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REVIEW

Controversies in cardiovascular medicine

When should we measure lipoprotein (a)?

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Recently published epidemiological and genetic studies strongly suggest a causal relationship of elevated concentrations of lipoprotein (a) [Lp(a)] with cardiovascular disease (CVD), independent of low-density lipoproteins (LDLs), reduced high density lipoproteins (HDL), and other traditional CVD risk factors. The atherogenicity of Lp(a) at a molecular and cellular level is caused by interference with the fibrinolytic system, the affinity to secretory phospholipase A2, the interaction with extracellular matrix glycoproteins, and the binding to scavenger receptors on macrophages. Lipoprotein (a) plasma concentrations correlate significantly with the synthetic rate of apo(a) and recent studies demonstrate that apo(a) expression is inhibited by ligands for farnesoid X receptor. Numerous gaps in our knowledge on Lp(a) function, biosynthesis, and the site of catabolism still exist. Nevertheless, new classes of therapeutic agents that have a significant Lp(a)-lowering effect such as apoB antisense oligonucleotides, microsomal triglyceride transfer protein inhibitors, cholesterol ester transfer protein inhibitors, and PCSK-9 inhibitors are currently in trials. Consensus reports of scientific societies are still prudent in recommending the measurement of Lp(a) routinely for assessing CVD risk. This is mainly caused by the lack of definite intervention studies demonstrating that lowering Lp(a) reduces hard CVD endpoints, a lack of effective medications for lowering Lp(a), the highly variable Lp(a) concentrations among different ethnic groups and the challenges associated with Lp(a) measurement. Here, we present our view on when to measure Lp(a) and how to deal with elevated Lp(a) levels in moderate and high-risk individuals.

Keywords Atherosclerosis • Myocardial infarction • Stroke • Guidelines • Assay

Introduction

There is currently a surge in lipoprotein (a) [Lp(a)] research that is reflected by numerous review articles published recently.[1–4] Lipoprotein (a) has been detected by Berg[5] in 1963 and has been considered a genetic variant of β-lipoproteins [low-density lipoproteins (LDLs)]. Later it has been recognized as a distinct lipoprotein class. Despite intensive research, the physiological function of Lp(a) remains elusive. There is mounting evidence that elevated plasma Lp(a) levels contribute significantly to the incidence of cardiovascular diseases (CVDs).[1–4,6] First reports in the 1970s were based on case–control studies with only few patients and on observations in single families. Based on the results of more recent large prospective studies and meta-analyses, it has become clear that the risk of developing coronary artery diseases (CAD) in Caucasians is more than two times higher in individuals with increased Lp(a).[7,8] In addition, a causal relationship between Lp(a) concentrations and CAD or myocardial infarction (MI) has been postulated using the strategy of Mendelian randomization.[9] So far, further progress in Lp(a) research has significantly been slowed by the lack of generally accepted high throughput methods to quantify plasma Lp(a) and to determine apo(a) isoforms.

Structure of lipoprotein (a) and apo(a)

Lipoprotein (a) consists of an LDL-like core lipoprotein and glycoprotein apo(a) covalently linked by a disulfide bridge (*Figure 1*). The disulfide bridge links Cys4326 in apoB-100 with the only free Cys4057 in apo(a), located in kringle four (K-IV) type 9, see below. The lipid core of Lp(a) is virtually indistinguishable from that of LDL. Apo(a), the characteristic glycoprotein component of Lp(a), has a unique structure; it consists of repetitive protein segments, so-called kringles (K) that are highly homologous to

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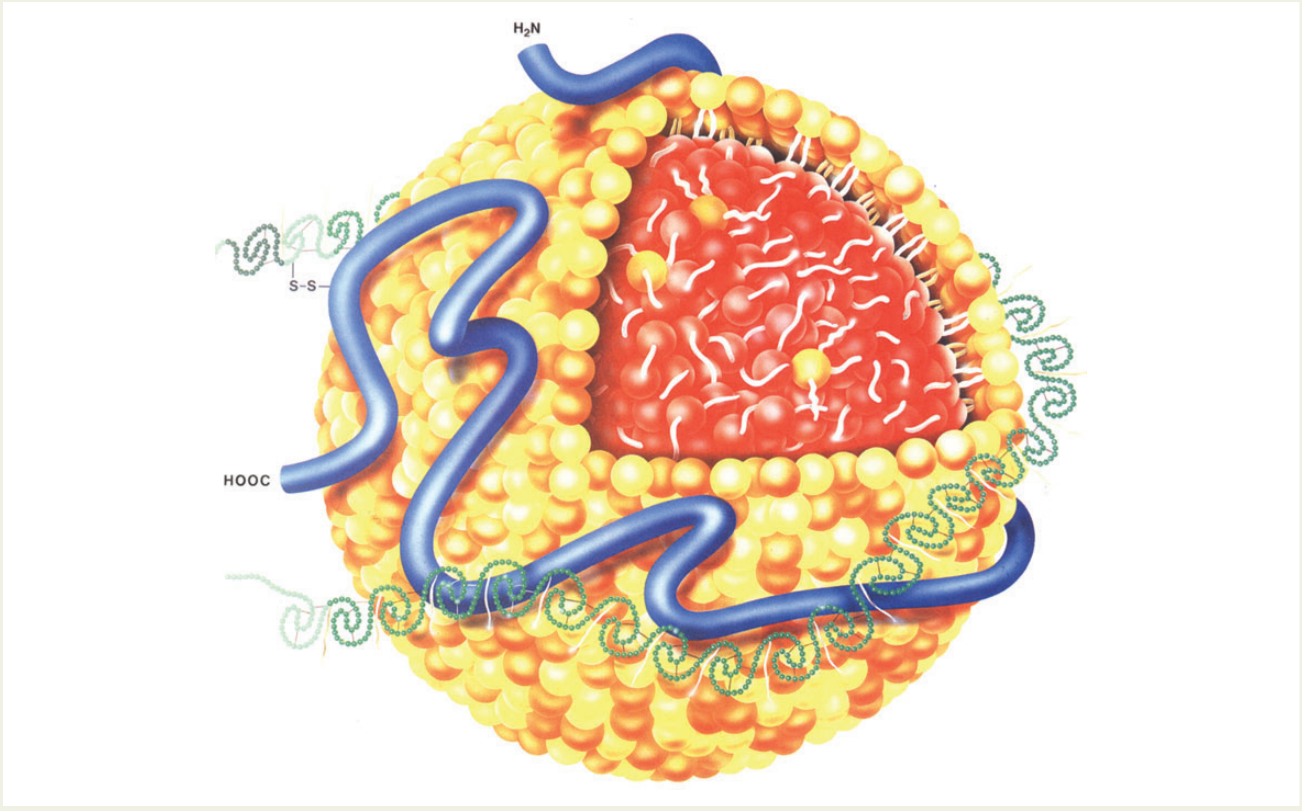

