

Review

Leveraging knowledge of HDLs major protein ApoA1: Structure, function, mutations, and potential therapeutics



Aishwarya Sudam Bhale, Krishnan Venkataraman *

Centre for Bio-Separation Technology, Vellore Institute of Technology, Vellore 632014, Tamil Nadu, India

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ABSTRACT

Apolipoprotein A1 (ApoA1) is a member of the Apolipoprotein family of proteins. It's a vital protein that helps in the production of high-density lipoprotein (HDL) particles, which are crucial for reverse cholesterol transport (RCT). It also has anti-inflammatory, anti-atherogenic, anti-apoptotic, and anti-thrombotic properties. These functions interact to give HDL particles their cardioprotective characteristics. ApoA1 has recently been investigated for its potential role in atherosclerosis, diabetes, neurological diseases, cancer, and certain infectious diseases. Since ApoA1's discovery, numerous mutations have been reported that affect its structural integrity and alter its function. Hence these insights have led to the development of clinically relevant peptides and synthetic reconstituted HDL (rHDL) that mimics the function of ApoA1. As a result, this review has aimed to provide an organized explanation of our understanding of the ApoA1 protein structure and its role in various essential pathways. Furthermore, we have comprehensively reviewed the important ApoA1 mutations (24 mutations) that are reported to be involved in various diseases. Finally, we've focused on the therapeutic potentials of some of the beneficial mutations, small peptides, and synthetic rHDL that are currently being researched or developed, since these will aid in the development of novel therapeutics in the future.

1. Introduction

Lipoproteins are a group of biological particles that carry lipids across cells and tissues while triggering intracellular signaling cascades [1,2]. A hydrophobic core with triacylglycerol, esterified cholesterol, a monophasic layer of phospholipids with non-esterified cholesterol, and apolipoproteins on the surface make up these particles [3]. Based on density, size, relative lipid (cholesterol and triglyceride) content, and protein composition, lipoproteins are been classified into five subtypes: chylomicron (CL), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL),

and high-density lipoprotein (HDL) [3,4]. Among all the lipoproteins HDL is the smallest (7–12 nm) and densest (1.063–1.21 g/ml) due to the highest ratio between proteins and lipids [5,6]. As compared to other lipoproteins, proteins make up the majority of the structural components of HDL particles [7,8]. Recent advancement in proteomic technology has revealed the HDL composition; apolipoproteins, lipid transport proteins, acute-phase response proteins, complement components, proteinase inhibitors, and other protein components [9–11]. HDL has long been considered "good cholesterol," beneficial to the whole body and, in specific, to cardiovascular health [12]. It represents a very heterogeneous assembly of lipids and proteins i.e. ~ 80 % of proteins

Abbreviations: ABCA1, ATP binding cassette transporter A1; ADP, Adenosine diphosphate; AKT, serine-threonine kinase; ANS, 8-aniline-1-naphthalene acid; APBP, ApoA1 binding protein; ApoA1, Apolipoprotein A1; ATP, Adenosine triphosphate; CETP, Cholesteryl ester transport protein; CL, Chylomicrons; D-HDL, Discoidal HDL; EL, Endothelial lipase; eNOS, Endothelial nitric oxide synthase; FAMP, Fukuoka University ApoA1 mimetic peptide; HDL-C, High-density cholesterol; HDL, High-density lipoprotein; HL, Hepatic lipase; HNF4, Hepatocyte nuclear factor 4; HREs, Hormone response elements; HUVECs, Human umbilical vein endothelial cells; ICAM-II, Intracellular adhesion molecule II; IDL, Intermediate density lipoprotein; IHD, Ischemic heart disease; LCAT, Lecithin-cholesterol acyl-transferase; LDLR, Low-density lipoprotein receptor; LDL, Low-density lipoprotein; LPS, Lipopolysaccharides; LRH1, Liver receptor homology 1; MAPK, Mitogen-activated protein kinase pathway; MI, Myocardial infarction; NF- κ B, Nuclear Factor kappa-light-chain-enhancer of activated B cells; P2Y1, Purinergic receptor; PI3K, Phosphoinositide 3-kinases; PLTP, Plasma phospholipid transfer protein; PPAR γ , Peroxisome proliferator activated receptor; RCT, Reverse cholesterol transport; rHDL, Reconstituted HDL; RXR α , Retinoid X-receptor; SRB1, scavenger receptor class B type 1; STAT3, Signal transducer and activator of transcription 3; TLR4, Toll like receptor 4; VCAM-I, Vascular cell adhesion molecule I; VLDL, Very low-density lipoprotein.

* Correspondence to: Centre for Bio-Separation Technology, Vellore Institute of Technology, Vellore 632014, India.

E-mail addresses: bmkrishna1@yahoo.com, krishnan.v@vit.ac.in (K. Venkataraman).

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that are involved in the lipid transfer/metabolism [13]. Decades of research have deliberated HDL, as a plasma cholesterol carrier, possessing anti-atherogenic and anti-inflammatory properties in its natural condition, but that when it becomes dysfunctional due to systemic and vascular inflammation, it functions as pro-atherogenic and pro-inflammatory [14,15]. Furthermore, it acts as an anti-apoptotic, and shields lipids from oxidation, besides restoring endothelial function, all of which contribute to HDL's ability to defend against a range of disorders [16,17]. Recent advances in understanding the structural minutiae of HDL have revealed apolipoprotein as a major component involved in the assembly of HDL molecules [18]. Moreover, the Davidson and Shah groups have been working diligently to reveal the HDL proteome; as of May 21, 2021, they have reported 936 proteins that makeup HDL [19,20]. However, in 2009, Wiesner and coworkers reported groundbreaking research that described the HDL lipidome, demonstrating the presence of phospholipids, sphingolipids, neutral lipids, and minor lipids in human HDL particles [21,22].

Apolipoproteins are the major protein part of HDL that regulates cholesterol transport and metabolism [23]. In humans, the HDL encompasses 14 different types of apolipoprotein molecules such as ApoA-I, ApoA-II, ApoA-IV, ApoC-I, ApoC-II, ApoC-III, ApoC-IV, ApoD, ApoE, ApoF, ApoH, ApJ, ApoL-I, and ApoM [24]. These apolipoproteins are important for preserving lipoprotein structural integrity, and enhancing solubility in aqueous environments, including activating and inhibiting lipid metabolic enzymes due to their amphipathic properties [25]. ApoA1, ApoA-II, ApoA-IV, and ApoE are structural and functional apolipoproteins, however, ApoA1 and ApoC-I are involved in the activation of Lecithin cholesterol acyl transferase (LCAT) [9,26].

ApoA1 was one of the first apolipoproteins discovered. It has long been considered a critical component for cholesterol metabolism and HDL synthesis [27]. It is the most structurally and functionally important protein in HDL, accounting for roughly 70 % of the total HDL protein content [28,29]. It primarily interacts with cellular receptors, activates LCAT, and equips HDL with several anti-atherogenic properties [30]. RCT is HDL's major function, which involves absorbing cholesterol from tissues and returning it to liver for bile excretion, hence ApoA1 aids HDL in this endeavor [31]. Furthermore, it has been predominantly involved in protecting against cardiovascular diseases, regulating inflammatory and immunological responses. Several decades of research on ApoA1 suggest that mutations, enzymatic modifications, and metabolic alterations cause a shift in ApoA1 level, ultimately lowering the HDL quality, and endorsing the onset of numerous diseases [16,32]. In light of ApoA1's importance, this article reviews its normal biological function and provides a detailed summary of its gene and protein structure. Additionally, it focuses on structural and functional consequences of ApoA1 mutations involved in various ailments especially cardiovascular diseases and amyloidosis. Finally, we have highlighted the beneficial effect of certain ApoA1 therapeutic mutations as well as mimetic peptides and their prospective use in future treatment techniques.

2. Diverse role of ApoA1

ApoA1 is an essential component of HDL and one of the most common proteins in human plasma, with typical levels ranging from 100 to 150 mg/dl [33]. It comprises stable α -helices related to HDL and is detected in the plasma in 95 % of cases [34]. It is predominantly produced by the liver (80 %) and the gut (20 %), with the liver playing an important role in HDL synthesis and RCT [16]. In order to carry out HDL production and RCT, ApoA1 interacts structurally with a range of cellular receptors, as explained in the following section.

2.1. ApoA1 in HDL biogenesis and RCT

RCT is the process through which HDL transfers excess cholesterol from peripheral tissues to the liver for clearance [35,36]. Lipidation

occurs when ApoA1 is released from the liver, resulting in HDL production and cholesterol transfer to the liver [37]. RCT starts from forkhead box proteins of the liver and intestine, which secrets lipid-free ApoA1 in the interstitial fluid. Whereas circulating ApoA1 interacts with serum phospholipids and forms discoidal HDL (D-HDL) [38,39]. Once D-HDL is generated, ApoA1 triggers cholesterol efflux by interacting with the transporter molecule ATP-binding cassette transporter (ABCA1) which is a membrane transporter protein found in macrophages and hepatocytes. This interaction favors the transfer of cellular cholesterol and phospholipids to be passed to D-HDL [40,41]. ApoA1 acts as a primary substrate in the relationship of ApoA1 and ABCA1, with its C-terminal domain involved in this process. Later on, LCAT present in plasma interacts with the ApoA1 residing on D-HDL after traveling in the blood through the lymphatic system. On D-HDL, ApoA1 activates LCAT, which catalyzes the conversion of lecithin's 2-acyl group to the free hydroxyl residue of unesterified cholesterol, resulting in the production of esterified cholesterol. Activation of LCAT by ApoA1 favors the transformation of D-HDL into spherical mature HDL particles [42,43]. These particles appear to embrace free cholesterol, resulting in the formation of larger, lipid-rich mature HDL particles, which are 7.2–12.9 nm in diameter, contain two, three, or four ApoA1 molecules, and have α -mobility on electrophoresis [44]. In humans, unesterified cholesterol and cholestry esters are transmitted from mature HDL to the liver through two essential pathways: directly, after reversibly binding to SRB1 in the liver, and indirectly, by being transferred to triglyceride-rich lipoproteins (VLDLs, IDLs, and LDLs) through cholesterol ester transfer protein (CETP), accompanied by the absorption of chylomicron remnants and LDLs by the low-density lipoprotein receptor (LDLR) in hepatocyte. Hepatic lipase (HL), endothelial lipase (EL), and plasma phospholipid transfer protein (PLTP) catalyze the remodeling of mature HDL, resulting in the development of lipid-poor HDL particles from which ApoA1 sheds and can bind with ABCA1 in the next lipidation step [45,46]. The liver then removes mature HDL from circulation by two mechanisms firstly through scavenger receptor class B type 1 (SRB1) and later via an unspecified HDL receptor (HDLR), causing hepatocytes to degrade cholesterol esters and excrete them into the bile [37]. A detailed schematic representation of this process has been shown in (Fig. 1).

