



Apolipoprotein C-III and cardiovascular diseases: when genetics meet molecular pathologies

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Abstract

Cardiovascular diseases (CVD) have overtaken infectious diseases and are currently the world's top killer. A quite strong linkage between this type of ailments and elevated plasma levels of triglycerides (TG) has been always noticed. Notably, this risk factor is mired in deep confusion, since its role in atherosclerosis is uncertain. One of the explanations that aim to decipher this persistent enigma was provided by apolipoprotein C-III (apoC-III), a small protein historically recognized as an important regulator of TG metabolism. Preeminently, hundreds of studies have been carried out in order to explore the *APOC3* genetic background, as well as to establish a correlation between its variants and dyslipidemia-related disorders, pointing to an earnest predictive power for future outcomes. Among several polymorphisms reported within the *APOC3*, the SstI site in its 3'-untranslated region (3'-UTR) was the most consistently and robustly associated with an increased CVD risk. As more genetic data supporting its importance in cardiovascular events aggregate, it was declared, correspondingly, that apoC-III exerts various atherogenic effects, either by intervening in the function and catabolism of many lipoproteins, or by inducing endothelial inflammation and smooth muscle cells (SMC) proliferation. This review was designed to shed the light on the structural and functional aspects of the *APOC3* gene, the existing association between its SstI polymorphism and CVD, and the specific molecular mechanisms that underlie apoC-III pathological implications. In addition, the translation of all these gathered knowledges into preventive and therapeutic benefits will be detailed too.

Keywords CVD · Atherosclerosis · Hypertriglyceridemia · apoC-III · SstI polymorphism

Abbreviations

ApoC-III	Apolipoprotein C-III	EC	Endothelial cells
ASO	Antisense oligonucleotides	FCS	Familial chylomicronemia syndrome
bp	Base pairs	G	Guanine
C	Cytosine	has-miR	Homo Sapiens-micro ribonucleic acid
CVD	Cardiovascular diseases	HDL	High-density lipoproteins
DAG	Diacylglycerol	HTG	Hypertriglyceridemia
DNA	Deoxyribonucleic acid	LDL	Low-density lipoproteins
		LPL	Lipoprotein lipase
		miRNA	Micro ribonucleic acid
		mRNA	Messenger ribonucleic acid
		NFκB	Nuclear factor kappa B
		PC	Phospholipase C
		PKC	Protein kinase C
		RNA	Ribonucleic acid
		SMC	Smooth muscle cells
		TG	Triglycerides
		TRL	Triglyceride rich lipoproteins
		UTR	Untranslated region
		VLDL	Very low-density lipoproteins

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Introduction

At the beginning of the 21st century, CVD have become a significant issue on the global health agenda. As early as 2001, these diseases have been documented as the largest single cause of mortalities on the planet [1]. Today, despite the huge growth of biological knowledge and advances in medication, CVD remain at the world's top rank of all human death reasons [2]. The data leave no room for doubt: Annually, around 17.5 million people die from CVD [3], and this number is predicted to increase dramatically in the next years. By 2030, more than 23.3 million individuals will succumb to this growing burden each year [4], and hence, this pandemic will continue to dominate the mortality trends in the future.

“Cardiovascular diseases” is an umbrella term that encompasses a broad spectrum of cardiologic diagnoses, affecting heart and circulatory system [5]. Disorders under this term primarily comprise coronary heart diseases, cerebrovascular accidents and peripheral vascular diseases [6]. The major underlying cause of CVD appears to be atherosclerosis [7], also known as the hardening of arteries [8]. It is defined as an immunoinflammatory fibroproliferative disease, in which fatty deposits called atheromatous plaque develops, over many decades, inside the inner layers of the arterial wall, and over time, it narrows the artery depriving the vascularized tissue of oxygen [8]. This process has been recognized as a human pathology for more than 3500 years: It occurred in ancient Egyptian mummies, showing the same salient features observed in the modern time [9].

Historically, a broad variety of factors have been linked to the etiology of atherosclerosis and CVD. Its incidence is ascribable to polygenic inheritance coupled with environmental exposures that add to the complexity of the disease [10]. Interestingly, most of the classic risk factors which can intensify and provoke a cardiovascular event are the result of people's lifestyles and behaviors; therefore, they are modifiable and avoidable [11]. These variables include unhealthy blood lipid profile, hypertension, smoking, type 2 diabetes, obesity and physical inactivity [8]. Other determinants cannot be changed such as increased age, gender, race and genetic background [8]. In order to improve the prognosis of CVD and provide better preventive strategies, it is critical to develop a greater understanding of these factors, especially, the genetic predisposition to these diseases.

