



Protein changes in non-LDL-lipoproteins in familial hypercholesterolemia: implications in cardiovascular disease manifestation and outcome

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Purpose of review

Familial hypercholesterolemia, represents one of the most extreme clinical entities associated with premature coronary artery disease (CAD). However, clinical manifestation of CAD varies across cohorts and individual patients suggesting the existence of additional non-LDL factors potentially contributing to their cardiovascular burden.

Recent findings

Changes in HDL-associated proteins appear as one of the potential additional factors contributing to the cardiovascular risk in familial hypercholesterolemia. Specifically, the content of Apo M-SP1 in HDL3 has been directly associated with cholesterol efflux capacity. In addition, a coordinated decrease in the content of Apo L1 and LCAT in HDL3 has been related to the presence of corneal arcus and to bad prognosis in familial hypercholesterolemia patients after an acute ischemic event. In fact, HDL3 particles of familial hypercholesterolemia patients have diminished antioxidant and anti-inflammatory function.

Summary

The identification of the specific changes in HDL-associated proteins that contribute to the increased cardiovascular risk of familial hypercholesterolemia patients could be useful for the development of novel therapeutic targets. These novel strategies, in combination with current lipid-lowering therapies, may help to reduce the residual risk found in these patients.

Keywords

apolipoproteins, atheroprotection, cardiovascular risk, familial hypercholesterolemia, HDL

INTRODUCTION

Many experimental and epidemiological studies have proven the causal involvement of low-density lipoproteins (LDL) in the development of atherosclerosis and in the onset of atherothrombotic complications such as coronary artery disease (CAD). LDL can induce endothelial dysfunction, activate monocytes, change vascular smooth muscle cell phenotype, and increase thrombogenicity [1]. An extreme situation of sustained high LDL-cholesterol (LDL-C) levels is familial hypercholesterolemia. This pathology is caused by different mutations in the LDL-receptor (LDL-R) gene (>95% described), in the apolipoprotein B (APOB) gene (2–11% described) and in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene (less than 1%) that lead to a life-long exposure to very high plasma LDL-C levels. These patients have an important increase in cardiovascular disease (CVD) frequency with sudden death and myocardial infarction as the principal

causes of mortality [2,3]. Indeed, the majority of patients with homozygous familial hypercholesterolemia (HoFH) without treatment suffer severe myocardial infarctions in the first decades of life [4]. The use of statins has significantly reduced mortality and morbidity in these patients; however, an unacceptably high residual risk still exists, making necessary the search for additional therapies to further improve their management. Besides statin treatment, LDL apheresis has shown to significantly

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KEY POINTS

- Familial hypercholesterolemia patients, in addition to having very high LDL-C levels, show an altered HDL protein composition.
- Specific changes in apolipoproteins such as Apo A-I, Apo M or Apo L1 and in key enzymes such as LCAT and PON1 affect the functionality of the HDL particle diminishing its atheroprotective capacity.
- The qualitative changes in HDL particles are an additional factor contributing to CVD burden in familial hypercholesterolemia patients.
- The development of novel therapeutic strategies targeting HDL qualitative changes, in combination with currently available lipid lowering therapies, could help to reduce the persisting risk in familial hypercholesterolemia patients and by extension in the entire population suffering from CVD.

reduce the levels of Apo B-containing particles including LDL, lipoprotein(a) (Lp(a)) and very-low-density lipoproteins (VLDL) delaying atherosclerosis progression, stabilizing atherosclerotic plaques and reducing clinical event presentation [5,6].

However, a key unresolved issue in the management of familial hypercholesterolemia patients is why individuals with the same genetic background show important differences in CAD prevalence [3,7,8]. This differential progression of CAD among familial hypercholesterolemia patients underscores the existence of additional factors contributing to the CVD burden in familial hypercholesterolemia patients.