Figure 1 Schematic view of lipoprotein (a). Lipoprotein (a) is a low-density lipoprotein molecule complexed with apolipoprotein(a). Green: repetitive kringle structure of apo(a); blue: apo B-100; yellow: phospholipids; orange: free cholesterol; red: core lipids, cholesteryl esters. Apo(a) and apo B-100, the major protein constituent of low-density lipoprotein, are linked to each other by a disulfide bond.

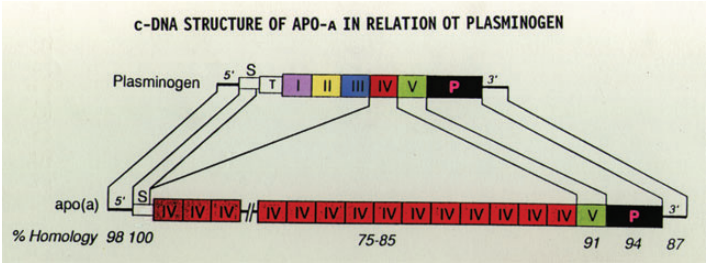

Figure 2 Structure of the cDNAs for apolipoprotein(a) and plasminogen. The 5′ and 3′ non-coding regions are shown as lines at both the ends of the cDNAs. S denotes the signal sequences, T the N-terminal portion of plasminogen (tail), the roman numerals the kringle domains. The apo(a) cDNA encodes a protein that consists of a variable number of kringle IV domains, a kringle V domain and a protease domain. There are 10 types of krigle IV domains. Types 1 and 3–10 occur once while the number of kringle IV domains of type 2 varies from 3 to >40. The variable number of kringle domains of type IV type 2 is responsible for the two mass polymorphism of apo(a). Kringles I to III of the plasminogen molecule are not present in apo(a).

K-IV of plasminogen. One K-IV contains 110 amino acids forming a secondary structure, which resembles 'Danish kringles'.[10] The N-terminal part of apo(a) consists of multiple repetitive copies of these kringle-IVs. Apo(a) in addition has one copy of a K-V like and a protease-like domain similar to plasminogen. The protease domain in apo(a), however, lacks enzymatic activity. In humans, 30 or more genetically determined apo(a) isoforms exist, giving rise to substantial size heterogeneity. The smallest apo(a) isoform contains the protease domain, one copy of K-V and 11 K-IVs of which K-IV type 1 and K-IV types 3 to 10 occur once, whereas K-IV type 2 is present in two identical copies (*Figure 2*). Larger isoforms differ by the number of K-IV type 2s; the largest

apo(a) described so far has 52–54 K-IVs. The K-IVs are connected by linkers that are highly glycosylated with *N*- and *O*-linked sugars. Although apo(a) is predominantly associated with LDL, there are small and variable amounts of 'free' apo(a) present in the plasma[11] which are found in the 'bottom' (i.e. non-lipoprotein) fraction after ultracentrifugation. Free apo(a) is prone to proteolytic degradation and the generated fragments are found in high amounts in the urine.

Metabolism of lipoprotein (a)

Biosynthesis

Apo(a) is produced almost exclusively in the liver and follows classical steps of glycoprotein biosynthesis. Since plasma Lp(a) levels highly correlate with apo(a) production, the regulation of apo(a) transcription is in the focus of current research. The transcription of genes involved in lipid and lipoprotein metabolism is strongly influenced by nuclear receptors including peroxisome proliferator-activated receptors, hepatocyte nuclear factors (HNFs), retinoid X receptor, liver X receptor, farnesoid X receptor (FXR), and others (reviewed in[12]). In our recent studies, we have observed that patients suffering from obstructive cholestasis with high plasma bile acid concentrations had comparatively low plasma Lp(a) levels. Since bile acids are ligands for FXR, we focused in our studies on the role of FXR signalling on apo(a) transcription (*Figure 3*).[13,14] Bile acids have a dual effect on apo(a) expression. Apo(a) expression is driven by the canonical liver transcription factor HNF4α. The apo(a) promoter has several putative-binding sites for HNFs. In the first regulatory pathway driven by bile salts, the HNF4α-binding site at −826 to −814 is involved. Activation of FXR by bile salts or synthetic ligands leads to a translocation to the nucleus and a competitive displacement of HNF4α from this binding site. The second pathway is mediated by FXR stimulation of fibroblast growth factor 19 (FGF-19) expression in the intestine. Fibroblast growth factor 19 migrates to the liver, binds to the FGF-receptor 4, and down-regulates apo(a) expression by mitogen-activated protein kinase (RAS-ERK1/2) signalling and binding of phosphorylated ELK-1 to the ETS promoter segment at −1603 to −1615. Since FXR ligands down-regulate apo(a) transcription to almost zero, we believe that the clarification of these pathways may serve as a basis for developing new medications to treat individuals with elevated plasma Lp(a).