Pathogenesis of atherosclerosis

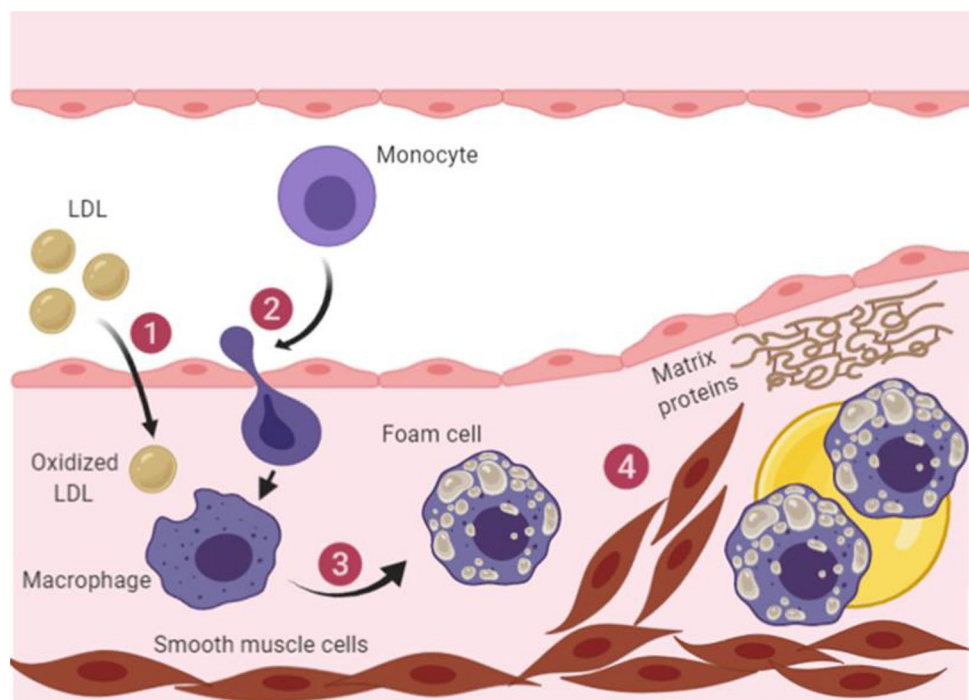
Although our current understanding of atheroma formation is fragmentary, a straightforward illustration of atherogenesis steps was outlined. It was established that the root cause of this pathogenesis is the deposition of lipids, or more precisely the apolipoprotein B-containing lipoproteins, inside the arterial wall, sparking an inflammatory response and promoting atherosclerosis [12]. Circulating low-density lipoprotein (LDL) particles, that are the most abundant atherogenic lipoproteins in the bloodstream, can penetrate via transcytosis across the endothelial cells (EC) monolayer into the sub-endothelial space [13], where they are retained and oxidized [12]. The EC become activated by atherogenic stimuli, and as a result, their expression of adhesion molecules is upregulated, and monocytes are recruited [12]. Within the intima, the monocytes differentiate into macrophages and internalize the oxidized LDL, resulting in the formation of lipid-loaded macrophages called foam cells [12]. With a containing supply of modified lipoproteins, these foam cells eat until they die [12]. This progressively destroys the normal architecture of the intimal layer, and the accumulated cells, lipids and debris, coalesce into enlarging pools, forming a soft and destabilizing core in the center of the plaque [12]. Concomitantly, SMC migrate from the adventitia to the intima, and secrete collagen and elastin adding a thick fibrous cap over the core, directly at the blood interface [8] (Fig. 1). At this point, the rupture of the raised patch favors the platelets to stick to the surface of injury, leading to the formation of a blood clot (thrombosis), and subsequently, the vessel becomes completely obstructed [8].

Hypertriglyceridemia and CVD: a renewed interest

Despite several decades of research, the implication of hypertriglyceridemia (HTG) in CVD, either as a direct promoter or merely as a biomarker, remains a topic of debate [14]. For a long time, elevated TG levels have been considered as an important risk factor for CVD [15], but randomized controlled trials aiming to reduce it failed to show any significant medical benefit [16, 17], rendering it a non-persuasive biomarker in cardiovascular pathologies [18]. This long-standing controversy stems from the heterogeneity of TG-rich lipoproteins (TRL) [19], as well as the complexity of TG metabolism described below.

Due to its hydrophobic nature, TG must combine with proteins forming lipoprotein particles that allow its transport through the circulation [20]. In accord with this

Fig. 1 Schematic of atherogenesis. The atherosclerotic plaque consists of a fibrous cap rich in collagen, surrounding a lipid-rich core comprising lipids, foam cells and debris. Atheroma plaque formation involves a series of steps initiated by the (1) lipid infiltration into the sub-endothelial space, followed by (2) EC dysfunction and activation with increased expression of adhesion molecules on their surface, promoting the attachment and penetration of inflammatory cells into the intima. (3) Monocytes differentiate into macrophages, engulf modified lipids forming foam cells. (4) Meanwhile, SMC proliferate, migrate and synthesize matrix proteins to form a fibrous cap that encloses the growing core



concept, plasma TG are mostly found in TRL [21], including very low-density lipoproteins (VLDL) which transport it from the liver, and chylomicrons which transport dietary fat from the intestine [22]. Along the luminal surface of capillaries, they are hydrolyzed by the action of lipoprotein lipase (LPL), generating free fatty acids that can be taken up by muscles and adipose tissues [23]. During this phenomenon, an enormous fraction of apolipoproteins and phospholipids moves from TRL into the high-density lipoproteins (HDL), converting VLDL and chylomicrons into remnant particles [23]. Ultimately, these later should be rapidly removed from the plasma, through a specific receptor mediated mechanism in the liver [24]. Under pathological conditions, HTG results from the accumulation of TRL in the bloodstream [25], either by excessive production, or by decreased catabolism [26] due to an LPL dysfunctionality, or to a grave alteration of another LPL-independent pathways, such as the hepatic removal of remnants. In this regard, it is difficult to separate TG from the complicated network of confounding variables in the experimental systems based on cellular mechanisms, clinical trials or epidemiological data [21]. However, renewed interest in this factor is brought by recent observations suggesting that the loss of function mutations in the gene encoding apolipoprotein C-III (*APOC3* gene; apoC-III protein), an important regulator of TG homeostasis, is beneficially associated with decreased CVD risk [27, 28]. Based on these findings, the *APOC3* gene and its genetic variants have been a great focus of research in the last years.

