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# Preventing CVD: treatment of elevated lipoprotein(a) with respect to LPA gene isoform type

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BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

**PREVENTING CVD:**

**TREATMENT OF ELEVATED LIPOPROTEIN(A)**

**WITH RESPECT TO LPA GENE ISOFORM TYPE**

by

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B.S., The University of Texas at Austin, 2022

Submitted in partial fulfillment of the

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**PREVENTING CVD:  
TREATMENT OF ELEVATED LIPOPROTEIN(A)  
WITH RESPECT TO LPA GENE ISOFORM TYPE**

**ALEXIS BROOKS**

**ABSTRACT**

Lipoprotein(a) [Lp(a)] is a unique low density lipoprotein consisting of an apolipoprotein(a) and apolipoprotein B subunit that is an established genetic risk factor for atherosclerotic cardiovascular disease (ASCVD). Current therapeutic options for inherited elevated circulating Lp(a) are generally limited to off-target effects of other cardiovascular therapies, making treatment for this condition difficult. This work evaluates a variety of established cardiovascular treatments and their impact on Lp(a) plasma concentration while taking into consideration potential impacts of Lp(a) isoform types on treatment response. It also assesses the value of Lp(a) lowering therapies based on their relative cardioprotective benefit, lowering or preventing occurrences of major adverse cardiovascular events (MACE) and ASCVD.

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## LIST OF ABBREVIATIONS

|                  |   |
|------------------|---|
| apo(a) .....     | Apolipoprotein(a)                             |
| apoB .....       | Apolipoprotein B                              |
| ASCVD .....      | Atherosclerotic Cardiovascular Disease        |
| CVD .....        | Cardiovascular Disease                        |
| KIV2 .....       | Kringle IV type 2                             |
| LDL .....        | Low Density Lipoprotein                       |
| LDLR .....       | LDL Receptor                                  |
| Lp(a) .....      | Lipoprotein(a)                                |
| <i>LPA</i> ..... | apo(a) gene                                   |
| MACE .....       | Major Adverse Cardiovascular Events           |
| OxPL .....       | Oxidized Phospholipid                         |
| PCSK9 .....      | Proprotein Convertase Subtilisin/Kexin Type 9 |
| SMC .....        | Smooth Muscle Cell                            |
| SNP .....        | Single Nucleotide Polymorphism                |

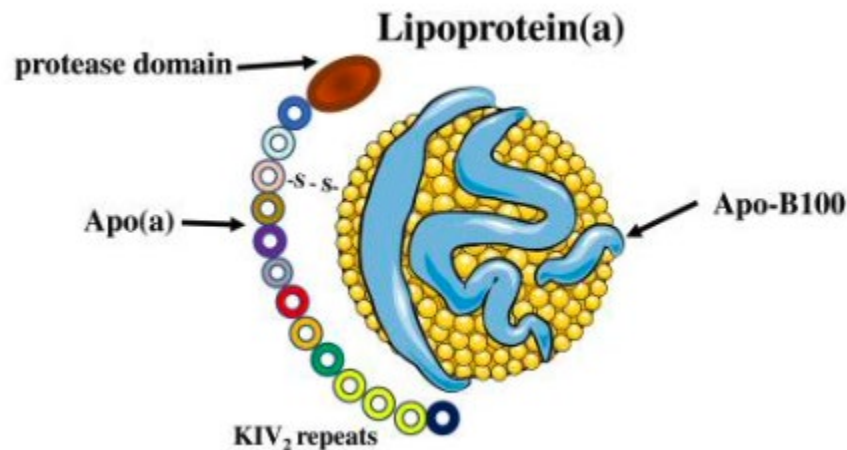
## INTRODUCTION

Certain lipoprotein(a) gene variants are recognized as a significant hereditary risk factor for cardiovascular disease and are estimated to impact more than 20% of the world's population (Wilson *et al.*, 2019). These variants result in elevated plasma Lp(a) concentration which currently has no direct targeted therapies despite being modulated by a single gene. Other lipoproteins such as LDL have been well defined in cardiovascular studies and their heritability is determined by more than 160 genes yet they have been awarded a myriad of therapies (Graham *et al.*, 2021). Regardless, high Lp(a) levels pose a threat for ASCVD, MACE, and early mortality independent of LDL levels and should be established as a necessary subject for treatment.

### *Structure and Genetic Background of Lipoprotein(a)*

Lipoprotein(a) is a complex of lipids and proteins found in blood plasma, consisting of a low-density lipoprotein core swathed in an apolipoprotein B with a single disulfide bond linking it to an encircling apolipoprotein(a) molecule. Though similar in approximate size, Lp(a) distinguishes itself from standard LDL cholesterol by its highly variable apo(a) segment but is represented in measurements of LDL serum level due to its core. The apo(a) molecule maintains

a standard structure comprised of 10 different types of kringle IV domains (KIV) and one kringle V bound to an inactive protease domain (Figure 1) (Reyes-Soffer *et al.*, 2022).

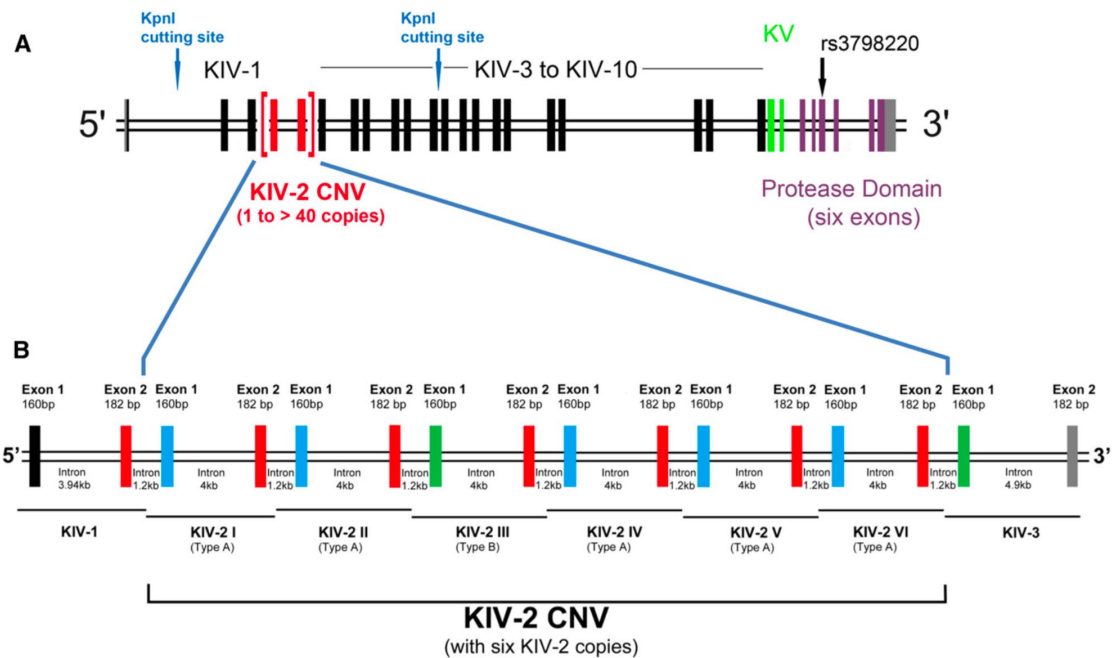


**Figure 1. Lipoprotein(a) structure**

Lipoprotein(a) shown with all essential components: LDL core, apoB subunit, apo(a) subunit, KIV<sub>2</sub> repeats, protease domain, and disulfide bond. Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (<https://creativecommons.org/licenses/by/3.0/>).

The Lp(a) glycoprotein is only afforded a singular copy of KIV<sub>1</sub> and KIV<sub>3</sub>-KIV<sub>10</sub> (Figure 2A) but can have a multitude of KIV<sub>2</sub> copies which create its distinct structural signature (Koschinsky *et al.*, 1997). Apo(a) is encoded by the *LPA* gene, a more than 130 kb stretch that resides on the reverse strand of chromosome 6q27 and is synthesized in liver hepatocytes; the Lp(a) molecule is then fully assembled in a manner still yet to be defined (Schmidt *et al.*, 2016). Size polymorphisms created by variation in KIV<sub>2</sub> copy number are directly

responsible for apo(a)'s isoforms which have been shown to impact the level of circulating Lp(a), establishing the *LPA* gene as a primary determinant of Lp(a) size and plasma concentration (Romagnuolo *et al.*, 2017). Plasma concentrations of lipoprotein(a) are 75% to 95% heritable and vary between individuals based on their specific isoform type (Trinder *et al.*, 2020). The number of KIV<sub>2</sub> repeats present in the *LPA* gene ranges from 10 to 50 (Figure 2B) which can correlate to a plasma Lp(a) concentration of 0.1 mg/dl to more than 200 mg/dl (Schmidt *et al.*, 2016). Variations in KIV<sub>2</sub> copy number have produced more than 40 distinct alleles and are of particular interest because the direct heritability of plasma Lp(a) concentration is so potent and is not significantly impacted by changes in diet and exercise (Yeang *et al.*, 2016).