2.2. Roles of ApoA1 in the anti-inflammation

Atherosclerotic heart disease has lately been linked to inflammation [47]. The stimulation of pro-inflammatory signals in endothelial cells is one of the initial stages of atherosclerotic lesions. This results in the formation of endothelial adhesion molecules such as vascular cell adhesion molecules (VCAM) and intracellular adhesion molecules (ICAM) [48]. HDL-associated ApoA1 has been discovered to have anti-inflammatory properties in the endothelium by releasing miRNA223 into endothelial cells, which suppresses cell adhesion molecules such as VCAM-II, ICAM-I, and E-selectin [49]. It has recently been discovered to reduce inflammation in endothelial cells by increasing annexin A1 expression ultimately dwindling phospholipase A2 activation [50,51]. Mitochondrial ATP synthase, also known as beta ATPase, has lately been discovered in endothelial cells. Activation of beta ATPase by ApoA1, results in the conversion of ATP to ADP, which then activates the purinergic receptor (P2Y1) on the surface of endothelial cells [52]. Additionally, this activation of ectopic beta-ATPase by ApoA1 reduces apoptosis, promotes cell proliferation, and initiates phosphorylation of nitric oxide synthase (eNOS) via P2Y1 and serial phosphorylation of PI3K and AKT in human umbilical vein endothelial cells (HUVECs) [53] (Fig. 2). On macrophages, ApoA1 has been discovered to exhibit anti-inflammatory properties. It interacts with the ABCA1 receptor, phosphorylates the JAK2 protein to trigger conformational changes, and opens the ABCA1 receptor to allow free cholesterol to flow out and towards ApoA1. Later, JAK2 also initiates the STAT3 pathway, which enhances the suppression of apoptosis and inflammatory cytokines [54,55]. The association between ABCA1 and ApoA1 on macrophages is

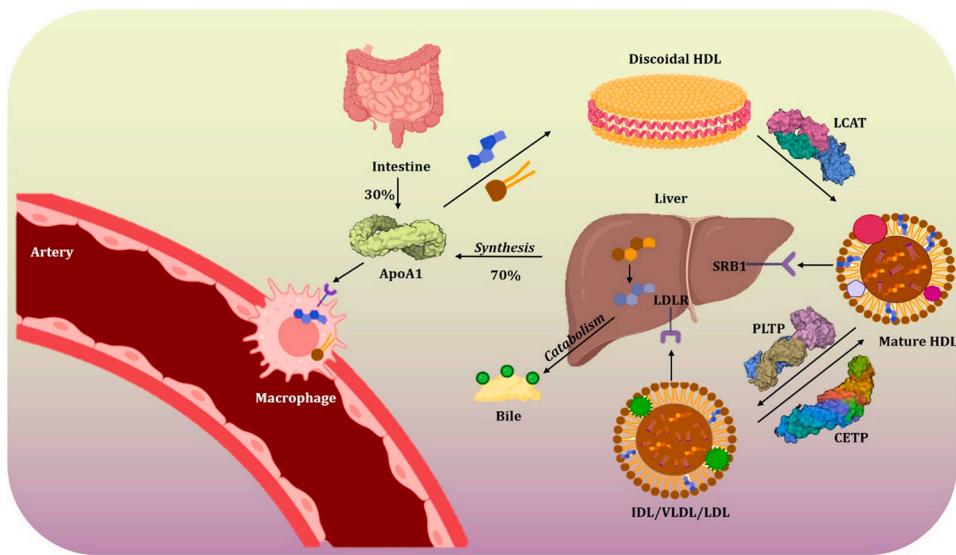


Fig. 1. RCT and HDL biosynthesis in the ApoA1-centric conventional paradigm: Hepatocytes secrete over 70 % of the ApoA1 protein, with epithelial cells of the small intestine providing the remaining 30 %. The interaction of ApoA1 with ABCA1 in circulation causes unesterified cholesterol and phospholipids to be effluxed to ApoA1, resulting in the synthesis of Discoidal/pre β -HDL. LCAT is activated by ApoA1 on pre β -HDL, resulting in the conversion of unesterified cholesterol to cholesteryl esters and the formation of mature HDL. Mature HDL particles can interact directly with the SRB1 receptor residing in the liver to transfer esterified cholesterol, which is then transferred to bile. Due to the action of CETP and PLTP, mature HDL can interchange esterified cholesterol with other lipoproteins, and these lipoproteins can carry it to the liver via LDLR.

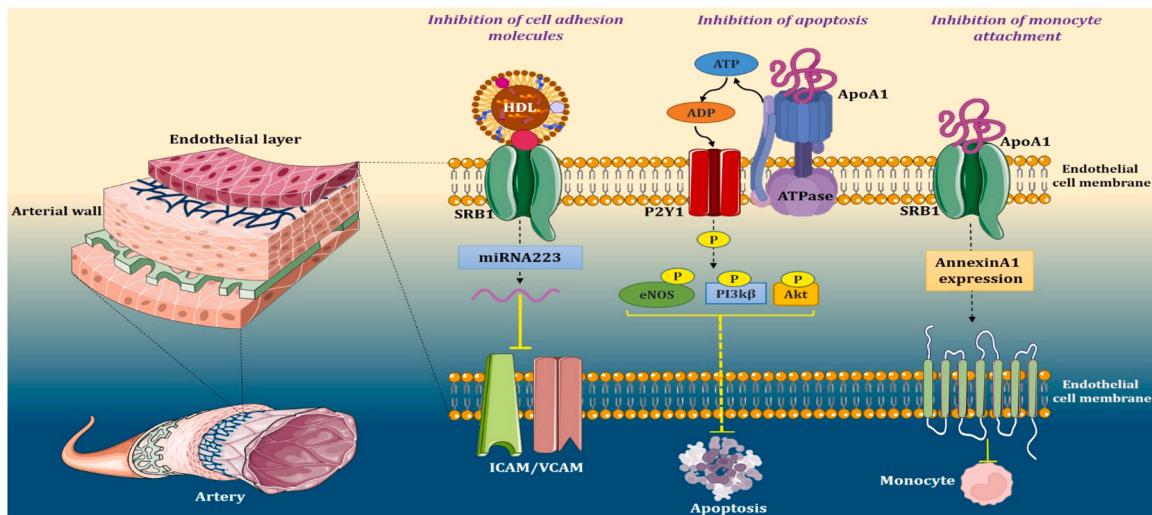


Fig. 2. An illustration of an endothelial cell's anti-inflammatory responses mediated by ApoA1: Cell adhesion molecules are suppressed when HDL-associated ApoA1 and SRB1 interact, as shown in the zoomed area of the endothelium membrane. This interaction introduces miRNA223 into the endothelium, which suppresses ICAM and VCAM expression on the endothelial membrane and prevents monocyte adherence to the endothelium. Additionally, ApoA1 binds alone to the endothelium's SRB1 receptor, promoting the overexpression of annexin A1 and inhibiting the activation of phospholipase A2, which causes monocytes to detach from the endothelial membrane. Moreover, the phosphorylation of eNOS, PI3K, and Akt begins when ApoA1 binds to the ATPase on the endothelium membrane, converting ATP into ADP and activating the P2Y1 receptor, all of which promote the proliferation of endothelial cells and prevent apoptosis.

enhanced by ApoA1 binding protein (APBP), which promotes cholesterol efflux and inhibits macrophage degeneration [56]. When ApoA1 and APBP simultaneously translocate TLR4 from the macrophage's lipid-raft and stop it from binding LPS, the MAPKs pathway and the response of pro-inflammatory cytokines are suppressed [31,57] (Fig. 3). In conclusion, all of these data exhibit that ApoA1 can limit the production of inflammatory cytokines in both endothelial cells and macrophages, presenting it as an anti-inflammatory molecule.

3. Genomic organization of ApoA1

The ApoA1 gene resides on the negative strand of the 11th chromosome at loci 11q23-q24 of humans, where it shares a gene section and exists in a cluster with ApoC-III, ApoA-VI, and ApoA-V. This cluster is crucial for regulating the expression of lipids and lipoproteins and serves as an exchangeable apolipoprotein aggregate [58]. An insight into the ApoA1 gene structure divulges that it primarily comprises 8870 base

pairs (bps) and 4 exons. The Exon-1 ranges from 5001 bps to 5018 bps and it is the smallest exon amongst all exons, while the Exon-2 starts from 5216 bps and ends at 5278 bps, the Exon-3 fields from 5466 bps to 5622 bps, whereas Exon-4 ranges from 6212 bps to 6870 bps [59–61]. The regulation of the human ApoA1 gene is intricate and controlled at different stages (Fig. 4). The transcription of the human ApoA1 gene is mostly dependent on two hormone response elements (HREs) that bind members of the hormone nuclear receptor superfamily and are located close to the transcription start site [62]. Amongst them, peroxisome proliferator-activated receptor- γ (PPAR γ) appears to have a prominent role in ApoA1 transactivation by interacting with HREs and retinoid X receptor- α (RXR α) as a heterodimer. Other transcription factors in the regulation of ApoA1 promoter include the hepatocyte nuclear factor 4 (HNF4), liver receptor homolog (LRH1), and the ApoA1 regulatory protein 1 (ARP1/NR2F2) which activate and repress the ApoA1 promoter, respectively. HNF4 operates together with Sp1 in the communication of ApoA1 promoter with enhancer sequence that facilitates the