Spotlight on the *APOC3* gene

The *APOC3* gene was discovered and characterized nearly 50 years ago [29], but firstly, it did not attract sufficient attention [24]. This gene resides within the *APOA5/APOA4/APOC3/APOA1* multigene cluster on the long arm of the human chromosome 11q23 [30]. It comprises 4 exons and 3 introns [31], and encoding a 99 amino acid glycoprotein called apoC-III [32]. This apolipoprotein is mostly expressed in hepatocytes and enterocytes, where it undergoes an intracellular cleavage, yielding the mature 79 amino acid protein [32]. Furthermore, it undergoes a post-translational modification leading to the formation of three distinct isoforms containing zero (apoC-III₀), one (apoC-III₁) or two (apoC-III₂) sialic acid residues, and importantly, all these isoforms exhibit the same plasma half-life and catabolic mechanisms, suggesting similar physiological implications [33]. However, important modifications in their normal distributions were detected in different diseased cases, but it remains unknown if these variations have a pathological consequence [23]. At the transcriptional level, the *APOC3* gene expression is tightly regulated by several proposed pathways. A series of in vivo and in vitro studies have demonstrated that its expression is downregulated by insulin [34], peroxisome proliferator-activated receptor α [35], Rev-erb [36] and farnesoid X receptor [37]. Conversely, the positive responsiveness of the *APOC3* promoter to glucose was reported. This factor

stimulates the gene expression by the activation of the carbohydrate-responsive element binding protein, as well as the hepatocyte nuclear factor-4 α [38]. Hence, the opposite interplay between insulin and glucose on modulating *APOC3* transcriptional activity may induce an enhanced apoC-III secretion under an insulin-resistant condition associated with hyperglycemia (as in type 2 diabetes) [38]. Also, the total apoC-III levels can be significantly modulated in hyperlipidemic individuals by the dietary intake of low saturated fat and high amounts of monosaturated and omega-3 polyunsaturated fatty acids [39].

Once secreted, apoC-III is released into the plasma as a major component of circulating TRL and HDL [40]. Today, we know that it is a multifaced apolipoprotein, with a major physiological concern [24]. The prevailing is that apoC-III impairs the LPL-mediated lipolysis of chylomicrons and VLDL, by displacing the lipase from TRL, as well as by antagonizing apolipoprotein C-II, the main protein responsible for the enzyme activation [41]. In addition, apoC-III affects TG catabolism by other LPL-independent pathways. It delays the clearance of remnants, by interfering with their specific binding to hepatic receptors [33]. Besides, it has been proposed to directly influence plasma VLDL levels, by stimulating their intrahepatic secretion [42]. A further

illustration of this point comes from the increased VLDL synthesis in genetically modified mice overexpressing apoC-III [43]. However, the reduction of apoC-III levels using antisense oligonucleotides (ASO) in mice did not affect VLDL production [31]. Thus, the biological implication of this protein in VLDL assembly and secretion is still obscure [24], but overall, the combination of all its actions renders it an essential actor in the regulation of TRL metabolism [23] (Fig. 2). Therefore, the overexpression of apoC-III causes an unwanted accumulation of TG inside the vessels, resulting in HTG [44].

***APOC3* genetic variants and CVD: is there a link?**

Although interest in apoC-III has continued, it appears theoretically plausible that the deficiency of this protein could be a cardioprotective phenotype, given the relationship that exists between unfavorable TG profiles and cardiovascular events. Correspondingly, two parallel large-scale epidemiological studies have revealed that a striking decrease in ischemic CVD and coronary heart disease risk can be influenced by a series of typical *APOC3* mutations (Table 1)

Fig. 2 The functions of apolipoprotein C-III in TRL metabolism and their impacts on atherogenesis. The red arrows depict the inhibition of LPL activity and TRL remnants hepatic uptake. The suggested intrahepatic effect of apoC-III as an enhancer of VLDL assembly and secretion is indicated with a red plus sign. The dashed blue arrow depicts the resulting accumulation of atherogenic TRL in the atherosclerotic artery