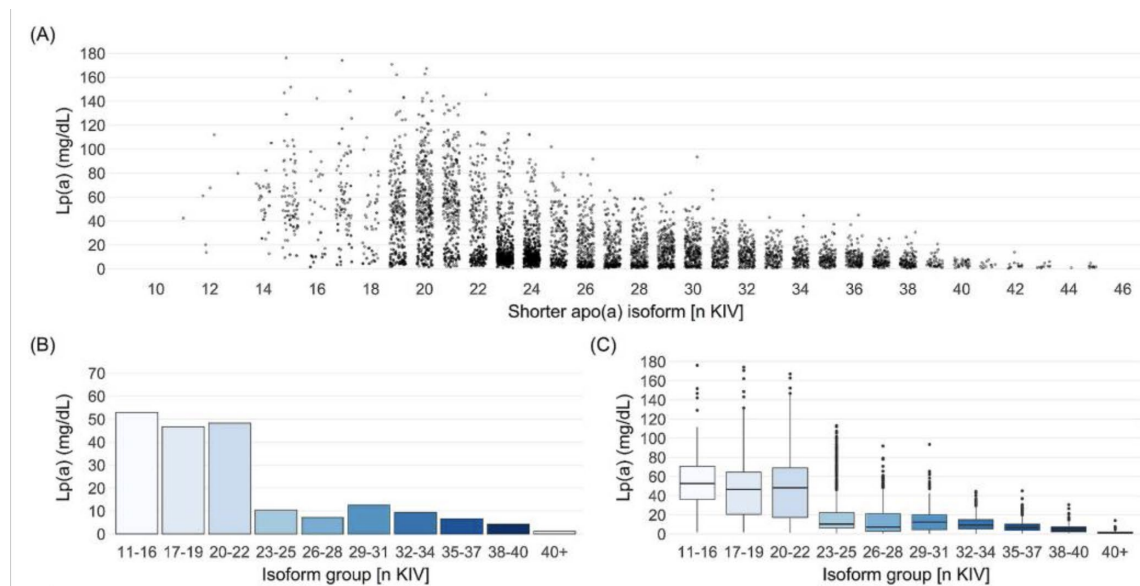
## Figure 2. *LPA* gene exon-intron structure

A. *LPA* gene structure with exons of each KIV domain. All KIV domains contain 2 exons while the protease domain contains 6.

B. Portion of *LPA* gene containing 6 KIV<sub>2</sub> copy number variants. Each one consists of a 4 kb intron flanked by two exons and these units are separated by a 1.2 kb intron. This example shows several variations of exon 1 that have distinct synonymous mutations while the majority of copies of exon 2 are identical. One KIV<sub>2</sub> copy contains an exon 1 that is identical to that of KIV<sub>3</sub> while all copies of KIV<sub>2</sub> contain copies of exon 2 that are identical to that of KIV<sub>1</sub>. Taken from (Schmidt *et al.*, 2016).

It has been demonstrated that apo(a) isoforms with greater KIV<sub>2</sub> copy number (over 22) result in lower overall Lp(a) plasma concentration whereas those with fewer copies of KIV<sub>2</sub> result in a marked increase in Lp(a) plasma concentration (Figure 3). The inverse relationship between isoform size and Lp(a) plasma concentration is a result of the hepatocyte's ability to produce smaller apo(a)

particles at a much higher rate than large particles as well as experiencing an increased rate of secretion (Lampsas *et al.*, 2023). Greater KIV<sub>2</sub> copy number and lower KIV<sub>2</sub> copy number can also be referred to as high molecular weight (HMW) and low molecular weight (LMW) isoforms, respectively.



**Figure 3. Isoform variation across a European population.**

(A) Lp(a) concentration by isoform group. (B) Median isoform groups. (C) Variance in each group based on data shown in 1B. Taken from (Coassin *et al.*, 2022).

When discussing the impact of allele heritability it is important to consider the role of heterozygosity in the expressed concentration of Lp(a) in circulation.

Inheritance of alleles of different isoform sizes does not result in expression of only one copy and instead produces two distinct types of Lp(a) lipoprotein in circulation with the LMW allele appearing at a significantly greater concentration than that of the HMW allele (Kraft *et al.*, 1992). Risk for atherosclerotic

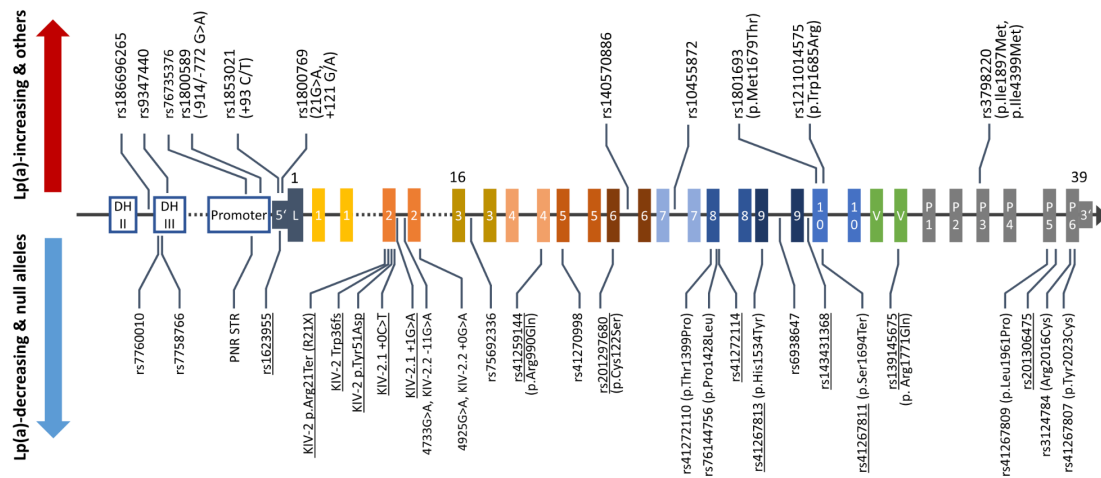
cardiovascular disease associated with plasma Lp(a) concentration has been established by the ACC/AHA 2018 Clinical Practice Guidelines as concentrations  $\geq 50$  mg/dL but has been more recently broken down into borderline risk 14-30 mg/dL, high risk 31-50 mg/dL, and highest risk greater than 50 mg/dL (Grundy *et al.*, 2018 & Farzam *et al.*, 2022).

### ***Role of SNPs***

The inverse relationship illustrated between isoform size and Lp(a) plasma concentration has been continuously observed across populations throughout the study of Lp(a) with some discrepancies due to genetic variation caused by single nucleotide polymorphisms (SNPs) (Coassin *et al.*, 2022). These SNPs often cause a dramatic decrease in Lp(a) concentrations (Figure 4) but are generally constrained to specific isoforms with frequencies that are specific to certain ethnic gene pools (Schmidt *et al.*, 2016). Notorious SNPs rs10455872 and rs3798220 have been recorded to show up most commonly in LMW isoforms, resulting in higher plasma Lp(a) concentration; rs10455872 being associated with alleles containing 17-20 KIV<sub>2</sub> repeats and rs3798220 with alleles containing 19-21 KIV<sub>2</sub> repeats. rs10455872 is found within intron 25 of the *LPA* gene with an allele frequency of 7% while rs3798220 is an Ile4399Met missense mutation in the

region containing the inactive protease domain and has an allele frequency of only 2%. Interestingly, rs10455872 and rs3798220 have been reported to not be in linkage disequilibrium despite only being 49 kb apart (Yi *et al.*, 2011). Each of these SNPs have been found to be independently linked with an increased risk of coronary disease as indicated by the Precocious Coronary Artery Disease (PROCARDIS) study where rs10455872 accounted for 25% and rs3798220 accounted for 8% of variation in plasma Lp(a) levels analyzed but collectively explained 36% of total variation in plasma Lp(a) levels during joint analysis (Clarke *et al.*, 2009). Carriers of either one or two copies of the rs3798220 SNP see a 57% increase in their risk for CHD whereas rs10455872 SNP carriers see a 42% increase in risk per copy. rs3798220 allele carriers face significant CHD risk compared to those without the allele as rs3798220 has been linked to severe coronary stenosis based on angiographic data as well as association with myocardial infarction. rs3798220 SNP carriers are also reported to have higher levels of oxidized phospholipids, thought to be a result of a longer half-life that allows Lp(a) more time to remain in circulation and thus further the chance for oxidation (Arai *et al.*, 2010). Other theories suggest that the oxidation of the specific methionine residue of this allele might thwart Lp(a) catabolism

analogous to the behavior of methionine oxidation in apolipoprotein A-I (Yi *et al.*, 2011).



**Figure 4. Prominent Lp(a) SNPs**

Lp(a) gene labeled with the most common SNPs and their locations within the gene. Portions of the gene representing different kringle domains are labeled by color and number. Whether the SNP causes relative increase or decrease in Lp(a) concentration is labeled.

Taken from (Coassin *et al.*, 2022).

SNP rs186696265 has had the largest impact on Lp(a) concentrations to date, raising them by about 49 mg/dL and increasing the likelihood of coronary artery disease by 1.73 fold but fortunately has a low minor allele frequency of about 1.5% (Mack *et al.*, 2017). Some SNPs such as rs1800769 have been found to increase promoter activity yet result in lower Lp(a) plasma concentration due to the SNP's association with HMW isoforms in Europeans and Mexican Americans while associating with smaller isoforms and higher Lp(a) plasma concentration in African Americans (Coassin *et al.*, 2022).

### *Plasminogen Homology*

Central to understanding the threat of Lp(a) is the remarkable homology of the apo(a) segment with plasminogen, suggesting a recent divergence of their respective controlling genes. The plasminogen *PLG* gene contains a serine protease, kringle IV, and kringle V akin to those of Lp(a) but differs with the additional kringles I-III in its structure (Figure 5A). The KIV<sub>10</sub> of Lp(a) is most closely related to the KIV of plasminogen as both contain a lysine binding site, allowing for binding to a variety of substrates (Koschinsky *et al.*, 1997).

Additionally, the *PLG* gene resides approximately 40 kb from the *LPA* locus on chromosome 6q26-27 (Figure 5B), where the two exhibit strong genetic linkage (Drayna *et al.*, 1988).



### *Atherosclerotic and Prothrombotic Behavior*

Mainstream discussions of atherosclerotic plaquing and ASCVD have historically placed blame on LDL, cementing it as the main perpetrator of poor patient outcomes. However, this ideology has overlooked the unique role of Lp(a) in atherosclerotic plaque formation and the fact that these plaques have been shown to contain more apo(a) than apoB as well as result in an elevation in circulating apo(a) levels as atherosclerotic plaquing progresses (Sabarinath *et al.*, 2015). As apo(a) is a unique feature of Lp(a), this directly confirms that Lp(a) poses a more significant threat in plaque formation than other apoB containing molecules such as LDL (van Dijk *et al.*, 2014).

