PORTLAND PRESS

Review Article

Lipoprotein(a) and cardiovascular disease

Michael B. Boffa[1,2] and Marlys L. Koschinsky[2,3]

[1]Department of Biochemistry, University of Western Ontario, London, Ontario, Canada; [2]Robarts Research Institute, University of Western Ontario, London, Ontario, Canada; [3]Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

Correspondence: Michael B. Boffa (mboffa@uwo.ca)

OPEN ACCESS

Elevated plasma levels of lipoprotein(a) (Lp(a)) are a prevalent, independent, and causal risk factor for atherosclerotic cardiovascular disease and calcific aortic valve disease. Lp(a) consists of a lipoprotein particle resembling low density lipoprotein and the covalently-attached glycoprotein apolipoprotein(a) (apo(a)). Novel therapeutics that specifically and potently lower Lp(a) levels are currently in advanced stages of clinical development, including in large, phase 3 cardiovascular outcomes trials. However, fundamental unanswered questions remain concerning some key aspects of Lp(a) biosynthesis and catabolism as well as the true pathogenic mechanisms of the particle. In this review, we describe the salient biochemical features of Lp(a) and apo(a) and how they underlie the disease-causing potential of Lp(a), the factors that determine plasma Lp(a) concentrations, and the mechanism of action of Lp(a)-lowering drugs.

Introduction

Lipoprotein(a) (Lp(a)) was first reported in 1963 as an antigenic variant of low-density lipoprotein (LDL), and was detectable in plasma from approximately one third of a Northern European population [1]. While numerous case-control studies dating back to the 1970s identified elevated Lp(a) (>30 mg/dl; >75 nmol/L) in coronary heart disease (CHD) patients, the contribution of Lp(a) to cardiovascular disease (CVD) risk remained unclear until many decades later. In three landmark studies published in 2009, Lp(a) was shown to be an independent and casual risk factor for atherosclerotic cardiovascular disease (ASCVD) [2–4], and in 2013, the importance of Lp(a) as a potent risk factor for the initiation and progression of aortic valve stenosis was reported [5].

Lp(a) contains an apoB-100-associated lipid component that is similar (but not identical) to LDL [6,7]. Importantly, a hallmark feature of Lp(a) is the presence of apolipoprotein(a) (apo(a)), which is unique amongst apolipoproteins both in its structure and its biochemical properties. Characterization of a human liver-derived apo(a) cDNA in 1987 revealed that apo(a) is very similar to the fibrinolytic proenzyme plasminogen [8] (Figure 1). In this regard, apo(a) contains numerous kringle domains that closely resemble several of those found in plasminogen. Kringle domains are structural motifs primarily found in proteins involved in the coagulation and fibrinolytic pathways. Each kringle domain contains three invariant disulfide bonds. Kringles are not present in any other apolipoproteins, and evidence continues to accrue that the kringles in apo(a) impart the majority of pathophysiological properties that are associated with Lp(a). The unexpected finding of a high level of sequence similarity between apo(a) and plasminogen led to speculation that apo(a) may interfere with normal functions of plasminogen in the breakdown of fibrin clots. Therefore, it was hypothesized that Lp(a) could provide a unique link between atherosclerosis (due to the apoB-containing lipid component of Lp(a)) and thrombosis (attributable to the apo(a) component of Lp(a)). This potential duality of function has sparked many studies and debates on the role of Lp(a) in cardiovascular pathology.

Plasma levels of Lp(a) vary widely in the global population, ranging from <1 mg/dl (2.5 nmol/L) to >250 mg/dl (625 nmol/l). Cardiovascular risk thresholds have been defined as >30–50 mg/dl, (75–125 nmol/l) with over 20% of the global population having levels of Lp(a) in excess of 50 mg/dl (125 nmol/l) [9]. Unlike LDL, a large component (more than 90%) of Lp(a) levels is genetically determined, and plasma Lp(a) levels reflect a co-dominant inheritance pattern [10]. The strong genetic
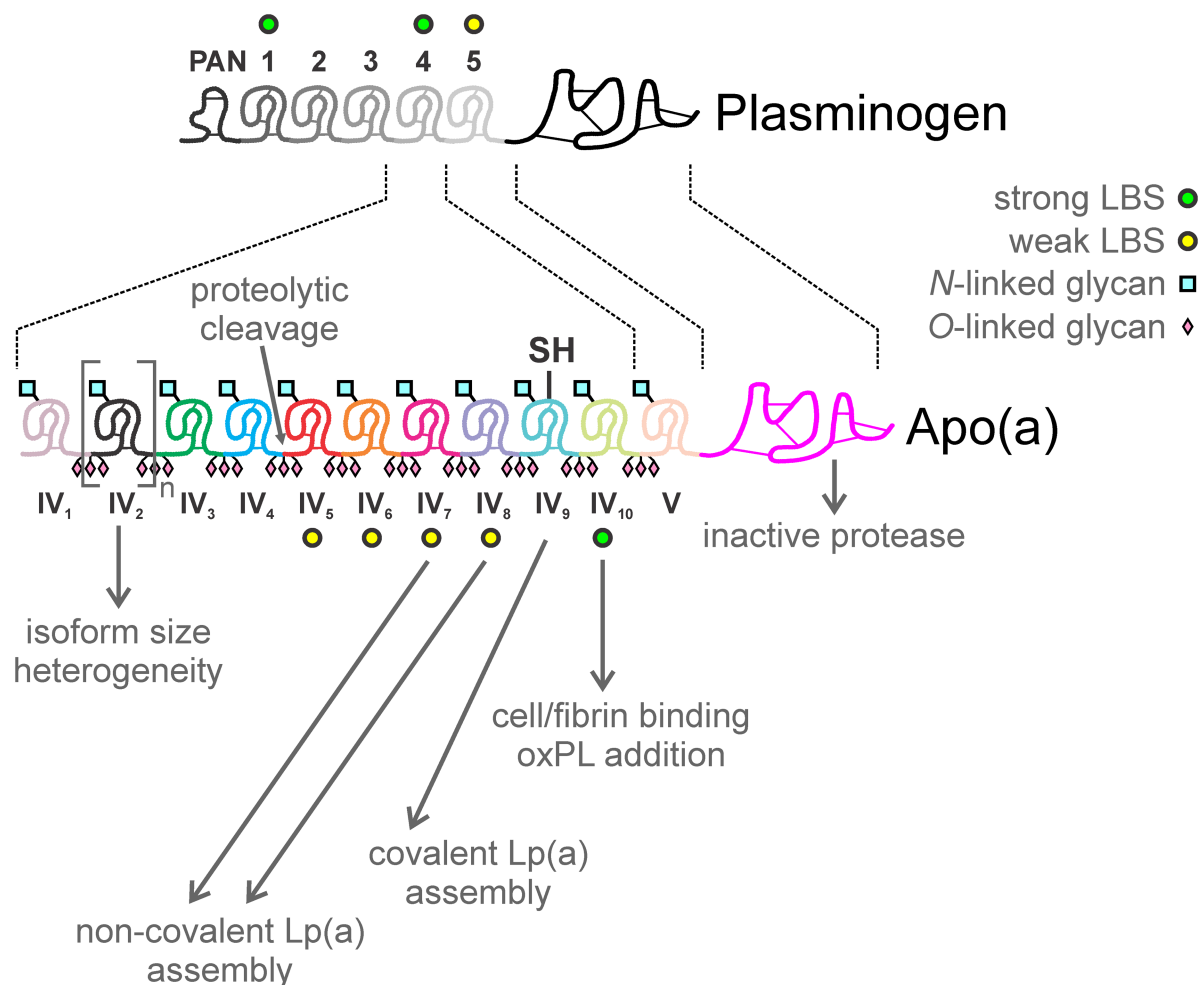
Figure 1. Kringle organization and functional domains of apo(a).

Apo(a) consists of multiply-repeated domains homologous to plasminogen kringle 4, followed by a single kringle 5-like domain and an inactive protease-like domain. Kringle UIV type 2 in apo(a) (KIV2) is present in between 3 and over 40 copies and underlies Lp(a) isoform heterogeneity in the population. Some apo(a) KIV types contains weak or strong lysine binding sites (LBS) that play roles in Lp(a) assembly, binding to Lp(a) to substrates, and addition of oxidized phospholipid (oxPL). Apo(a) KIV9 contains the single unpaired cysteine that mediates disulfide bonding to apoB100 in the Lp(a) particle. Also indicated is the presence of *N*- and *O*-linked glycosylation in each kringle and in the interkringle regions, respectively, and the site of proteolytic cleavage by elastase or MMP-12.

determination of Lp(a) levels cannot be attributed solely to *LPA* isoform size variability. Other genetic variants in *LPA* have been identified — including nonsense mutations and splice site mutations — that help explain vast differences in Lp(a) levels in individuals with identical isoform sizes [11]. Non-genetic factors (diet and hormones, for example) can also affect Lp(a) levels although the contributions are relatively modest [12,13].

Structure-function relationships in apo(a)
Structure of Lp(a) and apo(a)

Lp(a) is a spherical particle of ∼250 angstroms in diameter that floats in the higher density range of LDL particles [14]. It has been shown that apo(a) and apoB-100 exist in a 1:1 molar ratio in the Lp(a) particle [15]. The apo(a) component of Lp(a) is heavily glycosylated (∼28% carbohydrate by weight) with a high content of sialic acid [6].

The hallmark of the apo(a) protein is the presence of multiply-repeated kringle IV (KIV) domains that are very similar to plasminogen kringle 4 (75–85% sequence identity); these are followed by a single copy of sequences that closely resemble the plasminogen kringle 5 (KV) and protease domains of plasminogen (91 and 84% sequence identity, respectively) (Figure 1) [8]. Kringles are structural motifs containing three invariant disulfide bonds forming a compact tri-loop structure generally lacking any discernable regular secondary structure [16]. Typically, kringles are present in proteins involved in the fibrinolytic (e.g. tissue-type plasminogen activator (tPA) and plasminogen) and coagulation (e.g. prothrombin) pathways. Many kringles are involved in ligand interaction through a binding cleft characterized by an anionic center and a cationic center separated by a hydrophobic trough. Several of the five kringle domains in plasminogen contain specificity for lysine binding; the strength of the interaction is K1 > K4 > K5. In apo(a), the KV and several KIV domains each contain binding clefts which differ in their specificity for lysine and other amino acids compared with plasminogen [17–21].

Early studies demonstrated heterogeneity in apo(a) sizes from Lp(a) isolated from different individuals; it was observed that plasma Lp(a) isolates varied in density, with smaller-sized apo(a) species corresponding to less dense Lp(a) particles. In 1992 the Koschinsky group reported the core kringle organization of apo(a) using a PCR-based approach [22]. In this hallmark study, it was determined that apo(a) contains 10 kringle invariant plasminogen KIV-like domains (KIV1–KIV10) that are highly similar to each other but contain some key differences in amino acid sequence; the KIV domains are followed by a single copy of each of the KV and protease-like domains. The apo(a) KIV2 domain is present in different numbers of identically-repeated copies in human Lp(a), from 3 to >30 [23]. This corresponds to apo(a) isoform sizes ranging from <200 to >800 kDa that represent copy-number variation of the KIV2-encoding sequence in the *LPA* gene. This forms the molecular basis of plasma Lp(a) isoform size heterogeneity which is a hallmark of Lp(a).

The protease-like domain of apo(a) was shown to lack proteolytic activity despite the retention of the canonical catalytic triad residues found in serine proteases [24]. Modeling of the protease domain showed that an 8 amino acid deletion in the protease-like domain of apo(a) causes collapse of the active site, with a resulting lack of enzymatic activity. Additionally, the tPA cleavage site in plasminogen (arginine) that is required for plasminogen activation to plasmin is replaced by a serine residue in apo(a). Studies have shown that apo(a) contains no inherent proteolytic activity which is reflective of these differences [24]. The lack of catalytic activity in apo(a) analogous to that of plasmin(ogen) has led to speculation that apo(a) can compete with the proteolytic function of plasmin in cleaving fibrinogen to fibrin. This, in turn, has led to speculation that Lp(a) possess antifibrinolytic properties, which contribute to its pathogenic effects in the vasculature.

Lysine-binding properties of apo(a) kringles

Extensive structure/function analyses have been carried out on the kringle domains of apo(a) through the use of recombinant protein engineering and expression of apo(a). Expression of a 17-kringle containing isoform of apo(a) was reported in 1991 [25]. Following that, many recombinant apo(a) proteins have been generated to study the roles of specific kringle domains. We know, for example, that apo(a) KIV types 5–8 (KIV5–8) each contain a weak lysine-binding site (LBS), several of which are important in the biogenesis of the Lp(a) particle (Figures 1 and 2; see section 'Assembly of Lp(a) particles', below) and that the KIV type 10 (KIV10) domain in apo(a) has a LBS of comparable affinity to that in plasminogen KIV [27]. It was also reported that the single covalent bond that links apo(a) to apoB-100 in the Lp(a) particle is located in apo(a) KIV type 9 (KIV9) [28]; this is the only unpaired cysteine residue present in the apo(a) molecule. The identity of the corresponding cysteine in human apoB-100 is Cys4326 which is located in the C-terminal region of the molecule [29].

The crystal structures of apo(a) KIV2, KIV7 and KIV10, as well as KV have been solved [17,19–21]. While the overall structure of full-length apo(a) has not been reported, a model of the structure of three KIV domains (KIV types 8, 9 and 10) has been published [30]. Interestingly, the trigonal arrangement of these three kringles in the resulting model resembles the asymmetric unit of three KIV_2 molecules observed in the crystal structure of KIV2 [21].