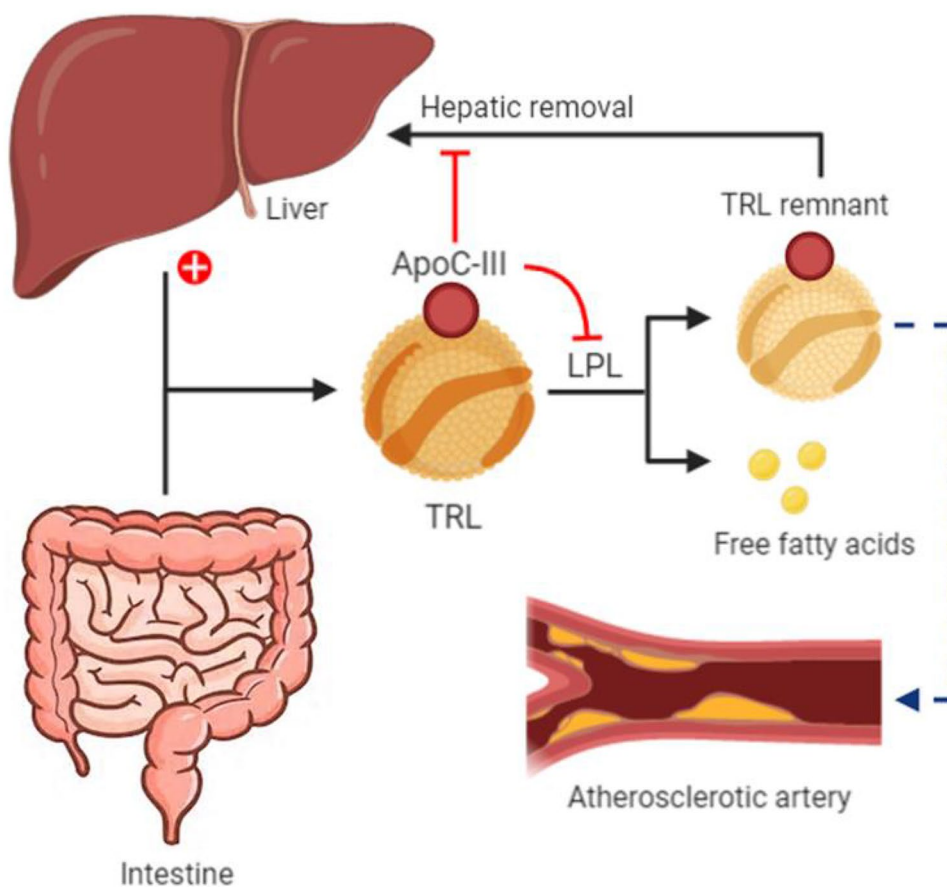


Table 1 Typical cardioprotective loss-of-function mutations in *APOC3* gene and their effects on plasma apoC-III levels

Mutation	rs ID	Chromosome position	Mutation type	Effect on plasma apoC-III levels	References
A43T	rs147210663	11:116,701,560	Missense	↓ 46–50%	Crosby et al. [27] Khaternal et al. [99]
R19X	rs76353203	11:116,701,353	Nonsense	↓ 46–50%	Crosby et al. [27] Reyes-Soffer et al. [100]
IVS2 + 1G→A	rs138326449	11:116,701,354	Splice site	↓ 46%	Crosby et al. [27]
IVS3 + 1G→T	rs140621530	11:116,701,613	Splice site	↓ 46%	Crosby et al. [27]

[27, 28]. In the first study, four mutations were identified in an Exome Sequencing Project, including a missense mutation (A43T), and three loss-of-function mutations: The null mutation R19X, as well as the two splicing site mutations IVS2 + 1G→A and IVS3 + 1G→T [27]. Even if only one in every 150 persons carried some of these alleles, their existence was coupled with a 39% decrease in TG levels, corresponding to a 40% reduction of CVD risk, as compared with non-carriers [27]. Likewise, the second cohort study (n = 75.725) has shown similar results [28]. Loss-of-function mutations in the *APOC3* gene have a conferred 41% decrease in the risk of ischemic vascular disease, and a 36% reduction in ischemic heart disease [28].

On the other side, the important biological functions of apoC-III, together with the aggregated data indicating that a null allele in its gene can lead to a healthy life, deem it necessary to explore the full genetic background of DNA polymorphisms at the *APOC3* locus, and how they may correlate to the susceptibility of dyslipidemia and other related disorders, such as CVD. Remarkably, several biallelic polymorphisms in this gene have been investigated as possible markers of HTG [45]. In particular, special attention has been paid to the SstI site, which represents the most extensively studied polymorphism in this gene [45]. Here, we focused specifically on this variant, since it was consistently linked to many pathological traits in the literature.

***APOC3* SstI polymorphism (rs5128)**

The SstI polymorphism, also recognized as SacI, 3238C>G or 3175C>G [40], was the first polymorphic site discovered within the *APOC3* gene [46]. Arising from a Cytosine (C) to Guanine (G) transversion in the 3' UTR [40], this variant distinguishes between 2 alleles: S1 (frequent allele) and S2 (rare allele) [47]. It involves the formation of a restriction site for the endonuclease SstI [48] that facilitated its exploration. Consequently, the genotype can be easily detected using the polymerase chain reaction-restriction fragment length polymorphism technique.

The correlation of *APOC3* SstI polymorphism with several phenotypic variables has been the subject of numerous association studies that have covered many ethnic groups.

Even though the published data provided some conflicting results, a recent meta-analysis confirmed that carriers of the rare allele exhibit high plasma concentrations of apoC-III and TG [40]. This polymorphism was also associated with CVD risk, through a large-scale genome wide association study [49]. The discrepancies reported in few populations are not explained, but they strongly postulate a gene-environment interaction [50].

Possible effects of *APOC3* SstI polymorphism on the transcriptional activity

Because of its localization in the 3'UTR, the SstI polymorphism was unexpected to have a functional significance [47]. However, elevated *APOC3* mRNA levels were detected in 3/5 of hepatic biopsies collected from heterozygotes carriers of the S2 allele [51]. Although the specific molecular mechanism explaining this overexpression is poorly understood [40], different potential processes have been hypothesized.

