (48) |
| c.55C>T; p. Arg19Ter (rs76353203) | LOF | lower plasma ApoCIII (- 50 %) lower TG levels (- 46 %) lower LDL-C levels (- 17 %) median TG level = 31 (mg/dl) median HDL-C level = 67 (mg/dl) lower TG levels (- 50 %) higher HDL levels (+ 20 %) mean TG level = 45.5 (mg/dl) mean HDL-C level = 56.8 (mg/dl) mean LDL-C level = 152.5 (mg/dl) lower TG levels (- 48 %) | Lancaster Amish 19,613 Americans + 1,113 Old Order Amish from Ohio and Indiana 3,734 individuals of European or African ancestry 75,725 Danish descents | 39 31 2 33 | Pollin et al. (36) Crawford DC et al (37) Crosby et al. (39) Jorgensen et al. (40) |

| | | | | | |
|---|------------|--|--|----------------------|--------------------------------|
| | | <p>near-absent plasma ApoCIII (- 88.9 %) lower TG levels (- 59.6 %) higher HDL-C levels (+ 26.9 mg/dl)</p> | <p>10,503 adult Pakistan participants (PROMIS)</p> | <p>4 homozygotes</p> | <p>Saleheen et al. (41)</p> |
| | | <p>lower plasma ApoCIII (- 50 %) lower TG levels (- 35 %) VLDL TG (- 45 %) VLDL-apoB100 (- 36 %) significantly higher rate of conversion of VLDL to LDL and reduction of remnant cholesterol</p> | <p>Lancaster Amish (kinetic study)</p> | <p>5</p> | <p>Reyes-Soffer et al (46)</p> |
| <p>c.55+1G>A (IVS2+1G>A) (rs138326449)</p> | <p>LOF</p> | <p>lower TG levels between 0.5 and 1.5 (mmol/L)</p> | <p>16,033 Europeans</p> | <p>na</p> | <p>Timpson et al. (38)</p> |
| | | <p>mean TG level = 71.0 (mg/dl) mean HDL-C level = 59.6 (mg/dl) mean LDL-C level = 111.7 (mg/dl)</p> | <p>3,734 individuals of European or African ancestry</p> | <p>10</p> | <p>Crosby et al. (39)</p> |
| | | <p>lower TG levels (- 44%)</p> | <p>75,725 Danish descents</p> | <p>209</p> | <p>Jorgensen et al. (40)</p> |
| | | <p>lower TG levels</p> | <p>926 South Dakota Hutterites</p> | <p>30</p> | <p>Igartua et al. (42)</p> |
| <p>c.179+1G>T (IVS3+1G>T) (rs140621530)</p> | <p>LOF</p> | <p>mean TG level = 82.8 (mg/dl) mean HDL-C level = 67.0 (mg/dl) mean LDL-C level = 154.2 (mg/dl)</p> | <p>3,734 individuals of European or African ancestry</p> | <p>5</p> | <p>Crosby et al. (39)</p> |
| <p>c.127G>A; p. Ala43Thr (rs147210663)</p> | <p>LOF</p> | <p>mean TG level = 79.0 (mg/dl) mean HDL-C level = 64.5 (mg/dl) mean LDL-C level=110.4 (mg/dl)</p> | <p>3,734 individuals of European or African ancestry</p> | <p>12</p> | <p>Crosby et al. (39)</p> |

| | | | | | |
|--|-----|---|---|--------------|---|
| | | lower TG levels (- 47 %) | 75,725 Danish descents | 18 | Jorgensen et al. (40) |
| rs5128 (SstI, sacI, c.C3238G, c.C3175G) | GOF | higher plasma levels of ApoCIII higher levels of TG, TC and LDL-C | 20 studies comprising 15591 participants (meta-analysis) 42 eligible studies with 23846 subjects (meta-analysis) | na na | Lin et al. (44) Song et al. (45) |
| rs2854117 (5' UTR C-482T) rs2854116 (5' UTR T-455C) rs4520 (c.C1100T; p. Gly34Gly) | GOF | higher plasma levels of ApoCIII controversial data regarding the effect on plasma lipids | 20 studies comprising 15591 participants (meta-analysis) | na | Lin et al. (44) |
| Gln38Lys | GOF | higher level of aApoCIII in the VLDL fraction (+ 34 %) higher TG levels (+ 32 %) increased VLDL secretion | Family Study Mexican - | 17 - | Pullinger et al. (43) Sundaram et al. (50) |

Variants are reported at cDNA or protein level or as rsID if available. Ethnic origin and number of carriers in which *APOC3* variants were found are also reported according to each study.

LOF, loss-of-function; GOF, gain-of-function; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; VLDL, very low density lipoproteins.