The structure of the LBS in kringle domains generally consists of a hydrophobic trough on the surface of the kringle, with clusters of anionic and cationic residues present on either end. The apo(a) KIV_{10} LBS consists of seven essential residues comprising the hydrophobic trough (Trp^{60}, Phe^{62} and Trp^{70}; numbering is relative to the first Cys in the kringle), the cationic center (Asp^{54} and Asp^{56}) and the anionic center (Arg^{35} and Arg^{69}). Other apo(a) KIV types contain substitutions in one or more of these residues that weaken or eliminate lysine binding. These include substitutions at position 56 which is cationic (Asp or Glu) in the lysine binding
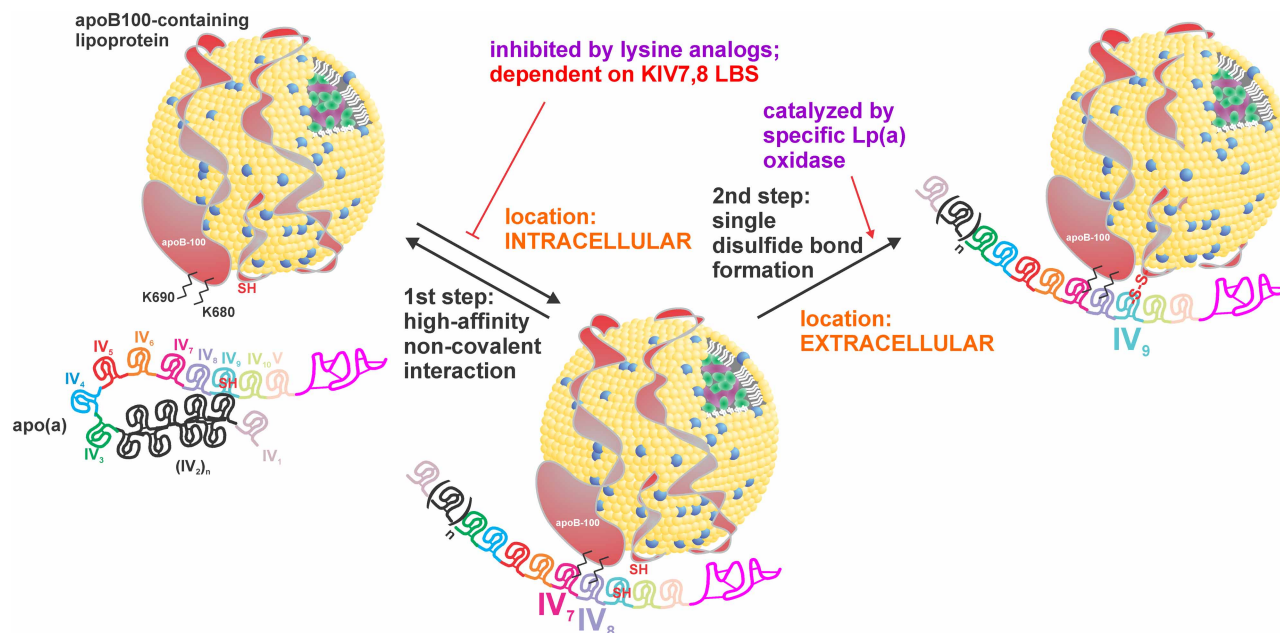
Figure 2. Assembly of Lp(a).
Within the hepatocyte, apo(a) KIV7 and 8 form an initial non-covalent interaction with specific lysine residues in the amino-terminal domain of apoB100. Covalent bond formation occurs extracellularly and is catalyzed by a specific oxidase enzyme. Also depicted is the ligand-induced conformation change in apo(a) that accelerates covalent Lp(a) assembly [26].

kringles, but neutral (Val, Gly) in the others. The X-ray crystal structure of KIV_7 revealed a smaller LBS compared with KIV_{10}, resulting from mutations of the critical residues Asp^{56} and Tyr^{62} in the LBS to Glu and Phe, respectively [17]. The Asp^{56} à Glu substitution is also present in KIV_{5-8} each of which bind lysine analogues more weakly compared with apo(a) KIV_{10} or plasminogen kringle 4. These differences in affinity to lysine may underlie (or at least be compatible with) the distinct functional roles of the weak and strong LBS in apo(a) in Lp(a) assembly and binding to lysine-containing substrates, respectively (see below) [31].

The kringle V domain in apo(a) is very similar (91% amino acid sequence identity) to the corresponding domain in plasminogen and has comparable weak lysine binding properties. It is interesting to note that *LPA* in many Old World monkeys lacks the KV domain in the apo(a) protein. There may be implications for apo(a) structure and function based on whether or not this domain is present. For example, it has been reported that in baboon apo(a), which lacks the KV domain, the LBS in KIV10 is non-functional despite the presence of the residues identified as essential for the binding of lysine [32].

Post-translational modifications of apo(a)
Glycosylation
Analysis of the structures of the *N*- and *O*-linked glycans in apo(a) has been performed using exoglycosidase digestion and mass spectroscopy analysis [33]. it was reported that two major *N*-glycans account for ∼17% of the oligosaccharide structures. These *N*-glyans are complex biantennary structures that exist in either a mono-or disialylated state. The *O*-linked glycans, representing the bulk of the carbohydrate modification in apo(a), were reported to be primarily monosialylated core type I structures (NeuNAcα2-3Galβ1-3GalNAc), with smaller amounts of disialylated and non-sialylated structures also present.

The KIV domains in apo(a) are separated by interkringle sequences that are between 30 and 36 amino acids in length. Interestingly, the plasminogen interkringle sequences are significantly shorter. Each of the interkringle regions in apo(a) contains six sites for *O*-linked glycan attachment, with at least one *N*-linked glycosylation site present in each KIV domain [8]. It is interesting to note that apo(a) is very highly glycosylated compared with plasminogen: the latter contains only one *N*-linked and one *O*-linked glycosylation site. The significance of the extensive glycosylation of apo(a) remains unclear.

Proteolysis

Proteolytic fragments of apo(a) have been identified in human plasma and urine corresponding to the N-terminal region of apo(a) (KIV_{1-4}); these fragments are not attached to apoB-100 [34]. Several studies have reported the sensitivity of plasma Lp(a) to elastase cleavage: the cleavage site has been identified as the Ile-Leu bond in the interkringle sequence between apo(a) KIV_4 and KIV_5 [35]; there is also a macrophage metalloelastase (MMP-12) cleavage site (Asn-Val bond) immediately upstream of the elastase cleavage site [36]. The origin and significance of the fragments of apo(a) remain unclear: it is possible that they arise in the vessel wall at the site of atherosclerotic lesions where Lp(a) accumulates, and can diffuse back into the plasma compartment. Interestingly, the interkringle region between apo(a) KIV_4 and KIV_5 is deficient in O-linked glycans due to amino acid substitutions in serine and/or threonine residues; this renders the sequence more susceptible to proteolytic cleavage compared other interkringle sequences in apo(a). Although the significance of the proteolytic cleavage remains unknown, is tempting to speculate that the role of the extensive O-linked glycosylation modification in the interkringle regions serve to protect apo(a) from proteolytic degradation.

Oxidized phospholipid addition to Lp(a) and apo(a)

Studies have firmly established that Lp(a) is the preferential carrier of oxidized phospholipids in human circulation [37]. The majority (∼85%) of oxPL in plasma is carried by Lp(a), and of this, 50% is associated with the apo(a) component of the particle [37]. These studies used E06, a murine immunodominant IgM antibody isolated from apoE knockout mice, that binds to the phosphocholine head group of oxidized (but not native) oxPL derived from phosphatidylcholine (PC) [38]. E06 recognizes oxPL on an equimolar basis when present either as a PC salt, or as PC on oxPL such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC), or attached to a variety of different peptides regardless of amino acid sequence [39]. These oxidized species are highly proinflammatory, and have been linked to early events in atherogenesis through their ability to promote inflammatory cascades in the vessel wall [40]. OxPL are associated with plaque vulnerability and destabilization, and can accumulate at high levels in lesions relative to their plasma concentrations.

The covalent addition of oxPL to proteins can occur through Schiff base formation with the ε-amino group of lysine residues, or by Michael addition to cysteine, histidine, or arginine residues [41]. Studies using a wide variety of r-apo(a) variants pinpointed a role for KIV10 and, specifically, the strong LBS (sLBS) in this kringle in mediating E06-reactive covalent oxPL addition (Figure 1) [42]. In transgenic mice expressing a truncated form of human apo(a) and human apoB-100 (i.e. Lp(a)-transgenic mice), oxPL was present on Lp(a) containing wild-type KIV10, but not the KIV10 sLBS mutant [42]. A role for the KIV10 sLBS in oxPL modification of apo(a) is also implied by the fact that non-human primates lack a functional KIV10 LBS as well as E06-reactive oxPL on their Lp(a). However, whether the sLBS plays a direct or indirect role in oxPL addition to apo(a) is unclear. Although the E06-detectable oxPL corresponds to oxidized phosphatidylcholine, the identity of the presumably oxidized sn-2 fatty acyl chain and the nature of its covalent linkage to apo(a) remain to be determined. Additionally, whether the OxPL modification of apo(a) occurs intra-or extracellularly is not known. Finally, little is known about the nature of the oxPL that are not covalently associated with apo(a), and are therefore extractable from Lp(a) using organic solvents [37]. Moreover, a recent lipidomic study showed that Lp(a) is enriched in (non-oxidized) diacylglycerols and lysophosphatidic acid, and that these species are able to evoke pro-inflammatory responses in monocytes [43].

Comparative evolution of the gene encoding apo(a)

The *LPA* gene, located on the long arm of chromosome (6q21) very likely arose from a gene duplication of the *PLG* gene: the two genes are in a head-to-head arrangement on the distal end of the long arm of chromosome 6 (6q25.3-q26). Interestingly, the *LPA* gene is absent in all species except for Old World monkeys, great apes and humans. This suggests that the gene duplication event giving rise to human *LPA* occurred <6 million years ago, subsequent to the divergence of Old and New World monkeys [44]. Like humans, the Old World monkeys and apes all lack functional protease domains owing to amino acid substitutions that inactivate this domain in a variety of ways in different species [32,45,46]. Additionally, none of the Lp(a) species in Old World Monkeys and apes contain a functional LBS in their respective KIV10 domains: this results from a combination of amino acid substitutions in the strong LBS and/or the lack of a sequence encoding apo(a) kringle V [32]. All Old World monkey and ape species exhibit apo(a) isoform size variability arising from different numbers of identically repeated copies of the apo(a) KIV2 domain as seen in human Lp(a).

A DNA sequence was cloned from hedgehogs (primitive insectivores) that contains multiple repeats of a sequence that is similar to plasminogen kringle 3, but lacks sequences corresponding to the plasminogen K4, K5 and protease-like domains of apo(a) [47]. Although this also likely arose from a gene duplication of the K3 encoding sequence in *PLG*, the similarity to human apo(a), apart from the presence of a multiply-repeated plasminogen kringle domain, is relatively low. Interestingly, however, there is a free cysteine in hedgehog apo(a) that allows it to form a covalent bond with apoB-100 in the context of hedgehog plasma Lp(a) particles [47]. Apart from that found in hedgehogs, Lp(a)-like species do not appear in evolution prior to the second plasminogen gene duplication event that gave rise to Lp(a) in monkeys, apes and humans. This suggests that the *LPA* gene was lost from the genome early in mammalian evolution.

The biosynthesis and catabolism of Lp(a)

It was reported in the 1990s that levels of Lp(a) are determined primarily at the level of production rather by the rate of removal from the circulation. These studies were performed in humans using injections of radiolabelled Lp(a) [48,49]. However, there are many steps that comprise production of Lp(a) at which regulation may occur. These include evidence for transcriptional regulation, regulation of secretion, and the process of Lp(a) particle summary, which are summarized below (Figure 3). Understudied areas include possible regulation of *LPA* transcript stability and translation efficiency. With respect to the role of catabolism in determining plasma Lp(a), changes in Lp(a) removal from circulation may be altered in response to disease states including renal disease.

Transcriptional regulation of *LPA*

The *LPA* gene, although expressed constitutively, is responsive to transcriptional regulation, with regulatory elements located throughout the intergenic region between *PLG* and *LPA*. Regulation of *LPA* expression by
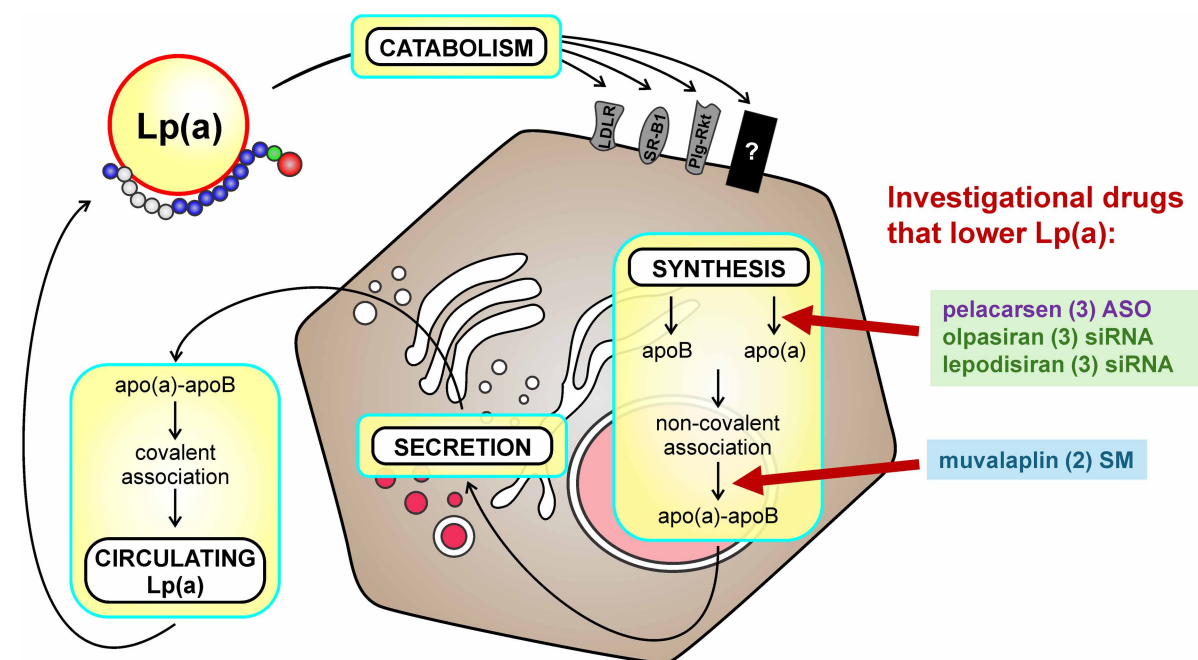

Figure 3. Biosynthesis and catabolism of Lp(a).
Novel therapeutics that specifically lower Lp(a) act by destabilizing the *LPA* mRNA that encodes apo(a) or by blocking the initial non-covalent interaction between apo(a) and apoB. After secretion of the non-covalent apo(a)-apoB complex, covalent Lp(a) is formed, and the circulating particle is taken up by the liver by one or more receptors. Candidates for which there is the most evidence are LDL receptor (LDLR), scavenger receptor-B1 (SR-B1) and the plasminogen receptor Plg-R_{KT}. The current phase of clinical trial development is listed n parentheses after the drug name. ASO, antisense oligonucleotide; siRNA: small interferening RNA; SM, small molecule.

estrogen through estrogen receptor binding to a negative enhancer estrogen response element in the *LPA* promoter has been reported [50]. Estrogen therefore decreases *LPA* expression which is reflected in the increase in Lp(a) (up to 27%) that occurs post-menopause [51]. Consistent with this, Lp(a) levels also decrease in response to hormone replacement therapy [52–54]. The FXR (Farnesoid X receptor) ligand binds to a negative control element in the *LPA* promoter and decreases *LPA* expression [55]. Additionally, Fibroblast growth Factor-19 (FGF-19) binding to hepatocytes results in ELK-1 translocation to the nucleus that in turn decreases *LPA* expression [56]. However, neither of these agents have been shown to decrease Lp(a) levels in humans in a clinical context. Activators of *LPA* expression have also been identified: both NHF1A and IL-6 induce *LPA* transcription [57], and an IL-6 receptor blocker (tocilizumab) lowers *LPA* in response to IL-6 [58].