Firstly, it was believed that the SstI site is located in a sequence that is involved in the regulation of gene expression or mRNA stability [52], on account of the well-known mechanisms of the 3'UTR region [53]. Additionally, it is conceivable that the S2 allele could be acting as a marker for other functional variants, within the *APOC3* or in neighboring genes [52]. Accordingly, an indirect functionality was proposed through the strong linkage disequilibrium of this allele with two *APOC3* promoter polymorphisms, the -455 T>C (rs2854117) and the -482C>T (rs2854116) [54]. These two substitutions are located within an insulin response element [52], and their presence leads to an attenuated suppression by insulin, and subsequently, an increased production of apoC-III [55]. Nevertheless, the analyses of haplotypes combination within the *APOA4/APOC3/APOA1* cluster provided evidence for a dominant effect of the SstI variant on lipid abnormalities [56].

Recently, the literature has suggested a possible post-transcriptional regulation of *APOC3* mRNA, involving the rare allele of the SstI polymorphic site [54]. In silico assays predicted potential binding losses for 5 miRNAs (has-miR-138, has-miR-185, has-miR-564, has-miR-1207-5p, has-miR-1270), all expressed in human hepatic

and intestinal tissues [54]. MiRNAs are a group of small non-coding RNA, that post-transcriptionally down-regulate gene expression, usually by binding target 3'-UTR mRNA sequences [57]. Unfortunately, the *in vitro* trials aiming to validate this hypothesis have failed [54]. However, it is not ruled out that the S2 allele could suppress the binding sites corresponding to other miRNAs, that are not detected by the bioinformatic analysis, nor expressed in the cell models used [54].

More recently, a new hypothesis was provided explaining the functionality of the SstI polymorphism. It was declared that the SstI site lies within 40 nucleotides from the 3' poly-A tail [58], a long chain of adenine bases involved in mRNA stability, nucleocytoplasmic transport and translation [59]. Generally, the shortening of the poly-A tail is one of the key steps initiating the decay of the messenger body, when it is no longer being used for translation [60]. In this respect, the inhibition of the binding of the specific proteins that remove the poly-A tail, in the presence of the S2 allele, was hypothesized to explain a possible prolonged half-life of the messenger [58]. Nevertheless, this suggestion requires empirical confirmation. Altogether, the unclear transcriptional regulatory effect of the SstI variant remains to be fully elucidated.

ApoC-III and cardiovascular pathophysiology: more than just a hypothesis

A growing evidence from epidemiological data supported a strong correlation of apoC-III with CVD risk [61]. In addition, the current body of literature contains numerous methodologically sound investigations, that can provide together a consistent proof for the deep implication of this protein in cardiovascular complications. It is now well known that the variation in plasma apoC-III levels is directly related to the atherogenicity of lipoprotein particles in which it resides, and to an alarming increase in cardiovascular risk [62]. This connection was confirmed in an important meta-analysis conducted by Wyler et al., based on the overall published data of 12 eligible studies [63]. Furthermore, apoC-III concentration was also reported, in a 15-year follow-up study, as a potent predictor of cardiovascular mortalities [64]. Accordingly, several clinical reports validated the usefulness of this protein in the prediction of future CVD outcomes, especially coronary artery events [33]. This relationship appears to be extremely complex, taking into consideration the pleiotropic impacts of this apolipoprotein on the cardiovascular system. As discussed previously, apoC-III keeps bad company: It inhibits the clearance of TRL remnants, augmenting their plasma levels and prolonging their residence time in the blood. Consequently, the causal association of apoC-III with CVD has been firstly attributed to its effect on TRL

catabolism [41]. While the previous theory is undoubtedly true in part, the apoC-III finds itself, recently, at the scene of the crime. An increasing body of researches reflected that it may be the primary mediator of disease onset and progression, exerting several atherogenic properties [62]. Accordingly, it has been identified as an independent risk factor for CVD [46].

ApoC-III links dyslipidemia with CVD

Circulating lipoproteins normally flux into and out of the arterial wall by transcytosis, a transport system by which these particles can be transported, via specialized clathrin-coated vesicles, across the endothelium [65]. Furthermore, the transport vesicles are approximately 100 nm in diameter, making the transcytotic system restricted to lipoproteins smaller than 70 nm, thereby excluding large VLDL and chylomicrons [66]. However, their remnants are small enough to exceed the size limitation and penetrate to the arterial intima [67] (Fig. 2). Despite being atherogenic, it is not their TG content that participates in the evolution of atheroma [68]. Besides TG, TRL remnants also contain enriched amounts of cholesterol, 40 times more than an LDL particle [69]. Thus, they directly mediate cholesterol deposition within the intima, and facilitate the activation of various inflammatory pathways [67]. Additionally, these particles do not need to be modified/oxidized to become atherogenic [70]. Hence, they can be taken up, in an unregulated fashion, by the arterial macrophages [67], resulting in accelerated foam cells generation. According to these mechanisms, TRL remnants may have a stronger atherogenic effect than LDL [67], and may further increase the risk. As apoC-III is elevated in CVD, it was reasonable to postulate that it might enhance atherosclerosis by enriching the plasma components with these plaque-promoting particles. Nonetheless, it was suggested that apoC-III can facilitate the sub-endothelial accumulation of apolipoprotein B-containing lipoproteins, by influencing their affinity for the artery wall proteoglycans [71]. This mechanism was firstly elusive since apoC-III itself is not able to bind the proteoglycans, but a possible explanation could be that it seems to provoke a modification in the lipid composition of lipoproteins, allowing the apolipoprotein B to adopt a specific conformation, which is more favorable for the binding to the artery wall proteoglycans [71].