Higher levels of Lp(a) have been linked to an increased risk of cardiovascular disease and stroke, often attributed to the atherosclerotic and thrombotic characteristics of this lipoprotein. Thought to occur because of the strong structural homology of its apo(a) segment, Lp(a) has been found to thwart fibrinolysis by competing with plasminogen for binding to t-PA and reducing plasminogen activation and therefore plasmin production (Boffa *et al.*, 2016). This binding competition also extends to the endothelial cell surface receptors annexin II and alpha enolase, preventing pericellular plasminogen activation (Pellegrino *et al.*, 2004) as well as increasing plasminogen activator inhibitor 1 (PAI-1)

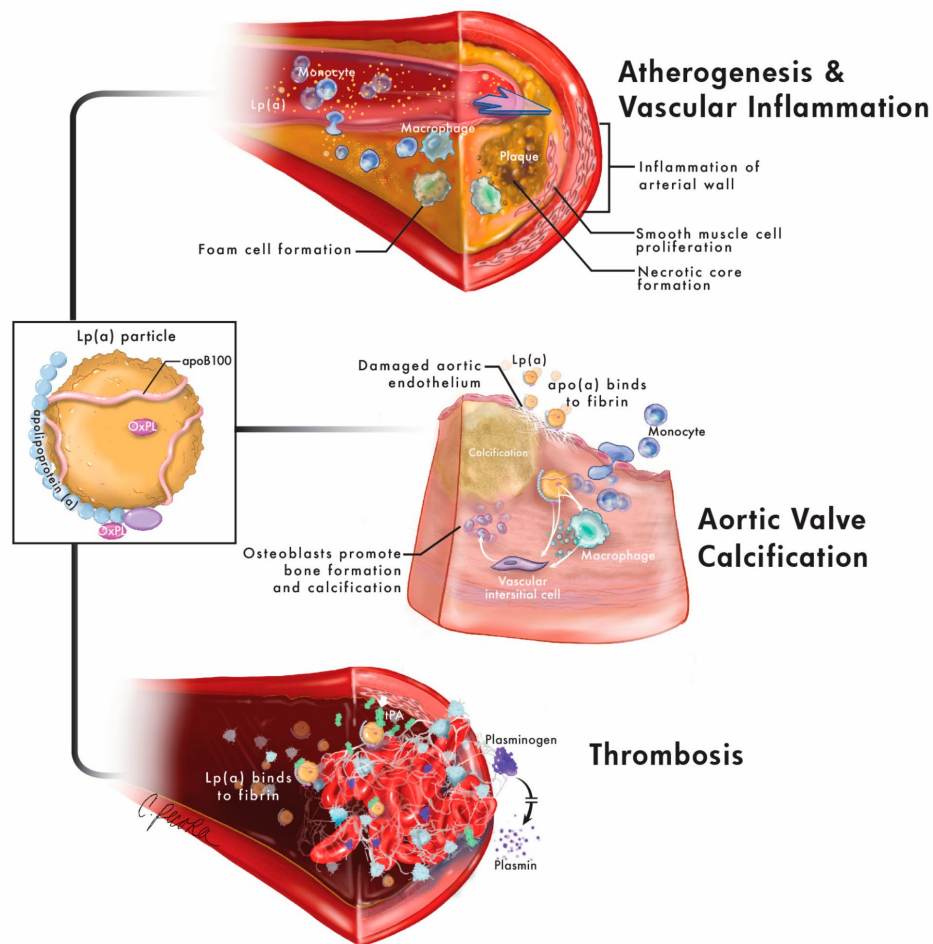
expression within endothelial cells. Alternatively, Lp(a) further promotes thrombogenesis by adhering to plasminogen binding sites on fibrin, colocalizing to the arterial wall, and preventing fibrin degradation as well as taking up residence within the intima (Kreuzer *et al.*, 1993).

Lp(a)'s affinity for fibrin is determined by isoform size and illustrates clear preference for LMW isoforms but independently requires the presence of the lysine binding site of KIV10; absence of this lysine binding site completely negates Lp(a)'s ability to bind fibrin and t-PA irrespective of isoform size (Koschinsky *et al.*, 1997). Lp(a) has also been observed to enhance Tissue Factor (TF) production within cell membranes as well as facilitating binding to TF pathway inhibitor in a lysine-dependent manner, directly disrupting cell surface TFPI activity without involving plasminogen (Caplice *et al.*, 2001). By thwarting endothelial thrombolytic functions, Lp(a) is able to migrate through the endothelial gaps and accumulate in intima and subintima, where fibrin turnover is most frequent (Kreuzer *et al.*, 1993). Inhibition of plasminogen activation by t-PA has also been noted to increase smooth muscle cell proliferation and migration by terminating latent transforming growth factor B (TGF-B) activation (Koschinsky *et al.*, 1997). Additionally, the plasminogen receptor KT has also been thought to be involved in the internalization of Lp(a) by cells within the

liver, subsequently leading to the recycling of the apo(a) component (Sharma *et al.*, 2016).

Beyond fibrin perturbation (Figure 6), Lp(a) utilizes a variety of mechanisms to impede endothelium integrity and promote atherosclerotic plaque formation.

Lp(a) has a distinct preference for extracellular matrix components (ECM) of the vessel wall and its ability to anchor to them is made possible by the apo(a) lysine binding sites. Such components include the proteoglycan decorin whose core forms a hydrophobic bond to the apo(a) segment, indicating a distinct affinity for Lp(a) within the arterial wall separate from plain LDL. Additionally, defensin proteins tend to aggregate with Lp(a) within plaques and the complex is then unable to migrate through the cytoplasmic membrane of endothelial cells which maintains Lp(a) extracellularly (Lampsas *et al.*, 2023). Lp(a) demonstrates such pathogenicity when it migrates from circulation into the arterial wall, concentrating in the extracellular space of the tunica intima and subintima (Kreuzer *et al.*, 1993).



**Figure 6. Lp(a) role in plaque formation and thrombogenesis**

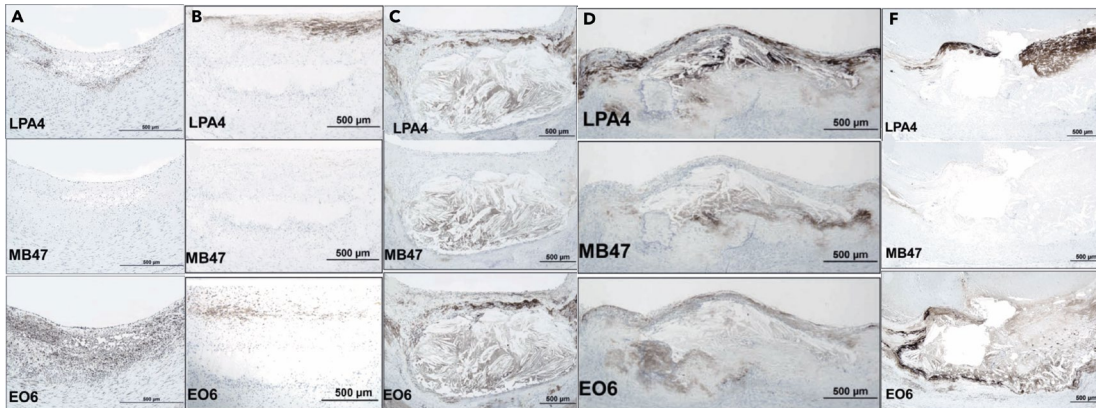
Lp(a) exacerbates thrombogenesis by binding to plasminogen sites on fibrin, co-localizing in the arterial wall, impeding fibrin degradation, and establishing itself within the intima. Taken from (Bhatia *et al.*, 2022)

The apo(a) component of Lp(a) has been shown to activate a Rho/Rho kinase dependent signaling cascade within the endothelial cells of the vasculature that triggers an increase in myosin light chain phosphorylation and rearrangement of actin cytoskeleton as well as disruption of VE-cadherin and reappportioning of focal adhesion molecules (Pellegrino *et al.*, 2004). The unique disruption of the

actin cytoskeleton, VE-cadherin, and focal adhesion molecules by apo(a) collectively permit endothelial transmigration of the Lp(a) glycoprotein through the adherens junctions between endothelial cells. This activity was experimentally compared to LDL, whose long established method of transfer through the endothelium is transcytosis, which failed to exhibit the same behavior (Pellegrino *et al.*, 2004).

Lp(a) has been termed the preferential carrier for phosphocholine containing oxidized phospholipids (OxPL) which together act to induce apoptosis in vascular macrophages as well as stimulating inflammation in endothelial cells (Lamon-Fava *et al.*, 2011). The atherosclerotic function of Lp(a) appears to be influenced primarily by its capacity to transport and distribute OxPL directly to tissue targets rather than its LDL moiety. These distributed OxPL are then converted to lysophosphatidic acid by autotaxin so that they may traverse the endothelium and induce inflammation (Koschinsky *et al.*, 2022). This action is exhibited most often by smaller Lp(a) isoforms as they appear to adhere to OxPLs to a greater degree and can move through vessel wall components more efficiently because of their enhanced binding affinity for lysine and fibrin (Erqou *et al.*, 2010) inducing human endothelial cells to release monocyte chemotactic protein.

When Lp(a) interacts with ECM components such as fibronectin and heparin it becomes integrated into macrophages, forming foam cells. Macrophages secrete sphingomyelinase, which, in conjunction with lipoprotein lipase, promotes the binding of LDL and Lp(a) to smooth muscle cells (SMCs) and ECM (Lampsas *et al.*, 2023). SMCs migrate within the arterial wall to create the fibrous cap (Figure 6) and are able to proliferate as Lp(a) blocks plasminogen activation thereby disabling plasmin facilitated activation of TGF- $\beta$ , a necessary inhibitor of vascular SMC growth. Foam cells containing large amounts of OxPL secrete inflammatory components such as IL-8, IL-1 $\beta$ , and TNF- $\alpha$  that amplify inflammatory response elements within the arterial wall, further promoting vascular cell wall damage and leading to necrosis. Together, these mechanisms contribute to the formation of atherosclerotic plaques and their subsequent necrosis and instability.