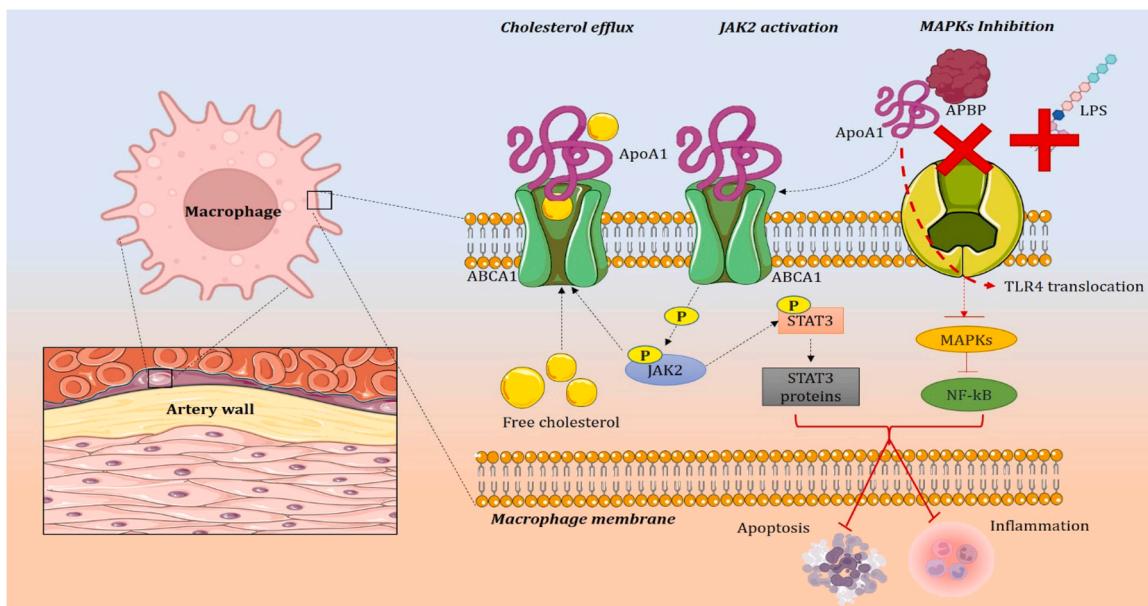


Fig. 3. Info-graphic demonstrating how ApoA1 is important for cholesterol efflux and anti-inflammatory functions in macrophages: ABCA1 and ApoA1 interaction causes JAK2 to be phosphorylated, which causes ABCA1 to alter conformation and open, allowing free cholesterol to flow out and into ApoA1. Additionally, phosphorylated JAK2 promotes signalling independently of ABCA1's lipid transfer pathway, which inhibits macrophage apoptosis and the generation of pro-inflammatory cytokines. When APBP binds to ApoA1 on macrophages, it improves ApoA1's interaction with ABCA1, which boosts cholesterol efflux and prevents macrophage degeneration. The MAPKs pathway and the response of pro-inflammatory cytokines are inhibited when ApoA1 and APBP concurrently translocate TLR4 from the macrophage's lipid-raft and prevent it from binding LPS.

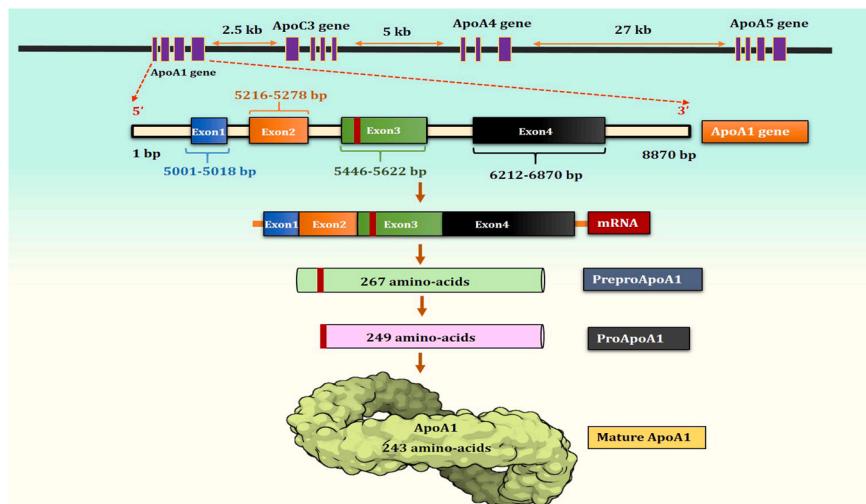


Fig. 4. ApoA1 gene structure and protein translation model: The human ApoA1 gene is located at locus 11q23-q24 on the negative strand of the 11th chromosome some, where it shares a gene section and is found in a cluster with the ApoC-III, ApoA-VI, and ApoA-V genes. The 8870 bp long apoA1 gene contains 4 exons. The ApoA1 protein is created by these exons. PreproapoA1, which is 267 amino acids (aa) long, is the main form of apoA1 that is produced in the liver and small intestine. The initial 18 aa long N-terminal peptide is cleaved later on during the post-translational process, culminating in the creation of proApoA1 (249 aa), which is released in plasma. This proApoA1 has six extra amino acids that, after being secreted, are cleaved by post-translational proteases to create mature ApoA1 (243aa).

recruitment of the basal transcriptional machinery [63]. The ApoA1 gene regulation is a crucial step if gets affected by any internal or external stimuli that can cause a hindrance in the production of ApoA1 protein eventually lowering the formation of HDL and its quality.

4. Structure of ApoA1 protein

ApoA1 is primarily synthesized as a preproapoA1 (267 amino acid (aa) long) in the liver and small intestine. Later during the post-translational process, the first 18aa long N-terminal peptide is cleaved resulting in the formation of proApoA1 (249aa) which is secreted in plasma. This proApoA1 contains additional six amino acids which undergo post-translational proteolytic cleavage after secretion, resulting formation of mature ApoA1 (243aa) with a molecular weight of 28 kDa in humans [64,65]. ApoA1 contains 90 % amphipathic α -helical

content, facilitating HDL synthesis and stabilization. Additionally, they are critical for biophysical properties aiding lipid solubilization in an aqueous environment [66]. The human ApoA1 features 10 tandem (11 and 22 aa) repeats that total (44–243 aa) residues. Each of these repeats, contributes significantly to the exchangeable binding of lipids, besides being critical for interacting with phospholipids and organizing an HDL particle [67,68]. The proline residues have been shown to have a very important role in the structural integrity of ApoA1 (Fig. 5B). They are located in the functionally important regions that assist ApoA1's optimal interaction with the membrane, which is an initial step in cholesterol efflux and HDL production [69,70]. The N-terminal region of ApoA1 (1–180aa) is important for the stabilization of lipid-free ApoA1 and facilitates increasing interaction with lipids by decreasing the helix bundle stability. Additionally, in 2004 Hongli L.Zhu and David Atkinson proposed a role of 1–44 N-terminal region residues in lipid binding and

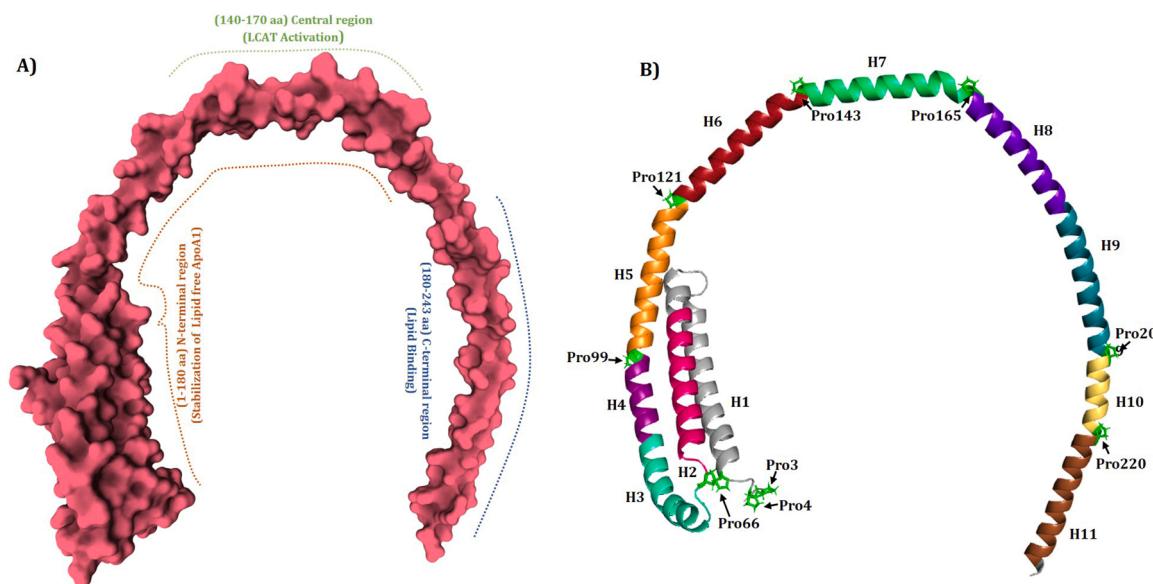


Fig. 5. ApoA1 protein structure: A) Showcases the comprehensive structural features of ApoA1 protein that includes N terminal region (1–180aa), Central region (140–170aa) and C terminal region (180–243aa). B) Portrays 11 helices (each one is shown with a different color) make up each ApoA1 monomer, and proline residues at the junctions between the helices cause the structure to kink.

highlighted its involvement in forming discoidal structure [71,72]. The ApoA1's central region (140–170 aa) is mostly associated with the activation of LCAT whereas the C-terminal domain region (181–243 aa) plays a key role in lipid binding [73,74] (Fig. 5A). Recently the in vitro studies carried out by a group of researchers have suggested a role for 181–220aa in enhancing the ability of ApoA1 to bind and solubilize lipid by the formation of α -helix (Fig. 5B). Moreover, they also demonstrated that a combination of the low lipid affinity region and high lipid affinity region of ApoA1 is required for efficient ABCA1-dependent HDL formation [56].

5. Potential role of ApoA1 in diseases

Inflammatory responses cause significant structural and functional changes in HDL, which ultimately influence ApoA1 amount and quality [16]. Reduced ApoA1 synthesis, accelerated HDL breakdown, ApoA1 substitution by other protein moieties, and several alterations of ApoA1 such as oxidation, carbamylation, glycation, and replacement of ApoA1 by serum amyloid A are all factors that impact ApoA1 structure [16,17]. Additionally, naturally occurring mutations in the ApoA1 protein structure also affect the properties of the proteins [75]. As a result, these alterations might change how ApoA1 interacts with LCAT and CETP, worsening cholesterol efflux and RCT. These modifications to ApoA1 have been associated with a variety of disorders, including hypoalphalipoproteinemia, Tangier disease, atherosclerosis, amyloidosis, and cardiovascular diseases [76–79]. A comprehensive list of potential disorders associated with ApoA1 are highlighted in Table 1.

6. Mutations of ApoA1

The ApoA1 has been deliberated to play a vital role in various diseases such as cardiovascular diseases, neurological disorders, amyloid formation, etc. The mutations and genetic recombination may arise naturally throughout the cell cycle and at the same time might be instigated due to various extrinsic factors. The mutations can render the normal function of the protein and could be responsible for causing many diseases. Since the first reports of spontaneous mutations of ApoA1 reported, scientists have been trying to figure out what causes the pathological accumulation of human ApoA1 leading to cause organ failure. There are reports stating ApoA1 mutations have been linked to

Table 1
Involvement of ApoA1 in various diseases.