Assembly

Lipoprotein (a) is biosynthesized only in humans and old world monkeys, which complicates in depth studies of its metabolism. Apo(a) is expressed primarily in the liver, yet small amounts of apo (a) mRNA have also been detected in the testis and the brain, the role of which in the overall Lp(a) metabolism is unknown. Hepatocytes from primates have been found to synthesize a pre-form of apo(a) with a lower degree of glycosylation. Upon maturation, intracellular apo(a) reaches the Golgi and is secreted in a mature form as a glycoprotein, most probably without being attached to LDL. The genetically determined size of apo(a) reflecting the number of K-IV repeats correlates with the intracellular residence time and thus, small isoforms are secreted much faster when compared with large isoforms. This appears to be the reason for the inverse correlation between apo(a) size and plasma Lp(a). We and others have found that the assembly of Lp(a) from apo(a) and LDL is a two-step process.[15] In the first step, specific K-IVs of apo(a), mostly K-IV types 3–6 non-covalently bind to lysine groups of apo B in LDL. This binding is still reversible.[16] It has been argued that by interfering with this step of assembly, Lp(a) levels may be reduced, as free apo(a) is degraded faster than LDL-bound apo(a). *In vivo* and *in vitro* experiments, however, have disproved this assumption.[16] We actually could demonstrate that cell-bound apo(a) dissociates upon treatment with lysine analogues and assembles with LDL more efficiently. The assembly of Lp(a) does not depend on any enzymatic activity. Interestingly, apo(a) preferentially binds to apo B-100 from humans and few animal species; yet, apoB-100 from rodents hardly forms any Lp(a) upon incubation with apo(a). Thus, the metabolism of Lp(a) can be studied only in double transgenic human apo(a):apoB-100 mice or in monkeys. Although the evidence for an extracellular assembly is favoured by many investigators, there are data that support an intracellular assembly.[17] Alternative mechanisms are given by Jenner *et al.*[18] who suggested several dissociation and re-association steps of apo(a) and apoB containing lipoproteins.

Turnover in man

In an early study, nine probands with Lp(a) concentrations between 5 and 75 mg/dL were investigated.[19] Lp(a) concentrations strongly and significantly correlated with the production rate, while there was no correlation with Lp(a) catabolism (*Figure 4*). Our results have been confirmed subsequently using radioactive Lp(a) or stable isotope precursors.[20] The liver also appears to be the major organ of Lp(a) degradation. This has been confirmed by turnover studies in animals including rats, rabbits, mice, and hedgehogs. The latter animal model has particularly been used since it synthesizes a lipoprotein that resembles Lp(a) yet with an apo(a) consisting of kringle-III instead of K-IV repeats.[21] *In vivo* approximately 50% of Lp(a) is taken up by the liver, followed by the kidney, the spleen, and the muscle. In the kidney, a 10% arteriovenous difference in Lp(a) concentrations has been reported[22] and in healthy humans apo(a) is found in the urine as 'free' apo(a) fragments which are 50–160 kD in size.[23] It is not fully clear where and how these fragments are formed, but it appears that a large portion is formed extra-renally, and in turn is selectively excreted by the kidney. Reduction of plasma Lp(a) by LDL apheresis leads to an immediate reduction of the urinary apo(a) fragment concentration.[24]

Genetics of lipoprotein (a)

The apo(a) gene is located on chromosome 6q26-q27and is among the most polymorphic genes in humans. Different types of variants are independently associated with Lp(a) concentrations. Utermann *et al.*[25] have first recognized that apo(a) occurs in isoforms differing in their molecular masses. The mass heterogeneity of apo(a) is attributable to different numbers of exon sequences encoding kringle IV type 2 repeats (see above)[10] and correlates with plasma Lp(a) levels, whereby large isoforms give rise to low plasma Lp(a) and vice versa. Mechanistically, larger apo(a) isoforms