**Figure 7. Morphometric patterns of immunostaining in atherosclerotic plaques**  
 (A) Immunostaining of human coronary artery intima early superficial lesion; Lp(a) select macrophage expression, apoB-100 absence, OxPL high expression. (B) Early fibroatheroma; Lp(a) ECM fibrous cap expression, apoB-100 absence, OxPL expression in macrophages of fibrous cap. (C) Fibroatheroma with late necrosis; Lp(a) expression ECM of fibrous cap and necrotic core, apoB-100 weak staining at necrotic core, OxPL expression ECM of fibrous cap and necrotic core. (D) TCFA or high-risk plaque for rupture; Lp(a) high expression in fibrous cap and ECM of necrotic core, apoB100 localized at necrotic core/acellular areas, OxPL fibrous cap macrophages and ECM in periphery of necrotic core. (E) Plaque rupture; Lp(a) high fibrous cap expression, apoB-100 negative, OxPL expression macrophages in periphery of necrotic core. Taken from (Van Dijk *et al.*, 2014).

In analyzing components of arterial lesions at different points of progression, apo(a), macrophages, and OxPL were found to increase over time but their relative composition among plaques was dependent on plaque type (van Dijk *et al.*, 2014). Apo(a) was detected in both early and advanced lesions utilizing the IgG antibody LPA4, with a particular concentration observed in foam cell and macrophages and the necrotic core, although early lesions saw insignificant presence (Figure 7B and A). Areas with prominent smooth muscle cell and macrophage localization showed significant apo(a) and OxPL (detected with

antibody E06) expression. Alternatively, immunostaining showed hardly any apoB-100 (detected with antibody MB47) expression in the fibrous cap compared to apo(a) and OxPL (Figure 7B). Specifically, staining of the necrotic core of ruptured plaques exhibited the presence of apo(a) in 57% of the area followed by OxPL at 60% (Figure 7E) (van Dijk *et al.*, 2014).

### ***CLEARANCE***

Lp(a) clearance is defined as an ongoing investigation that has been best informed by investigating the efficacy of various cardiovascular treatments designed for other conditions. Research shows that Lp(a) plasma concentration demonstrates significant correlation with Lp(a) production rate yet appears negligible in relation to the fractional catabolic rate. This behavior is functionally distinctly from LDL, whose plasma concentration is modulated specifically by clearance rather than production (Reyes-Soffer *et al.*, 2017). In both human and cell culture studies, synthesis rates of apo(a) have been shown to be dependent on isoform size thereby directly regulating plasma Lp(a) concentration. In contrast, Lp(a) catabolism rates showed no statistical difference across isoform sizes thus affirming that the size of the apo(a) protein does not influence the catabolic rate of its resultant Lp(a) particle (Rader *et al.*, 1994).

However, investigation of the mechanisms behind Lp(a) clearance has remained a topic of interest. Studies have pinpointed the plasminogen receptor with a C-terminal lysine (PlgRKT) as a facilitator of Lp(a) uptake in liver cells, unveiling a new pathway for Lp(a) endocytosis by incorporating various receptors for plasminogen, which amplify surface binding and provoke macropinocytosis. This finding provides significant insight into mechanisms that might be utilized for development of targeted therapies for elevated plasma Lp(a) that are independent of plasma LDL clearance (Siddiqui et al., 2023).

## SPECIFIC AIMS

This work aims to outline the relevance of Lipoprotein(a) as a contributor to CVD and MACE as well as advocate for consideration of patient LPA gene isoform type when investigating interventional treatment options to ensure the greatest reduction in cardiovascular risk for every individual patient.

Additionally, the efficacy of current and future potential treatment options with regard to isoform type is assessed and evaluated in order to shed light on the necessity of individual approach to treatment. In this work, ASCVD can be defined as peripheral arterial disease, coronary artery disease, myocardial infarction, ischemic stroke, and cardiovascular mortality. Additionally, standards for measuring Lp(a) concentrations include units of mg/dL and nmol/L; both measurements are used throughout the studies referenced in this discussion for the sake of continuity and comparability. To convert between either measurement, nmol/L concentration is multiplied by 0.4 to achieve mg/dL concentration while mg/dL is divided by 0.4 to achieve nmol/L concentration.

## DISCUSSION

At present, there are no specific targeted therapies available to address elevated Lp(a) levels, although the efficacy of established treatments for ASCVD in reducing Lp(a) concentrations have been investigated. It is important to consider that Lp(a) reduction alone is not the ultimate fix for lowering ASCVD risk and that while protective it should be combined with other preventative measures. Studies have shown that there is a positive correlation between increases in Lp(a) and established risk for ASCVD. Specifically, every 20 mg/dL (50 nmol/L) increase in plasma Lp(a) results in a direct 11% increase in risk for ASCVD, regardless of differences in risk factors. While the average range of Lp(a) concentrations is not uniform spanning different ancestry groups, the percent ASCVD risk attributed to increases in concentration has been shown to be congruent, corresponding to hazard ratios of 1.07-1.11 per 20 mg/dL increase (Patel *et al.*, 2021).

A variety of studies utilizing mendelian randomization have investigated the relative reduction level in Lp(a) concentration required in order to achieve significant decreases in the risk of ASCVD and MACE. While no precise amount has been determined, researchers suggest that absolute reduction should fall between 50 to 100 mg/dL minimum in order to obtain cardioprotective benefits

that are clinically relevant (Kronenberg *et al.*, 2022). Measurements for Lp(a) concentration (n=69,764), LPA KIV<sub>2</sub> repeats (n=98,810), and LPA rs10455872 genotype (n=119,094) were conducted in two studies involving the general population of Denmark. An elevated Lp(a) level exceeding 93 mg/dL (233 nmol/L), compared to levels below 10 mg/dL (25 nmol/L), demonstrated an associated hazard ratio of 1.50 for CVD mortality and 1.20 for all-cause mortality. Additionally, an increase of 50 mg/dL (125 nmol/L) in Lp(a) levels corresponded to a hazard ratio of 1.16. Stroke risk hazard ratio was 1.60, with each 50 mg/dL (125 nmol/L) rise in Lp(a) levels associated with a hazard ratio of 1.20 (Lamon-Fava *et al.*, 2011). In all, Lp(a) presents a very real threat to cardiovascular health and currently has little to no viable treatment options, making it arguably a greater hazard than LDL.

## Treatments

| Treatment                  | Mechanism of action   | Lowers Lp(a) level | Overall cardioprotective benefit |
|----------------------------|---|--------------------|----------------------------------|
| Niacin                     | Inhibits triacylglycerol synthesis in hepatocytes thereby increasing apoB degradation | Yes                | No                               |
| Apheresis                  | Immunoabsorption column with Lp(a) antibody   | Yes                | Yes                              |
| Statins                    | Inhibits HMG-CoA reductase in hepatocytes thereby reducing cholesterol synthesis      | No                 | Yes                              |
| Ezetimibe                  | Inhibits dietary cholesterol absorption by enterocytes of small intestine             | Somewhat           | Yes                              |
| Aspirin                    | Prevents platelet aggregation and potentially inhibits <i>LPA</i> gene transcription  | Yes                | Yes                              |
| Monoclonal Antibodies      | Blocks PCSK9 protein from binding to LDLR and tagging it for degradation              | Yes                | Yes                              |
| Antisense Oligonucleotides | Targets <i>LPA</i> mRNA within hepatocyte and degrades it                             | Yes                | Not yet known                    |

**Table 1. Summary of Treatments**

### *Niacin*

For many years, Niacin (nicotinic acid) has been utilized as a therapy for raising HDL and lowering LDL, generally added to an established statin regimen.

Mechanistically, the action of Niacin with respect to Lp(a) is ill informed but is hypothesized to lower Lp(a) concentrations by reducing the ability of hepatocytes to secrete lipoproteins with apoB components as it does with LDL (Fazio *et al.*, 2012). However, in the HPS2-THRIVE randomized clinical trial it

was confirmed that Niacin had no significant benefit in preventing cardiovascular events or lowering risk for CVD (Landray *et al.*, 2014). In fact, niacin was determined to pose a greater risk to patients due to adverse side effects and was officially withdrawn from care recommendation by the European Medicines Agency (Cegla *et al.*, 2019). Despite this, niacin was one of the only established treatments for elevated Lp(a) concentration for some time so it is worth investigating its impact.

In the AIM-HIGH trial Niacin was shown to reduce Lp(a) levels by an overall average of 21% across 1,424 patients in the treatment group; patients in the 90th percentile for Lp(a) concentration (greater than 50 mg/dL or 125 nmol/L) saw a 64% decrease while those in the 50th percentile saw a decrease of 20%. It is important to note that these patients had no significant reduction in cardiovascular events after three years despite reductions in Lp(a) as well as maintaining target LDL cholesterol levels through simvastatin administration and raising HDL cholesterol (Albers *et al.*, 2013).

The Lp(a) substudy of the HPS2-THRIVE randomized clinical trial organized nearly 4,000 patients into 5 subgroups established by isoform type informed by KIV<sub>2</sub> domains as well as baseline Lp(a) level. The trial included 2,227 white

participants from the United Kingdom where the largest established subgroup was those with less than 18 KIV<sub>2</sub> domains and 1,701 participants from China where the largest established subgroup was those with more than 30 KIV<sub>2</sub> domains. Percentage reduction of log<sub>e</sub> Lp(a) level after treatment with niacin-laropiprant for one year was evaluated and showed significant variation across subgroups where those with isoforms containing 18 to 22 or less than 17 KIV<sub>2</sub> domains saw a 15% or 16% reduction from baseline while subgroups containing 23 to 26, 27 to 30, or more than 30 KIV<sub>2</sub> domains saw a 29%, 38%, and 50% reduction from baseline, respectively. Assessing percentage reduction of Lp(a) by subgroups sorted by baseline Lp(a) concentration revealed an 18% or 22% reduction from baseline for those with an initial Lp(a) concentration of 42 to 128 nmol/L or greater than 128 nmol/L, while reductions of 35%, 40%, and 36% were seen in those with Lp(a) concentrations of 17 to 42 nmol/L, 17 to 7 nmol/L, or less than 7 nmol/L, respectively. Ultimately, the trend in Lp(a) reduction with respect to isoform type illustrated greater significance with a  $P_{\text{trend}}$  of  $4 \times 10^{-29}$  than that of baseline Lp(a) concentration with a  $P_{\text{trend}}$  of  $2 \times 10^{-8}$ . From these trends, the overall percent reduction was determined to be 31% but when data was separated by geographic region, the percent reduction was significantly higher in China at

38% than in the United Kingdom at 25% due to the propensity of large isoforms in those with Chinese ancestry (Parish *et al.*, 2018).