ApoC-III is directly involved in atherosclerosis

The plethora of observational studies has provided a mean for testing whether apoC-III is the real disease culprit or an innocent bystander in the manifestation of CVD. Biologists have struggled with this issue, however, due to the rapid evolution of molecular genetics technologies, the apoC-III pathogenicity is uncovered [61]. Today, it is becoming clear

that apoC-III has an atherogenic potency, beyond its ability to raise TG levels [62]. It was detected in the site of atherosclerotic lesions of major arteries, such as the coronary artery, the carotid artery and the aorta [72], adding more weight to its direct implication in CVD. Also, it was found that patients with elevated levels of apoC-III-containing lipoproteins have an increased risk of both fatal and nonfatal myocardial infarction [73]. In the light of all these facts, arduous efforts have been made to explore the exact molecular mechanisms, by which this protein can induce numerous atherogenic conditions.

CVD are not only a complication of a disordered lipid profile, but they are also considered as inflammatory diseases. As aforementioned, the development of atheroma requires, in addition to the lipoproteins accumulation, a concomitant inflammation in the arterial wall. Interestingly, several inflammatory effects have been ascribed to apoC-III [74], opening entirely new prospects on its participation in disease progression. This protein was found to be involved in crucial events of the early pathological stage, including endothelial dysfunction, recruitment of blood monocytes to the site of injury, and their subsequent extravasation through the endothelium into the sub-endothelial area [75]. Indeed, a series of interactions between immune and endothelial cells can be promoted by the action of apoC-III, alone or as a lipoprotein component [74]. It is deemed to activate the protein kinase C- β (PKC- β), and posteriorly the nuclear factor kappa B (NF κ B) in the vascular EC, pushing them to express two types of adhesion molecules, the vascular cell adhesion molecule 1 and the intracellular adhesion molecule 1, on their surface [76]. Remarkably, a specific antibody targeting apoC-III was able to abolish this effect [46]. Moreover, because apoC-III-containing particles hold other apolipoproteins, many assays were designed in order to validate that the increased adhesiveness of EC is attributed to the apoC-III itself [77].

In addition to exerting effects on the EC layer, apoC-III can enhance the attachment of circulating monocytes to the vascular wall, by acting directly on these immune cells. It was shown to increase their surface expression of the β 1-integrin [18], a member of the receptors family that mediates cell–cell and cell–extracellular matrix contact [78]. Eminently, the contribution of Kawakami et al. has delved into the intricacies of the induced signal transduction axis [79]. They have demonstrated that this process is fulfilled through the activation of the pertussis toxin-sensitive G protein pathway in monocytic cells [79]. Particularly, it was revealed that apoC-III stimulates the phosphatidylcholine-specific phospholipase C (PC) activity, and this later, in turn, catalyzes the PC to generate phosphatidylcholine and diacylglycerol (DAG) [79]. Then, DAG triggers the activation of PKC- α , resulting in the translocation of NF κ B into the nuclei, where it increases the transcription of the gene

encoding the β 1-integrin [79]. Also, Ras homolog family member A may be partially involved [76]. Eventually, the net result of this long cascade is an enhanced crawling of monocytes on the endothelial surface [18]. Although these findings seem to have illustrated a complete and compelling figure concerning the inflammatory role of apoC-III, they leave some unanswered questions. For example, the specific signaling pathway by which apoC-III can activate the PKC- β in EC, as well as the possible involvement of other mediators in PKC- α activation in monocytes, present an unexplained conundrum that provides fertile ground for future research.

Simultaneously, laboratory assays have continued to unravel additive mechanistic information about the pathophysiology of apoC-III. From an attractive standpoint, the study of its impact on SMC has yielded a new elucidation of its atherogenic capacity. Various transgenic and knocked-out mouse models have been created, and they were used to demonstrate that the apoC-III can trigger the proliferation of SMC, participating in the worsening of atherosclerosis [80]. ApoC-III takes part in this process via the stimulation of the Akt signaling pathway in these cells [80]. Nonetheless, it was examined whether the apoC-III-poor particles could elicit similar effects, since this apolipoprotein resides typically on the surface of TRL. It was indicated that apoC-III-enriched TRL induce aortic SMC proliferation and arterial stenosis, while apoC-III-poor TRL don't [80]. In summary, all these advances have robustly deepened our understanding of apoC-III atherogenicity.

ApoC-III alters HDL functionality

To more complicate this issue, the direct contribution of the apoC-III in atherosclerosis was not limited to the previously mentioned scenarios. Looking beyond inflammation and fibroproliferation, apoC-III emerged as an actor in the profound modification of the functionality of HDL. This lipoprotein is an intriguing particle that has attracted the attention of the scientific community for many years, mostly by its atheroprotective potential [81]. Besides its considerable anti-inflammatory and anti-apoptotic properties, it can promote classically the reverse efflux of cholesterol from the lipid-loaded macrophages in the plaque, to the liver [82]. Unluckily, all of these characteristics can be altered in many pathological conditions, including CVD [18]. As a matter of fact, a strong correlation of this dysfunctionality with the presence of apoC-III has been well established [18]. Based on proteomics analyses with different methodologies, HDL particles isolated from CVD patients were remarkably enriched by this atherogenic apolipoprotein [83, 84]. Further, apoC-III in these lipoproteins was found to be responsible for the activation of the p38-mitogen activated protein kinase signaling pathway in EC, followed by an excessive expression of the pro-apoptotic protein truncated-BID,

therefore, converting HDL from cytoprotective into proapoptotic molecules [83]. The significant value of this result comes from the usual EC turn over and death in the atherosclerotic injury-prone regions within the vasculature [84]. Overall, these newly discovered functions of the apoC-III have confirmed that this protein is a causal factor for CVD. Importantly, they have also highlighted a new target for therapeutic interventions, allowing the development of apoC-III based treatments with respect to HTG and atherosclerosis.