Interestingly, *LPA* can be up-regulated by proinflammatory mediators such as IL-6 as mentioned above, and can also itself serve to increase the expression of inflammatory factors such as IL-8 [59]. This has led to speculation that elevated Lp(a) levels may pose a major risk to patients with COVID-19 in response to the ensuing 'cytokine storm' associated with this virus [60].

Secretion of apo(a) from hepatoctyes

Plasma Lp(a) contains apo(a) and apoB-100, both of which are produced in hepatocytes. Given the lack of detectable apo(a) in both primary and cultured hepatocytes, much of our understanding of apo(a) synthesis and secretion has been obtained using either immortalized cells lines or primary hepatocytes derived from baboon liver.

The baboon liver hepatocyte model has provided critical information regarding the posttranslational control of apo(a) secretion [61]. Apo(a) is synthesized as a precursor containing high-mannose *N*-linked glycans that have a prolonged residence time in the ER prior to maturation and secretion. Maturation occurs in the Golgi and involves modification of *N*-linked glycans and the addition of *O*-linked carbohydrate side chains including sialic acid [62].

White et al. [63,64] showed that the strong inverse correlation between plasma Lp(a) levels and apo(a) isoform sizes could be explained in large part by the increased ER retention time for larger apo(a) species. This reflects greater complexity in folding requirements of larger isoform sizes, resulting in increased degradation through the ERAD pathway in which misfolded species are translocated across the ER membrane followed by ubiquitination. Whether or not other processes regulate apo(a) presecretory degradation remains unclear since it has been reported that only a portion of apo(a) is targeted for proteasomal degradation [65].

Using the same model, the White group showed that apo(a) folding begins co-translationally during import into the ER lumen. The nascent protein progresses through a series of intermediately-folded species during which multiple chaperones such as PDI, calnexin and calreticulin play key roles in ensuring that protein folding is complete [65,66]. It is important to note that *N*-linked glycan addition and trimming in the ER is required for apo(a) secretion, and mediates critical interactions with calnexin and calreticulin that ensure proper folding of nascent apo(a) prior to its export from the ER. Notably, White et al. [63] showed that all apo(a) isoforms folded at the same rate regardless of their size. The size-sensitive process controlling the rate of ER exit remains unknown, but is dependent on the trimming of *N*-linked glucose.

Assembly of Lp(a) particles

It has been firmly established that Lp(a) particle assembly proceeds as a two-step process: the first step involves non-covalent interactions that occur between apo(a) and apoB-100 prior to the second step of assembly that involves the formation of a single disulfide bond between apo(a) and apoB-100 (Figure 2) [31]. The non-covalent step of Lp(a) assembly involves lysine-dependent interactions between weak LBSs present in each of apo(a) KIV types 7 and 8, with lysine residues present in the amino-terminal globular domain of apoB-100 [67]. As such, Lp(a) assembly can be effectively inhibited by lysine analogues *in vitro* through interference with the first step of the process. The second step of Lp(a) assembly involves formation of a covalent bond between the free cysteine in apo(a) KIV9 and the unpaired cysteine at amino acid position 4326 in human apoB-100.

An active field of Lp(a) assembly has involved determination of whether the process occurs intracellularly or extracellularly. A number of investigators have reported the inability to detect intracellular covalent Lp(a) particles using cultured hepatocyte cell models and human liver tissue [28,62,68]. White et al. [62,68] work in cultured baboon hepatocytes suggested that assembly of Lp(a) occurs extracellularly, on the hepatocyte cell surface. Becker et al. [69] showed that an enzyme with oxidase activity present in cultured hepatocyte cell medium is responsible for extracellular covalent bond formation between apo(a) and apoB-100. They also

demonstrated that covalent bond formation requires opening of the apo(a) structure using a lysine analogue [26]. This presents an intriguing possibility where secreted apo(a) may bind to a receptor(s) on the cell membrane in a lysine dependent manner, thereby opening the apo(a) structure to allow covalent Lp(a) formation. *In vivo* human kinetics models have provided evidence to support all possible scenarios for intracellular assembly, extracellular assembly and components of the process occurring in both locations, as well as use of apo(a) or apoB recycled from internalized Lp(a) [70–76]. Intriguingly, however, it has been reported using this approach that there exists a separate pool of apoB-100 intracellularly that binds to apo(a) to form Lp(a) particles, and that the rate of production of Lp(a)-apoB is different than that of either VLDL-apoB or LDL-apoB [70]. Our own evidence from a cultured hepatocyte model is consistent with this concept, where we found that apo(a) and apoB interact non-covalently within the cell, and that this interaction dictates the secretion rate of apo(a) [77].

Removal of Lp(a) from the circulation
Role of the liver in Lp(a) catabolism
Although steps in the production of Lp(a) are the major determinant of Lp(a) levels, a role for catabolism has been identified using *in vivo* human kinetics in which plasma Lp(a) levels are negatively correlated with the fractional catabolic rates (FCRs) of both apo(a) and apoB-100 components of Lp(a) [72]. In humans, the FCR of Lp(a) is ∼40% higher for LDL than for Lp(a) [78]. The liver is the primary site of Lp(a) catabolism, with apo(a) playing the major role in mediating Lp(a) particle uptake [79]. A number of receptors have been suggested to play a role in Lp(a) catabolism including the LDL receptor (LDLR; apoB/E receptor) and related family members including LRP-1 (LDLR-related protein-1) and the VLDL receptor, as well as SR-B1 (Scavenger Receptor Type B Class 1), megalin/gp330, the asialoglycoprotein receptor, and plasminogen receptors (Figure 3) [80,81].

The role of the LDLR in Lp(a) catabolism has been highly debated, with evidence both for, and against a role for this receptor [80]. *In vitro* data using different cell types have been relatively consistent in identifying high affinity binding and uptake of Lp(a) through the LDLR [82–85], although kindred studies from FH patients have reported conflicting data with respect to whether or not Lp(a) levels are elevated in patients with LDLR mutations [81]. Nonetheless, it had generally been accepted that Lp(a) is not an effective ligand for the LDLR in humans given that statins do not lower Lp(a) but may, in fact, slightly increase it [86]. It therefore came as a surprise when therapeutic antibody inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9) lowered Lp(a) by 15–30% [87,88]. Romagnuolo et al. [83] subsequently showed that PCSK9 could reduce Lp(a) internalization by LDLR using cultured hepatoma cells and function blocking monoclonal antibodies against the LDLR; similar results were found using fibroblasts from FH patients versus healthy control and using primary liver hepatocytes from mice [83,84]. We also found that Lp(a) clearance was accelerated in PCSK9 knockout mice and delayed in LDLR knockout mice, with double-knockout animals having the same Lp(a) clearance rate as LDLR knockout mice (i.e. the defect in clearance could not be rescued by deletion of PCSK9) [89]. *In vivo* human kinetic studies were also performed to probe the basis for Lp(a)-lowering by PCSK9 inhibitors. Interesting, effects on Lp(a) production rate and/or FCR were observed, in some cases depending on whether patients were also receiving a statin [90–92]. It possible that Lp(a) may function as a ligand for the LDLR under specific conditions where Lp(a) levels are significantly elevated, LDL levels are low, and LDLR is substantially up-regulated by statins and PCSK9 inhibitors. This may explain results from catabolic studies using injected radiolabeled Lp(a) in mice overexpressing the LDLR; in these animals, Lp(a) clearance rates were dramatically increased, suggesting a role for the LDLR in Lp(a) uptake [93]. Importantly, however, mice also have very low levels of competing LDL, thereby favoring Lp(a) uptake through the LDLR. Nonetheless, the balance of the evidence generated to date is consistent with the idea that the LDLR is not the major clearance receptor for Lp(a) in liver.

The LRP (LDL related receptor) is a hepatic receptor in the LDL receptor (LDLR) family that mediates the hepatic uptake of chylomicrons and apoE-containing VLDL remnants. *In vitro* data suggest that LRP specifically may play a role in catabolism of high molecular mass Lp(a) isoforms [94]. However, this has not been confirmed in other studies [95,96]. It has also been reported that SR-BI can participate in Lp(a) catabolism: as is the case for both HDL and LDL, the SR-B1 receptor is involved in selective uptake of cholesteryl esters from the Lp(a) particle [97,98]. Additionally, it has been shown that the apo(a) and apoB-100 components of Lp(a) are can also internalized by this receptor [97]. Although the SR-B1 expression in liver is modest compared

with adrenal glands and placenta, Remaley and colleagues have reported genetic variants of *SCARB1* that are associated with both elevated Lp(a) and HDL levels [98]. These include exonic variants with diminished SR-B1 activity that are associated with reduced Lp(a) binding and uptake.

The asialoglycoprotein receptor, present in high levels on the liver, is responsible for desialylated protein clearance. However, despite the extensive sialic acid modification of apo(a), there is no experimental evidence to suggest a role for this receptor in Lp(a) catabolism [79]. The megalin/gp330 receptor has been shown to function as a receptor for Lp(a) through the apoB100 moiety [99], but is not found in liver. However, this receptor is present in proximal tubules of the kidney which has been proposed as a site for Lp(a) catabolism [100].

Tam et al. [82] also identified a low-affinity, high-capacity receptor on hepatocytes which they suggested could correspond to members of the plasminogen receptor family. A role for the plasminogen receptor PLG-R$_{KT}$ in Lp(a) catabolism has also been reported [101]. This receptor appears to participate in binding and internalization of Lp(a) based on *in vitro* studies in established hepatocyte cell lines. Although interesting, it is not clear what role this receptor plays *in vivo*, and whether other members of the plasminogen receptor family are also involved in Lp(a) catabolism. The PLG-R$_{KT}$ receptor is expressed in many different tissues including brain, vasculature, and epithelial tissues such as lung and kidney and has a number of functions ascribed to it; the functions are predominantly centered on the role of this receptor in plasminogen activation to plasmin [97]. The potential ability for Lp(a) to compete with plasminogen to bind to this receptor clearly requires further study.

Most recently, a role for PLG-R$_{KT}$, in addition to two other plasminogen receptors on liver cells (annexin A2, and S100A10 (S100 calcium-binding protein A10)) have been reported to facilitate the uptake of Lp(a) *in vitro* through the process of macropinocytosis [102]. Since these are not internalization receptors, the authors proposed that PLG-R$_{KT}$ interacts with the apo(a) moiety of Lp(a) at the cell surface, followed by uptake of Lp(a) by macropinocytosis through the cell surface binding activity of the annexin A2 tetramer. Although potentially interesting, a number of questions remain. Hepatocytes are not known to perform macropinocytosis; this process is primarily associated with non-receptor mediated engulfment of cargo in immune cells [103]. Currently, no *in vivo* data have been presented to support either a physiological role for these plasminogen receptors in Lp(a) uptake, or the involvement of macropinocytosis in Lp(a) catabolism. Considering all the available evidence, we believe that the true Lp(a) receptor, if it exists at all, remains unidentified.

Genetics of Lp(a)
Regulation of Lp(a) levels by the *LPA* gene

Lp(a) is the most common monogenic cause of inherited CHD. In this regard, plasma Lp(a) concentrations are primarily determined at the level of the *LPA* gene with estimates of heritability of Lp(a) concentrations exceeding 90% [10]. Plasma Lp(a) concentrations reflect a co-dominant inheritance pattern where Lp(a) levels correspond to the sum of the contribution of each *LPA* allele. The majority of the variability in Lp(a) levels arises from the *LPA* gene itself [104]; this is primarily conferred by differences in *LPA* size where smaller alleles are strongly associated with higher plasma Lp(a) levels as well as by SNPs. Given the strong contribution of the *LPA* gene to plasma Lp(a) concentrations, levels are relatively resistant to lowering through alterations in diet and lifestyle or most lipid-lowering therapies [12].

Both median Lp(a) levels and the distribution of levels vary between different ancestry groups. It is known that Blacks have the highest Lp(a) level of all ancestry groups, followed by South Asians, European Caucasians, Hispanics, and East Asians [105]. Interestingly, the distribution of Lp(a) levels in the Black population are near-normal in their distribution, versus the positively skewed distribution in other ancestry groups. Lp(a) levels in European Caucasians are highly positively-skewed, while South Asians have a distribution intermediate to that observed in Whites and Blacks. Haplotype studies have shown that both *LPA* SNPs and KIV2 copy number are genomic determinants of plasma Lp(a) concentration, but that the extent of these contributions differs across ethnic groups [106]. SNPs that have been identified in *LPA* haplotypes are linked to both plasma Lp(a) levels and KIV2 copy number in Caucasian, Asian and South Asian populations. However, the KIV2 copy number explains a larger proportion of variability in plasma Lp(a) levels in European Caucasians, compared with that observed in the Asian or South Asian populations [106]. This may reflect the distribution of the rs10455872 SNP, which is only found in European Caucasians, and which is significantly associated with both KIV2 copy number and Lp(a) levels. Importantly, many SNPs are common in some ancestry groups but absent in others,

indicating that they may play a role in the different relationships between *LPA* allele size and Lp(a) levels in these groups [11].

The KIV2 repeat region has been comparatively impenetrable to discovery of mutations because of its highly repetitive nature. However, advances in next-generation sequencing have allowed recent insights into the mutation burden in this region. Deep sequencing of this region has revealed the presence of many mutations (missense mutations as well as a substantial number of loss-of-function and splice-site mutations) that affect Lp(a) levels beyond that attributable solely to the number of KIV2-encoding sequences [11]. Several mutations resulting in 'null' *LPA* alleles have been reported: these can take the form of either nonsense codons or splice-site mutations [107,108]. Importantly, null alleles are strong predictors of both lower Lp(a) levels and reduced risk for CHD [107].

A strong implication of the finding of a very high mutation burden in the KIV2-encoding sequence is that *LPA* has characteristics of an expressed pseudogene. This, in turn, raises the question as to whether there is a critical physiological role for Lp(a), separate from its contributions to the pathophysiology of CVD. The remains highly debated since no physiological function(s) of Lp(a) to date have been confirmed.

Relationship between *LPA* and other genes

GWAS has revealed three protein-encoding genes: *APOE*, *APOH* and *CETP*, outside the *LPA* gene cluster on chromosome 6, that are significantly associated with Lp(a) levels [104,109]. A 65% increase in Lp(a) mass was found to be associated with the apoE4-encoding ε4/ε4 genotype compared with apoE2-encoding ε2/ε2 genotype [110]. The ε2/ε2 genotype is associated with elevated triglycerides due to the impaired association of apoE2 with the LDLR; he basis of this association with Lp(a) levels remains unclear. The *APOH* locus encodes the protein beta2-glycoprotein I (β2GP1.) This plasma protein has a high affinity for negatively charged phospholipids. *In vitro*, β2GP1 has been reported to bind to Lp(a), both through the phospholipids on LDL as well as through the KIV domain of apo(a) [111]. However, the effect of these interactions on Lp(a) levels remains unclear. The identification of *CETP* in this GWAS analysis is consistent with the ability of CETP inhibitors to lower human Lp(a) levels in plasma [112,113]. However, the mechanism of action underlying these observations remains unclear and may reflect effects on either production or catabolism of Lp(a).