It is important to recognize that the absolute reduction of Lp(a) concentration demonstrated a different variation by subgroup, where those with large isoforms saw reductions between 4 and 8 nmol/L while those with small isoforms saw reductions between 13 and 30 nmol/L. Similarly, those with low baseline Lp(a) concentration saw absolute reductions between 1 and 7 nmol/L while those with high baseline Lp(a) concentration saw absolute reductions between 16 and 34 nmol/L. In all, the absolute reduction in this case displayed increases as baseline Lp(a) concentration increased but did not trend proportionately, leaving the overall absolute reduction at 12.2 nmol/L. The study concluded that Lp(a) reductions by niacin-laropiprant were significantly associated with isoform size and that patients with larger isoforms saw the greatest percent reduction with this specific treatment but this activity did not provide significant protection from cardiovascular events (Parish *et al.*, 2018).

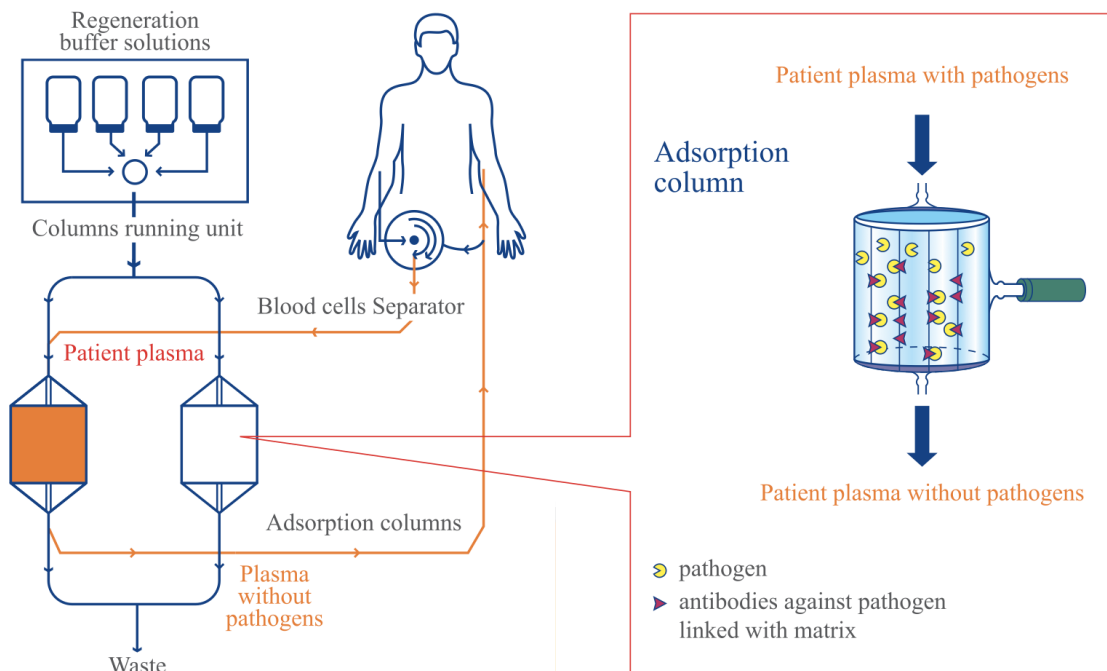
These absolute reductions inform the percent reductions but can also be used to address the relative benefit of treatment administration by comparing the absolute reduction from baseline Lp(a) concentration post treatment to the

clinical practice guideline cutoffs (the study measured Lp(a) levels in nmol/L which differs from the guidelines but these measurements can be converted to these approximate levels: borderline risk 35-75 nmol/L, high risk 77.5-125 nmol/L, and highest risk greater than 125 nmol/L) to establish whether or not individual patients with a specific size isoform size are the best candidate for such a treatment. Although Niacin was a standard available therapy for elevated Lp(a) for some time, its use was ultimately discontinued due to severe adverse effects and the revelation that it provided no significant protection against ASCVD (Julius *et al.*, 2013).

### *Apheresis*

Lipoprotein apheresis is a filtration technique defined by the separation of blood cells from plasma and subsequently lipoproteins and other macromolecules by using molecule specific filters. While lipoprotein apheresis follows a general structure, the specific macromolecule filters allow for various lipoproteins to be withheld based on their molecular structure. Specifically, Lp(a) is most commonly retained using broad apoB-100 filter systems that retain molecules based on size or charge like heparin-induced extracorporeal LDL precipitation (HELP), direct adsorption of lipoproteins (DALI), Liposorber, and TheraSorb.

These systems are useful for patients with both elevated LDL and Lp(a) that have failed other lipoprotein lowering therapies and meet the criteria for apheresis indication. For patients with dangerously high plasma Lp(a) concentrations who have LDL levels under control (usually by way of statin therapy), the immunoabsorption column Lp(a) Lipopak can be used to selectively remove Lp(a) particles via an apo(a) antibody without causing significant disruption to the levels of other blood lipoproteins (Feingold *et al.*, 2023). This process is defined by an initial separation of blood cells from plasma via a venous access followed by movement of the plasma into one of two absorption columns containing agarose beads with sheep polyclonal antibodies against apo(a). There the antibodies expunge Lp(a) particles from the plasma and the stripped plasma is reintegrated with the blood cells exiting the original cell separator. As the processed whole blood is returned to the patient through an opposing venous access point, the second adsorption column begins this process on another cycle of separated plasma while the first column is cleansed of Lp(a) using regeneration buffer solution and prepared to complete another cycle (Figure 8). The use of two adsorption columns allows for continuous filtration without concern for overwhelming the binding capacity of the apo(a) antibody (Pokrovsky *et al.*, 2020).



**Figure 8. Immunoadsorption lipoprotein apheresis model (Lp(a) Lipopak)**

Lipopak employs venous access to separate blood cells from plasma, utilizing agarose beads coated with sheep polyclonal antibodies against apo(a). In this system, the antibodies remove Lp(a) particles from the plasma and purified plasma is then reintegrated with the blood cells which exit the initial cell separator. The processed whole blood is then returned to the patient through an opposing venous access point. Simultaneously, the second adsorption column initiates a new cycle of plasma separation, while the first column undergoes cleansing of Lp(a) using a regeneration buffer solution preparing it for another cycle. Taken from (Lp(a) Lipopak® www.pocard.ru).

In a small cohort of 30 participants with established CHD, plasma Lp(a) concentration greater than 50 mg/dL, and LDL below 100 mg/dL on atorvastatin; 15 patients were concurrently administered weekly lipoprotein apheresis using the Lp(a) Lipopak system while the other 15 remained on atorvastatin monotherapy for 18 months. Following each apheresis treatment session, plasma Lp(a) concentrations decreased by an average of  $73 \pm 12\%$  from baseline, yielding

an average concentration of  $29 \pm 16$  mg/dL. The concluding apheresis treatment resulted in a terminal reduction of  $31.7 \pm 22.3$  mg/dL in the Lp(a) Lipopak group whereas the atorvastatin monotherapy control group saw an overall increase of  $4.8 \pm 10.8$  mg/dL from baseline Lp(a). To assess the efficacy of apheresis treatment beyond just a reduction in Lp(a) levels, the mean intima media thickness of the carotid arteries was measured at baseline and then again at 9 and 18 months on treatment. The Lp(a) Lipopak treatment group saw a significant reduction in carotid mean intima media thickness of  $-0.03 \pm 0.09$  mm at 9 months and  $-0.07 \pm 0.15$  mm at 18 months from a baseline of  $0.88 \pm 0.30$  mm. By comparison, the control group saw no significant changes during the study period. Importantly, regression of the carotid mean intima media thickness was apparent in 17 out of 30 measured carotid segments of the treatment group while the control only saw regression in 8 out of 30 segments. Two years after the completion of the study and cessation of apheresis treatments, carotid mean intima media thickness was once again measured. The treatment group saw an average increase of  $0.02 \pm 0.08$  mm while the control saw an average increase of  $0.06 \pm 0.10$  mm. Regression or stabilization was seen in 20 out of 28 carotid segments in Lp(a) Lipopak treatment patients (one patient had inadequate imaging and was removed from analysis) and in 11 out of 30 carotid segments

in control patients. The majority of apheresis patients experienced improved angina symptoms and half no longer had positive stress tests by the end of the study whereas controls saw an increase in angina and majority maintained positive stress test status (Ezhov *et al.*, 2015). Prior literature revealed that changes in carotid mean intima media thickness up to 0.1 mm translated to elevation in risk for myocardial infarction or stroke by 15% and 18%, respectively (Lorenz *et al.*, 2007). It has been demonstrated that among patients with elevated Lp(a) levels and no targeted treatment there is a yearly rate of CIMT progression of 0.03 mm. A direct correlation was identified between the decline in Lp(a) concentration during scheduled visits and the reduction in the mean CIMT (Ezhov *et al.*, 2015).

Unlike other treatments, lipoprotein apheresis has shown no isoform specific activity and appears to lower plasma Lp(a) concentration by more than 50% (Howell *et al.*, 2015). While it has proven to be very effective at reducing cardiovascular risk by way of physically removing lipoproteins from the blood, it is still considered a last resort treatment because it is both time consuming and expensive. The indications for lipoprotein apheresis include stringent requirements such as 6 months of failing maximum tolerated drug therapy while having familial hypercholesterolemia and LDL greater than 300 mg/dL, familial

hypercholesterolemia and LDL greater than 200 mg/dL with Lp(a) greater than 50 mg/dL, or familial hypercholesterolemia and LDL greater than 160 mg/dL with severe CHD, CVD, or diabetes. These conditions are modified by the presence of additional risk factors and ultimately create a significant barrier to approval for treatment (Feingold *et al.*, 2023).