HIGH-DENSITY LIPOPROTEIN PROTEIN CHANGES IN FAMILIAL HYPERCHOLESTEROLEMIA

Owing to their known role as guardians against the excess in LDL-C, high-density-lipoproteins (HDL) appear as one of the additional factors potentially contributing to CVD in familial hypercholesterolemia patients. Indeed, HDLs represent the first line of defence against the accumulation of high LDL-C levels not only through their role in reverse cholesterol transport (RCT), but also because of their antioxidant, anti-inflammatory, antithrombotic, immunomodulating and cytoprotective properties [1]. Although the inverse association between HDL-cholesterol (HDL-C) levels and the risk of CAD has been consistently shown in primary prevention [9], therapeutic attempts to raise HDL-C levels have failed to reduce cardiovascular risk in secondary prevention [10,11]. Even a U-shape correlation

between HDL-C levels and cardiovascular mortality has been shown in specific groups of patients [12]. Thus, it seems that high cardiovascular risk situations modify the functionality of HDL disrupting the association between HDL-C levels and protection [13]. All this evidence underscores the fact that HDL-C levels do not reflect HDL protective properties. This is also true in the context of familial hypercholesterolemia whereas increasing evidence points towards the presence of an altered HDL metabolism and function as an additional factor, not related to the LDL-R locus, contributing the CVD burden in this specific group of patients.

Defective atheroprotective reverse cholesterol transport pathway in familial hypercholesterolemia

Some studies have demonstrated the presence of a defective atheroprotective RCT pathway in familial hypercholesterolemia [14]. HoFH patients seem to exhibit a reduced cholesterol efflux capacity compared to healthy controls [15]. Furthermore, this diminished RCT ability in familial hypercholesterolemia patients has been specifically related to the HDL2 sub-class [16]. Interestingly, a recent study [17] has demonstrated an association between this decreased cholesterol efflux capacity and the presence of the main clinical features of atherosclerotic CVD development in familial hypercholesterolemia patients, such as the presence of corneal arcus and Achilles tendon xanthomas and carotid intima-media thickness [18]. Moreover, the same authors demonstrated that the levels of Apo A-I, the main protein component of HDL, were inversely associated with the presence of these clinical read-outs. Apo A-I levels were a stronger predictor than RCT capacity for the development of atherosclerosis in familial hypercholesterolemia patients. In fact, several previous reports have shown decreased Apo A-I levels in familial hypercholesterolemia [19–21] partly due to an increased transfer of triglycerides (TGL) to the HDL particles. Enrichment with TGL induces instability in these micelles and leads to an increased clearance and reduced content of Apo A-I [22,23]. These results highlight a direct implication of the changes of Apo A-I in the CVD burden in familial hypercholesterolemia patients.

However, HDL particles contain several proteins other than Apo A-I. In fact, high throughput proteomic approaches have demonstrated that HDL particles contain proteins involved not only in lipid metabolism, but also in complement regulation, acute phase response and proteinase inhibition, among others [24]. In addition to changes in Apo A-I, a recent study has suggested an association

between the presence of a higher content of the bioactive sphingolipid sphingosine-1-phosphate (SP1) and its carrier protein Apo M in HDL3 and a higher plasma cholesterol efflux capacity in asymptomatic familial hypercholesterolemia patients [25²²]. A potential role of Apo M in the protection against the development of symptomatic CHD in familial hypercholesterolemia patients was recently proposed [25²²]. Importantly, Apo M is a key molecule for the HDL ability to induce cholesterol efflux [26]. However, there is some controversy on the role of Apo M, because another study showed a lack of influence of familial hypercholesterolemia on the SP1 content in HDL [27²³]. It has to be acknowledged that in this latter study, total HDL and not specific HDL subclasses were analyzed and that no differences were made between asymptomatic familial hypercholesterolemia patients and those that had developed symptomatic CHD. Thus, the cholesterol efflux capacity of HDL particles appears as a key factor contributing to disease progression in familial hypercholesterolemia patients. However, further studies in larger cohorts of patients are still needed, in order to identify the HDL changes that are responsible for this diminished RCT capacity in familial hypercholesterolemia patients.