Mechanism of action of Lp(a) in CVD

Based on Mendelian randomization studies, a causal relationship between elevated levels of Lp(a) and ASCVD or calcific aortic valve disease (CAVD) has been demonstrated [114]. However, the mechanism of action of Lp(a) at the cellular and molecular levels that underlies these observations remains unclear. This reflects the lack of an animal model that has the human *LPA* gene, making it suitable for manipulation in a disease model. As such, much of our mechanistic insights into Lp(a) pathogenicity have come from *in vitro* vascular cell studies (Figure 4). While transgenic apo(a)/Lp(a) mouse and rabbit models have been reported, they usually have significant limitations including Lp(a) levels below the pathogenic range, non-physiological truncated apo(a) variants, and the absence of human apoB100 to allow formation of a covalent Lp(a) particle in plasma [118]. Most recently, a novel transgenic mouse expressing a 13-kringle form of human apo(a) and human apoB100 has been reported that features very high plasma levels of bona fide human Lp(a) [119]. After 12 weeks of a high-fat high-cholesterol diet in the presence of antisense oligonucleotide-mediated *Ldlr* knockdown, female Lp(a) transgenic mice had higher aortic sinus plaque area, as well as increased calcification, oxidized phospholipid deposition, and necrotic core area compared with control mice expressing only human apoB100. The increased vulnerable plaque features seen in these animals will make them a useful model for understanding the role of Lp(a) in the initiation and progression of atherosclerosis.

Determining a role for Lp(a) in thrombosis

When the cDNA sequence of apo(a) was published by McLean et al. [8], a striking similarity between apo(a) and the proteolytic precursor plasminogen was observed. When plasminogen is activated to plasmin by either tPA or uPA action, the resulting enzyme cleaves many substrates including fibrin, resulting in dissolution of thrombi through fibrinolysis. Based on its similarity with plasminogen, it was hypothesized that apo(a) and Lp(a) could inhibit this process. However, the data supporting a role for Lp(a) in fibrinolysis *in vivo* have been largely disappointing [115]. For example, Mendelian randomization studies suggested against a causal role of elevated Lp(a) in venous thromboembolism [120]. Although apo(a) alone is very effective at inhibiting fibrinolysis and plasminogen activation [121–123], the same does not appear to be true for the intact Lp(a) particle
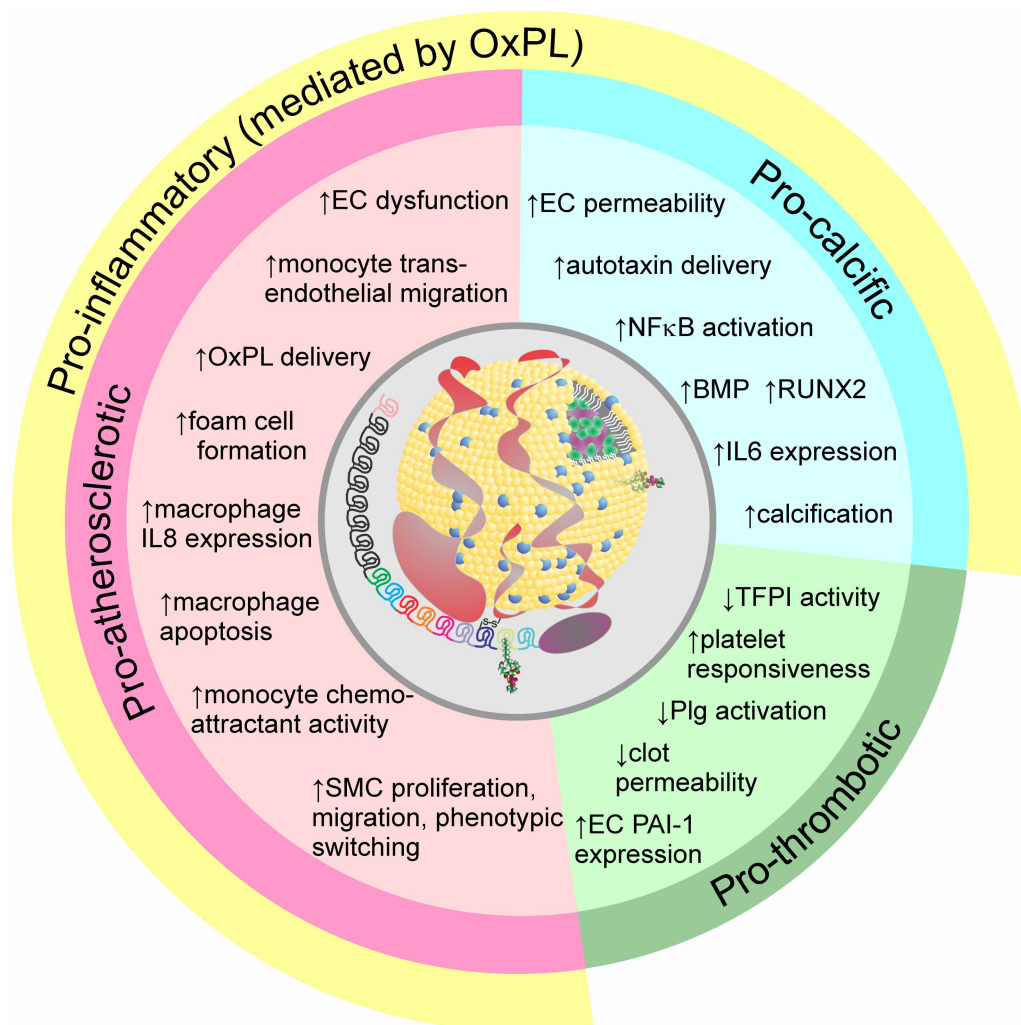
Figure 4. Potential pathogenic mechanisms of Lp(a).

Lp(a) has pro-atherosclerotic, pro-calcific (primarily leading to calcific aortic valve disease), and pro-thrombotic mechanisms of action. Virtually all of the pro-atherosclerotic and pro-calcific effects have been shown to be mediated by the oxPL covalently associated with apo(a) KIV10. The individual pathogenic mechanisms have been primarily demonstrated through *in vitro* and *ex vivo* studies (see [115–117] for details). EC, endothelial cells; oxPL: oxidized phospholipids; IL: interleukin; SMC, smooth muscle cell; TFPI, tissue factor pathway inhibitor; Plg: plasminogen; PAI-1: plasminogen activator inhibitor type 1.

[124]. Indeed, in an *ex vivo* plasma clot lysis study using samples from patients in clinical trials of a potent Lp (a)-lowering antisense oligonucleotide, no effect of Lp(a) lowering on clot lysis time was observed [124]. However, this does not rule out a potential role for Lp(a) in the promotion of thrombus formation in the vasculature. For example, Lp(a) may modulate platelet reactivity and/or stimulate the pathways that result in thrombus formation and/or clot stabilization [115]. These mechanisms would operate independently of the similarity between apo(a) and plasminogen, pointing to a unique role for Lp(a) in ASCVD. In this regard, it has been shown that Lp(a) can inhibit tissue factor pathway inhibitor, thereby promoting coagulation [125]. Additionally, it has been reported that Lp(a) can increase PAI-1 expression in endothelial cells [126], and may play a role in promoting platelet aggregation based on *in vitro* findings [127]. In this regard, patients with elevated Lp(a) levels show greater benefit from prolonged dual antiplatelet therapy than patients with low Lp(a) levels [128]. In the ASPREE trial of aspirin in primary prevention in older adults, the benefit of this intervention was restricted to those subjects with a genetic signature of elevated Lp(a), without a significant increase in bleeding events [129]. Also consistent with a procoagulant role for Lp(a) are the results of a Mendelian

randomization study which showed that elevated Lp(a) is in fact associated with a lower risk of major bleeding in the brain and airways [130].

Mechanism of Lp(a) action in atherosclerosis and aortic valve disease — a role for Lp(a)-oxPL

In a series of studies involving ∼30 000 subjects [37,131–135], a very strong correlation between the oxPL/apoB ratio (measured using E06 and an apoB antibody in a plate assay) and Lp(a) concentrations was observed (r = 0.8–0.9) with the strength of the correlation inversely proportional to apo(a) isoform size [134]. Elevated oxPL/apoB levels have been shown to be associated with worse cardiovascular outcomes [136] and are positively associated with CAD as well as the progression of carotid, coronary and femoral atherosclerosis [133,134]. In the prospective 10-year follow up of the Bruneck study, oxPL/apoB was shown to strongly predict new CVD events and was independent of all known risk factors except for Lp(a) [137]. Importantly, levels of oxPL/apoB are predictive of new future CVD events beyond those predicted by the Framingham Risk Score [138]. Subsequent reports have linked the oxPL in Lp(a) to the pathogenesis of CAVD [139,140], which is notable since CAVD is not an atherothrombotic disease, despite sharing some risk factors [139]. These findings underscore the emerging concept that the effects on vascular cells of the apo(a) moiety contribute to the pathogenicity of Lp(a), while also serving to highlight oxPL as a mechanistic link between these observations.

The key observation that Lp(a), and not LDL, is the preferential carrier of oxPL in plasma has provided a different lens through which to consider the mechanistic basis of Lp(a) pathogenicity (Figure 4) [116]. OxPL species are highly proinflammatory, and their specific interaction with Lp(a) provides a potential avenue for delivery and retention of these species in the vessel at the site of atherosclerotic lesions. In addition of oxPL species present on the LDL component of Lp(a), apo(a) contains a covalently-linked oxPL moiety within its KIV10 domain, the addition of which requires the strong LBS present in this kringle [42]. The mechanism underlying the role of the LBS in mediating oxPL coordination is a subject of ongoing investigation.

Lp(a) accumulates and is retained in the site of developing atherosclerotic lesions to greater extent than LDL; the extent of accumulation is proportional to the plasma Lp(a) concentration [141]. The retention of Lp(a) in the intima is likely facilitated by its ability to bind to several extracellular matrix proteins including collagen, fibrinogen, and fibronectin. This provides a route by which Lp(a) can deliver oxPL to arterial lesions [142]. The oxPL present on apo(a) has been linked to a number of proinflammatory effects of Lp(a) including increased expression of inflammatory mediators in cultured macrophages and human monocytes [59,143]. The oxPL on apo(a) has also been implicated in alteration of vascular endothelial cell phenotype through activation of glycolysis, promoting adhesion and transmigration of monocytes across the endothelium [144]. This is in keeping with previous studies that have demonstrated a role for Lp(a) in endothelial cell signaling pathways that result in cellular contraction and disruption of cell-cell adhesion molecules, leading to endothelial dysfunction [145,146]. It has recently been shown that Lp(a), and to a lesser extent apo(a), increases caspase activation and release of IL-1B and IL-18 in human monocyte-derived macrophages and cultured THP-1 macrophages [7]. Interestingly, the oxPL moiety in apo(a) has also been shown to play a role in the development of CAVD through its ability to stimulate osteogenic differentiation in isolated valve interstitial cells [147]. As such, it has been hypothesized that OxPL-Lp(a) provides a link between ASCVD and CAVD in large part due to its proinflammatory properties [117].

The exact composition of the OxPL associated with apo(a) and the lipid portion of Lp(a) is not known. However, in addition to covalently-linked OxPL on apo(a), it is likely that OxPL associated with the lipid component of the Lp(a) particle may also contain oxidized adducts that contribute to proinflammatory responses. Although the lipidome of Lp(a) remains poorly characterized, it has been shown that it is enriched in diacylglycerols and lysophosphatidic acid (LPA), which contribute to proinflammatory responses in monocytes [43].

The mechanism by which Lp(a) contributes to phenotypic changes in aortic valve interstitial cells, vascular endothelial cells and monocytes/macrophages remains unclear with respect to initiation of signaling pathways that lead to programs of proatherosclerotic and procalcific gene expression in these cells. A role for Toll-like receptors has been suggested in monocytes and macrophages [116]. In VICs, oxPL bound to Lp(a) appears to be activated by phospholipase A2 to form lysophosphatidyl choline (LPC); autotaxin, also associated with Lp(a), then converts LPC to lyosphophatidic acid [140]. LPA, in turn binds to the LPA receptor on valve endothelial cells, initiating a cascade that leads to mineralization and osteogenic transition of valve interstitial cells, resulting in valve calcification [140].

Taken together, the role of Lp(a) in the progression of atherosclerosis is and CAVD is likely dependent in large part on its unique proinflammatory properties. This helps to explain the independent nature of the risk associated with Lp(a) and vascular diseases.

Pharmaceutical approaches to Lp(a) lowering

It has been a major challenge to develop compounds that can specifically and effectively lower plasma Lp(a) levels. Most existing lipid-lowering medications do not lower Lp(a), or lower Lp(a) to a lesser extent than they do LDL-C [148]. As a consequence, it has not been possible thus far to demonstrate a specific CVD benefit from Lp(a) lowering.

In general, compounds that lower apoB secretion also lower plasma Lp(a) levels, but to a much lesser extent than LDL-C; this includes, for example, mipomersin [149] and lomitapide [150]. Although niacin lowers LDL and Lp(a), the effect on Lp(a) lowering is more effective for larger isoforms of apo(a) [151], which generally correspond to lower plasma levels. Amongst therapies that increase LDLR expression, statins do not lower Lp(a) (and may in fact slightly raise it [86]). PCSK9 inhibitors have an Lp(a) lowering effect, but much less than that observed for LDL-C [152].

A new approach to Lp(a) lowering has been ushered in with the use of RNA-directed therapies (Figure 3). These agents specifically, and very effectively, lower Lp(a) as they target the *LPA* mRNA encoding apo(a). The first of these agents was pelacarsen (formerly known as IONIS-APO(a)-L_{Rx} and TQJ230), an antisense oligonucleotide with chemically modified phosphate backbones and ribose moieties to increase its stability by decreasing nuclease digestion and to enhance its affinity for the target mRNA; pelacarsen also contains a triantennary GalNAc modification at the 5- end to facilitate specific uptake into hepatocytes via the asialoglycoprotein receptor [153]. Pelacarsen is able to achieve up to 70% reduction in Lp(a) levels with once-monthly dosing; in Phase II studies in subjects with highly elevated Lp(a), Pelacarsen was able to lower Lp(a) to below 50 mg/dl (125 nmol/l) in over 90% of subjects [154]. Pelacarsen is currently being tested in several Phase 3 trials, including Lp(a)HORIZON (NCT04023552), a randomized, double-blind, placebo-controlled cardiovascular outcomes trial. Subjects ($n = 8323$) with Lp(a) ≥ 70 mg/dl (175 nmol/l) and pre-existing ASCVD were randomized to receive 80 mg pelacarsen subcutaneously every 4 weeks or placebo, with the primary endpoint of time to expanded major adverse cardiovascular events (MACE; cardiovascular death, non-fatal MI, non-fatal stroke and urgent coronary re-vascularization requiring hospitalization) in patients with Lp(a) ≥ 70 mg/dl (175 nmol/l) or ≥90 mg/dl (225 nmol/l) Recruitment has been completed and the anticipated study reporting date is May 2025.