In a study specifically focused on patients with high Lp(a) levels, 170 participants were entered based on Lp(a) levels above 60 mg/dL and history of progressive CVD in the two years prior to the study. The majority of patients were men with a history of statin administration at the time of admission to the study and had an average Lp(a) concentration of approximately 105 mg/dL as well as an average LDL cholesterol of 99 mg/dL. They were then observed during two years of lipoprotein apheresis (using Lipidfiltration, HELP, DALI, Monet, Liposorber LA & DL, and TheraSorbLDL techniques) for occurrences of MACE and had plasma lipoproteins measured every 6 months. Immediately after each session, recorded Lp(a) concentrations showed reduction by almost 70% from pretreatment levels but tended to recover to around 80% of pretreatment levels (Leebmann *et al.*, 2013). This behavior highlights the temporary benefit of apheresis and its drawback as a permanent source of treatment for patients with severe Lp(a), especially as some patients still experience outcomes that fall

outside the standard response to lipoprotein apheresis treatment. Despite this, lipoprotein apheresis remains a valuable last resort treatment for patients suffering from severe elevated Lp(a) and offers cardioprotective benefits regardless of isoform type.

### ***Statins***

The standard preliminary treatment for elevated LDL cholesterol is statin-based therapy which provides a significant reduction in CVD risk. However, statins have been shown to actually increase Lp(a) blood plasma concentration by 10% to 20% with a mean increase of 11% but the mechanistic action behind this activity is still under investigation (Yeang *et al.*, 2016). More recent studies using the human hepatoma cell line (HepG2) treated with 5 or 10  $\mu\text{M}$  of atorvastatin or pravastatin for 24 hours revealed significant changes in the expression of *LPA* and *PCSK9*. Expression of *LPA* mRNA increased by 50% in atorvastatin treated cells under both doses while 5  $\mu\text{M}$  of pravastatin increased expression by 90% and 10  $\mu\text{M}$  of pravastatin increased expression by 70% compared to controls. Atorvastatin appeared to increase expression of the *PCSK9* mRNA by 50% while pravastatin more than doubled expression (Tsimikas *et al.*, 2020). These pronounced increases in *LPA* and *PCSK9* mRNA synthesis may yet be

responsible for the upward trend in Lp(a) concentration upon administration of statins. Additionally, this can create issues in patients with undiagnosed high Lp(a) plasma concentration as they may experience adequate LDL cholesterol reduction but are unknowingly increasing their risk for ASCVD and potential for MACE. Instances in which patients fail to sufficiently respond to statin therapy and LDL levels remain elevated might even be attributed to undiagnosed excessive Lp(a) plasma concentration because Lp(a) is included in circulating LDL measurements. It is established that statins upregulate expression of the LDL receptor in order to increase LDL clearance but even with its LDL core, Lp(a) does not appear to be positively impacted by this upregulation. In the case that significant reductions in LDL cholesterol are attained, elevated Lp(a) still creates strong risk for ASCVD, making statin treatment alone inadequate for managing elevated Lp(a). In fact, individuals with baseline Lp(a) blood plasma above 180 mg/dL are thought to have a lifetime risk for severe atherosclerosis and CVD that parallels those with familial hypercholesterolemia, a more well known genetic cholesterol issue (Ruscica *et al.*, 2020).

When considering whether or not statin therapy is truly right for patients with high levels of Lp(a), a variety of statin based clinical trials can be compared in their individual assessments of LDL versus Lp(a) lowering and consequent

cardiovascular protection. The AIM-HIGH trial reported that individuals who attained LDL levels of 65.2 mg/dL (from statin therapy alone or with niacin) and possessed Lp(a) levels exceeding 50 mg/dL experienced an 89% increased likelihood of experiencing MACE compared to those with comparable LDL levels with lower Lp(a). In the JUPITER trial, individuals who reached an LDL-C level of 55.0 mg/dl and had Lp(a) exceeding 54 nmol/l (approximately 21 mg/dL) faced a 71% elevated risk of MACEs; whereas the LIPID study was defined by patients who attained LDL-C levels above 112 mg/dl and had Lp(a) exceeding 73.7 mg/dL experienced a 23% higher incidence of MACEs. When comparing data across these statin based clinical trials, the role of Lp(a) as an independent contributor to MACE can be clearly established. This assessment stems from a weighted hazard ratio of 1.61 that is associated with levels of LDL at 89.1 mg/dL and Lp(a) of 54.9 mg/dL. At this level of Lp(a), risk reduction can only be demonstrated by an average reduction of more than 50%, that in which statin therapy simply cannot achieve (Tsimikas *et al.*, 2017). The ILLUMINATE trial affirmed that atorvastatin administration resulted in a positive dose dependent increase in circulating Lp(a) levels in patients with significant CVD risk. It specifically found that patients with higher baseline LDL who required higher atorvastatin doses also had high baseline Lp(a) and their reduction of free LDL

came at the expense of elevated Lp(a) (Arsenault *et al.*, 2017). Yet even with the established detriments of statin usage on Lp(a) concentration and the evidence that elevated Lp(a) poses a significant threat regardless of whether LDL is controlled, a statement by the European Atherosclerosis Society has evaluated the cardioprotective benefit of statin therapy as outweighing any dangers from elevated Lp(a) (Kronenberg *et al.*, 2022).

While broad genetic studies have not been executed for statin based therapy, the SNP rs10455872 in the *LPA* gene has been shown to have strong influence in lowering LDL in patients on statin therapy. Specifically, patients with the minor allele of rs10455872 have demonstrated reductions in LDL that are 5.9% smaller than noncarriers. This activity has even been shown to differ between those heterozygous and homozygous for this minor allele, having a doubling effect in homozygous carriers. However, this reduction was analyzed in a breakdown of LDL subfractions (large, medium, small and very small) and there was no association between the allele's impact on reduction and any changes in LDL subfractions (Postmus *et al.*, 2014). Similarly, the rs10455872 SNP has been shown to increase the risk of coronary heart disease events by 58% (Wei *et al.*, 2018). When compared to Lp(a) level reductions, researchers determined that there was congruent LDL reduction and that the observed relative change in LDL was due

to the nature of Lp(a) reduction impacting the overall measurement of LDL and not the rs10455872 SNP being associated with individual LDL particles itself. The rs10455872 SNP was also recognized as being strongly associated with circulating plasma Lp(a) levels and copy number variants of KIV<sub>2</sub>. It was followed by higher Lp(a) levels and a higher percentage of Lp(a) falling into total LDL measurements because of Lp(a)'s LDL core which prevents any benefit from statin therapy such as increased clearance from being realized (Postmus *et al.*, 2014).

### ***Ezetimibe***

Ezetimibe, known more commonly under the brand name Zetia, is a blood cholesterol lowering medication that acts by inhibiting cholesterol absorption through the transmembrane sterol transporter Niemann Pick C1-Like1 in enterocytes of the small intestine. This activity prevents cholesterol from being dispatched to the liver and lowers the available cholesterol stored in hepatocytes. While ezetimibe has been shown to reduce plasma LDL cholesterol by 14-25% when administered in addition to a statin, it still provides significant reduction when used as a monotherapy, achieving a reduction of about 17% (Sahebkar *et al.*, 2018). Few studies have effectively evaluated ezetimibe's impact on plasma

Lp(a) concentration but one analysis suggested that daily administration of 10 mg ezetimibe resulting in a 7% reduction should be considered significant despite acknowledging the lack of CVD protection this level of reduction provides (Awad *et al.*, 2018).

A variety of clinical trials have established the benefit of ezetimibe as a cardioprotective drug, both with or without a statin. The IMPROVE-IT trial specifically noted that rates of myocardial infarction and ischemic stroke decreased significantly in ezetimibe treatment groups and plasma LDL levels fell by 16% when 10 mg was added to daily 40 mg simvastatin (Cannon *et al.*, 2015). Because the structural nature of Lp(a) includes an LDL particle as its core, researchers hypothesized that part of the observed LDL reduction might include decreases in Lp(a) that registered under plasma LDL level measurements as well (Sahebkar *et al.*, 2018).

A comprehensive meta analysis of 10 studies evaluated the effect of ezetimibe on plasma Lp(a) concentrations, assessing this for ezetimibe as a monotherapy as well as in combination with a statin. The analysis included randomized controlled clinical trials with 5,188 total study participants who received either treatment or placebo over periods of 5 to 12 weeks. From these studies, effect size was calculated using the formula: (measure at the end of follow-up in the

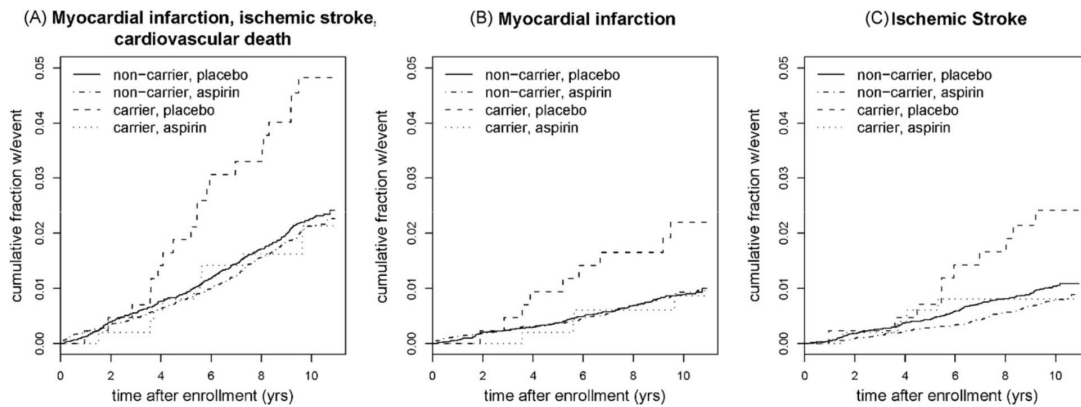
treatment group – measure at baseline in the treatment group) – (measure at the end of follow-up in the control group – measure at baseline in the control group) (Sahebkar *et al.*, 2018). To effectively evaluate this data, the resulting values were then communicated as the weighted mean difference (WMD) with a 95% confidence interval and subjected to a sensitivity analysis to ensure accurate analysis. From this it was determined that ezetimibe produced no significant change in plasma Lp(a) concentration with an overall WMD of -2.59%, suggesting that there was little to no difference in plasma Lp(a) concentration before and after treatment across all 10 studies. Similarly, trials that focused on ezetimibe as a monotherapy versus placebo produced a WMD of -4.64% while those where ezetimibe was combined with a statin versus plain statin therapy produced a WMD of -1.04%. Interestingly, this study suggests that differences in the Lp(a) lowering effect of ezetimibe may be attributed to participant BMI as certain studies where individuals had an average BMI of 29 reported reductions that were significant while those where individuals had an average BMI of 26 reported that ezetimibe had a negligent impact on Lp(a) levels (Sahebkar *et al.*, 2018). Overall, ezetimibe has not been shown to provide clinically significant reductions in plasma Lp(a) concentration but remains a useful cardioprotective medication otherwise.