Altered high-density lipoprotein functionality in familial hypercholesterolemia

In addition to the cholesterol efflux capacity, other HDL atheroprotective properties that play a key role in CVD progression and outcomes, such as their anti-inflammatory and antioxidant potential, could be altered in familial hypercholesterolemia patients. Specifically, a diminished ability to inhibit intracellular adhesion molecule (ICAM)-1 expression in human umbilical vein endothelial cells after tumor necrosis factor- α (TNF- α) induction has been demonstrated in HDL3 of familial hypercholesterolemia patients with high BMI and premature CAD [28]. These results indicate that an altered HDL anti-inflammatory function could be associated to the differential distribution of clinically manifested CAD across familial hypercholesterolemia individuals. In fact, the same authors demonstrated the presence of increased circulating levels of soluble ICAM-1 in familial hypercholesterolemia patients compared with non-familial hypercholesterolemia controls. Thus, it seems that those familial hypercholesterolemia patients with an altered HDL3 anti-inflammatory function would less effectively counteract the increased pro-inflammatory milieu caused by hypercholesterolemia and therefore be more prone to develop CVD clinical manifestations.

In this regard, we have recently demonstrated for the first time the association between an altered HDL proteomic profile and CVD progression and outcome in familial hypercholesterolemia patients treated as per guidelines to control their LDL-C levels [29²⁴]. Specifically, HDL particles isolated from familial hypercholesterolemia patients show a differential proteomic distribution of Apo L1 forms in association with a decrease in total Apo L1 serum levels when compared to non-familial hypercholesterolemia relatives. Beside its role in lipid metabolism [30], this yet unexplored apolipoprotein, has been associated to HDL3 antiinfectious properties [31] and furthermore, it has been shown to correlate with HDL capacity to attenuate LDL oxidation [32]. Furthermore, lower Apo L1 levels have shown to be predictive for acute ischemic events presentation in familial hypercholesterolemia patients at follow-up, including acute myocardial infarction (AMI) or unstable angina. Interestingly, no association was observed in the same study between Apo A-I levels and the presentation of acute ischemic events. Indeed, the predictive value of Apo L1 was independent of the effect of other markers such as the type of LDL-R mutation. Additionally, our specific analysis of the HDL3 subfraction revealed a coordinated decrease in the content of Apo L1 and lecithin-cholesterol acyltransferase (LCAT), a key enzyme in HDL metabolism, in those FH patients that suffered a fatal AMI at follow-up. This result highlights that specific protein changes in HDL3 particles could hamper HDL atheroprotective properties predisposing familial hypercholesterolemia patients to suffer cardiovascular events and death. Indeed, the differential impact of HDL subclasses in prognosis was previously suggested based on the specific association of HDL3-C, but not HDL2-C, with an increased risk for clinical event presentation in secondary prevention [33]. It is important to highlight that HDL3 particles are those having the most potent ability to protect LDL from oxidative damage [31], and this antioxidant property has been mainly attributed to LCAT [34]. Thus, a decreased LCAT content in HDL3 particles could reduce their antioxidant potential and favour LDL oxidation and progression of atherosclerosis, significantly increasing the cardiovascular risk of familial hypercholesterolemia patients. In fact, familial hypercholesterolemia patients with corneal arcus, that is an independent predictor for cardiovascular risk [35²⁵], have a reduced content of Apo L1, LCAT and paraoxonase-1 (PON1) in HDL particles [29²⁴], showing a coordinated protein change contributing to an increased CVD burden.

In line with these observations, a recent study has demonstrated that HDL3 particles of familial

hypercholesterolemia patients show decreased antioxidant and anti-inflammatory activity compared to HDL3 particles of normolipidemic controls [36^{***}]. However, these authors did not analyze in depth the protein composition of the defective HDL3 particles. They only analyzed Apo A-I levels and found a decreased content in HDL3 of familial hypercholesterolemia patients. Interestingly, this study demonstrated that LDL apheresis, despite reducing total HDL-C levels, was able to partially restore HDL3 functionality in familial hypercholesterolemia patients by increasing the Apo A-I content, further attenuating systemic oxidative stress and endothelial activation. The exact mechanism by which LDL apheresis selectively improves Apo A-I levels and HDL3 functionality remains to be fully investigated. It is possible that LDL apheresis by its preferential removal of Apo E containing lipoproteins selectively depletes Apo A-I-poor/large HDL particles, known to contain higher amounts of Apo E [37]. In fact, we have previously shown that familial hypercholesterolemia patients have a decreased HDL content of transthyretin [38], that is known to be involved in

the stability of these particles [39]. Transthyretin is the transporter of plasma retinol binding protein (RBP4) [40], which is also known to be associated to HDL particles. Importantly, familial hypercholesterolemia patients show lower RBP4 levels prior to the presentation of an acute ischemic event [41] underscoring a potential implication of this protein in the increased cardiovascular burden of familial hypercholesterolemia patients. Interestingly, RBP4 induces vasculoprotection by the induction of NO and PGI₂ production in physiological conditions.