Sr no.	ApoA1 associated diseases	Ref.
1	Amyloidosis	[85]
2	Membranous glomerulonephritis	[158]
3	Tangier disease	[159]
4	Hypoxia	[160]
5	Carcinoma of breast	[161]
6	Peripheral neuropathy	[162]
7	Hypoalphalipoproteinemia, primary, 1	[163]
8	Liver carcinoma	[164]
9	Inflammation	[165]
10	Diabetic Cardiomyopathies	[166]
11	Lung cancer	[167]
12	Gastric cancer	[168]
13	Hepatitis	[169]
14	Ovarian cancer	[170]
15	Hypercholesterolemia, Familial	[171]
16	Ataxia	[172]
17	Hypertensive disorder	[173]
18	Coronary artery disease	[174]
19	Behcet disease	[175]
20	Lipid metabolism disorder	[25]
21	Acute lung injury	[176]
22	HIV infection disease	[177]
23	Edema	[178]
24	Adenocarcinoma	[77]
25	Pediatric obesity	[179]
26	Atherosclerosis	[31]
27	Psoriasis	[180,181]
28	Metabolic syndrome X	[182]
29	Apolipoprotein A-I deficiency	[76]

low plasma HDL levels, and defective LCAT activation, which can contribute to atherosclerosis and amyloidosis [80]. In addition to that mutations of ApoA1, are responsible for amyloid self-recognition, aggregation, and overall amyloigenic propensity [81]. Given the importance of mutations in the onset of several diseases, we've highlighted 24 ApoA1 single nucleotide polymorphisms in this section, along with their structural position and involvement in causing structural and functional changes (Table 2 and Fig. 6).

Table 2

Structural and Functional consequences of ApoA1 mutants.

Sr no.	Mutation	Location	Shifted charge and polarity	Hydrophobicity	Structural effects	Functional effects	Ref.
1	GLY26ARG	N-terminal	Positive	GLY > ARG	Helix bundle disruption and higher solvent exposure	Amyloidosis	[86]
2	GLU34LYS	N-terminal	Positive	GLU < LYS	Electrostatic equilibrium at the hinge area is disrupted.	Amyloidosis	[89]
3	TRP50ARG	N-terminal	Positive	TRP > ARG	Interaction between W50 and K23 is hampered.	Amyloidosis	[90]
4	LEU60ARG	N-terminal	Positive	LEU > ARG	Unstable and susceptible to cleavage	Amyloidosis	[92]
5	PHE71TYR	N-terminal	Polar	PHE > TYR	Disrupts bottom aromatic cluster	Amyloidosis	[94]
6	LEU75PRO	N-terminal	Neutral	LEU > PRO	Destabilizes the four-segment bundle	Amyloidosis	[95]
7	LEU90PRO	N-terminal	Neutral	LEU > PRO	Kinks the α helix	Amyloidosis	[75]
8	TYR100HIS	N-terminal	Positive	TYR > HIS	Loses ability to self-associate	Hinders LCAT Activity	[105,130]
9	GLU110LYS	N-terminal	Positive	GLU < LYS	Electrostatic abnormalities	Do not affect LCAT activity	[131]
10	LEU141ARG	N-terminal	Positive	LEU > ARG	Affect LCAT activity	Cardiovascular disease	[101]
11	PRO143ARG	N-terminal	Positive	PRO > ARG	Change in the orientation of amphipathic α -helices, defect in LCAT activity	Hyperlipoproteinemia	[113]
12	LEU144ARG	N-terminal	Positive	LEU > ARG	Affect LCAT activity	Cardioprotective	[116]
13	ARG151CYS	N-terminal	Neutral	ARG < CYS	Affect LCAT and lipid binding	Cardioprotective	[118]
14	ARG153PRO	N-terminal	Neutral	ARG < PRO	Affect LCAT binding	Cardiovascular disease	[104]
15	VAL156GLU	N-terminal	Negative	VAL > GLU	Affect LCAT binding, dimerization, and orientation	Cardiovascular disease	[106]
16	LEU159PRO	N-terminal	Neutral	LEU > PRO	Enhances hydrophilicity, and decreases the alpha-helix formation	Cardiovascular disease	[108]
17	LEU159ARG	N-terminal	Positive	LEU > ARG	Affect LCAT activation	Cardiovascular disease	[110]
18	ARG160LEU	N-terminal	Neutral	ARG < LEU	Affects lipid binding	Hinders LCAT activity	[133]
19	HIS162GLN	N-terminal	Polar	HIS < GLN	Affects LCAT activation	Hinders LCAT activity	[129]
20	ALA164SER	N-terminal	Polar	ALA > SER	Lower binding affinity to lipids	Amyloidosis, Atherosclerosis, IHD, and MI	[99]
21	PRO165ARG	N-terminal	Positive	PRO > ARG	Instability in lipid-bound ApoA1	Hinders LCAT activity	[134]
22	ARG173CYS	N-terminal	Neutral	ARG > CYS	Formation of disulphide bond and stable dimer	Cardioprotective	[121,124, 125]
23	LEU178PRO	N-terminal	Neutral	LEU > PRO	Disrupts an α -helix when not located at one of the first 3 positions of that helix	Cardiovascular disease	[111]
24	GLU198LYS	C-terminal	Positive	GLU < LYS	Do not interfere with the amphipathic characteristics	Hyperlipoproteinemia.	[114]

6.1. ApoA1 mutations in amyloidosis

Amyloidosis is a cluster of numerous heterogeneous diseases caused due to extracellular deposition of amyloid proteins in various organs ensuing in organ dysfunction and failure [82]. ApoA1 is involved in causing amyloidosis which is characterized by depositions of ApoA1 in various organs and can be either hereditary or nonhereditary [81,83]. The clinical manifestations of ApoA1 instigated amyloidosis mostly involves the larynx, skin, liver, kidney, and myocardium. Moreover, in some rare cases, it has been noticed to form amyloids in the testes and adrenal glands. Several mutations of ApoA1 have been reported to cause structural and functional changes leading to excess deposition in various organs eventually instigating amyloidosis. Most of these mutations are located in two major amyloidogenic hotspot regions (50–93, 170–178) [84]. The reports say that the mutations from 1 to 75 codons are mostly responsible for instigating renal and hepatic amyloidosis whereas the region from 173 to 178 is responsible for cardiac, cutaneous, and laryngeal amyloidosis [85]. Hence in this section, we have discussed some of the important mutations of ApoA1 which apt in triggering amyloidosis.

6.1.1. Gly26Arg (G26R)

The first and most common amyloidogenic mutation found in ApoA1 is G26R which is well known as “Iowa”. According to experiments carried out by Nichols et al. in the late 1980s the G26R mutation was inherited for 3 generations and was associated with the development of systemic amyloidosis [86]. When it comes to ApoA1 protein structure: the G26 position forms a kink in the middle of the first helix in ApoA1 confers the curvature of ApoA1 and HDL. Recent understanding of G26R mutations has shown to disrupt the helix bundle structure of full-length ApoA1. As this mutation is based on the replacement of a strong basic arginine residue into the nonpolar face of the amphipathic helix, which hinders the helical segment from packing optimally, resulting in N-terminal helix bundle destabilization and increased solvent exposure. Furthermore, the G26R substitution provides a positive charge to K23, replacing the favorable cation-π interaction between K23 and W50 with a repulsive interaction between K23 and R26, destabilizing the extended region L44–S55, where residue 50 is found. Regardless of a slight change in the net helical content, Emi Adachi et al. through their experimental evidence demonstrated that the G26R mutation causes broad structural reconfiguration of the residue in the central region of the ApoA1

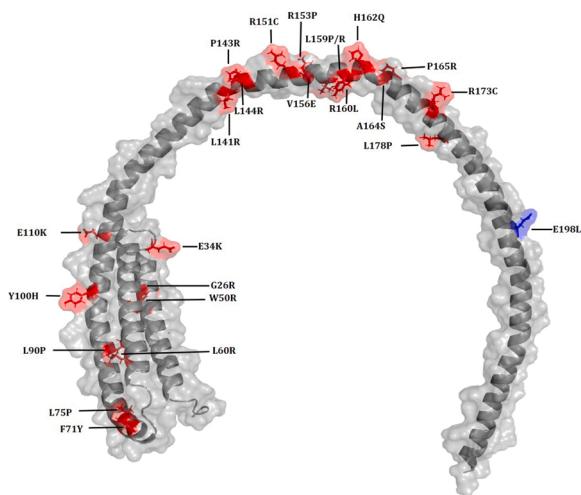


Fig. 6. The mutations in ApoA1 protein structure: Image portrays the ApoA1 protein mutations, with red residues indicating N-terminal and central region mutations and blue residues denoting C-terminal mutations.

molecule [87]. They also observed that the G26R mutation in ApoA1 has a dual impact on protein structure and stability, increasing the tendency to generate amyloid fibrils by boosting intermolecular aggregation.

6.1.2. Glu34Ilys (E34K)

The E34K a rare mutation of ApoA1 has been reported in severe systemic amyloidosis by numerous studies in the past. Unlike the other mutations of ApoA1, E34K is the only known charge inversion mutation that has been located at the top of the four-helix bundle in lipid-free ApoA1. ApoA1 hydration is determined by charged residues, which impacts protein stability and interaction with other molecules. Therefore this charge inversion allows ApoA1 to get aggregated. This mutation is located in the hinge region at the top of the helix bundle and is known to disrupt the overall electrostatic balance of this flexible region. Furthermore, Andeen NK et al. conducted a case study on a 26-year-old man with ApoA1 E34K mutation who had no family history of amyloidosis but presented with testicular, conjunctival, and renal involvement along with lipid and amyloid deposits. The clinical study conducted by Yoshinga et.al on the 43 yrs. old male patient who exhibited hepatomegaly with splenic-testicular enlargement along with amyloidosis [88]. The proteomics analysis for this patient revealed that the amyloid was composed of the mutant ApoA1 (E34K). In 2021, Sagawa T and colleagues conducted a study on a 45-year-old male patient having E34K mutation who showed liver failure and had previously experienced renal disease and infertility at the age of 35 and 42, respectively, and found amyloid buildup. The testis biopsy which was taken at the age of 42 revealed the infertility was caused by the accumulation of amyloid. Later the amyloid and genetic profile led to a definitive diagnosis of hereditary ApoA1 amyloidosis caused by E34K mutation [89].

6.1.3. Trp50Arg (W50R)

The ApoA1 W50R mutation has been identified as a naturally occurring mutant linked to human amyloidosis [90]. This mutation, like others seen in amyloidosis, involves the replacement of single neutral amino acids with the cationic residue arginine, implying a similar mechanism of amyloidogenesis. This mutation resides in the middle of the extended strand 44–55aa. The experimental studies carried out by Das M et.al showed that W50R slightly increases protein stability to recruit lipids and form reconstituted HDL. Furthermore, the W50R mutation does not destabilize ApoA1 on reconstituted HDL and does not augment free protein dissociation which suggests that it does not alter the population distribution from HDL-bound to free ApoA1. When

compared to other non-amyloidogenic mutations, this one has a lower protein destabilizing effect, indicating that instability isn't enough to produce amyloidosis. The W50R lowers ApoA1's net negative charge, notably at its acidic N-terminus, which encourages β -aggregation. Additionally, this mutation epitomized its role in causing cardiac amyloid deposition leading to cardiac and renal failure [90].