Several siRNA-based Lp(a) lowering agents are at various stages of clinical development. These agents are able to achieve an even more dramatic lowering of Lp(a) (>90%) with less frequent subcutaneous dosing [155]. Olpasiran is a GalNAc-modified siRNA that is currently being tested in the OCEAN(a) (NCT05581303) cardiovascular outcomes trial. Approximately 7000 patients with a history of ASCVD and Lp(a) ≥ 200 nmol/l were randomized to receive either 75 mg olpasiran subcutaneously every 12 weeks or placebo. The primary endpoint is time to CHD death, myocardial infarction, or urgent coronary revascularization, and the anticipated study completion date is December 2026.

Lepodisiran, another GalNAc-modified siRNA, is currently being tested in the ACCLAIM-Lp(a) (NCT06292013) randomized, placebo-controlled cardiovascular outcomes trial. Approximately 12 500 patients with a history of ASCVD or at high risk for ASCVD and Lp(a) ≥ 175 nmol/l will be enrolled, with the primary endpoint of time to first event in a composite MACE (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, and urgent coronary revascularization). Mean follow-up time will be 4.5 years, and the anticipated study completion date is in 2029.

A completely different approach to Lp(a) lowering has recently been reported, in which small molecules were developed to bind to KIV7 and KIV8 and thus inhibit the non-covalent first step of assembly of the Lp(a) particle (Figure 3) [156]. The ultimate product of this program, muvalaplin (LY3473329) is tripartite molecule with each arm able to bind tightly to the LBS in these kringles, affording an exceedingly potent ability to inhibit Lp(a) assembly *in vitro* (IC50 of 0.09 nmol/l). The crystal structure of muvalaplin with isolated KIV8 shows each arm bound to one KIV8 molecule, invoking the possibility of multivalent binding of muvalaplin to apo(a) to potentiate its Lp(a)-lowering ability [156]. In a Phase 1 ascending-dose study, muvalaplin lowered Lp(a) levels as early as 24 h after the first dose, and a maximum Lp(a) lowering of ~65% was observed at doses >100 mg/day [157]. Importantly, as was the case for the RNA-directed therapies no safety or tolerability issues were noted in this study.

With the development of these effective Lp(a)-lowering therapies, we are at last at the cusp of answering the long-standing question of whether specific Lp(a) lowering can reduce risk of cardiovascular events. Given the prevalence of elevated Lp(a) in patients with existing ASCVD (10–15% of such patients would have Lp(a) levels high enough to be eligible for the cardiovascular outcomes trials), these agents could have a major impact on residual risk of ASCVD events in secondary prevention. It will take larger and longer studies to assess if Lp(a) lowering could also reduce risk in the primary prevention population; as we understand more about the mechanisms through which elevated Lp(a) causes ASCVD events, such studies will likely take on a greater urgency.

Although we are on the cusp of being able to effectively and specifically lower Lp(a), an ongoing question in the field is whether or not Lp(a) has a physiological function that might be affected by Lp(a) lowering. This has been a difficult question to address due to the lack of animal models that express endogenous Lp(a) containing all of the critical aspects of its structure that are found in humans. For example, KIV10 in Old World monkeys and apes lacks the strong LBS in KIV10 and consequently does not contain OxPL bound to this kringle. Many of these species also lack the KV domain, the function of which remains unclear. Based on its ability bind OxPL, early speculation suggested that human Lp(a) may play a protective role by functioning as a preferential carrier for plasma OxPL in plasma, thereby reducing the abundance of these inflammatory species on LDL [158]. It has also been proposed that Lp(a) may have evolved to prevent excessive bleeding through its pro-thrombotic properties [130,159]. Although population-based genetic analyses suggest that that lifelong exposure to low Lp(a) levels is linked to longevity [160], increased risk for new onset type 2 diabetes as well as metabolic dysfunction-associated liver disease in individuals with low Lp(a) levels (<3.8 nmol/l) has been reported [161]. However, these relationships may not be causal in nature and require further study. Clearly, there is much left to learn about Lp(a) structure and its mechanisms of action in both the vasculature and more broadly in the human body. Increased fundamental knowledge of Lp(a) will help to address the many remaining questions related to its role in human health and disease.

Competing Interests

M.B. Boffa has no competing interests to declare. M.L. Koschinsky holds a research contract with Eli Lilly & Co. and is a consultant for Novartis and Eli Lilly & Co.

CRediT Author Contribution

Michael B. Boffa: Conceptualization, Writing — original draft. **Marlys L. Koschinsky**: Conceptualization, Writing — original draft, Writing — review and editing.

Abbreviations

ASCVD, atherosclerotic cardiovascular disease; CAVD, calcific aortic valve disease; CHD, coronary heart disease; CVD, cardiovascular disease; FCR, fractional catabolic rate; KIV, kringle IV; LBS, lysine-binding site; LDL, low density lipoprotein; LPC, lysophosphatidyl choline; MACE, major adverse cardiovascular event; PCSK9, proprotein convertase subtilisin/kexin type 9; tPA, tissue-type plasminogen activator.

References

1   Berg, K. (1963) A new serum type system in man–the Lp system. *Acta Pathol. Microbiol. Scand.* **59**, 369–382 https://doi.org/10.1111/j.1699-0463.1963.tb01808.x

2   Clarke, R., Peden, J.F., Hopewell, J.C., Kyriakou, T., Goel, A., Heath, S.C. et al. (2009) Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N. Engl. J. Med.* **361**, 2518–2528 https://doi.org/10.1056/NEJMoa0902604

3   Erqou, S., Kaptoge, S., Perry, P.L., Di Angelantonio, E., Thompson, A., White, I.R. et al. (2009) Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA* **302**, 412–423 https://doi.org/10.1001/jama.2009.1063

4   Kamstrup, P.R., Tybjaerg-Hansen, A., Steffensen, R. and Nordestgaard, B.G. (2009) Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA* **301**, 2331–2339 https://doi.org/10.1001/jama.2009.801

5   Thanassoulis, G., Campbell, C.Y., Owens, D.S., Smith, J.G., Smith, A.V., Peloso, G.M. et al. (2013) Genetic associations with valvular calcification and aortic stenosis. *N. Engl. J. Med.* **368**, 503–512 https://doi.org/10.1056/NEJMoa1109034

6   Fless, G.M., ZumMallen, M.E. and Scanu, A.M. (1986) Physicochemical properties of apolipoprotein(a) and lipoprotein(a-) derived from the dissociation of human plasma lipoprotein (a). *J. Biol. Chem.* **261**, 8712–8718 https://doi.org/10.1016/S0021-9258(19)84439-3

7   Lorey, M.B., Youssef, A., Aikas, L., Borrelli, M., Hermansson, M., Assini, J.M. et al. (2023) Lipoprotein(a) induces caspase-1 activation and IL-1 signaling in human macrophages. *Front. Cardiovasc. Med.* **10**, 1130162 https://doi.org/10.3389/fcvm.2023.1130162

8   McLean, J.W., Tomlinson, J.E., Kuang, W.J., Eaton, D.L., Chen, E.Y., Fless, G.M. et al. (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* **330**, 132–137 https://doi.org/10.1038/330132a0

9   Tsimikas, S., Fazio, S., Ferdinand, K.C., Ginsberg, H.N., Koschinsky, M.L., Marcovina, S.M. et al. (2018) NHLBI Working Group recommendations to reduce lipoprotein(a)-mediated risk of cardiovascular disease and aortic stenosis. *J. Am. Coll. Cardiol.* **71**, 177–192 https://doi.org/10.1016/j.jacc.2017.11.014

10 Schmidt, K., Noureen, A., Kronenberg, F. and Utermann, G. (2016) Structure, function, and genetics of lipoprotein (a). *J. Lipid Res.* **57**, 1339–1359 https://doi.org/10.1194/jlr.R067314

11 Coassin, S. and Kronenberg, F. (2022) Lipoprotein(a) beyond the kringle IV repeat polymorphism: the complexity of genetic variation in the LPA gene. *Atherosclerosis* **349**, 17–35 https://doi.org/10.1016/j.atherosclerosis.2022.04.003

12 Enkhmaa, B. and Berglund, L. (2022) Non-genetic influences on lipoprotein(a) concentrations. *Atherosclerosis* **349**, 53–62 https://doi.org/10.1016/j.atherosclerosis.2022.04.006

13 Kronenberg, F., Mora, S., Stroes, E.S.G., Ference, B.A., Arsenault, B.J., Berglund, L. et al. (2022) Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. *Eur. Heart J.* **43**, 3925–3946 https://doi.org/10.1093/eurheartj/ehac361

14 Utermann, G., Menzel, H.J., Kraft, H.G., Duba, H.C., Kemmler, H.G. and Seitz, C. (1987) Lp(a) glycoprotein phenotypes. Inheritance and relation to Lp (a)-lipoprotein concentrations in plasma. *J. Clin. Invest.* **80**, 458–465 https://doi.org/10.1172/JCI113093

15 Albers, J.J., Kennedy, H. and Marcovina, S.M. (1996) Evidence that Lp[a] contains one molecule of apo[a] and one molecule of apoB: evaluation of amino acid analysis data. *J. Lipid Res.* **37**, 192–196 https://doi.org/10.1016/S0022-2275(20)37647-1

16 Atkinson, R.A. and Williams, R.J. (1990) Solution structure of the kringle 4 domain from human plasminogen by 1H nuclear magnetic resonance spectroscopy and distance geometry. *J. Mol. Biol.* **212**, 541–552 https://doi.org/10.1016/0022-2836(90)90330-0

17 Ye, Q., Rahman, M.N., Koschinsky, M.L. and Jia, Z. (2001) High-resolution crystal structure of apolipoprotein(a) kringle IV type 7: insights into ligand binding. *Protein Sci.* **10**, 1124–1129 https://doi.org/10.1110/ps.01701

18 Chitayat, S., Kanelis, V., Koschinsky, M.L. and Smith, S.P. (2007) Nuclear magnetic resonance (NMR) solution structure, dynamics, and binding properties of the kringle IV type 8 module of apolipoprotein(a). *Biochemistry* **46**, 1732–1742 https://doi.org/10.1021/bi061814g

19 Mochalkin, I., Cheng, B., Klezovitch, O., Scanu, A.M. and Tulinsky, A. (1999) Recombinant kringle IV-10 modules of human apolipoprotein(a): structure, ligand binding modes, and biological relevance. *Biochemistry* **38**, 1990–1998 https://doi.org/10.1021/bi9820558

20 Sandmark, J., Tigerstrom, A., Akerud, T., Althage, M., Antonsson, T., Blaho, S. et al. (2020) Identification and analyses of inhibitors targeting apolipoprotein(a) kringle domains KIV-7, KIV-10, and KV provide insight into kringle domain function. *J. Biol. Chem.* **295**, 5136–5151 https://doi.org/10.1074/jbc.RA119.011251

21 Santonastaso, A., Maggi, M., De Jonge, H. and Scotti, C. (2020) High resolution structure of human apolipoprotein (a) kringle IV type 2: beyond the lysine binding site. *J. Lipid Res.* **61**, 1687–1696 https://doi.org/10.1194/jlr.RA120001023

22 van der Hoek, Y.Y., Wittekoek, M.E., Beisiegel, U., Kastelein, J.J. and Koschinsky, M.L. (1993) The apolipoprotein(a) kringle IV repeats which differ from the major repeat kringle are present in variably-sized isoforms. *Hum. Mol. Genet.* **2**, 361–366 https://doi.org/10.1093/hmg/2.4.361

23 Marcovina, S.M., Hobbs, H.H. and Albers, J.J. (1996) Relation between number of apolipoprotein(a) kringle 4 repeats and mobility of isoforms in agarose gel: basis for a standardized isoform nomenclature. *Clin. Chem.* **42**, 436–439 https://doi.org/10.1093/clinchem/42.3.436

24 Gabel, B.R. and Koschinsky, M.L. (1995) Analysis of the proteolytic activity of a recombinant form of apolipoprotein(a). *Biochemistry* **34**, 15777–15784 https://doi.org/10.1021/bi00048a023

25 Koschinsky, M.L., Tomlinson, J.E., Zioncheck, T.F., Schwartz, K., Eaton, D.L. and Lawn, R.M. (1991) Apolipoprotein(a): expression and characterization of a recombinant form of the protein in mammalian cells. *Biochemistry* **30**, 5044–5051 https://doi.org/10.1021/bi00234a029

26 Becker, L., Webb, B.A., Chitayat, S., Nesheim, M.E. and Koschinsky, M.L. (2003) A ligand-induced conformational change in apolipoprotein(a) enhances covalent Lp(a) formation. *J. Biol. Chem.* **278**, 14074–14081 https://doi.org/10.1074/jbc.M212855200

27 Rahman, M.N., Becker, L., Petrounevitch, V., Hill, B.C., Jia, Z. and Koschinsky, M.L. (2002) Comparative analyses of the lysine binding site properties of apolipoprotein(a) kringle IV types 7 and 10. *Biochemistry* **41**, 1149–1155 https://doi.org/10.1021/bi010964i

28 Koschinsky, M.L., Cote, G.P., Gabel, B. and van der Hoek, Y.Y. (1993) Identification of the cysteine residue in apolipoprotein(a) that mediates extracellular coupling with apolipoprotein B-100. *J. Biol. Chem.* **268**, 19819–19825 https://doi.org/10.1016/S0021-9258(19)36587-1

29 McCormick, S.P., Ng, J.K., Taylor, S., Flynn, L.M., Hammer, R.E. and Young, S.G. (1995) Mutagenesis of the human apolipoprotein B gene in a yeast artificial chromosome reveals the site of attachment for apolipoprotein(a). *Proc. Natl Acad. Sci. U.S.A.* **92**, 10147–10151 https://doi.org/10.1073/pnas.92.22.10147

30 Gonen, A., Yang, X., Yeang, C., Alekseeva, E., Koschinsky, M., Witztum, J.L. et al. (2020) Generation and characterization of LPA-KIV9, a murine monoclonal antibody binding a single site on apolipoprotein (a). *J. Lipid Res.* **61**, 1263–1270 https://doi.org/10.1194/jlr.RA120000830