All these evidences point towards a coordinated change in several HDL-associated proteins as one additional factor potentially contributing to the phenotypic variations seen in familial hypercholesterolemia patients (Fig. 1).

CLINICAL IMPLICATIONS IN THE MANAGEMENT OF FAMILIAL HYPERCHOLESTEROLEMIA PATIENTS

Changes in HDL composition and functionality are contributing factors to cardiovascular risk in familial

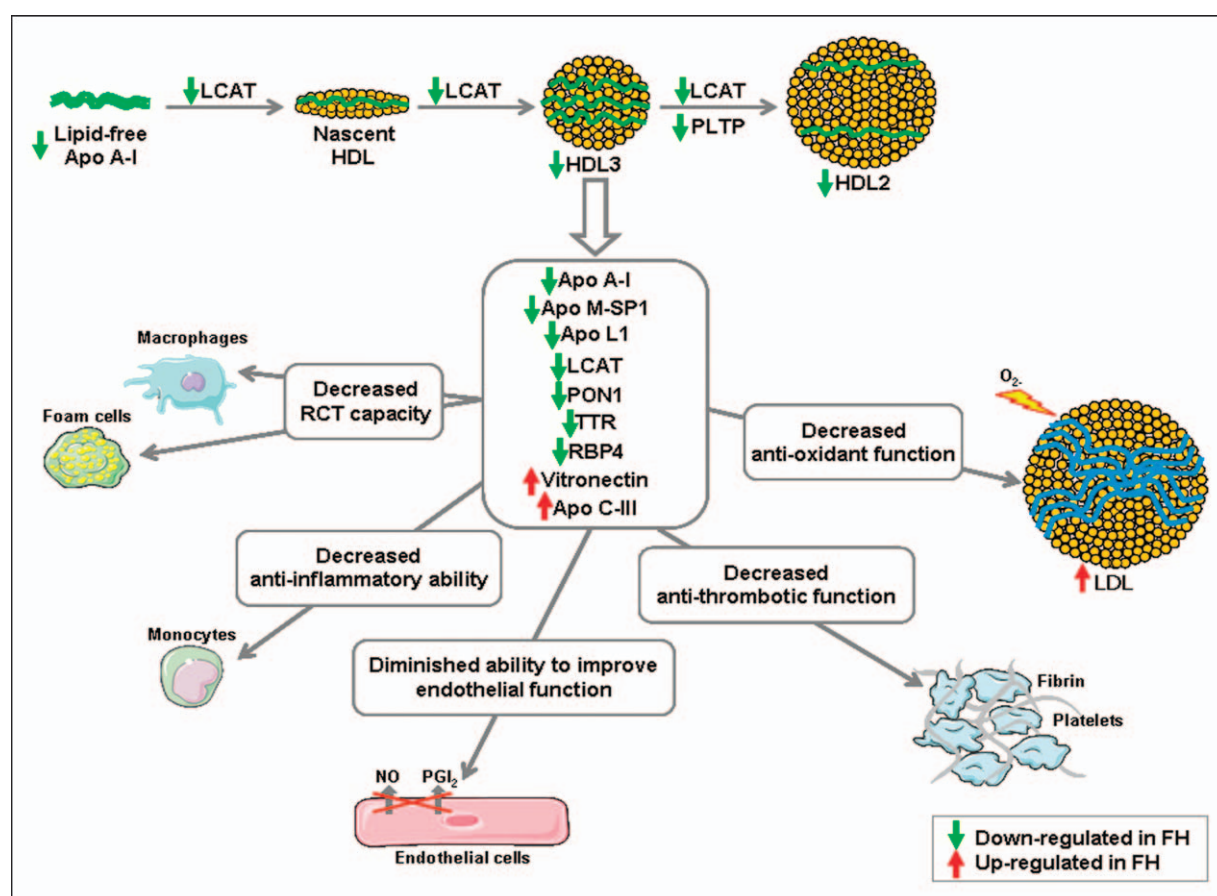


FIGURE 1. Schematic figure showing the coordinated change in HDL protein composition in familial hypercholesterolemia patients. The decrease (green arrows) and the increase (red arrows) in several key high-density lipoproteins in familial hypercholesterolemia patients have a direct impact on HDL functionality decreasing their atheroprotective capacity.