6.1.4. Leu60Arg (L60R)

The L60R variation is one of the rarest, and it's noteworthy that the neutral residue is replaced by charged residue in this mutation. It's also interesting to note that this mutation has received relatively little attention. According to a case report by Soutar AK et al., an English family with autosomal dominant non-neuropathic systemic amyloidosis had the ApoA1 L60R mutation. This family has been diagnosed with visceral amyloidosis for three generations. The 24 years old male from the family who was completely asymptomatic showed extensive splenic and hepatic amyloidosis, later he developed progressive hypertension, thrombocytopenia, and easy bruising [91]. Another investigation was conducted in 2020 by Gaddi et al. to provide light on the structural disruption of the L60R mutation. They employed a range of analytic tools to compare the structural behavior of the mutant protein to that of the natural protein and found that the L60R mutation made ApoA1 protein unstable and more susceptible to cleavage. They also discovered that the mutant protein's N-terminus was more disordered and tended to aggregate at physiological pH and it preserved the dimeric conformation and lipid-binding capacity [92]. As a result of these experiments, we strongly indicate that the protein after this mutation is either cytotoxic or progenitors of amyloid confirmation due to conformational shift. Likewise, many more studies will be required in the future to fully comprehend the behavior of mutations in diverse diseases.

6.1.5. Phe71Tyr (F71Y)

The F71Y is the most conservative amyloidogenic mutation in ApoA1 that is located in the middle of the minor predicted hot spot 69–72aa [93]. The F71 resides on the bottom of the aromatic cluster of a highly hydrophobic environment formed by residues W8, L14, L60, L64, F71, W72, L75, L170, L174, L178, & L181. It has an orthogonal phenolic ring that interacts with the indole rings of W8 and W72 to generate a stacking interaction. When tyrosine is changed at this site, adding an OH group to this densely-packed hydrophobic cluster results in its destabilization. As a result of this, the side chain flips out into a more polar environment, disrupting the bottom aromatic clusters. According to a recent in vitro experiment, this mutation results in a reduction in circular dichroism signal at 185–200 nm, which is comparable to the phenomenon reported in the G26R and W50R mutants. It also showed a modest increase in ANS emission, which was similarly identified for the G26R and W50R mutations, both of which are important in amyloidosis [94]. Furthermore, little deuteration in C-terminal was also been discovered with F71Y mutation.

6.1.6. Leu75Pro (L75P)

The L75P is a prevalent amyloidogenic mutation that exists in the center of short helix 70–76aa, which determines the relative helical orientation in the four-segment bundle. In normal protein, L75 interacts with L14 which is located at the bottom of the hydrophobic cluster. Hence mutation at this position disrupts the interaction with L14 and also affects the orientation of the bundle-forming helices, which untimely destabilizes the four-segment bundle. Obici L et al. published a case report in 2004 in which they took 13 subjects of Italian ancestry and discovered that 7 of them had amyloid fibrils specifically immunoreacting with anti-apoA1 antibody, indicating that this mutation segregates with disease and strongly supporting its pathogenic role [95]. Moreover, they proposed a link between this mutation in the onset of systemic amyloidosis, which primarily affects the kidney, liver, and testis. Raimondi S et al. observed that the L75P mutant increased the rate of aggregation and produced 2–3 times more amyloid [96].

Furthermore, the investigation by Gomaraschi M et al. on L75P mutant carrier subjects, revealed that this mutation was associated with hypoalphalipoproteinemia. In these subjects, they also noticed decreased levels of HDL particles containing only ApoA1 with a partial defect in cholesterol esterification [97]. Gregorini G et al. reported this mutation in tubulointerstitial nephritis in a case study involving 253 subjects carrying this mutation. Among the 253 subjects examined, 219 were found to have undergone clinical, laboratory, and instrumental tests, with 62 % being diagnosed with renal, hepatic, and testicular illnesses, and 38 % being asymptomatic. According to their observation, the disease showed age-dependent penetrance. Tubulointerstitial nephritis was found in 49 % of carriers, with 13 % progressing to renal failure and necessitating dialysis. Hepatic association with elevated cholestasis indices was found in 30 % of the carriers, with portal hypertension developing in 38 % of them. In 68 % of carriers, impaired spermatogenesis and hypogonadism were discovered. In 80 % of mutation carriers, cholesterol levels were lower than normal [98]. As a result, these investigations clearly show that the L75P mutation in ApoA1 has a role in illness onset and progression.

6.1.7. *Leu90Pro (L90P)*

Hamidi AL et al. were the first to discover this ApoA1 variation in the N-terminal region. This mutation was discovered via a structural analysis of the amyloid protein isolated from the members of the family with amyloid deposits in their hearts. Additionally, they observed that members with the L90P mutation died due to heart failure in their sixth or seventh decade, according to the investigators. According to the study, this mutation is not found inside the amyloid plaque protein fibrils, but it is likely connected to metabolic alterations [75]. As a result, further research is needed in the future to fully comprehend the function of this mutation.

6.1.8. *Ala164Ser (A164S)*

Hasse et al. identified this mutation in the general population of Danish descent. According to their observation, this mutation was associated with an increased risk of Ischemic heart disease (IHD) and myocardial infarction (MI). Moreover, the median survival time of the subjects with this mutation was reduced by 5–10 years [80]. The LCAT activation, plasma levels of lipid, lipoproteins including HDL-C, and ApoA1 remained normal in subjects with this mutant. The increased risk of the IHD, MI, and associated mortality with this mutation, was not related to the decrease in the HDL-C as seen in the many mutations of ApoA1. The explanations for this might be as follows; 1) it might obstruct the intramural coronary vascular amyloidosis with symptomatic IHD; 2) amyloidosis accelerated by amyloidosis; 3) or it might be due to dysfunctional HDL particle caused by the loss of proposed anti-inflammatory and anti-oxidative properties of HDL. The structural studies carried out by Dalla-Riva J et al., on A164S using various biophysical techniques like circular dichroism spectroscopy and electron microscopy suggested the similarity in structural properties between a normal ApoA1 and A164S mutant [99]. Additionally, they observed the A164S mutant shows lower binding affinity to lipids but forms similarly sized HDL particles as produced by the normal ApoA1.

6.2. *ApoA1 mutations in cardiovascular diseases*

Cardiovascular diseases are a major cause of mortalities globally holding the highest disease burden. Numerous molecular factors contribute to the development of cardiovascular diseases [80,100]. One of these is lower levels and bad quality of HDL, which has been linked inadvertently to an increase in the risk of cardiovascular disease. According to numerous research, familial HDL deficiency spurred on by ApoA1 mutations increases the risk of developing cardiovascular diseases. As a result, we have highlighted some ApoA1 mutations in this section that have been associated with several cardiovascular disorders.

6.2.1. *Leu141Arg (L141R)*

The L141R mutation, often known as "Pisa," residing in the central region of ApoA1. It was initially discovered in a case study conducted by Miccoli et al., in 1996 on patients who had a lipid metabolism deficiency and showed symptoms of coronary heart disease [101]. They discovered that the L141R mutation blocks ApoA1 secretion and induces full HDL deficiency, which increases the risk of early atherosclerosis. They also reported that the L141R mutation prevents the formation of lipid-rich ApoA1 but not lipid-free ApoA1, and that it affects LCAT function, resulting in a reduction in cholesterol efflux capability [102]. Later the in vitro and in vivo experiments presented that this mutation is secreted efficiently from the cells but had shown reduced activation capacity of LCAT. Additionally, they proposed that L141R causes a considerable decrease in levels of total plasma cholesterol, HDL, and ApoA1 which eventually results in inhibition of HDL biogenesis [102]. Furthermore, researchers found that transgenic mice carrying the L141R mutation had changed HDL shape and function, making them more susceptible to atherosclerosis. Besides they found that mice with this mutation had lower HDL-C levels, as well as impaired anti-oxidant capabilities and total cholesterol efflux capacity of HDL, which could explain the human carriers of this mutation's tendency in promoting atherosclerosis [103].

6.2.2. *Arg153Pro (R153P)*

The R153P mutant was reported by Esperón et al., in patients with premature coronary artery disease [104]. This mutant is referred to as "Montevideo" and is located in the COOH-terminal of ApoA1 polypeptide. At positions 149, 151, 153, and 160 in the COOH-terminal region, four arginines are required for LCAT activation. Arginine at 153 is one the most highly conserved residues amongst several species of ApoA1 including humans. Substitutions at this position cause a tenfold decrease in maximal reaction velocity of the mutant protein. Additionally, it causes several changes in the protein by altering the electrostatic charge of a buried site in an α -helix or affecting its interaction with LCAT. According to the researcher, this mutation was associated with familial hypoalphalipoproteinemia. Additionally, it seemed to have impaired HDL formation and hindered efflux promoting ability. The phenotypic changes caused by this mutation are associated with instability and consequently high turnover rate in the reverse cholesterol pathway. As the significance of this variant in altering the normal function of ApoA1, more research in the future is needed to evaluate it in this context.

6.2.3. *Val156Glu (V156E)*

Huang et al. were the first to describe the V156E mutation in a homozygous subject with low ApoA1 and HDL levels, as well as corneal opacities and coronary artery disease. In addition, they revealed that when compared to the normal subjects, LCAT activity, LCAT mass, and cholesterol esterification rate were all reduced by half in subjects with this mutation. They also observed that this ApoA1 variation is predominantly seen in small HDL or ApoA1 that is lipid poor. Because of this mutation, ApoA1 is removed from plasma faster than normal HDL, resulting in ApoA1 deficiency, with a decrease in levels of HDL in patients [105]. V156E does not contribute to cholesterol esterification as it does not activate LCAT to any significant extent. This mutation causes changes in protein structure and function. The mutant in the lipid free-state of ApoA1 is more stable compared to the lipid bounded form. The change in hydrophobic residue valine to negatively charged glutamic acid at 156 positions interferes with the formation of protein-protein links that lead to self-association. The work by Kyung-Hyun Cho et al., has shown that deletion of the COOH-terminal sequence of ApoA1 also hinders the self-association of ApoA1 [106]. Therefore signifies that V156E mutation affects the dimerization and oligomerization of ApoA1. The stability of the mutant is due to the introduction of glutamic acid at this position resulting in favorable hydration and stabilization of the mutant. Various models have been proposed to explain the structural and functional role of this mutant, but none of them fully explains the

changes. As a result, there is still an opportunity to investigate this mutation in depth.