Lesson learned from genomics: why the *APOC3* fingerprint should be pursued?

While the picture is less hazy in the era of “Mendelian” diseases that typically arise from a single gene rare mutation, the heritable component of complex conditions is attributable to a constellation of several variants, spreading across different genomic loci [85]. Altogether, they can disrupt a key physiological mechanism, in a clear illustration of the so-called “polygenic inheritance” pattern [86]. In other words, a genetic variation, credibly correlated to a common disease, is not necessarily the straight cause of illness [10], but it can account for only a minuscule fraction of the trait in question [85]. However, it can be used as a marker that will help in the prediction of a future pathological event [10].

The past few years witnessed substantial progress in the genomic field, especially in the horizon of high throughput analyses of single nucleotide polymorphisms, leading to the identification of a large number of CVD-associated genetic loci [87], and providing an unprecedented opportunity to assess these widespread disorders, that clinicians encounter daily. Owing to the small effect of each polymorphism, the theory of the “genetic risk score” has been conceived [88]. Fundamentally, aggregating information about diverse sets of risk-conferring alleles into a single algorithm, has become a useful tool for examining the cumulative predictive ability of these variants on subclinical CVD-related phenotypes [88].

Generally speaking, the determination of the personal genetic makeup in candidate genes may improve the overall cardiovascular outcomes, by identifying relative at-risk patients before that the symptoms manifest, motivating them to change their health behaviors [5]. Moreover, this genotype-guided approach can lower the burden of CVD by bringing the attention of clinicians to medical follow-up for these subjects, and helping them to make better treatment decisions [5], under a personalized medicine mode. In this context, the harnessing of the available *APOC3* genetic information, considering its serious contribution in the onset, as well as the aggravation of atherosclerosis, may yield further advances in risk stratification. The “cardiogenomic profiling”, or the “Heart health tests”, are methods

used to evaluate single nucleotide polymorphisms in genes that modulate lipid balance, blood pressure, inflammation and oxidative stress, and they continue to be offered in the health care market [5]. They are already advertised on the Internet, and it can be ordered online, without the direct involvement of a physician [5]. Interestingly, the *APOC3* is included in some of these panels, besides other relevant genes [5, 89]. Additionally, Palomaki et al. have summarized the CVD-associated variants observed in the white population [5]. In this study, the SstI polymorphism was mentioned with moderate credibility [5], making it an important high-risk predictor, that may provide discriminatory power in the assessment of cardiovascular disorders.

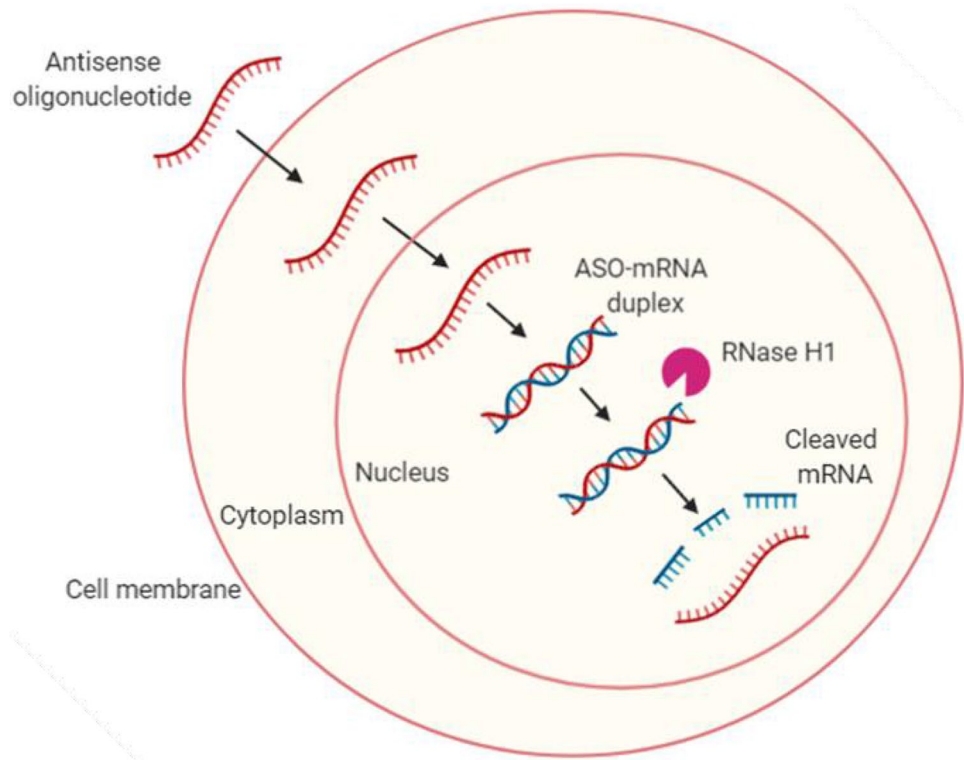
ApoC-III as a therapeutic target

While multiple lipid-lowering drugs, such as atorvastatin [90], fibrates [35] and others, decrease moderately the plasma apoC-III levels by 10–30% [23], there is still no effective compound that can suppress robustly this protein overexpression [91]. The apoC-III emerging roles have been the catalyst for a remarkable endeavor in the therapeutic field. Currently, a promising strategy in the treatment of hyperlipidemia, based on the ASO technology, is under development [91].