hypercholesterolemia patients; hence the identification of novel therapies targeting HDL quality for preventing atherosclerosis development and clinical event presentation may become a new area of research. This strategy would involve two steps:

- (1) The identification of reliable biomarkers indicative of HDL functionality, in order to obtain a panel of HDL-associated proteins that could be used as surrogate markers of HDL quality. This strategy will enable the identification of patients at higher risk of suffering ischemic events and death due to the presence of dysfunctional HDL particles that cannot be improved by current therapeutic approaches targeting LDL-C levels.
- (2) The development of novel therapeutic approaches able to reverse the deleterious changes in HDL composition and functionality, in order to reduce the persistent high atherosclerotic risk in specific subgroups of familial hypercholesterolemia patients.

In this context, LDL apheresis has shown to exert additional beneficial effects in addition to reducing LDL levels. Apheresis can reduce the content of proteins with key roles in atherogenesis, thrombosis and inflammation. Specifically, it has been reported that LDL apheresis significantly decreases serum levels of vitronectin and apolipoprotein C-III [42]. Interestingly, both proteins have been identified in the HDL proteome [24], and more specifically, increased vitronectin levels have been associated to CAD severity [43]. Thus, the concomitant decrease of vitronectin serum levels induced by LDL apheresis could be an additional factor contributing to the beneficial effects of this treatment in familial hypercholesterolemia patients. In addition, LDL apheresis has demonstrated to reduce circulating levels of several pro-inflammatory mediators (cytokines, CRP and pentraxin-3, among others) and fibrinogen [44,45], further reinforcing the relevance of non-LDL associated proteins on familial hypercholesterolemia pathophysiology.

Due to the relevance of HDL composition in functionality, the search for novel HDL-directed therapies has gained significant interest as an additional approach on top of LDL-C lowering therapies [46]. In this context, one of the most investigated targets is the improvement of the ability of RCT by increasing Apo A-I circulating levels. In heterozygous familial hypercholesterolemia patients, the infusion of a recombinant form of proapolipoprotein A-I has been shown to enhance RCT by increasing cholesterol mobilization to plasma, thus increasing fecal sterol excretion [47]. Similarly, 6-month infusions of a

recombinant human Apo A-I-containing HDL-mimetic particle has shown to significantly reduce the mean vessel wall area measured by magnetic resonance imaging in HoFH patients [48], suggesting that additional therapeutic approaches targeting non-LDL proteins, on top of maximal LDL-lowering therapy, may reverse the atherogenic process [49] in familial hypercholesterolemia patients.

However, due to the high plasticity of HDL particles and the important influence of all the exchangeable protein components on their functionality, the improvement of HDL atheroprotective function needs to target not only Apo A-I, but also other key HDL-associated proteins. Specifically, in the context of familial hypercholesterolemia it would be necessary to reverse the depletion of atheroprotective proteins that seems to occur in those patients that exhibit a higher cardiovascular risk [29^o]. Therapies aimed to improve HDL content of key enzymes such as LCAT and PON1, or other yet less known proteins such as Apo L1 or Apo F (Fig. 1), would be crucial to improve not only HDL metabolism, but also HDL antioxidant ability decreasing LDL oxidative damage and atherosclerotic burden in familial hypercholesterolemia patients.

FUTURE DIRECTIONS AND CONCLUSION

Despite the unquestionable importance of LDL-C in the pathogenesis of familial hypercholesterolemia, increasing evidence highlights the existence of additional factors contributing to the high cardiovascular risk of familial hypercholesterolemia patients. These factors help to explain the differential clinical expression of the pathology across individuals with the same genetic background.

HDL particle composition, and hence functionality, appears as one of these factors. The identification of the protein changes that occur in the HDL particle in familial hypercholesterolemia patients and their association to CVD progression and outcome is an unmet clinical need. The identification of these HDL protein changes could be useful for the development of novel therapeutic targets that, in combination with currently available lipid lowering therapies, could help to reduce the persisting risk in familial hypercholesterolemia patients and by extension in the entire population suffering from CVD.

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Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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