6.2.4. *Leu159Pro (L159P)*

Miller et al., 1988 identified a novel mutation L159P in the ApoA1 gene in large US families primarily residing in Ohio and Texas [107]. This mutation is well known as "Zavalla" [108]. In their study, they observed that changes in leucine to proline affected the concentration level of HDL-C and normal ApoA1. Based on structural examination this mutation significantly enhances hydrophilicity, decreases the α -helix formation potential, and increases the beta turns forming potential. Additionally, it affected the stability of the protein structure, reduced the LCAT activity, and altered the post-translational degradation. This mutation disturbs the domain structure and makes the protein vulnerable to catabolism. Reduction in the levels of HDL-C due to this mutant develops premature coronary artery disease when accompanied by additional cardiovascular risk factors.

6.2.5. *Leu159Arg (L159R)*

Miettinen et al. identified the L159R mutation of the ApoA1 gene and designated it as "Fin" [109]. They noticed that patients carrying this mutation had dominant hypoalphalipoproteinemia with reduced serum HDL-C and ApoA1 concentration. According to their study, heterozygous carriers of mutation possessed half the HDL-C concentration. Moreover, they demonstrated that mutant carriers have one normal allele and one mutant allele forming ApoA1 "Fin". Later on, the study conducted by the same group revealed that this mutation not only affects the HDL levels but also IDL and LDL levels and has a vital role in reducing the LCAT activation by 60 %. Besides, it maintained the ability to promote cholesterol efflux from fibroblasts [110]. Therefore future studies are needed that can evaluate whether ApoA1 "Fin" has an atherogenic or anti-atherogenic potential, as well as its role in RCT and its impact on coronary artery disease development.

6.2.6. *Leu178Pro (L178P)*

Hovingh et al. were the first to report this mutation and determined the consequences of this mutant on HDL metabolism, endothelial function, carotid arterial wall intima-media thickness, and coronary artery disease risk in 54 heterozygous carriers. This mutation was identified in six families from the Netherlands' northeastern region. In their study, they observed half the number of the variant and normal ApoA1. Moreover, they found a reduction in the levels of HDL-C by 62 % in the heterozygous carriers of L178P. They also noticed that triglycerides levels were not affected due to this mutation. The amyloidosis was not observed in carriers with this mutation. The reason behind this might be the preponderance of ApoA1 mutations that cause amyloidosis has been linked to a change in the protein's isoelectric point, which does not occur in the case of the L178P mutant. From their study, they concluded that L178P mutation has been linked to causing endothelial dysfunction, increased arterial wall thickness, and finally premature coronary artery disease [111,112]. From our perspective, some more studies need to be performed to reveal and justify the consequences of such mutations conclusively.

6.3. *ApoA1 mutations in hyperlipoproteinemia*

The inability of the body to break down lipids or fats, particularly cholesterol and triglycerides, is the hallmark of the disease condition known as hyperlipoproteinemia. Additionally, it increases the risk of cardiovascular, brain, and atherosclerotic illnesses. Similarly, a number of laboratory investigations have linked hyperlipoproteinemia to a range of gastrointestinal and urinary disorders. Therefore, some ApoA1 mutations that are important in the development of hyperlipoproteinemia are briefly reviewed in the section that follows.

6.3.1. *Pro143Arg (P143R)*

The P143R mutation in ApoA1 commonly referred to as "Giessen" can be found in the protein's central region. Utermann G et al. were the first to describe this mutation in a family with vertical transmission [113]. All of the subjects in their study had heterozygous forms of normal and mutant ApoA1, which was linked to hyperlipoproteinemia and dyslipoproteinemia. According to their observation, this mutation does not affect the LCAT activity in vivo. Whereas they found a mild deficiency of HDL and hypertriglyceridemia in the subjects which indicated their perturbation in lipoprotein metabolism. As a result of this mutation, the total charge of the ApoA1 protein changes, becoming more positive. Apart from that, no significant changes in the mutant ApoA1's lipid-binding properties were found. When looking at the structural details, this mutation is found in the putative β turn region of ApoA1 that divides two amphiphilic helices. Proline 143 in normal ApoA1 is required to maintain the 3D structure of this region because proline breaks the α -helix at this point. Due to the replacement of the arginine at 143, this helix did not break and it relatively changed the orientation of the two flanking helical segments eliminating the β turn. This change in orientation of the amphiphilic helices affects the cofactor properties, ultimately causing the change in the function of the ApoA1 [113]. As a result, extensive research in elucidating the mechanism of this mutation is urgently needed, especially in the context of studies exhibiting structural abnormalities produced by this mutation.

6.3.2. *Glu198Lys(E198L)*

Strobl W et al. carried out a clinical screening of ApoA1 in 530 probands of unrelated Austrian origin which included 168 children and 362 adults. In the study, they described six subjects possessing this mutant and reported the genetic transmission of this mutant which was to be inherited in an autosomal co-dominant way. Moreover, they wanted to investigate if this mutant was related to dyslipoproteinemia or atherosclerosis. And they found that the subjects were having hyperlipoproteinemic of ApoAIIa, ApoAIIb, and ApoAIV and there was no such relationship seen between this mutant and hyperlipoproteinemia. According to their observation, they did not find any solid evidence that this mutant helps in accelerating atherosclerosis. Besides there was a congenital deficiency of ApoA1 and ApoCIII was associated with severe premature arterial disease. The structural examination of this mutant presented that this mutation does not disrupt the structure of ApoA1. The amphipathic α -helical region is considered to be important as the polar face of this region interacts with polar head groups of phospholipids. The E198L mutant occurs on the polar side but it does not interfere with the amphipathic characteristic of the ApoA1 helix. The lipid-binding capacity due to this mutation does not change it remains intact as seen in normal ApoA1 subjects [114]. Therefore new insights exploring the role of this mutation are required in the future.

6.4. *ApoA1 mutations in cardio protection*

A number of studies have suggested that some ApoA1 mutations have cardiac protective effects [115]. Leu144Arg, Arg151Cys, and Arg173Cys have primarily been observed to have this impact; hence, a brief review of their cardio protective role has been provided in the following section.

6.4.1. *Leu144Arg (L144R)*

Recalde D et al. first discovered the L144R mutation in a Spanish family with low HDL-C levels and normal ApoA1 levels in 2000, and named it "Zaragoza" [116]. They found that the subjects in their study were heterozygous and that none of them had any indications of coronary artery disease. According to their findings, this ApoA1 variant causes heterozygosity in abnormal HDL particle composition and increases ApoA1 and ApoAII catabolism, affecting the secretion rate of these proteins and LCAT activation. Therefore this mutation is considered as atheroprotective as subjects possessing this mutant have low

levels of HDL-C without cardiovascular disease or atherosclerosis. In contrast to a previous study by Recalde D et al., Haase et al. in 2011 found that LCAT activity was reduced in mice with the L144R mutation due to a lower cholesteryl ester ratio, implying that more research is needed to determine the exact mechanism [80]. Due to the increased positive charge (arginine), this mutation has a distinct monomeric or lipid-bound shape, and could be possibly due to additional intra or intermolecular interaction [117]. The structural conformation of this mutant ApoA1 in both lipid-free and lipid-bound states is unknown. As a result, the scarcity of structural information for this mutant in both conformations must be addressed. In the hunt for better rHDL-infusion therapy treatments, the beneficial phenotype reported in carriers of the L144R mutation suggests that this mutant protein may have better functional capabilities than normal ApoA1. As a result, extensive research is needed in the future to identify the unique mechanism of this mutation, due to its atheroprotective functions.

6.4.2. Arg151Cys (R151C)

Bruckert E et al. were the first to report rare mutation R151C of ApoA1 in the French family which presented very low serum HDL-C levels hence this mutation was referred to as "Paris". In their observation, they have found that R151C can form homodimers as well as heterodimers. Moreover, they presented that heterozygotes with hypalphalipoproteinemia have a prevalence of smaller, phospholipid-rich HDL particles with partial LCAT deficiency [118]. This mutation resides in a region of ApoA1 that is thought to be critical for early lipid binding and LCAT activity. The typical function of ApoA1 activating LCAT has been observed to be defective in the R151C mutant. They also noticed that this mutant had the normal ability to promote cholesterol efflux either in the lipid-free or in the lipid-bound form. Moreover, they reported that R151C shows a resemblance of the physicochemical and functional features with the R173C "Milano" mutant of ApoA1 [118]. Considering R151C mutant anti-atherogenic properties more studies are required, focusing its anti-atherogenic properties and potential therapeutic applications in the future.

6.4.3. Arg173Cys (R173C)

The mutation of ApoA1 was discovered by Franceschini et al., in the Italian family which belongs to north Italy. This mutation was carried by 3 members of the family having hypertriglyceridemia [119]. Later Wesigraber KH et al., in a clinical study conducted identified that the protein (ApoA1) content of the HDL isolated from these patients was different than normal. After biochemical analysis of the serum, they found the presence of cysteine and isoleucine which were not present in the normal ApoA1 [120,121]. They named the R173C variant of ApoA1 "Milano". According to their observation, this mutation had an atheroprotective role i.e. it is assumed to have protective functional capabilities against the accumulation of cholesterol in the arterial wall. Franceschini et al., reported the subjects with this mutation exhibited lower levels of HDL-C and importantly low incidences of atherosclerosis [119]. The Gualandri et al. later determined this mutant in 33 carriers from the ages 2–81 years old having the heterozygous amount of R173C in autosomal dominant trait [122]. The plasma analysis of the mutant carriers by Wesigraber et al. has presented that normal and mutant ApoA1 in the carrier was approximately 25 % and 75 % respectively. Additionally R173C mutant exhibits the phenotypes with reduced levels of plasma HDL, ApoA1, HDL-C, cholesterol, and increased HDL triglycerides [123]. When looking into the structural consequences of this mutant, the replacement of cysteine at the 173 positions allows forming the cysteine-cysteine disulfide-bonded dimer. The presence of the cysteine at the 173 positions also helps in destabilizing the helix bundle domain conformation, because cationic arginine residue, forms an intrahelical salt bridge with the anionic 169 glutamic acids in normal ApoA1 in both lipids free and lipid bounded form. In normal ApoA1 there are intermolecular salt bridges between the N-terminal helix bundle residue and helix number 7. It is also been observed that the

carriers of HDL R173C have low incidences of atherosclerosis or cardiovascular disease with changes in HDL particle composition and size. The reason for this might be the enhanced RCT process. Moreover, R173C mutation has resistance to forming amyloid structures and therefore this might be the additional reason for its atheroprotective potential [124–127]. In animal studies, a recombinant ApoA1 Milano/phospholipid complex significantly reduced coronary atherosclerosis [128]. Taken together with its importance as an anti-atherogenic mutation more studies on exploring its endurance in therapeutic need to be performed in the future.