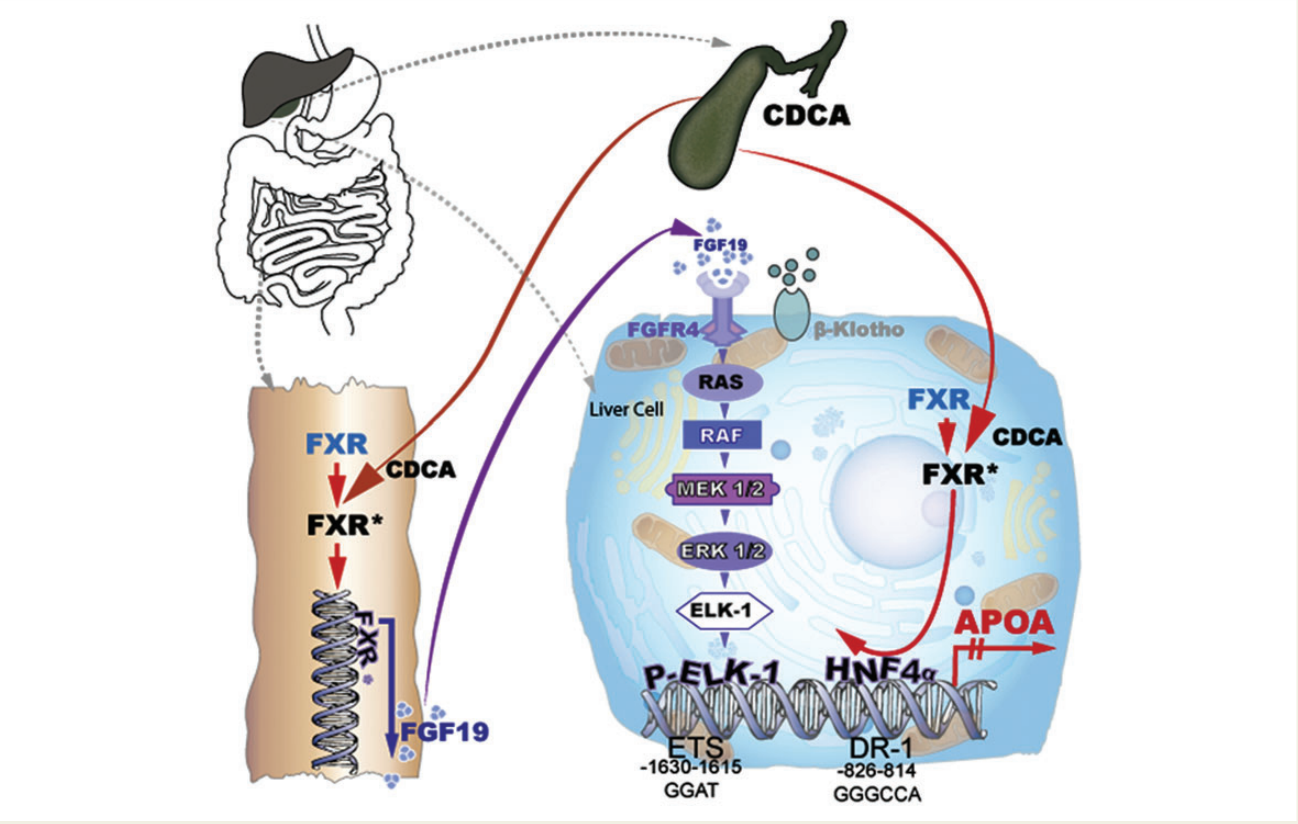

Figure 3 Inhibition of apo(a) biosynthesis by farnesoid X receptor ligands. Farnesoid X receptor ligands such as chenodeoxy cholic acid interfere with apo(a) transcription in a dual manner: (i) Farnesoid X receptor activated by chenodeoxy cholic acid is translocated to the nucleus and competitively inhibits the binding of hepatocyte nuclear factor4α, thereby repressing apo(a) transcription. (ii) Activation of FXR up-regulates FGF-19 biosynthesis in the intestine that in turn binds to its cognate receptor on liver cells. By RAS-RAF-MEK1/2 signalling ELK-1 is phosphorylated, binds to a specific ETS site in the apo(a) promoter and down-regulates apo(a) transcription. The dotted lines indicate the gall bladder, the liver cell, and the small intestine.

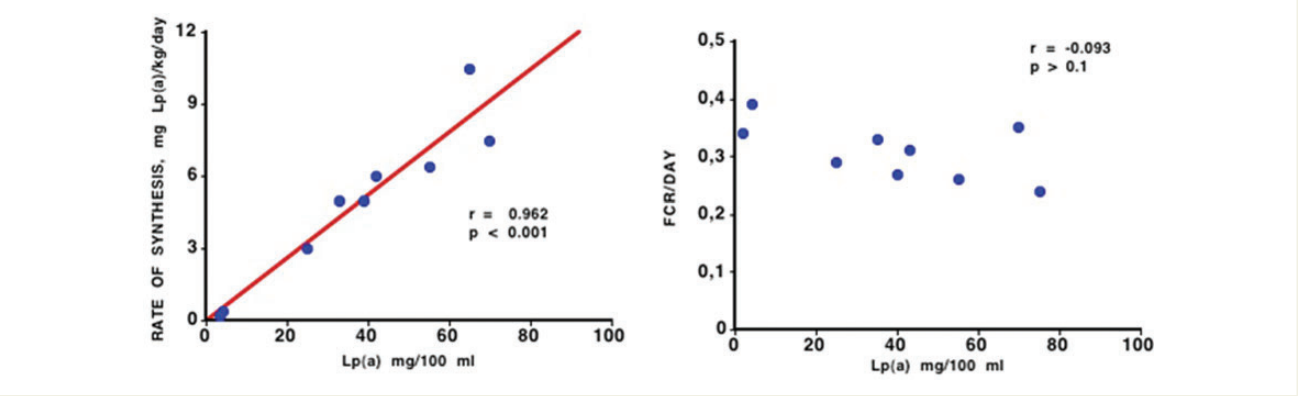

Figure 4 Turnover of lipoprotein (a) in humans. ^{125}I radiolabelled lipoprotein (a) was injected into nine volunteers with plasma concentrations ranging from 5 to 75 mg/dL and the decay of the specific radioactivity was followed over time (for details see[19]).

are most likely trapped and degraded immediately following biosynthesis in the rough endoplasmic reticulum or in the Golgi at a higher rate than smaller ones.[26] The promoter region of the apo(a) gene contains a variable number of a pentanuclotiode repeats (TTTTA)[27–30] and a +93 C/T polymorphism[27,30–32] in the untranslated region of the apo(a) gene. Further mutations and polymorphisms are abundant in the apo(a) gene and explain part of the variance of Lp(a) concentrations.[33–38] Ichinose[39]

identified two functional SNPs in the distal enhancer region 20 kB upstream of the apo(a) gene. In addition, numerous polymorphisms were identified in the K-IV domains of the apo(a) gene impacting significantly on apo(a) concentrations. Lipoprotein (a) concentrations in human plasma range from <1 to 250 mg/dL and more. Early linkage studies had suggested that up to 90% of the variance of the Lp(a) concentration is determined by genetic factors.[40,41] More recent estimates suggest that approximately one-third of the variation in Lp(a) concentrations is due to genetic heterogeneity of the Lp(a) locus.[37,42–44]