## *Aspirin*

Aspirin is a nonsteroidal anti-inflammatory drug that prevents the aggregation of platelets. Given the prothrombotic nature of Lp(a), aspirin is recommended for patients with elevated plasma Lp(a) due to its antiplatelet properties. It has also been suggested that aspirin might modestly decrease Lp(a) levels by inhibiting *LPA* gene transcription and thereby apo(a) expression but these findings are limited in scope and warrant additional validation through larger studies (Saeed *et al.*, 2021). A well studied SNP within the *LPA* gene known as rs3798220 has been established as an isoleucine to methionine substitution within the protease-like domain of apo(a) and has been associated with both elevated plasma levels of Lp(a) and increased risk for cardiovascular disease. While the protease-like domain is inactive, it heavily resembles the active domain within the plasminogen gene and mutations within it have been suggested to potentially contribute to the prothrombotic role of Lp(a) (Boffa *et al.*, 2016). The rs3798220 SNP has been linked to elevated Lp(a) levels, heightened clot density, and prolonged clot lysis times specifically in heterozygous carriers of Caucasian descent. However, the opposite is seen in non Caucasian carriers, who display increased clot permeability, shorter lysis times, and lack notable rise in Lp(a) levels (Rowland *et al.*, 2014).

In a randomized clinical trial, 25,131 initially healthy caucasian women 45 years or older were monitored over 10 years for cardiovascular events. The focal population for analysis in this study was characterized by both their rs3798220 SNP genotype and baseline Lp(a) level. Of these women, 906 (3.6%) were heterozygotes while 15 (0.06%) were homozygotes for the minor allele (those possessing the rs3798220 SNP) of rs3798220, resulting in a minor allele frequency of 1.9%. On the other hand, the median Lp(a) levels in heterozygous and homozygous carriers of the minor allele were 79.5 mg/dL and 153.9 mg/dL, while the median Lp(a) level of non-carriers was only 10 mg/dL. This meant that possessing a copy of the minor allele could result in Lp(a) levels 8 to 15 times higher than that of non-carriers, putting them at significantly greater risk for MACE (Chasman *et al.*, 2008). Because only a small number of women among this population were actually carriers of the minor allele, a subset was formed that had 486 minor allele carriers randomly assigned to aspirin and 417 minor allele carriers randomly assigned to placebo. Subsequently, all women in the study were prospectively tracked for the occurrence of an initial major cardiovascular event, defined as myocardial infarction, ischemic stroke, and/or cardiovascular death. These participants were administered aspirin or placebo in order to assess the efficacy of aspirin for primary prevention of cardiovascular

disease as well as how it was impacted by the presence or lack of the rs3798220 SNP.



**Figure 9. MACE in rs3798220 SNP patient groups**

Cumulative fraction estimations using Kaplan-Meier for Caucasian women experiencing an initial major cardiovascular event with respect to rs3798220 SNP carrier status and treatment group. (A) Myocardial infarction, ischemic stroke, cardiovascular death (B) Myocardial infarction (C) Ischemic stroke. Taken from (Chasman *et al.*, 2008).

To evaluate the link between the rs3798220 SNP and the risk of major incident cardiovascular disease, this study utilized Cox proportional hazards models.

These models estimated hazard ratios by comparing homozygotes of the major allele (those lacking the rs3798220 SNP) to either only heterozygotes containing one minor allele and one major allele, or all carriers of the minor allele. In the 10 years following the trial assignments, 510 women experienced a first ever major cardiovascular event of myocardial infarction, ischemic stroke, and/or cardiovascular death. Of the 12,379 women in the placebo group, there were 270 that experienced a major cardiovascular event; 251 were homozygous for the

major allele and 19 were heterozygous carrying one minor allele. Importantly, there was no report of major cardiovascular events for those homozygous for the minor allele. Compared to non-carriers, women carrying the minor allele with the rs3798220 SNP were more than twice as likely to experience a major cardiovascular event with an age adjusted hazard ratio of 2.22; whereas women carrying the minor allele with the rs3798220 SNP who were administered aspirin had an age adjusted hazard ratio of 0.44. This indicated that aspirin usage offered significant protective benefit for SNP carriers, providing a 56% reduction in risk of major cardiovascular events. The absolute risk for experiencing major cardiovascular events associated with being a carrier of the rs3798220 SNP after ten years was 2.14% for participants treated with aspirin in contrast to those treated with placebo, who had an absolute risk of 4.83% (Figure 9). Conversely, participants not carrying the rs3798220 SNP allele had an age adjusted hazard ratio for aspirin compared to placebo of 0.91. In addition, the absolute risk for participants treated with aspirin was 2.13% while for those treated with placebo it was 2.25% (Chasman *et al.*, 2008).

This study provided significant insight into the benefit of genotype specific medication administration, illustrating how patients with the highest risk for MACE due to rs3798220 SNP allele carrier status and high Lp(a) plasma

concentration saw the greatest benefit from treatment with aspirin, reducing their absolute risk from 4.83% to 2.14%, bringing event risk rate to a level comparable to non carriers who also had low plasma Lp(a) concentration.

### *Monoclonal antibodies*

At present, there are no specific targeted therapies available to address elevated Lp(a) levels, but research indicates that inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9) may lead to a significant reduction in plasma Lp(a). However, the unintended effects of PCSK9 inhibitors on Lp(a) clearance have been studied and the varying response profiles observed across patient populations emphasize the need to consider Lp(a) isoforms when determining the best course of action for treatment in patients at risk for ASCVD.

The exact mechanisms by which circulating Lp(a) is cleared from plasma is a source of contention amongst researchers; fluctuations in clearance, evident in patients using PCSK9 inhibitors, prompt further exploration into the involvement of the LDLR in this process. PCSK9 inhibitors function by impeding the interaction of PCSK9 proteins with the active site, preventing the destruction of hepatocyte surface LDLR, and subsequently enhancing LDL uptake from plasma. While the mechanism is well understood for LDL, the clearance of Lp(a)

remains an area of investigation, with its primary contributors still unidentified and the LDLR showing only situational participation. (Romagnuolo *et al.*, 2017). To tackle this issue, a research investigation employed established human cell lines, along with human and mouse hepatocytes. These cells underwent transient transfection using plasmids that carried different types of the LDLR, recombinant LPA genes, and recombinant PCSK9 genes. Subsequently, an internalization assay with purified Lp(a) was administered to assess the outcomes. This study employed western blotting, using either mouse-anti human apo(a) a5 antibody or mouse-anti human  $\beta$ -actin, to assess the success of internalization followed by qRT-PCR to measure expression of mRNA in hepatocytes. The clearance of apo(a) isoforms 12K, 17K, 23K, and 30K was observed, while 23K and 30K isoforms exhibited less clearance in the presence of PCSK9 compared to smaller isoforms, without significant difference in mean decreases. Overexpression of the LDLR when PCSK9 was inhibited in human hepatocytes was found to increase apo(a) internalization by about 60% while Lp(a) internalization was shown to increase in mouse hepatocytes by about 90%, with both cases decreasing significantly if PCSK9 is present (Romagnuolo *et al.*, 2017). However, this finding was compared to conclusions from PCSK9 inhibitor clinical trials where greater decreases in Lp(a) blood plasma concentration were

seen in patients with lower initial Lp(a) while absolute decrease was greater in those with high initial Lp(a).

The overexpression of LDLR, when PCSK9 was inhibited in human hepatocytes, resulted in a 60% increase in apo(a) internalization. Similarly, in mouse hepatocytes, Lp(a) internalization increased by about 90%. However, both cases showed a significant decrease if PCSK9 was present. This finding contrasts with conclusions from PCSK9 inhibitor clinical trials, where greater decreases in Lp(a) concentration were observed in patients with lower initial Lp(a) while the absolute decrease was greater in those with high initial Lp(a). With respect to apo(a) isoform size, this suggests a potential conflict with the results, as higher initial Lp(a) concentration correlates with smaller isoform size. The observed increase in apo(a) and Lp(a) clearance when LDLR is overexpressed or PCSK9 is inhibited underscores the LDLR's role as a viable means of uptake for Lp(a), but only when LDLR is abundant (Romagnuolo et al., 2017).

Similarly, the LDLR's involvement in Lp(a) uptake was highlighted in the FOURIER clinical trial, where the administration of the PCSK9 inhibitor Evolocumab was linked to a decrease in Lp(a) plasma concentration. The trial focused on patients with a history of CVD and other risk factors indicating a potential for CVD. Baseline profiles containing LDL levels and non-HDL levels

as well as current high-level statin therapy use, were collected from participants. Lp(a) assessment was conducted using an isoform-independent immunoturbidimetric assay on blood samples taken at 12, 24, and 48 weeks. The reductions in plasma Lp(a) concentration varied with regard to baseline levels; absolute reduction trended upward in patients with high baseline Lp(a) while percent reduction decreased with an average reduction of 27%. There was a significant reduction in MACE in cases with higher baseline Lp(a), particularly those exceeding the median and overall reduction amounted to 16%. The consideration of specific isoform sizes was not part of this study's analysis, as PCSK9 inhibitors were not originally designed to directly affect Lp(a) blood plasma concentration. However, the observed differences in absolute versus percent reduction in cases with high baseline plasma Lp(a) concentration were attributed to potential challenges in clearing small isoforms. This suggests a need for future investigations focusing on specific isoform types and their clearance levels. Additionally, it was theorized that a decrease in Lp(a) assembly could be an additional factor contributing to the decline in Lp(a) clearance. The secretion of free apo(a) was observed to significantly increase in the presence of PCSK9 proteins but subsequently decreased upon the administration of Evolocumab (O'Donoghue et al., 2019). Considering the variation in reduction concerning

baseline plasma Lp(a) concentration, a more in-depth exploration of the relationship between isoform types and the achieved reduction could enhance confidence in prescribing PCSK9 inhibitors for effectively treating high Lp(a) in suitable candidates.