In general, the ASO application is a newest type of pharmacological approaches pertaining to nucleic acid-based gene silencing [92]. Briefly, a heavily modified single-stranded deoxyribonucleotide can serve as a selective sequence that pairs to specific regions of a given mRNA, and regulates its translation into a functional protein [92]. The ASO can bind complementarily to the targeted mRNA by Watson–Crick hybridization and, in consequence, block the expression of this genetic material via many distinct mechanisms [92]. When the synthetic antisense sequence contains stretches of > 5 DNA nucleotides, it can elicit the activation of the ribonuclease H1 [92]. This enzyme is an endogenous endonuclease that degrades selectively the cognate RNA strand of an RNA–DNA heteroduplex [93] (Fig. 3), thus decreasing the amount of translated protein.

Fortunately, a chemically modified-second generation anti-apoC-III ASO (5'-AGCTTCTTGTCAGCTTTAT-3'), formally called ISIS 304801 or ISIS-ApoCIIIrx and now referred to as “Volanesorsen” [91], can be powerful in the management of HTG and future CVD. It has been shown to selectively reduce the biosynthesis of apoC-III and TG in all preclinical tested rodents and nonhuman primates [31]. Following these results, the drug was examined in healthy volunteers in a double-blind, placebo-controlled, dose-ranging trial, during the initial phase of clinical applications, revealing a dose-dependent reduction of apoC-III and TG concentrations [31].

Fig. 3 Mechanism of action of ASO. The ASO have been proposed to traverse cell and nuclear membrane, bind to cognate mRNA sequences, and activate RNase-H1. Upon its activation, this enzyme cleaves the mRNA to prevent translation of the targeted protein



Based on the previous proof-of-concept published data, the phase II trials were rapidly initiated. Gaudet et al. decided to test the effect of this agent in three hypertriglyceridemic patients with a familial chylomicronemia syndrome (FCS), and after treatment, these participants have benefited from more than 70% reduction in plasma apoC-III levels [94]. FCS is a rare autosomal recessive disorder, usually caused by loss of function mutations in the genes encoding LPL or LPL-modulator proteins, and it is characterized by high plasma concentrations of chylomicrons, severe hypertriglyceridemia, and consequently, an elevated risk for recurrent episodes of abdominal pain and pancreatitis [95]. The efficacy of the anti-apoC-III ASO in this case supported the physiological concept that apoC-III is involved in the regulation of TRL metabolism by LPL-independent pathways, besides its effect on LPL activity. In another larger trial, “Volanesorsen” was administered by patients with HTG, either as a monotherapy, or in combination with a stable fibrate-based therapy [96]. This cohort confirmed a dose-dependent reduction in both plasma apoC-III and TG levels. In addition, the compound was examined in a randomized double-blind study including 15 participants with type 2 diabetes and HTG [97], revealing that dyslipidemia, as well as the whole-body insulin sensitivity, were improved after this treatment [97]. Furthermore, it has been advantageous that “Volanesorsen” was generally safe and well-tolerated in phases I and II of investigations, and only mild local cutaneous

reactions at the injection site were reported at a low rate [91]. In the third phase of clinical trials, two important randomized, double-blind, placebo-controlled studies were designed. The first (APPROACH trial) included 66 FCS patients [98], and the second (COMPASS study) recruited 113 subjects with severe HTG [91]. About 70–80% reduction of apoC-III and TG concentrations were reported in the released data of these studies.

Even though there are not many treatment options for FCS patients, “Volanesorsen” was not approved by the FDA in August 2018 as a medication for this illness. This negative decision was only based on safety considerations, since the APPROACH study indicated that an unexplained grade 4 thrombocytopenia (low blood platelet counts) occurred in three enrolled patients, and it ended when they stopped the drug administration. Otherwise, the COMPASS study did not report a similar observation, therefore, the current thinking is that the underlying mechanism could be linked to the methodology of drug delivery, and not to the apoC-III itself. Recently, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) adopted an important positive opinion concerning “Volanesorsen” in March 2019, with respect to the treatment of genetically confirmed adult FCS patients, who are at a high pancreatitis risk and their response to diet interventions and lipid-lowering drugs was inadequate [24]. Till now, ongoing randomized trials are underway, and the prospect looks very hopeful.

Conclusion

Modern research has greatly expanded our knowledge on the sizable effect of apoC-III in TG homeostasis, inflammation and atherosclerosis. In addition, the emerging genetic results and association studies have shed light on the usefulness of specific *APOC3* variants in pursuing future CVD. Particularly, the S2 allele of SstI polymorphism was identified as a good predictor for the HTG susceptibility, implying notoriously the susceptibility to cardiovascular disorders. Moreover, the novel understanding of the apoC-III atherogenicity renders it an attractive target for treatment strategies, and by finishing the inaugurated phase III of clinical trials, an anti-apoC-III ASO could offer an intriguing therapy for cardiovascular protection.

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