As described above, the proximal apo(a) promoter region contains numerous regulatory sequences including response elements for HNF-1 and -4, interleukin 6 (IL-6), sterol regulatory element-binding proteins and cAMP response element-binding protein. Beyond the genetic variation of the apo(a) gene itself, variants in these transcription factors may therefore give rise to variations of plasma Lp(a) levels. It is in this context of interest that Lp(a) has been claimed to act as a positive acute phase reactant,[45,46] the expression being enhanced by IL-6.[47] Other authors, however, have seen sharp falls of Lp(a) following burns and during sepsis.[48] It is in line with a role of IL-6 in modulation of Lp(a) that tocilizumab, an inhibitor of IL-6 signalling, lowered Lp(a) levels by ~30% after 3 months of treatment in patients with rheumatoid arthritis[49] and that the IL-6 promoter polymorphism 174G/C is significantly associated with Lp(a) concentrations.[50]

Further, Lp(a) concentrations vary significantly among different ethnic groups.[35,42] African Americans, for example, have much higher plasma concentrations than Caucasians independent of isoform variations. In Chinese and some Asian populations, the opposite is true.

Non-genetic factors affecting lipoprotein (a)

There are numerous publications dealing with primary and secondary effects of metabolic pathways, drugs, and diseases that influence plasma Lp(a) levels. Here, we concentrate only on the most significant ones being aware that many of the reported effects are controversial and also dependent on apo(a) isoforms and initial plasma concentrations (cf. Table 1, for review also see[51]) (Table 2).

The role of the kidney

Both nephrotic syndrome and end-stage renal disease increase Lp(a) levels two- to three-fold, probably by different mechanisms. While in the nephrotic syndrome the rate of Lp(a) biosynthesis appears increased, reduced Lp(a) catabolism may occur in end-stage renal disease. Urinary apo(a) significantly decreases once the glomerular filtration rate becomes <70 mL/min[52,53] and this contributes to the increase in Lp(a) in chronic kidney disease.

Lp(a) in diabetes mellitus

Data on Lp(a) in type 1 diabetes mellitus (DM) are not consistent. No relationship has been found in young children with type 1 DM and it appears that the positive correlation with type 1 diabetes in adults is mostly indirect, because many patients at the same time suffer from impaired kidney function. Kollerits *et al.*[54] studied the role of Lp(a) as a predictor of CAD in >400 patients with type 1 diabetes and with normal kidney function; they concluded that Lp(a) >30 mg/dL significantly contributed to CAD risk.

The pathogenesis of type 2 diabetes is complex and it is unlikely that Lp(a) levels are affected in all patients to the same extent. This may explain that unaltered, higher, or lower Lp(a) plasma concentrations have been reported. In 2010 Mora *et al.*[55] published a study of >35 000 US and Danish participants. Lp(a) levels were inversely associated with the incidence rate of type 2 DM with hazard ratios for quintiles 2–5 vs. quintile 1 of 0.87, after adjusting for established CAD risk factors, HbA1c, and C-reactive protein. The authors concluded that Lp(a) is inversely related to the risk of type 2 DM independently of CAD risk factors. This is in discordance to previous reports that suggest positive associations of Lp(a) with CVD.

Liver diseases

Since the liver is the only organ for Lp(a) biosynthesis, it is not surprising that Lp(a) is decreased in liver diseases. This was first observed in patients with cholestasis; yet, their Lp(a) reduction remains transient if they are successfully treated. Other substances that are liver toxic including alcohol and several drugs have also been shown to significantly reduce Lp(a).

Hormones and drugs

Steroid hormones, including oestrogens, progesterone, testosterone, and synthetic sex-hormone-like compounds, reduce plasma Lp(a) up to 40%, yet these effects are partly transient. In particular, anabolic steroids reduce Lp(a), whereby the mechanism has not been elucidated so far. Thyroid hormones are also known to affect plasma Lp(a) levels. It has been found that hypothyroid patients had significantly higher and hyperthyroid patients had lower Lp(a) concentrations. Appropriate treatment of these patients led to Lp(a) changes towards levels of euthyroid controls.[56]

Conventional lipid-lowering drugs, including niacin, statins, fibrates, and drugs interfering with cholesterol absorption (neomycin, ezetimibe), have all been reported to reduce plasma Lp(a). Unfortunately, these effects have not been consistent. The only compound that consistently reduces Lp(a) by up to 35% by interfering with apo(a) transcription[57] is nicotinic acid or derivatives thereof.[58] While niacin mono-therapy may decrease cardiovascular events,[59] this is not clear in combination with statins.[60] The outcome of the HPS2-THRIVE study <http://clinicaltrials.gov/ct2/show/NCT00461630> that will be opened in March 2013 will certainly provide additional valuable information on this point. Angiotensin-converting enzyme inhibitors lower elevated Lp(a) plasma concentrations in proteinuric patients by reversing proteinuria and in turn reducing Lp(a) production by the liver.[61] The most effective therapy for lowering Lp(a) is extracorporeal elimination of Lp(a) with apheresis. Low-density lipoprotein apheresis and selective Lp(a) apheresis using antibodies coupled to columns, precipitation and complex formation at low pH, double filtration and direct absorption have been demonstrated to lower plasma Lp(a) to the same extent as LDL cholesterol by up to 80%.[62] Although randomized studies are not available, a recent retrospective