31 Koschinsky, M.L. and Marcovina, S.M. (2004) Structure-function relationships in apolipoprotein(a): insights into lipoprotein(a) assembly and pathogenicity. *Curr. Opin. Lipidol.* **15**, 167–174 https://doi.org/10.1097/00041433-200404000-00009

32 Belczewski, A.R., Ho, J., Taylor, Jr, F.B., Boffa, M.B., Jia, Z. and Koschinsky, M.L. (2005) Baboon lipoprotein(a) binds very weakly to lysine-agarose and fibrin despite the presence of a strong lysine-binding site in apolipoprotein(a) kringle IV type 10. *Biochemistry* **44**, 555–564 https://doi.org/10.1021/bi048156p

33 Garner, B., Merry, A.H., Royle, L., Harvey, D.J., Rudd, P.M. and Thillet, J. (2001) Structural elucidation of the N- and O-glycans of human apolipoprotein (a): role of o-glycans in conferring protease resistance. *J. Biol. Chem.* **276**, 22200–22208 https://doi.org/10.1074/jbc.M102150200

34 Mooser, V., Marcovina, S.M., White, A.L. and Hobbs, H.H. (1996) Kringle-containing fragments of apolipoprotein(a) circulate in human plasma and are excreted into the urine. *J. Clin. Invest.* **98**, 2414–2424 https://doi.org/10.1172/JCI119055

35 Edelstein, C., Italia, J.A., Klezovitch, O. and Scanu, A.M. (1996) Functional and metabolic differences between elastase-generated fragments of human lipoprotein[a] and apolipoprotein[a]. *J. Lipid Res.* **37**, 1786–1801 https://doi.org/10.1016/S0022-2275(20)39122-7

36 Edelstein, C., Shapiro, S.D., Klezovitch, O. and Scanu, A.M. (1999) Macrophage metalloelastase, MMP-12, cleaves human apolipoprotein(a) in the linker region between kringles IV-4 and IV-5. Potential relevance to lipoprotein(a) biology. *J. Biol. Chem.* **274**, 10019–10023 https://doi.org/10.1074/jbc.274.15.10019

37 Bergmark, C., Dewan, A., Orsoni, A., Merki, E., Miller, E.R., Shin, M.J. et al. (2008) A novel function of lipoprotein [a] as a preferential carrier of oxidized phospholipids in human plasma. *J. Lipid Res.* **49**, 2230–2239 https://doi.org/10.1194/jlr.M800174-JLR200

38 Horkko, S., Bird, D.A., Miller, E., Itabe, H., Leitinger, N., Subbanagounder, G. et al. (1999) Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J. Clin. Invest.* **103**, 117–128 https://doi.org/10.1172/JCI4533

39   Friedman, P., Horkko, S., Steinberg, D., Witztum, J.L. and Dennis, E.A. (2002) Correlation of antiphospholipid antibody recognition with the structure of synthetic oxidized phospholipids. Importance of Schiff base formation and aldol condensation. *J. Biol. Chem.* **277**, 7010–7020 https://doi.org/10.1074/jbc.M108860200

40   Berliner, J.A., Leitinger, N. and Tsimikas, S. (2009) The role of oxidized phospholipids in atherosclerosis. *J. Lipid Res.* **50**, S207–S212 https://doi.org/10.1194/jlr.R800074-JLR200

41   Milic, I., Melo, T., Domingues, M.R., Domingues, P. and Fedorova, M. (2015) Heterogeneity of peptide adducts with carbonylated lipid peroxidation products. *J. Mass Spectrom.* **50**, 603–612 https://doi.org/10.1002/jms.3568

42   Leibundgut, G., Scipione, C., Yin, H., Schneider, M., Boffa, M.B., Green, S. et al. (2013) Determinants of binding of oxidized phospholipids on apolipoprotein (a) and lipoprotein (a). *J. Lipid Res.* **54**, 2815–2830 https://doi.org/10.1194/jlr.M040733

43   Dzobo, K.E., Cupido, A.J., Mol, B.M., Stiekema, L.C.A., Versloot, M., Winkelmeijer, M. et al. (2024) Diacylglycerols and lysophosphatidic acid, enriched on lipoprotein(a), contribute to monocyte inflammation. *Arterioscler. Thromb. Vasc. Biol.* **44**, 720–740 https://doi.org/10.1161/ATVBAHA.123.319937

44   Lawn, R., Patthy, L., Pesole, G. and Saccone, C. (1997) Apolipoproteins(a): a puzzling evolutionary story. *J. Mol. Evol.* **44**, 234–236 https://doi.org/10.1007/PL00013141

45   Tomlinson, J.E., McLean, J.W. and Lawn, R.M. (1989) Rhesus monkey apolipoprotein(a). Sequence, evolution, and sites of synthesis. *J. Biol. Chem.* **264**, 5957–5965 https://doi.org/10.1016/S0021-9258(18)83643-2

46   Chenivesse, X., Huby, T., Wickins, J., Chapman, J. and Thillet, J. (1998) Molecular cloning of the cDNA encoding the carboxy-terminal domain of chimpanzee apolipoprotein(a): an Asp57 −> Asn mutation in kringle IV-10 is associated with poor fibrin binding. *Biochemistry* **37**, 7213–7223 https://doi.org/10.1021/bi9721546

47   Lawn, R.M., Boonmark, N.W., Schwartz, K., Lindahl, G.E., Wade, D.P., Byrne, C.D. et al. (1995) The recurring evolution of lipoprotein(a). Insights from cloning of hedgehog apolipoprotein(a). *J. Biol. Chem.* **270**, 24004–24009 https://doi.org/10.1074/jbc.270.41.24004

48   Rader, D.J., Cain, W., Zech, L.A., Usher, D. and Brewer, Jr, H.B. (1993) Variation in lipoprotein(a) concentrations among individuals with the same apolipoprotein (a) isoform is determined by the rate of lipoprotein(a) production. *J. Clin. Invest.* **91**, 443–447 https://doi.org/10.1172/JCI116221

49   Rader, D.J., Cain, W., Ikewaki, K., Talley, G., Zech, L.A., Usher, D. et al. (1994) The inverse association of plasma lipoprotein(a) concentrations with apolipoprotein(a) isoform size is not due to differences in Lp(a) catabolism but to differences in production rate. *J. Clin. Invest.* **93**, 2758–2763 https://doi.org/10.1172/JCI117292

50   Boffelli, D., Zajchowski, D.A., Yang, Z. and Lawn, R.M. (1999) Estrogen modulation of apolipoprotein(a) expression. Identification of a regulatory element. *J. Biol. Chem.* **274**, 15569–15574 https://doi.org/10.1074/jbc.274.22.15569

51   Simony, S.B., Mortensen, M.B., Langsted, A., Afzal, S., Kamstrup, P.R. and Nordestgaard, B.G. (2022) Sex differences of lipoprotein(a) levels and associated risk of morbidity and mortality by age: the Copenhagen General Population Study. *Atherosclerosis* **355**, 76–82 https://doi.org/10.1016/j.atherosclerosis.2022.06.1023

52   Jenner, J.L., Ordovas, J.M., Lamon-Fava, S., Schaefer, M.M., Wilson, P.W., Castelli, W.P. et al. (1993) Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study. *Circulation* **87**, 1135–1141 https://doi.org/10.1161/01.CIR.87.4.1135

53   Jiang, Y. and Tian, W. (2017) The effects of progesterones on blood lipids in hormone replacement therapy. *Lipids Health Dis.* **16**, 219 https://doi.org/10.1186/s12944-017-0612-5

54   Anagnostis, P., Galanis, P., Chatzistergiou, V., Stevenson, J.C., Godsland, I.F., Lambrinoudaki, I. et al. (2017) The effect of hormone replacement therapy and tibolone on lipoprotein (a) concentrations in postmenopausal women: a systematic review and meta-analysis. *Maturitas* **99**, 27–36 https://doi.org/10.1016/j.maturitas.2017.02.009

55   Chennamsetty, I., Claudel, T., Kostner, K.M., Baghdasaryan, A., Kratky, D., Levak-Frank, S. et al. (2011) Farnesoid X receptor represses hepatic human APOA gene expression. *J. Clin. Invest.* **121**, 3724–3734 https://doi.org/10.1172/JCI45277

56   Chennamsetty, I., Claudel, T., Kostner, K.M., Trauner, M. and Kostner, G.M. (2012) FGF19 signaling cascade suppresses APOA gene expression. *Arterioscler. Thromb. Vasc. Biol.* **32**, 1220–1227 https://doi.org/10.1161/ATVBAHA.111.243055

57   Wade, D.P., Clarke, J.G., Lindahl, G.E., Liu, A.C., Zysow, B.R., Meer, K. et al. (1993) 5' control regions of the apolipoprotein(a) gene and members of the related plasminogen gene family. *Proc. Natl Acad. Sci. U.S.A.* **90**, 1369–1373 https://doi.org/10.1073/pnas.90.4.1369

58   Muller, N., Schulte, D.M., Turk, K., Freitag-Wolf, S., Hampe, J., Zeuner, R. et al. (2015) IL-6 blockade by monoclonal antibodies inhibits apolipoprotein (a) expression and lipoprotein (a) synthesis in humans. *J. Lipid Res.* **56**, 1034–1042 https://doi.org/10.1194/jlr.P052209

59   Scipione, C.A., Sayegh, S.E., Romagnuolo, R., Tsimikas, S., Marcovina, S.M., Boffa, M.B. et al. (2015) Mechanistic insights into Lp(a)-induced IL-8 expression: a role for oxidized phospholipid modification of apo(a). *J. Lipid Res.* **56**, 2273–2285 https://doi.org/10.1194/jlr.M060210

60   Moriarty, P.M., Gorby, L.K., Stroes, E.S., Kastelein, J.P., Davidson, M. and Tsimikas, S. (2020) Lipoprotein(a) and its potential association with thrombosis and inflammation in COVID-19: a testable hypothesis. *Curr. Atheroscler. Rep.* **22**, 48 https://doi.org/10.1007/s11883-020-00867-3

61   White, A.L. and Lanford, R.E. (1995) Biosynthesis and metabolism of lipoprotein (a). *Curr. Opin. Lipidol.* **6**, 75–80 https://doi.org/10.1097/00041433-199504000-00003

62   White, A.L., Rainwater, D.L. and Lanford, R.E. (1993) Intracellular maturation of apolipoprotein[a] and assembly of lipoprotein[a] in primary baboon hepatocytes. *J. Lipid Res.* **34**, 509–517 https://doi.org/10.1016/S0022-2275(20)40742-4

63   White, A.L., Guerra, B. and Lanford, R.E. (1997) Influence of allelic variation on apolipoprotein(a) folding in the endoplasmic reticulum. *J. Biol. Chem.* **272**, 5048–5055 https://doi.org/10.1074/jbc.272.8.5048

64   White, A.L., Hixson, J.E., Rainwater, D.L. and Lanford, R.E. (1994) Molecular basis for "null" lipoprotein(a) phenotypes and the influence of apolipoprotein(a) size on plasma lipoprotein(a) level in the baboon. *J. Biol. Chem.* **269**, 9060–9066 https://doi.org/10.1016/S0021-9258(17)37076-X

65   White, A.L., Guerra, B., Wang, J. and Lanford, R.E. (1999) Presecretory degradation of apolipoprotein [a] is mediated by the proteasome pathway. *J. Lipid Res.* **40**, 275–286 https://doi.org/10.1016/S0022-2275(20)33367-8

66   Wang, J. and White, A.L. (1999) Role of N-linked glycans, chaperone interactions and proteasomes in the intracellular targeting of apolipoprotein(a). *Biochem. Soc. Trans.* **27**, 453–458 https://doi.org/10.1042/bst0270453

67   Becker, L., McLeod, R.S., Marcovina, S.M., Yao, Z. and Koschinsky, M.L. (2001) Identification of a critical lysine residue in apolipoprotein B-100 that mediates noncovalent interaction with apolipoprotein(a). *J. Biol. Chem.* **276**, 36155–36162 https://doi.org/10.1074/jbc.M104789200

68   White, A.L. and Lanford, R.E. (1994) Cell surface assembly of lipoprotein(a) in primary cultures of baboon hepatocytes. *J. Biol. Chem.* **269**, 28716–28723 https://doi.org/10.1016/S0021-9258(19)61964-2

69   Becker, L., Nesheim, M.E. and Koschinsky, M.L. (2006) Catalysis of covalent Lp(a) assembly: evidence for an extracellular enzyme activity that enhances disulfide bond formation. *Biochemistry* **45**, 9919–9928 https://doi.org/10.1021/bi060283t

70   Reyes-Soffer, G., Ginsberg, H.N. and Ramakrishnan, R. (2017) The metabolism of lipoprotein (a): an ever-evolving story. *J. Lipid Res.* **58**, 1756–1764 https://doi.org/10.1194/jlr.R077693

71   Su, W., Campos, H., Judge, H., Walsh, B.W. and Sacks, F.M. (1998) Metabolism of Apo(a) and ApoB100 of lipoprotein(a) in women: effect of postmenopausal estrogen replacement. *J. Clin. Endocrinol. Metab.* **83**, 3267–3276 https://doi.org/10.1210/jcem.83.9.5116

72   Jenner, J.L., Seman, L.J., Millar, J.S., Lamon-Fava, S., Welty, F.K., Dolnikowski, G.G. et al. (2005) The metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein (a) in human beings. *Metabolism* **54**, 361–369 https://doi.org/10.1016/j.metabol.2004.10.001

73   Diffenderfer, M.R., Lamon-Fava, S., Marcovina, S.M., Barrett, P.H., Lel, J., Dolnikowski, G.G. et al. (2016) Distinct metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein(a). *Metabolism* **65**, 381–390 https://doi.org/10.1016/j.metabol.2015.10.031

74   Frischmann, M.E., Ikewaki, K., Trenkwalder, E., Lamina, C., Dieplinger, B., Soufi, M. et al. (2012) In vivo stable-isotope kinetic study suggests intracellular assembly of lipoprotein(a). *Atherosclerosis* **225**, 322–327 https://doi.org/10.1016/j.atherosclerosis.2012.09.031

75   Demant, T., Seeberg, K., Bedynek, A. and Seidel, D. (2001) The metabolism of lipoprotein(a) and other apolipoprotein B-containing lipoproteins: a kinetic study in humans. *Atherosclerosis* **157**, 325–339 https://doi.org/10.1016/S0021-9150(00)00732-2

76   Ooi, E.M., Watts, G.F., Chan, D.C., Pang, J., Tenneti, V.S., Hamilton, S.J. et al. (2015) Effects of extended-release niacin on the postprandial metabolism of Lp(a) and apoB-100-containing lipoproteins in statin-treated men with type 2 diabetes mellitus. *Arterioscler. Thromb. Vasc. Biol.* **35**, 2686–2693 https://doi.org/10.1161/ATVBAHA.115.306136