6.5. Other mutations of ApoA1

6.5.1. Tyr100His (Y100H)

Moriyama K et al. observed the ApoA1 Y100H polymorphism in a 25-year-old Japanese woman named "Karatsu" who had epigastralgia [129]. Thereafter this mutation was well known as "Karatsu". According to the observation, the substitution of histidine in place of tyrosine at the 100th position may affect the size and apolipoproteins composition of lipoprotein particles as well as metabolism and LCAT activity. Y100 residue is highly conserved and mutation at this position may affect the protein's overall functionality. The study conducted by Sviridov D et al. revealed that this mutation does not affect the ability of the mutant to bind lipids and promote cholesterol efflux. Additionally, this mutation does not affect the α -helicity of the lipid-free mutant. Besides the Y100H mutation of ApoA1 losses its ability to self-associate [105,130].

6.5.2. Glu110Lys (E110K)

Takada Y et al. were the first to report this mutation in a Japanese male at 57 years of age who was hospitalized for diabetic coma and it is well known as "Fukuoka" [131]. There were electrophoretic abnormalities detected in the biochemical studies performed on this variation. The glutamic acid and lysine both have a polar side chain the substitution at the 110 position does not alter the nature of the hydrophobic or hydrophilic face of the presumed α -helix of this mutant. Moreover, they noticed that it does not affect the LCAT activation property of the ApoA1. Since there are studies describing the consequences of this mutation, considerable research is needed to determine the impact of this mutation in the future.

6.5.3. Arg160Leu (R160L)

The R160L mutation of ApoA1 is well known as "Oslo" and was first observed by the Leren TP et al., in a Norwegian patient and his family who had 60–70 % low HDL-cholesterol along with 50–60 % of low ApoA1 when compared to healthy subjects [132]. This mutation resides in the essential domain of ApoA1 which is important for the regular function and metabolism of HDL. In a subject with this mutation, they observed that high concentration of ApoA1 in plasma compared to normal ApoA1. From their observation, they found that due to these mutations ApoA1 losses its property of binding to lipid and hence hinders the formation of HDL-cholesterol. Later various biochemical experiments were conducted to evaluate the effect of this mutation and found that this mutation does not affect the protein structure of ApoA1. Besides it causes a major inhibition of the LCAT reaction, as Arginine 160 residue has a direct role in LCAT activation [133]. Therefore many studies in the future are required for this mutation revealing its importance in causing various diseases.

6.5.4. His162Gln (H162Q)

Moriyama K et al. were the first to report this mutation in 34 years old Japanese men with palpitations. This mutation was named after this Japanese man "Kurume" [129]. It is one of the naturally occurring mutations of ApoA1 that resides in the LCAT activation region. According to a report, available H162Q inhibits the LCAT activation besides generating the same sized HDL particles as normal. This mutation does not affect the secondary structure of ApoA1. The histidine residue

in a neutral state participates in cation- π interaction that might be required for stabilizing antiparallel ApoA1 molecule and may aid in developing a favorable interaction with LCAT. When compared to histidine, glutamine has a more flexible, but less bulky side chain. Hence substitution may form hydrogen bonds elsewhere in LCAT or ApoA1 which might be the reason for the inhibition of LCAT activation. Aside from evidence indicating that this mutation lowers LCAT activation, there are also findings indicating that it preserves ABCA1-mediated cholesterol efflux [105]. Therefore further studies are required to clearly understand the nature of this mutation.

6.5.5. Pro165Arg (P165R)

A von Eckardstein et al. conducted a clinical screening of 32,000 samples from newborns, from the German population that reported P165R mutant of ApoA1 in four unrelated families. For this study, they found 12 heterozygous carriers of mutant and were associated with low levels of plasma ApoA1 and reduced levels of HDL compared to normal [134]. Later the research conducted on this mutations lipid binding and LCAT activation capabilities revealed that this mutant disturbs the lipid binding and moderately affects LCAT activation. Additionally, they proposed that Proline 165 of ApoA1 contributes to the formation of a domain that is very important for lipid binding and contributes to LCAT activation, and promotes the cholesterol efflux P165R mutant does not stabilize lipid-bound ApoA1. Because of the significance of the P165R mutation, much research is needed to completely understand its structural impact, which will contribute to developing better therapeutics.

7. Therapeutic potentials

HDL has been discovered as a therapeutic target to lower cardiovascular diseases. Recently, the focus has shifted to enhancing HDL quality by targeting the critical protein ApoA1 in a range of diseases. It was discovered that adenoviral overexpression of the human ApoA1 gene enhanced cardiac function in a mouse model of ventricular hypertrophy and heart failure. ApoA1 also guards against apoptosis in cardiomyocytes *in vivo* [135]. Treatment of mice with recombinant ApoA1 provided proof that the protein had anti-atherogenic properties [136,137]. Additionally, in the lymphatic system, ApoA1 exerts cardioprotective effects as well. It has been demonstrated that *in vivo* ApoA1 treatment can decrease blood platelet thrombotic potential while keeping the platelet activity necessary to maintain adequate lymphatic function [138]. These investigations suggest that ApoA1 based therapy may be effective in treating cardiovascular disorders, in addition to lowering atherosclerosis. Furthermore, it has been shown that ApoA1 Milano inhibits the formation of thrombi and platelet aggregation in rats [139]. ApoA1 infusion protected C57BL/6J wild-type mice from flow restriction-induced deep vein thrombosis, according to Brill et al. [140]. In order to cure or prevent deep vein thrombosis in patients, it is, therefore, possible that ApoA1 or its variant agonists may provide a cutting-edge medical strategy.

A single rHDL infusion unexpectedly increased HDL and ApoA1 levels in a small, randomized clinical study of Type 2 diabetes patients, offering the first evidence that doing so improves glycemic management. ApoA1 causes an increase in insulin secretion, according to a molecular mechanism described by Cochran et al. These findings provide insight into how therapies that increase plasma ApoA1 levels may help type 2 diabetes mellitus patients achieve improved glycemic control [141–143]. Gkouskou et al. showed the connection between lipid metabolism, colitis, and colitis-related cancer. ApoA1 defends against colitis and colitis-related carcinogenesis, according to gene profiling findings. Therapy with an ApoA1 mimicking peptide decreased the disease's phenotypic, histological, and inflammatory symptoms [144]. Furthermore, Su et al. revealed that a mouse model of ovarian cancer showed tumor development suppression [145]. All of these investigations suggested that ApoA1 could be therapeutically useful for treating a range of solid cancers. According to several researchers,

ApoA1 and HDLs lessen amyloid β plaques and inflammation in the brain's vascular system. Contrarily, chronic administration of lipid-free ApoA1 and recombinant ApoA1 Milano has been shown to lower levels of amyloid β plaques and neuroinflammation [146]. Taking into account ApoA1 as a key pharmacological target in a number of illnesses, the subsequent section of the review emphasizes some of the relevant mutations and small peptides as forthcoming treatment alternatives in a variety of diseases.

7.1. ApoA1 mutations in therapeutic

ApoA1 has several naturally occurring mutations, two of which are known for their therapeutic potential: Milano (R173C) and Paris (R151C) [127,147]. The substitution of cysteine for arginine (at 173 in Milano and 151 in Paris) leads ApoA1 to form a disulfide-linked dimer, making this ApoA1 mutant more functional than the normal ApoA1 [147]. The same characteristic features are shown by the Paris mutation of ApoA1. Additionally, the research on Milano in a rabbit model was shown to form a complex with phospholipids and brought rapid regression of a focal carotid atheroma, and protected the animals from myocardial infarction [148]. The carriers with Milano mutation have been presented to have reduced levels of LDL cholesterol and very low levels of HDL. Despite the pro-atherogenic profile of ApoA1 Milano; carriers appear to be reducing the risk of cardiovascular diseases. They're both linked to lowering the risk of vascular disease and providing a longer lifespan in their carriers. Additionally, Milano has also been noted to augment ABCA-1 mediated cholesterol efflux, anti-inflammation and presented to possess plaque stabilizing properties when compared with native ApoA1. Considering Milano's importance "Esperion therapeutics" developed an ETC-216 formulation which was recombinant ApoA1 Milano with phospholipid [128]. Moreover in phase-2 clinical trials, it was noticed that when ETC-216 is administrated to acute coronary syndrome patients showed regression of coronary atherosclerosis with serious side effects. Later "the Medicine Company" made a modification to ETC-216 formulation and named it MDCO-216 which exhibited to have addressed the side effects caused due to ETC-216. The clinical trial which was carried out on healthy human subjects found MDCO-216, to be more potent in stimulating cholesterol efflux by forming pre- β HDL particles. In contrast, MDCO-216 was reported to be less effective in regressing coronary atherosclerosis in acute coronary syndrome patients when compared to subjects undergoing statin therapy [149]. The Paris mutant (R151C) of ApoA1 has the same distinctive features, which requires further investigation.

7.2. ApoA1 based peptide therapy

Since ApoA1 is well-known for its anti-atherogenic and anti-thrombotic properties, many alternative strategies have been investigated to mimic this mechanism. The development of short, cost-effective ApoA1 mimic peptides is one such potential strategy. These peptides are structural analogs of the class A amphipathic helices and may be utilized alone or in combination with lipid to create recombinant HDL (Table 3). They have been proven in animal models to increase HDL synthesis, RCT, and atherosclerotic plaque reduction, as well as being easier to administer than full-length ApoA1. 18A (18 residues) is one of the peptides which mimic the ApoA1 and was synthesized as a class of amphipathic helix of ApoA1 but it does not share any sequence homology [141] (Fig. 7). In 18A polar face has a number of salt bridges that are 3–4 residues apart between positively and negatively charged residues, which also aids in stabilizing helix formation. Later some modifications were introduced in the 18A which gave rise to a peptide named 4F.

The 4F peptide possesses two major helices that are linked with proline and have four phenylalanine residues in its hydrophobic face. This 4F peptide is been synthesized from both D & L amino acids and is

Table 3
Details of ApoA1 mimetic peptides.