Table 1 Major factors affecting the concentration of lipoprotein (a) in humans

	Effect on Lp(a)[a]	Comment
Genes		
Apo(a)	Up to 90%	Size polymorphism accounts for 30 to 50% of the variation[25,27,30–42]
LDL-R	Two- to three-fold increase	The mechanism of Lp(a) elevation in FH patients is not fully understood[25]
MODY (HNF-4a)	3.3-fold increase	The R154X mutation in HNF4a was associated with a 3.3-fold increase in Lp(a)[94]
Non-genetic factors		
Acute phase	Up to two-fold increase	Maximal Lp(a) values are reported 6–8 days after the acute event, controversial findings reported in the literature[45,46]
Renal disease	Three-fold increase	Different mechanisms of the Lp(a) increasing effect exist for nephrotic syndrome and end-stage renal disease[20,22]
Diabetes mellitus	Indirect effect	The elevation of Lp(a) in diabetic subjects mostly relates to the degree of kidney dysfunction[54,55]
Liver diseases	Up to 90% reduction	Particularly, cholestatic patients show very low Lp(a) levels[13]
Alcohol	Up to 20–57% decrease	The greatest effect was seen in subjects consuming >50 g alcohol/day (reviewed in[51,96])
Hormones		
Thyroxine	10–25% reduction	Hypothyroid patients treated with T4 showed a reduction in plasma Lp(a). The effect of T4 on plasma Lp(a) is, however, controversial[56]
Pregnancy	2.5–3-fold elevation	During pregnancy, maximal Lp(a) elevations were seen at weeks 19–20 (reviewed in[51,96])
Oestrogens	37% reduction	This effect was seen in post-menopausal women receiving hormone replacement therapy (reviewed in[51,96])
Progesterone	3–5% reduction	During hormone replacement therapy the addition of gestagens to oestrogens showed a slight additional effect in some studies (reviewed in[51,96])
Tamoxifen	35% reduction	anti-oestrogen (reviewed in[51,96])
Tibolone	35% reduction	Agonist of type I steroid hormone receptor (reviewed in[51,96])
Raloxifene	18% reduction	Oestrogen receptor modulator (reviewed in[51,96])
Testosterone	30–40% reduction	Only few reports published (reviewed in[51,96])
Anabolic steroids	60–70% reduction	Not for clinical use (reviewed in[51,96])
ACTH	30–40% reduction	Relates to kidney function (reviewed in[51,96])
Drugs or apheresis		
Niacin	30–35% reduction	Currently most recommended drug for Lp(a) reduction[57]
Fibrates	Up to 20% reduction	The highest effect was seen with Gemfibrozil (reviewed in[51,96])
Statins	Inconsistent	may cause reduction but also significant Lp(a) elevation (reviewed in[51,96])
L-Carnitine	10–20% reduction	Mitochondrial fatty acid transporter (reviewed in[51,96])
N-acetyl-cysteine	Controversial	No effect in own studies (reviewed in[51,96])
Acetylsalicylic acid	10–20% reduction	Appears to be effective even at low doses (reviewed in[51,96])
LDL/Lp(a) apheresis	Up to 80% reduction	Apheresis has been shown to reduce the risk for CVD[63]

[a]The effects shown are the maximal changes reported.

analysis of the clinical course in patients undergoing apheresis treatment for elevated Lp(a) makes it likely that lowering Lp(a) would also reduce the risk for CVD.[63]

There are promising treatments in the pipeline of several drug companies that significantly reduce elevated plasma Lp(a) in addition to other lipoproteins. Their mechanisms of action on Lp(a), however, are poorly understood. One of these drugs, the antisense oligonucleotide mipomersen, originally created to treat therapy-resistant FH patients, reduces plasma Lp(a) levels to the same degree (up to 40%) as LDL and VLDL.[64] Other drugs that also lower Lp(a) include the microsomal triglyceride transfer protein (MTP) inhibitor lomitapide,[65] antibodies against proprotein convertase subtilisin/kexin type 9 (PSK9)[66] and the cholesterol ester transfer protein inhibitor anacetrapib.[67] In a recently published multicentre phase 2 trial, the addition of anti-PCSK9 (SAR236553) to either 10 or 80 mg atorvastatin daily caused a reduction of Lp(a) by 31 and 34.7% when compared with 80 mg atorvastatin alone.[66]

Lipoprotein (a) and atherosclerosis

Epidemiological evidence

Berg[68] originally denominated Lp(a) as 'sinking-pre-β' lipoprotein and reported a positive relationship of this fraction with CAD. We were the first to quantitatively measure Lp(a) using 'rocket

Table 2 Diagram of recommendations for lipoprotein (a) screening in different patient groups

Patient group	Patients characteristics	Comments
Premature CVD or stroke	Without evident risk factors	Measure Lp(a) at least once
Intermediate CVD risk group	According to Framingham, PROCAM, ESC Heart Score, or Australian/New Zealand risk scores	Patients with Lp(a) >50 mg/dL fall into the higher risk category
Recurrent or rapidly progressive vascular disease	Presence of various recognized risk factors	Measure Lp(a) repeatedly if patients are on drug treatment
FH, genetic dyslipidaemia or low HDL-C	Grossly elevated LDL-C; presence of β-VLDL; reduced levels of HDL-C	Measure Lp(a) in regular periods
Genetic defects of haemostasis, homocystein metabolism, as well as diabetes mellitus or auto-immune diseases	Defects in blood clotting or platelet aggregation; elevated levels of homoCys; insulin resistance; phospholipid antibodies	If on medication, Lp(a) levels should be followed at regular time intervals
Elevated CVD risk	10-year risk of fatal CVD ≥3% according to EAS guidelines or ≥10% 10-year risk of fatal + non-fatal CHD according to EAS or US guidelines	For details consult Nordestgaard et al. [1]

electrophoresis' and suggested a cut-off concentration of 30 mg/dL beyond which the risk for MI increased.[69] We also showed that individuals with both elevated Lp(a) plus LDL cholesterol were at a 10-fold or higher risk of MI.