77   Youssef, A., Clark, J.R., Marcovina, S.M., Boffa, M.B. and Koschinsky, M.L. (2022) Apo(a) and apoB interact noncovalently within hepatocytes: implications for regulation of Lp(a) levels by modulation of apoB secretion. *Arterioscler. Thromb. Vasc. Biol.* **42**, 289–304 https://doi.org/10.1161/ATVBAHA.121.317335

78   Rader, D.J., Mann, W.A., Cain, W., Kraft, H.G., Usher, D., Zech, L.A. et al. (1995) The low density lipoprotein receptor is not required for normal catabolism of Lp(a) in humans. *J. Clin. Invest.* **95**, 1403–1408 https://doi.org/10.1172/JCI117794

79   Cain, W.J., Millar, J.S., Himebauch, A.S., Tietge, U.J., Maugeais, C., Usher, D. et al. (2005) Lipoprotein [a] is cleared from the plasma primarily by the liver in a process mediated by apolipoprotein [a]. *J. Lipid Res.* **46**, 2681–2691 https://doi.org/10.1194/jlr.M500249-JLR200

80   Hoover-Plow, J. and Huang, M. (2013) Lipoprotein(a) metabolism: potential sites for therapeutic targets. *Metabolism* **62**, 479–491 https://doi.org/10.1016/j.metabol.2012.07.024

81   Chemello, K., Chan, D.C., Lambert, G. and Watts, G.F. (2022) Recent advances in demystifying the metabolism of lipoprotein(a). *Atherosclerosis* **349**, 82–91 https://doi.org/10.1016/j.atherosclerosis.2022.04.002

82   Tam, S.P., Zhang, X. and Koschinsky, M.L. (1996) Interaction of a recombinant form of apolipoprotein[a] with human fibroblasts and with the human hepatoma cell line HepG2. *J. Lipid Res.* **37**, 518–533 https://doi.org/10.1016/S0022-2275(20)37595-7

83   Romagnuolo, R., Scipione, C.A., Boffa, M.B., Marcovina, S.M., Seidah, N.G. and Koschinsky, M.L. (2015) Lipoprotein(a) catabolism is regulated by proprotein convertase subtilisin/kexin type 9 through the low density lipoprotein receptor. *J. Biol. Chem.* **290**, 11649–11662 https://doi.org/10.1074/jbc.M114.611988

84   Romagnuolo, R., Scipione, C.A., Marcovina, S.M., Gemin, M., Seidah, N.G., Boffa, M.B. et al. (2017) Roles of the low density lipoprotein receptor and related receptors in inhibition of lipoprotein(a) internalization by proprotein convertase subtilisin/kexin type 9. *PLoS One* **12**, e0180869 https://doi.org/10.1371/journal.pone.0180869

85   Raal, F.J., Giugliano, R.P., Sabatine, M.S., Koren, M.J., Blom, D., Seidah, N.G. et al. (2016) PCSK9 inhibition-mediated reduction in Lp(a) with evolocumab: an analysis of 10 clinical trials and the LDL receptor's role. *J. Lipid Res.* **57**, 1086–1096 https://doi.org/10.1194/jlr.P065334

86   Tsimikas, S., Gordts, P., Nora, C., Yeang, C. and Witztum, J.L. (2020) Statin therapy increases lipoprotein(a) levels. *Eur. Heart J.* **41**, 2275–2284 https://doi.org/10.1093/eurheartj/ehz310

87   Desai, N.R., Kohli, P., Giugliano, R.P., O'Donoghue, M.L., Somaratne, R., Zhou, J. et al. (2013) AMG145, a monoclonal antibody against proprotein convertase subtilisin kexin type 9, significantly reduces lipoprotein(a) in hypercholesterolemic patients receiving statin therapy: an analysis from the LDL-C Assessment with Proprotein Convertase Subtilisin Kexin Type 9 Monoclonal Antibody Inhibition Combined with Statin Therapy (LAPLACE)-Thrombolysis in Myocardial Infarction (TIMI) 57 trial. *Circulation* **128**, 962–969 https://doi.org/10.1161/CIRCULATIONAHA.113.001969

88   Gaudet, D., Kereiakes, D.J., McKenney, J.M., Roth, E.M., Hanotin, C., Gipe, D. et al. (2014) Effect of alirocumab, a monoclonal proprotein convertase subtilisin/kexin 9 antibody, on lipoprotein(a) concentrations (a pooled analysis of 150 mg every two weeks dosing from phase 2 trials). *Am. J. Cardiol.* **114**, 711–715 https://doi.org/10.1016/j.amjcard.2014.05.060

89   Roubtsova, A., Scipione, C.A., Garcon, D., Boffa, M.B., Seidah, N.G., Koschinsky, M.L. et al. (2023) Surface LDLR is a major receptor for lipoprotein(a) clearance in male mice lacking PCSK9. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1868**, 159288 https://doi.org/10.1016/j.bbalip.2023.159288

90   Watts, G.F., Chan, D.C., Somaratne, R., Wasserman, S.M., Scott, R., Marcovina, S.M. et al. (2018) Controlled study of the effect of proprotein convertase subtilisin-kexin type 9 inhibition with evolocumab on lipoprotein(a) particle kinetics. *Eur. Heart J.* **39**, 2577–2585 https://doi.org/10.1093/eurheartj/ehy122

91   Watts, G.F., Chan, D.C., Pang, J., Ma, L., Ying, Q., Aggarwal, S. et al. (2020) PCSK9 inhibition with alirocumab increases the catabolism of lipoprotein (a) particles in statin-treated patients with elevated lipoprotein(a). *Metabolism* **107**, 154221 https://doi.org/10.1016/j.metabol.2020.154221

92   Ying, Q., Chan, D.C., Pang, J., Marcovina, S.M., Barrett, P.H.R. and Watts, G.F. (2022) PCSK9 inhibition with alirocumab decreases plasma lipoprotein (a) concentration by a dual mechanism of action in statin-treated patients with very high apolipoprotein(a) concentration. *J. Intern. Med.* **291**, 870–876 https://doi.org/10.1111/joim.13457

93   Hofmann, S.L., Eaton, D.L., Brown, M.S., McConathy, W.J., Goldstein, J.L. and Hammer, R.E. (1990) Overexpression of human low density lipoprotein receptors leads to accelerated catabolism of Lp(a) lipoprotein in transgenic mice. *J. Clin. Invest.* **85**, 1542–1547 https://doi.org/10.1172/JCI114602

94   Marz, W., Beckmann, A., Scharnagl, H., Siekmeier, R., Mondorf, U., Held, I., et al. (1993) Heterogeneous lipoprotein (a) size isoforms differ by their interaction with the low density lipoprotein receptor and the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor. *FEBS Lett.* **325**, 271–275 https://doi.org/10.1016/0014-5793(93)81087-G

95   Reblin, T., Niemeier, A., Meyer, N., Willnow, T.E., Kronenberg, F., Dieplinger, H. et al. (1997) Cellular uptake of lipoprotein[a] by mouse embryonic fibroblasts via the LDL receptor and the LDL receptor-related protein. *J. Lipid Res.* **38**, 2103–2110 https://doi.org/10.1016/S0022-2275(20)37140-6

96   Bottalico, L.A., Keesler, G.A., Fless, G.M. and Tabas, I. (1993) Cholesterol loading of macrophages leads to marked enhancement of native lipoprotein(a) and apoprotein(a) internalization and degradation. *J. Biol. Chem.* **268**, 8569–8573 https://doi.org/10.1016/S0021-9258(18)52913-6

97   Yang, X.P., Amar, M.J., Vaisman, B., Bocharov, A.V., Vishnyakova, T.G., Freeman, L.A. et al. (2013) Scavenger receptor-BI is a receptor for lipoprotein (a). *J. Lipid Res.* **54**, 2450–2457 https://doi.org/10.1194/jlr.M038877

98   Yang, X., Sethi, A., Yanek, L.R., Knapper, C., Nordestgaard, B.G., Tybjaerg-Hansen, A. et al. (2016) SCARB1 gene variants are associated with the phenotype of combined high high-density lipoprotein cholesterol and high lipoprotein (a). *Circ. Cardiovasc. Genet.* **9**, 408–418 https://doi.org/10.1161/CIRCGENETICS.116.001402

99   Niemeier, A., Willnow, T., Dieplinger, H., Jacobsen, C., Meyer, N., Hilpert, J. et al. (1999) Identification of megalin/gp330 as a receptor for lipoprotein(a) in vitro. *Arterioscler. Thromb. Vasc. Biol.* **19**, 552–561 https://doi.org/10.1161/01.ATV.19.3.552

100   Albers, J.J., Koschinsky, M.L. and Marcovina, S.M. (2007) Evidence mounts for a role of the kidney in lipoprotein(a) catabolism. *Kidney Int.* **71**, 961–962 https://doi.org/10.1038/sj.ki.5002240

101   Sharma, M., Redpath, G.M., Williams, M.J. and McCormick, S.P. (2017) Recycling of apolipoprotein(a) after PlgRKT-mediated endocytosis of lipoprotein (a). *Circ. Res.* **120**, 1091–1102 https://doi.org/10.1161/CIRCRESAHA.116.310272

102   Siddiqui, H., Deo, N., Rutledge, M.T., Williams, M.J.A., Redpath, G.M.I. and McCormick, S.P.A. (2023) Plasminogen receptors promote lipoprotein(a) uptake by enhancing surface binding and facilitating macropinocytosis. *Arterioscler. Thromb. Vasc. Biol.* **43**, 1851–1866 https://doi.org/10.1161/ATVBAHA.123.319344

103   Kerr, M.C. and Teasdale, R.D. (2009) Defining macropinocytosis. *Traffic* **10**, 364–371 https://doi.org/10.1111/j.1600-0854.2009.00878.x

104   Mack, S., Coassin, S., Rueedi, R., Yousri, N.A., Seppala, I., Gieger, C. et al. (2017) A genome-wide association meta-analysis on lipoprotein (a) concentrations adjusted for apolipoprotein (a) isoforms. *J. Lipid Res.* **58**, 1834–1844 https://doi.org/10.1194/jlr.M076232

105   Mehta, A., Jain, V., Saeed, A., Saseen, J.J., Gulati, M., Ballantyne, C.M. et al. (2022) Lipoprotein(a) and ethnicities. *Atherosclerosis* **349**, 42–52 https://doi.org/10.1016/j.atherosclerosis.2022.04.005

106   Lanktree, M.B., Anand, S.S., Yusuf, S., Hegele, R.A. and Investigators, S. (2010) Comprehensive analysis of genomic variation in the LPA locus and its relationship to plasma lipoprotein(a) in South Asians, Chinese, and European Caucasians. *Circ. Cardiovasc. Genet.* **3**, 39–46 https://doi.org/10.1161/CIRCGENETICS.109.907642

107   Coassin, S., Erhart, G., Weissensteiner, H., Eca Guimaraes de Araujo, M., Lamina, C., Schonherr, S. et al. (2017) A novel but frequent variant in LPA KIV-2 is associated with a pronounced Lp(a) and cardiovascular risk reduction. *Eur. Heart J.* **38**, 1823–1831 https://doi.org/10.1093/eurheartj/ehx174

108   Di Maio, S., Gruneis, R., Streiter, G., Lamina, C., Maglione, M., Schoenherr, S. et al. (2020) Investigation of a nonsense mutation located in the complex KIV-2 copy number variation region of apolipoprotein(a) in 10,910 individuals. *Genome Med.* **12**, 74 https://doi.org/10.1186/s13073-020-00771-0

109   Hoekstra, M., Chen, H.Y., Rong, J., Dufresne, L., Yao, J., Guo, X. et al. (2021) Genome-wide association study highlights APOH as a novel locus for lipoprotein(a) levels-brief report. *Arterioscler. Thromb. Vasc. Biol.* **41**, 458–464 https://doi.org/10.1161/ATVBAHA.120.314965

110   Moriarty, P.M., Varvel, S.A., Gordts, P.L., McConnell, J.P. and Tsimikas, S. (2017) Lipoprotein(a) mass levels increase significantly according to APOE genotype: an analysis of 431 239 patients. *Arterioscler. Thromb. Vasc. Biol.* **37**, 580–588 https://doi.org/10.1161/ATVBAHA.116.308704

111   Kochl, S., Fresser, F., Lobentanz, E., Baier, G. and Utermann, G. (1997) Novel interaction of apolipoprotein(a) with beta-2 glycoprotein I mediated by the kringle IV domain. *Blood* **90**, 1482–1489 https://doi.org/10.1182/blood.V90.4.1482

112   Nicholls, S.J., Ruotolo, G., Brewer, H.B., Wang, M.D., Liu, L., Willey, M.B. et al. (2016) Evacetrapib alone or in combination with statins lowers lipoprotein(a) and total and small LDL particle concentrations in mildly hypercholesterolemic patients. *J. Clin. Lipidol.* **10**, 519–527 e4 https://doi.org/10.1016/j.jacl.2015.11.014

113   Thomas, T., Zhou, H., Karmally, W., Ramakrishnan, R., Holleran, S., Liu, Y. et al. (2017) CETP (cholesteryl ester transfer protein) inhibition with anacetrapib decreases production of lipoprotein(a) in mildly hypercholesterolemic subjects. *Arterioscler. Thromb. Vasc. Biol.* **37**, 1770–1775 https://doi.org/10.1161/ATVBAHA.117.309549

114   Arsenault, B.J. and Kamstrup, P.R. (2022) Lipoprotein(a) and cardiovascular and valvular diseases: a genetic epidemiological perspective. *Atherosclerosis* **349**, 7–16 https://doi.org/10.1016/j.atherosclerosis.2022.04.015

115   Boffa, M.B. (2022) Beyond fibrinolysis: the confounding role of Lp(a) in thrombosis. *Atherosclerosis* **349**, 72–81 https://doi.org/10.1016/j.atherosclerosis.2022.04.009

116   Koschinsky, M.L. and Boffa, M.B. (2022) Oxidized phospholipid modification of lipoprotein(a): epidemiology, biochemistry and pathophysiology. *Atherosclerosis* **349**, 92–100 https://doi.org/10.1016/j.atherosclerosis.2022.04.001

117   Boffa, M.B. and Koschinsky, M.L. (2019) Oxidized phospholipids as a unifying theory for lipoprotein(a) and cardiovascular disease. *Nat. Rev. Cardiol.* **16**, 305–318 https://doi.org/10.1038/s41569-018-0153-2

118   Yeang, C., Cotter, B. and Tsimikas, S. (2016) Experimental animal models evaluating the causal role of lipoprotein(a) in atherosclerosis and aortic stenosis. *Cardiovasc. Drugs Ther.* **30**, 75–85 https://doi.org/10.1007/s10557-015-6634-1