It's hypothesized that LDLRs primarily impact Lp(a) clearance when hepatic levels of the receptor are substantially elevated and LDL-C levels are low, such as in PCSK9 inhibitor therapy. Thus, there is potential for the length of apo(a) isoforms to affect how Lp(a) is cleared in blood plasma after LDLR upregulation (Julius *et al.*, 2019). However, it is suggested that a decrease in Lp(a) concentrations due to PCSK9 inhibitors might not be solely due to an impact on Lp(a) catabolism but rather they might attenuate assembly instead; this could provide a new window of explanation into the link between apo(a) isoform size and relative decreases in Lp(a) concentration by PCSK9 (Blanchard *et al.*, 2021). In a German study, 268 adults with hypercholesterolemia and extremely high CVD risk, heterozygous FH, or LDL level persistently above therapeutic target despite prior maximum therapy were recruited to investigate whether Lp(a) isoforms altered PCSK9 inhibitor efficacy in lowering plasma Lp(a) concentration.

Recruited patients had LDL levels of  $160 \pm 62$  mg/dL with total cholesterol at  $237 \pm 71$  mg/dL with measurements of Lp(a) concentration skewed amongst the cohort and apo(a) isoforms varying with a median of 24 KIV domains.

Concentrations of apoB100 were elevated but not with relation to Lp(a), instead with LDL and other lipoprotein forms. Patients were divided into subgroups based on PCSK9 inhibitor type and dosage; these included 126 patients receiving Evolocumab 140 mg, 89 patients receiving Alirocumab 150 mg, and 53 patients receiving Alirocumab 75 mg every two weeks. Of the 268 patients, 122 were recorded to have two different apo(a) isoforms while 129 had a single apo(a) isoform; only 17 patients were unable to genotype through western blotting.

When assessing the correlation between apo(a) isoform size and absolute reduction, the group of patients with a single apo(a) isoform and those with two different apo(a) isoforms were both reported to have a significant positive correlation as a result of PCSK9 inhibitor treatment.

Of the various sized isoforms, the smaller isoforms were found to have a more significant positive correlation with absolute reduction than that of the larger isoforms. Patients possessing two different isoform types were analyzed for the relative amount of Lp(a) particles for each isoform type before and after one month of treatment with PCSK9 inhibitors. This revealed that the larger isoform

was present at 29% at baseline and dropped to 15% after treatment. While the majority of patients saw a decrease in the larger isoform, a small group of patients saw a preferential decrease in the presence of the smaller isoform. There was an overall decrease in Lp(a) plasma concentration of  $36\% \pm 35\%$  and an absolute reduction of  $41\% \pm 62\%$ . Greater absolute and percent reductions were seen in patients treated with Evolocumab 140 mg than Alirocumab 150 mg or 75 mg. Collectively, this data suggests that apo(a) isoform size directly contributes to the reductions in Lp(a) concentration made by PCSK9 inhibitors (Blanchard *et al.*, 2021).

### *Antisense Oligonucleotides*

The future of treatments for Lp(a) has begun with the antisense oligonucleotide AKCEA-APO(a)-LR<sub>x</sub> also known as Pelacarsen. This drug acts by targeting *LPA* mRNA in the hepatocyte thereby preventing apo(a) molecule production and thereby Lp(a) assembly. In the 2B portion of the Lp(a) HORIZON trial, a total of 286 patients with established CVD and Lp(a) concentration exceeding 60 mg/dL (150 nmol/L) were enrolled in the study. Average baseline Lp(a) concentration ranged from 204.5 to 246.6 nmol/L (81.8 to 98.64 mg/dL) and average baseline LDL levels concentration measured at 70 to 80 mg/dL for this patient population

despite prior treatments with several different lipid lowering therapies at once. These patients were administered the hepatocyte directed antisense oligonucleotide Pelacarsen at various dosages: 20 mg, 40 mg, or 60 mg every 4 weeks; 20 mg every 2 weeks; or 20 mg every week (resulting in cumulative monthly doses of 20, 40, 60, and 80 mg) (Tsimikas *et al.*, 2020). Alternatively, some patients were administered a saline placebo subcutaneously over a duration of 6 to 12 months. The primary objective of the study was to evaluate the change in Lp(a) levels from baseline to the end of the study. The end was defined as week 25 for the groups receiving monthly doses or week 27 for the groups receiving more frequent doses (Tsimikas *et al.*, 2020).

| Lp(a) HORIZON Trial Patient Lp(a) Change From Baseline |                     |                      |                      |                      |                      |                         |
|--|---------------------|----------------------|----------------------|----------------------|----------------------|-------------------------|
| Mean Lp(a) concentration $\pm$ SD                      | 20 mg/Q4W (n = 48)  | 40 mg/Q4W (n = 48)   | 60 mg/Q4W (n = 47)   | 20 mg/Q2W (n = 48)   | 20 mg/QW (n = 48)    | Pooled placebo (n = 47) |
| Baseline (nmol/L)                                      | 246.6 (179.2-300.8) | 220.0 (176.5-283.3)  | 204.5 (163.8-286.5)  | 238.2 (183.7-298.4)  | 233.7 (193.1-275.3)  | 231.6 (194.9-317.7)     |
| Absolute reduction (nmol/L)                            | -95.9 ( $\pm$ 94.4) | -116.9 ( $\pm$ 71.7) | -149.5 ( $\pm$ 67.4) | -130.3 ( $\pm$ 66.1) | -187.8 ( $\pm$ 80.3) | -15.2 ( $\pm$ 34.6)     |
| Mean percent reduction                                 | 35%                 | 56%                  | 58%                  | 72%                  | 80%                  | 6%                      |
| Percent of patients below 125 nmol/L                   | 23%                 | 63%                  | 81%                  | 65%                  | 98%                  | —                       |
| Percent of patients below 75 nmol/L                    | 6%                  | —                    | —                    | —                    | 71%                  | —                       |

**Table 2. Lp(a) HORIZON Trial Patient Lp(a) Change From Baseline**

Mean Lp(a) concentration for participants at baseline followed by the absolute and percent reduction caused by Pelacarsen after 6 months of use. The percent of patients who attained an Lp(a) level below official risk related cutoffs is also shown. Data from (Tsimikas *et al.*, 2020).

After 6 months of treatment, patients saw mean decreases in Lp(a) concentration between 35 to 80% depending on Pelacarsen dose and frequency whereas the placebo group saw only a 6% reduction. These reductions occurred in a clear dose dependent manner, with the highest cumulative dose over time, 20 mg every week, resulting in an 80% mean decrease in Lp(a) concentration (Table 2). Within each of the dosage groups, the percent of patients who had their Lp(a) concentration drop below 50 mg/dL (125 nmol/L) and 30 mg/dL (75 nmol/L) was

also dependent on dose and was mostly reflected in line with that of the percent reductions. Similarly, mean reductions in OxPL residing on circulating apo(a) were recorded at 28% for 20 mg/Q4W, 49% for 40 mg/Q4W, 45% for 20 mg/Q2W, 63% for 60 mg/Q4W, and 70% for 20 mg/QW. In contrast, the placebo group saw a 20% decrease (Tsimikas *et al.*, 2020). Overall, Pelacarsen provides significant dose dependent reductions in Lp(a) concentrations for patients with elevated Lp(a) and ongoing clinical trials have provided promising evidence for antisense oligonucleotides as non isoform specific therapy. It also has great potential for prevention of ASCVD and MACE specifically because of its documented decrease of circulating OxPL with Lp(a). However, the therapeutic benefit of Pelacarsen has yet to be evaluated with respect to Lp(a) isoform types and future studies may consider taking that into account when testing patient response to certain doses to evaluate any link between the two.

## CONCLUSION

In all, Lp(a) has proven to be a complex issue in cardiovascular patient care and research due to its novel molecular behavior and limited treatment options. As discussed herein, available cardiovascular therapies provide limited and variable reductions in Lp(a) concentration, especially concerning certain isoform types or baseline levels. Of all the available on market treatments, Lipoprotein apheresis appears to provide the greatest non isoform specific overall reduction in Lp(a) concentration as well as ASCVD and MACE, but is not widely utilized because of its indication, cost, and time-consuming nature. Statins and ezetimibe have proven to have a negligible or paradoxical impact on Lp(a) concentration but are still essential tools for managing elevated LDL cholesterol and offer benefit in reducing cardiovascular risk in that regard.

Aspirin has been historically used to prevent thrombosis, thereby offering a cardioprotective benefit but has been shown to specifically reduce risk for cardiovascular events for carriers of the rs3798220 SNP by more than 50%. For many years, Niacin was a standard treatment for many cardiovascular issues but was the only drug that contributed a notable reduction in Lp(a) specifically in patients with larger isoforms. However, it was determined to have failed to

provide significant cardioprotective benefit in addition to causing moderate to severe side effects, resulting in its use being ultimately discontinued.

Monoclonal antibodies such as the PCSK9 inhibitors Evolocumab and Alirocumab have made significant strides in helping better understand the behavior of Lp(a) with regard to assembly and clearance. Their ability to lower Lp(a) concentration by up to 30% was an unintended benefit of their development and led researchers to investigate how this action was occurring, theorizing that assembly rather than clearance was impacted when PCSK9 protein was inhibited.

The future of Lp(a) treatment is on the horizon with the development of antisense oligonucleotide therapy. Clinical trials have shown that the antisense oligonucleotide Pelacarsen provides significant Lp(a) reductions but until long term studies can be done, its cardioprotective benefit will remain up for consideration. However, while the dose dependent reductions are significant they have also been seen to vary significantly based on individual baseline levels. This suggests that future investigations might consider analysis of isoform dependent reactions to certain doses in order to better personalize treatment for Lp(a).

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## CURRICULUM VITAE