Subsequently, a great number of reports were published examining the role of Lp(a) as a risk factor for atherosclerosis, MI, stroke, and peripheral vascular disease. The majority of them has confirmed Lp(a) as a risk factor.[70] As Lp(a) metabolism is distinct from that of other plasma lipoproteins, it is not surprising that Lp(a) has mostly been predictive independent of common established risk factors. A comprehensive meta-analysis of prospective studies involving >126 000 individuals by Erqou *et al.*[8] found incidence rates of coronary heart disease in the top and bottom tertiles of baseline Lp(a) of 4.4 (95% CI: 4.2–4.6) and 5.6 (95% CI: 5.4–5.9) per 1000 years. These estimates are close to the ones we obtained in our more recent meta-analysis (RR = 1.48 for top tertile compared with the bottom tertile; 95% CI: 1.26–1.74, $P = 0.001$) in asymptomatic individuals.[71] Erqou *et al.*[8] concluded that a 'continous, independent, and modest association of the Lp(a) concentration with risk of CHD and stroke existed.' We also analysed patients with a positive history of CAD and found a substantially greater risk ratio of 2.37 (95% CI: 1.41–3.97, $P = 0.001$) for the top compared with the bottom tertile, suggesting that Lp(a) is particularly crucial on the background of elevated global risk. This is also illustrated by an elegant investigation by von Eckardstein *et al.*[72] who found that Lp(a) increases the risk of CAD at high and less so at low global cardiovascular risk in almost 800 male participants of the PROCAM study. Closer scrutiny of the results presented by Erqou *et al.*[8] also reveals that the relationship between Lp(a) and CHD might not be strictly continuous, because significant increases of risk were only seen once Lp(a) exceed the threshold of 30 mg/dL, whereby further increases in risk occurred the higher the Lp(a) concentrations became.

Because of the significant correlation between plasma and urinary apo(a) it should be possible to discriminate CAD patients from normals by measuring urinary apo(a). Indeed, in a study of 225 patients and controls, urinary apo(a) turned out to be a better discriminator than plasma Lp(a).[73] Since the analysis of the kringle IV fragments found in urine is not biased by the apo(a) phenotype, it might be appropriate to include the measurement of apo(a) fragments into future studies. In this regard, it is noteworthy that the 'free' apo(a) in the plasma, which consists mostly of these fragments, may have a better diagnostic performance than total Lp(a).[74]

It should be mentioned at this point that some prospective studies, such as the Physicians Health Study, have shown contrasting results.[75] In some of the negative reports, Lp(a) was measured in long-term frozen samples and/or with insufficiently evaluated, possibly ill standardized methods. Moreover, due to the extremely wide range of plasma Lp(a) levels from <0.1 to > 300 mg/dL and the highly skewed distribution, studies that include small numbers of cases–controls are prone to random findings.

Very recently, variants in the apo(a) gene have been used as proxies for Lp(a) plasma concentrations in epidemiological studies and as instrumental variables in Mendelian randomization experiments to confirm causality.[76] The promoter pentanucleotide repeat polymorphism of apo(a) has been linked to MI.[7,29] Tregouet *et al.*[77] studied 2700 CAD patients and >4500 control individuals using the 500K Affymetrix whole genome array and identified the *LPA* gene cluster as a strong susceptibility locus for CAD. Kamstrup *et al.*[9] in the same year published data from the Copenhagen Heart study comprising >40 000 individuals. There was a significant correlation between plasma Lp(a) levels, KIV-2 genotype, and the risk of MI which they interpreted as proof for causality. Clarke *et al.*[37] described strong and independent associations between CHD and two *LPA* variants, rs10455872, which is a non-coding intronic SNP, and rs3798220, which is a missense variant replacing Ile4399 by Met in the apo(a) protease-like domain. The rs3798220 SNP has also been reported to be associated with angiographically defined coronary stenosis or MI[78,79] and appears to be in linkage disequilibrium with small apo(a) isoforms. Li *et al.*[38] confirmed these associations in a recent meta-analysis, with carriers of one or two minor alleles of rs3798220 being at 57% increased risk of CHD and each copy of rs10455872 increasing risk by 42%. Erqou *et al.*[80] finally performed a meta-analysis including 40 studies with > 58 000 participants and found that individuals

with smaller isoforms compared with larger isoforms are at a more than two-fold risk for coronary heart disease. It is completely in line with these findings that further hypothesis free genome-wide association studies identified the *LPA* gene locus as associated with CAD at genome-wide significance.[81,82]

Mechanisms of atherogenicity

Lipid oxidation is a hallmark for atherosclerotic diseases. Significantly increased levels of oxidized phospholipids that exert potent pro-inflammatory actions have been found in CVD patients and a majority of them is bound to Lp(a).[83] On the other hand, Lp(a) also carries the majority of platelet-activating factor—acetyl hydrolase, also called lipoprotein-associated phospholipase A2 that hydrolyzes short chain fatty acids in phospholipids.[84] The exact mechanism of the interplay of these compounds in relation to atherogenicity is currently not known and needs to be explored further. Lp(a) has been demonstrated by several research groups in atherosclerotic plaques of humans and in experimental animals.[85] Cerebral vascular disease, peripheral vascular disease, and more recently carotid atherosclerosis have also been associated with elevated Lp(a) levels. Finally, it appears that Lp(a) may be involved as a cofactor in essential hypertension.[86] Another important finding is that Lp(a) may play a role in acute coronary syndromes. Shindo *et al.*[87] found significantly higher apo(a) and PAI-1 stainable areas in atherectomy specimens of patients with unstable coronary disease than in those with stable angina.