119   Assini, J.M., Clark, J.R., Youssef, A., Xing, C., Doerfler, A.M., Park, S.H. et al. (2023) High levels of lipoprotein(a) in transgenic mice exacerbate atherosclerosis and promote vulnerable plaque features in a sex-specific manner. *Atherosclerosis* **384**, 117150 https://doi.org/10.1016/j.atherosclerosis.2023.05.019

120   Kamstrup, P.R., Tybjaerg-Hansen, A. and Nordestgaard, B.G. (2012) Genetic evidence that lipoprotein(a) associates with atherosclerotic stenosis rather than venous thrombosis. *Arterioscler. Thromb. Vasc. Biol.* **32**, 1732–1741 https://doi.org/10.1161/ATVBAHA.112.248765

121   Sangrar, W., Bajzar, L., Nesheim, M.E. and Koschinsky, M.L. (1995) Antifibrinolytic effect of recombinant apolipoprotein(a) in vitro is primarily due to attenuation of tPA-mediated Glu-plasminogen activation. *Biochemistry* **34**, 5151–5157 https://doi.org/10.1021/bi00015a028

122   Hancock, M.A., Boffa, M.B., Marcovina, S.M., Nesheim, M.E. and Koschinsky, M.L. (2003) Inhibition of plasminogen activation by lipoprotein(a): critical domains in apolipoprotein(a) and mechanism of inhibition on fibrin and degraded fibrin surfaces. *J. Biol. Chem.* **278**, 23260–23269 https://doi.org/10.1074/jbc.M302780200

123 Scipione, C.A., McAiney, J.T., Simard, D.J., Bazzi, Z.A., Gemin, M., Romagnuolo, R. et al. (2017) Characterization of the I4399M variant of apolipoprotein(a): implications for altered prothrombotic properties of lipoprotein(a). *J. Thromb. Haemost.* **15**, 1834–1844 https://doi.org/10.1111/jth.13759

124 Boffa, M.B., Marar, T.T., Yeang, C., Viney, N.J., Xia, S., Witztum, J.L. et al. (2019) Potent reduction of plasma lipoprotein (a) with an antisense oligonucleotide in human subjects does not affect ex vivo fibrinolysis. *J. Lipid Res.* **60**, 2082–2089 https://doi.org/10.1194/jlr.P094763

125 Caplice, N.M., Panetta, C., Peterson, T.E., Kleppe, L.S., Mueske, C.S., Kostner, G.M. et al. (2001) Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. *Blood* **98**, 2980–2987 https://doi.org/10.1182/blood.V98.10.2980

126 Etingin, O.R., Hajjar, D.P., Hajjar, K.A., Harpel, P.C. and Nachman, R.L. (1991) Lipoprotein (a) regulates plasminogen activator inhibitor-1 expression in endothelial cells. A potential mechanism in thrombogenesis. *J. Biol. Chem.* **266**, 2459–2465 https://doi.org/10.1016/S0021-9258(18)52266-3

127 Rand, M.L., Sangrar, W., Hancock, M.A., Taylor, D.M., Marcovina, S.M., Packham, M.A. et al. (1998) Apolipoprotein(a) enhances platelet responses to the thrombin receptor-activating peptide SFLLRN. *Arterioscler. Thromb. Vasc. Biol.* **18**, 1393–1399 https://doi.org/10.1161/01.ATV.18.9.1393

128 Cui, K., Wang, H.Y., Yin, D., Zhu, C., Song, W., Wang, H. et al. (2021) Benefit and risk of prolonged dual antiplatelet therapy after percutaneous coronary intervention with drug-eluting stents in patients with elevated lipoprotein(a) concentrations. *Front. Cardiovasc. Med.* **8**, 807925 https://doi.org/10.3389/fcvm.2021.807925

129 Lacaze, P., Bakshi, A., Riaz, M., Polekhina, G., Owen, A., Bhatia, H.S. et al. (2022) Aspirin for primary prevention of cardiovascular events in relation to lipoprotein(a) genotypes. *J. Am. Coll. Cardiol.* **80**, 1287–1298 https://doi.org/10.1016/j.jacc.2022.07.027

130 Langsted, A., Kamstrup, P.R. and Nordestgaard, B.G. (2017) High lipoprotein(a) and low risk of major bleeding in brain and airways in the general population: a Mendelian randomization study. *Clin. Chem.* **63**, 1714–1723 https://doi.org/10.1373/clinchem.2017.276931

131 Tsimikas, S., Bergmark, C., Beyer, R.W., Patel, R., Pattison, J., Miller, E. et al. (2003) Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J. Am. Coll. Cardiol.* **41**, 360–370 https://doi.org/10.1016/S0735-1097(02)02769-9

132 Tsimikas, S., Lau, H.K., Han, K.R., Shortal, B., Miller, E.R., Segev, A. et al. (2004) Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. *Circulation* **109**, 3164–3170 https://doi.org/10.1161/01.CIR.0000130844.01174.55

133 Tsimikas, S., Brilakis, E.S., Miller, E.R., McConnell, J.P., Lennon, R.J., Kornman, K.S. et al. (2005) Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. *N. Engl. J. Med.* **353**, 46–57 https://doi.org/10.1056/NEJMoa043175

134 Tsimikas, S., Kiechl, S., Willeit, J., Mayr, M., Miller, E.R., Kronenberg, F. et al. (2006) Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: five-year prospective results from the Bruneck study. *J. Am. Coll. Cardiol.* **47**, 2219–2228 https://doi.org/10.1016/j.jacc.2006.03.001

135 Rodenburg, J., Vissers, M.N., Wiegman, A., Miller, E.R., Ridker, P.M., Witztum, J.L. et al. (2006) Oxidized low-density lipoprotein in children with familial hypercholesterolemia and unaffected siblings: effect of pravastatin. *J. Am. Coll. Cardiol.* **47**, 1803–1810 https://doi.org/10.1016/j.jacc.2005.12.047

136 Fraley, A.E., Schwartz, G.G., Olsson, A.G., Kinlay, S., Szarek, M., Rifai, N. et al. (2009) Relationship of oxidized phospholipids and biomarkers of oxidized low-density lipoprotein with cardiovascular risk factors, inflammatory biomarkers, and effect of statin therapy in patients with acute coronary syndromes: results from the MIRACL (Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering) trial. *J. Am. Coll. Cardiol.* **53**, 2186–2196 https://doi.org/10.1016/j.jacc.2009.02.041

137 Kiechl, S., Willeit, J., Mayr, M., Viehweider, B., Oberhollenzer, M., Kronenberg, F. et al. (2007) Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. *Arterioscler. Thromb. Vasc. Biol.* **27**, 1788–1795 https://doi.org/10.1161/ATVBAHA.107.145805

138 Ravandi, A., Boekholdt, S.M., Mallat, Z., Talmud, P.J., Kastelein, J.J., Wareham, N.J. et al. (2011) Relationship of IgG and IgM autoantibodies and immune complexes to oxidized LDL with markers of oxidation and inflammation and cardiovascular events: results from the EPIC-Norfolk Study. *J. Lipid Res.* **52**, 1829–1836 https://doi.org/10.1194/jlr.M015776

139 Nsaibia, M.J., Mahmut, A., Boulanger, M.C., Arsenault, B.J., Bouchareb, R., Simard, S. et al. (2016) Autotaxin interacts with lipoprotein(a) and oxidized phospholipids in predicting the risk of calcific aortic valve stenosis in patients with coronary artery disease. *J. Intern. Med.* **280**, 509–517 https://doi.org/10.1111/joim.12519

140 Bouchareb, R., Mahmut, A., Nsaibia, M.J., Boulanger, M.C., Dahou, A., Lepine, J.L. et al. (2015) Autotaxin derived from lipoprotein(a) and valve interstitial cells promotes inflammation and mineralization of the aortic valve. *Circulation* **132**, 677–690 https://doi.org/10.1161/CIRCULATIONAHA.115.016757

141 Rath, M., Niendorf, A., Reblin, T., Dietel, M., Krebber, H.J. and Beisiegel, U. (1989) Detection and quantification of lipoprotein(a) in the arterial wall of 107 coronary bypass patients. *Arteriosclerosis* **9**, 579–592 https://doi.org/10.1161/01.ATV.9.5.579

142 van Dijk, R.A., Kolodgie, F., Ravandi, A., Leibundgut, G., Hu, P.P., Prasad, A. et al. (2012) Differential expression of oxidation-specific epitopes and apolipoprotein(a) in progressing and ruptured human coronary and carotid atherosclerotic lesions. *J. Lipid Res.* **53**, 2773–2790 https://doi.org/10.1194/jlr.P030890

143 van der Valk, F.M., Bekkering, S., Kroon, J., Yeang, C., Van den Bossche, J., van Buul, J.D. et al. (2016) Oxidized phospholipids on lipoprotein(a) elicit arterial wall inflammation and an inflammatory monocyte response in humans. *Circulation* **134**, 611–624 https://doi.org/10.1161/CIRCULATIONAHA.116.020838

144 Schnitzler, J.G., Hoogeveen, R.M., Ali, L., Prange, K.H.M., Waissi, F., van Weeghel, M. et al. (2020) Atherogenic lipoprotein(a) increases vascular glycolysis, thereby facilitating inflammation and leukocyte extravasation. *Circ. Res.* **126**, 1346–1359 https://doi.org/10.1161/CIRCRESAHA.119.316206

145 Cho, T., Jung, Y. and Koschinsky, M.L. (2008) Apolipoprotein(a), through its strong lysine-binding site in KIV(10'), mediates increased endothelial cell contraction and permeability via a Rho/Rho kinase/MYPT1-dependent pathway. *J. Biol. Chem.* **283**, 30503–30512 https://doi.org/10.1074/jbc.M802648200

146 Cho, T., Romagnuolo, R., Scipione, C., Boffa, M.B. and Koschinsky, M.L. (2013) Apolipoprotein(a) stimulates nuclear translocation of beta-catenin: a novel pathogenic mechanism for lipoprotein(a). *Mol. Biol. Cell* **24**, 210–221 https://doi.org/10.1091/mbc.e12-08-0637

147 Zheng, K.H., Tsimikas, S., Pawade, T., Kroon, J., Jenkins, W.S.A., Doris, M.K. et al. (2019) Lipoprotein(a) and oxidized phospholipids promote valve calcification in patients with aortic stenosis. *J. Am. Coll. Cardiol.* **73**, 2150–2162 https://doi.org/10.1016/j.jacc.2019.01.070

148 Schwartz, G.G. and Ballantyne, C.M. (2022) Existing and emerging strategies to lower lipoprotein(a). *Atherosclerosis* **349**, 110–122 https://doi.org/10.1016/j.atherosclerosis.2022.04.020

149 Santos, R.D., Raal, F.J., Catapano, A.L., Witztum, J.L., Steinhagen-Thiessen, E. and Tsimikas, S. (2015) Mipomersen, an antisense oligonucleotide to apolipoprotein B-100, reduces lipoprotein(a) in various populations with hypercholesterolemia: results of 4 phase III trials. *Arterioscler. Thromb. Vasc. Biol.* **35**, 689–699 https://doi.org/10.1161/ATVBAHA.114.304549

150 Samaha, F.F., McKenney, J., Bloedon, L.T., Sasiela, W.J. and Rader, D.J. (2008) Inhibition of microsomal triglyceride transfer protein alone or with ezetimibe in patients with moderate hypercholesterolemia. *Nat. Clin. Pract. Cardiovasc. Med.* **5**, 497–505 https://doi.org/10.1038/ncpcardio1250

151 Parish, S., Hopewell, J.C., Hill, M.R., Marcovina, S., Valdez-Marquez, E., Haynes, R. et al. (2018) Impact of apolipoprotein(a) isoform size on Lp(a) lowering in the HPS2-THRIVE study. *Circ. Genom. Precis. Med.* **11**, e001696 https://doi.org/10.1161/CIRCGEN.117.001696

152 Boffa, M.B. and Koschinsky, M.L. (2019) Proprotein convertase subtilisin/kexin type 9 inhibitors and lipoprotein(a)-mediated risk of atherosclerotic cardiovascular disease: more than meets the eye? *Curr. Opin. Lipidol.* **30**, 428–437 https://doi.org/10.1097/MOL.0000000000000641

153 Tsimikas, S., Moriarty, P.M. and Stroes, E.S. (2021) Emerging RNA therapeutics to lower blood levels of Lp(a): JACC focus seminar 2/4. *J. Am. Coll. Cardiol.* **77**, 1576–1589 https://doi.org/10.1016/j.jacc.2021.01.051

154 Tsimikas, S., Karwatowska-Prokopczuk, E., Gouni-Berthold, I., Tardif, J.C., Baum, S.J., Steinhagen-Thiessen, E. et al. (2020) Lipoprotein(a) reduction in persons with cardiovascular disease. *N. Engl. J. Med.* **382**, 244–255 https://doi.org/10.1056/NEJMoa1905239

155 Nicholls, S.J. (2024) Therapeutic potential of lipoprotein(a) inhibitors. *Drugs* **84**, 637–643 https://doi.org/10.1007/s40265-024-02046-z

156 Diaz, N., Perez, C., Escribano, A.M., Sanz, G., Priego, J., Lafuente, C. et al. (2024) Discovery of potent small-molecule inhibitors of lipoprotein(a) formation. *Nature* **629**, 945–950 https://doi.org/10.1038/s41586-024-07387-z

157 Nicholls, S.J., Nissen, S.E., Fleming, C., Urva, S., Suico, J., Berg, P.H. et al. (2023) Muvalaplin, an oral small molecule inhibitor of lipoprotein(a) formation: a randomized clinical trial. *JAMA* **330**, 1042–1053 https://doi.org/10.1001/jama.2023.16503

158 Berglund, L. and Ramakrishnan, R. (2004) Lipoprotein(a): an elusive cardiovascular risk factor. *Arterioscler. Thromb. Vasc. Biol.* **24**, 2219–2226 https://doi.org/10.1161/01.ATV.0000144010.55563.63

159 Wilson, D.P., Jacobson, T.A., Jones, P.H., Koschinsky, M.L., McNeal, C.J., Nordestgaard, B.G. et al. (2019) Use of lipoprotein(a) in clinical practice: a biomarker whose time has come. A scientific statement from the National Lipid Association. *J. Clin. Lipidol.* **13**, 374–392 https://doi.org/10.1016/j.jacl.2019.04.010

160 Arsenault, B.J. (2019) Exposure to low lipoprotein(a) levels: key to healthy aging? *J. Am. Coll. Cardiol.* **74**, 2995–2997 https://doi.org/10.1016/j.jacc.2019.06.083

161 Yeung, M.W., Said, M.A., van de Vegte, Y.J., Verweij, N., Dullaart, R.P.F. and van der Harst, P. (2024) Associations of very low lipoprotein(a) levels with risks of new-onset diabetes and non-alcoholic liver disease. *Atheroscler. Plus* **57**, 19–25 https://doi.org/10.1016/j.athplu.2024.07.001