Peptide	Sequence	Residue mass (AA)	Isoelectric point (pI)	Charge	Hydrophobicity (kcal * mol ⁻¹)	Structural features	Administration	Property	Potential treatment	Clinical phase	Ref.
18A	DWLKAFYDKVAEKLKEAF	18	2200.1531	6.86	0	+ 25.96	Formation of class A amphipathic α -helix	IV	Enhanced lipoprotein binding	Cardiovascular diseases	Preclinical [141]
D-4F	Ac-DWFKAFYDKVAEKFKEAF-NH ₂	18	2268.1219	6.86	0	+ 25.04	D-amino acids form the single helical shape known as D-4 F. Is closely similar to 18 A, but has a hydrophobic face with four phenylalanine residues and two helices joined by proline.	OL	Increases hydrophobicity over 18 A	Hypercholesterolemia	Phase2 [150]
L-4F/ APL180	Ac-DWFKAFYDKVAEKFKEAF-NH ₂	18	2268.1219	6.86	0	+ 25.04	L-4 F is a single helical structure made with L-amino acids	IV, SC	Similar to D-4 F but susceptible to proteolysis	Hypercholesterolemia	Phase2 [141]
5A	DWLKAFYDKVAEKLKEAFPDWAKAAYDKAAEKAKEAA	37	4215.1611	7.07	0	+ 53.04	The second helix of this bihelical peptide has five alanine residues	IV	ABCA1 specific and less cytotoxic	Cardiovascular diseases	Phase1 [152]
ETC-642	PVLDLFRELLNELLEALKQQLK	22	2621.5471	6.82	0	+ 22.73	Single helix complexed with SPPC	IV	Activates LCAT	Atheroprotective	Phase 1 [154]
ATI5-261	EVRSKLEEWFAAFREFAAEFLARLK	26	31.87.6403	4.77	-1	+ 30.49	It is fully made up of L-amino acids with a cap of acetyl and amide groups at the N- and C-terminal	IV	Reduced atherosclerosis and increased RCT	Cardiovascular diseases	Preclinical [155]
FAMP	H-ALEHLFTLSEKAKKAEDLLKKLL-OH	24	2750.6258	9.64	+ 1	+ 29.26	This peptide's single 24-amino-acid helix has D-alanine at the c-terminus	IV	Increased cholesterol efflux	Cardiovascular diseases	- [156]

Abbreviations: IV: Intravenous; OL: Oral; SC: Subcutaneous; AA: Amino Acid.

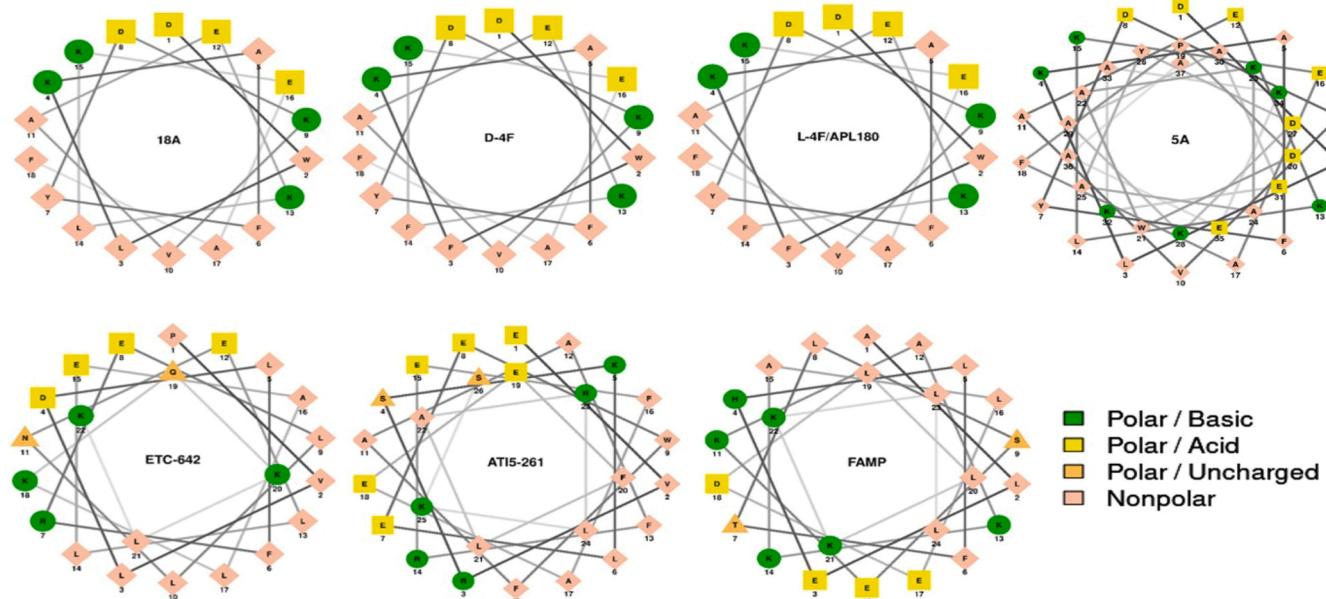


Fig. 7. Helical wheel plots of peptides that mimic ApoA1: The features of α -helices in proteins are displayed using a diagram or visual representation known as a helical wheel. The graphic displays how hydrophobic and hydrophilic residues are arranged in protein structure. The helical wheels seen above were made using the tool (<https://netwheels.herokuapp.com>). It includes 18A, D-4F, L-4F, 5A, ETC-642, ATI5-261, and FAMP, which are ApoA1 mimetic peptides. Basic residues are portrayed by the colour green, acidic residues are depicted by the colour yellow, uncharged residues by the colour orange, and non-polar residues by the colour pink.

named D-4F and L-4F respectively (Fig. 7). The D-4F peptide is also known as “Novartis APP018” and has proven potential clinical importance [150,151]. The D-4F peptide can be administrated orally in addition to that in the atherosclerotic mouse model this peptide has been effective in reducing aortic atherosclerosis. The reduction in aortic atherosclerosis by using D-4F was supplemented with the improved anti-inflammatory activity of HDL although the bioavailability of oral administration was low [141]. The phase 1 clinical trials of D-4F on patients with coronary artery diseases have shown improvement in the inflammatory index of HDL for the highest dose. Additionally, the lipid-binding efficacy of the D-4F peptide was observed to be better than the normal ApoA1 [141]. Besides L-4F and D-4F showed some limitations in oral bioavailability, as it is resistant to proteolysis in the intestine and has been found to be reducing atherosclerosis in animal models.

Later, as peptide synthesis progressed, tandem peptides were developed to better mimic the ApoA1 protein, and an example of such peptide was 5A (37 residues) (Fig. 7). This peptide followed the same functional feature as ApoA1; the cholesterol efflux is mediated through ABCA1 and the anti-inflammatory effect involved inhibited activation of NF- κ B which indicates that this peptide mimics the ApoA1 mechanism. Studies of 5A in a murine model with atherosclerosis have shown to reduce the atherosclerosis volume when complexed with phospholipid micelles. All these peptides have a greater affinity for lipids, therefore they appear to increase cholesterol efflux via a non-specific, passive pathway [152].

Fx-5A is a lipopeptide formulated from 5A peptide along with sphingomyelin. It was majorly designed to decrease macrophage cholesterol through the ABCA1 transporter, besides reducing inflammation associated with colitis, asthma, and chronic kidney disease [153]. The safety and tolerability of this peptide was assessed by National Heart, Lung, and Blood Institute (NHLBI) (NCT04216342).

Another ApoA1 mimetic peptide ETC-642 was designed to form an amphipathic helix and was found to activate the LCAT. ETC-642 is a 22 aa long peptide that forms a lookalike amphipathic helices of ApoA1 when bound with lipids (Fig. 7). The animal model study of ETC-642 has proven its atheroprotective and cardio protective effects. In addition, the in vivo and in vitro studies revealed its anti-inflammatory effect via modulating the NF κ B signaling pathway [154].

ATI-5261 is an ApoA1 mimetic peptide of length 26 aa which was derived from the c-terminal end of ApoAE (Fig. 7). In vitro studies carried out have demonstrated that it helps in promoting cellular cholesterol efflux through ABCA1 which is the similar activity of ApoA1. The study carried out by Liao et al. on the preeclampsia mouse model (COMT $^{-/-}$) presented ATI-5261 treatment reversed arterial stiffness associated with preeclampsia [155].

Fukuoka University ApoA1 mimetic peptide (FAMP) is one of the recent novel human ApoA1 mimetic peptides which is of 24 mer and it does not complex with phospholipids (Fig. 7). It has been found to significantly enhance the function of HDL and reduce the formation of aortic plaques by ~ 48 % in mice with a heavy fat diet. FAMP interacts specifically with ABCA1 without the involvement of the passive efflux pathway which is the same mechanism of ApoA1 protein. Hence the HDL-targeted therapies using FAMP have proven to have atheroprotective potential and represent a new therapeutic against cardiovascular disease [156]. Recently FAMP was modified to i-FAMP-D1, to increase its plasma half-life. The major difference between these two peptides was like in i-FAMP-D1, there was an addition of D-alanine to its C-terminal end which increased its plasma half-life.

There are some ApoA1 mimetic peptides that are being evaluated against idiopathic pulmonary fibrosis (NCT02315586) [157]. The estimated date for completion of the study is been described as around December 2029. The therapies based on recombinant HDL hold a promising therapeutic option for atherosclerosis. However, owing to the high costs involved in the production of therapeutic ApoA1, small peptides that mimic ApoA1 function may serve as a viable option. Therefore more research into this area is needed in the future, which also utilizes the knowledge of the important ApoA1 polymorphisms.

8. Concluding remarks

ApoA1, HDL’s signature apoprotein, has been a keen topic of interest as ApoA1 helps in the stabilization of the vulnerable plaques by the removal of cholesterol and reduction of lesion causing lipids from the atherosclerotic plaques. This contributes enormously towards the lowering of the risk of cardiac events. Numerous investigations on ApoA1 have focused predominantly on its ability to reduce

atherosclerosis and eventually cardiovascular diseases. Considering the importance of ApoA1, researchers are increasingly interested in understanding more about its 3D structure and its tendency to form oligomeric structures. Several mutations in this protein have been reported and found to be either linked with various diseases or reported to impart health benefits. The N-terminal regions of ApoA1 play a critical role in the overall function of protein alongside a majority of the mutations that are reported in this region. As a result, more research is needed focusing on the ApoA1 N-terminal mutations and their impact on the structure and function, which could aid in the development of effective therapeutic strategies in the future. Using the current understanding of ApoA1's beneficial effects, several synthetic peptides that replicate ApoA1's beneficial function have been produced and proven to significantly improve therapy choices in a range of disorders. However, there aren't enough experiments done on ApoA1 mimetic peptides in clinical settings, therefore further studies are required in this domain. The Milano (R173C) and Paris (R151C) mutations have gained a lot of attention because of their athero-protective properties. Although, experimental evidences of the Zaragoza (L144R) mutation revealed its athero-protective nature in mutant carriers with healthy cardiovascular systems, the structure-function, mechanistic basis of protection and clinical relevance need to be investigated further before developing it as a therapeutic molecule. Thus this review points out that there are very promising therapeutic candidates derived from ApoA1 (either the beneficial mutants or the synthetic peptides) in treating diseases like, cardiovascular disease, atherosclerosis, amyloidosis, and other ailments which afflict the human population.

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Aishwarya Sudam (Lead author). Carried out the review literature, wrote the manuscript, made figures and tables, Krishnan Venkataraman (Corresponding author) Carried out the review of literature, conceived the ideas, written and edited the review. Both the authors have read the manuscript and accepted it to be submitted to "Biomedicine & Pharmacotherapy".

Aishwarya Sudam Bhale: Review of literature, conception of ideas, writing and editing. **Krishnan Venkataraman:** Review of literature, conception of ideas, writing and editing.

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