Recommendations from guidelines

Today there is still no unanimous agreement when to measure Lp(a) and how to deal with increased Lp(a) values. The reasons for this include, that no commonly accepted assays and reference standards exist. There is also a lack of effective medications to lower Lp(a) applicable to large population groups, preventing intervention studies for the reduction in CVD endpoints. An argument that is also frequently raised is that many patients may suffer from CVD despite of a very low Lp(a). This led most of the experts to formulate the guidelines to cautious recommendations.

The expert panel that compiled the ATP-III NCEP 2002 guidelines http://www.nhlbi.nih.gov/guidelines/cholesterol/index.htm[88] emphasized several difficulties regarding the monitoring of Lp(a) as a risk factor for CHD. These relate to problems with the standardization of Lp(a) assays, the lack of proper medication, the great differences in Lp(a) levels in populations of different ethnic origin and more. Although ATP-III did not find strong evidence for Lp(a) as a significant risk factor they accepted the suggestion of experts to measure Lp(a) as an option for selected patients, particularly if they have a strong family history of premature CHD or suffer from familial hypercholesterolaemia (FH). The experts also agreed that high Lp(a) counts as an additional risk factor that justifies a lower goal for LDL-C.

In the ACCF/AHA 2010 Guidelines,[89] the committee considered 36 long-term prospective morbidity and mortality studies published between 1979 and 2009. They included >125 000 participants. The study revealed continuous, independent, yet modest associations of Lp(a) levels with increased risk for CHD and stroke.

The 2012 AACE guidelines[90] have concluded that the risk associated with elevated Lp(a) varies between ethnic groups, and the interpretation of data be further complicated by the lack of standardized measurement procedures. Yet a considerable body of evidence confirming the status of Lp(a) as a major CV risk factor independently of TG, LDL-C, and HDL-C has appeared over the past 10 years. 'Testing for lipoprotein (a) is not generally recommended, although it may provide useful information to ascribe risk in white patients with CAD or in those with an unexplained family history of early CAD.'

Very similar recommendations are given in the combined 2011 ESC/EAS guidelines[6] which state that Lp(a) might not be a target for risk screening in the general population, yet it should be considered in individuals with elevated cardiovascular risk or a family history of premature vascular diseases. Most recently, a report by the Emerging Risk Factors Collaboration[91] focusing on low to intermediate risk populations showed that Lp(a) slightly improves CVD prediction in addition to HDL cholesterol and total cholesterol. Interestingly, Lp(a) is not even mentioned in the AHA/ASA guidelines for prevention of stroke although Lp(a) came out to be significantly correlated with that disease.[92]

Lastly, we may point to the advise by the EAS Consensus Panel (detailed in[1]) to screen once for Lp(a) by isoform-insensitive methods in individuals at intermediate and high CVD risk including FH and hyper-Lp(a), and in patients with a family history of premature CVD and an ≥3% 10-year fatal CVD risk according to the EAS guidelines.

Laboratory methods and reference values

Quantitative Lp(a) measurements have been performed using all kinds of immunochemical methods including rocket immunoelectrophoresis, rate and endpoint nephelometry, turbidimetry, radio-immuno assays, enzyme immuno assays (ELISA), and dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA). In addition, Lp(a) values may semi-quantitatively be measured as Lp(a) cholesterol by lipoprotein electrophoresis. Problems inherent to all of these methods result from the unique features of Lp(a): (i) The molar mass and the hydrated density of Lp(a) vary over a relatively broad range, in part due to the size polymorphism of apo(a). This causes an underestimation of Lp(a) concentrations among individuals with small isoforms, and an overestimation at large isoforms when using a single reference standard. (ii) Most antibodies against apo(a) react with variable numbers of epitopes depending on the apo(a) isoform, except for very specific monoclonals. (iii) Purification of Lp(a) to obtain primary standards is not straight forward and purified Lp(a) is unstable; the larger the isoform, the higher is the degree of self-aggregation. (iv) Lp(a) forms mixed aggregates with LDL that are not always fully dissociable. To deal with all these problems, a Lp(a) standardization working group was formed under the guidance of S.M. and supported by grants from the WHO and the IFCC.[93] In a first survey, 42 self-made or commercial assays were evaluated using individual reference standards; four serum samples either in liquid, fresh or frozen state and two lyophilized secondary standards

were assayed in 29 different laboratories. In this study, nephelometric methods appeared to perform best followed by DELFIA and some ELISA assays. In a follow-up study, several commercial Lp(a) calibrators (secondary standards) were evaluated for precision, linearity, and method harmonization using various immunochemical assays.[94] In essence, there was a large between-method variation mainly caused by the lack of a common reference material. This is also the major challenge that persists until today, since comparisons of Lp(a) concentrations between laboratories can still hardly be made, with the consequence, that precise cut-off levels are hard to set. Despite of all these drawbacks, the diagnostic industry came out with new Lp(a) kits that accounted for many of the problems and are nowadays used in clinical laboratories. Although the accuracy (i.e. absolute calibration) of the current results may be questionable, the precision appears sufficient to use them for comparing Lp(a) concentrations measured in a given laboratory.

Another question is whether Lp(a) values should be expressed as mass or molar concentrations. Although molar concentrations are recommended for clinical assays, the available methodology hardly meets all requirements to express Lp(a) in molar values. In particular because the majority of individuals is heterozygous for apo(a) isoforms that are present in the plasma at different ratios.

Given all these considerations, the question arises whether a reliable cut-off value for CAD risk assessment exists. The answer is regrettably 'no' and thus each method and each laboratory might use its own reference values. It is noteworthy, however, that in the very first report on Lp(a) and MI,[69] a cut-off value of 30 mg/dL has been suggested. This value has been adopted in numerous subsequent reports or a less rigorous cut-off of 50 mg/dL has been suggested in the consensus report of the EAS.[1] For the accurate conversion of mass concentrations into molar concentrations, the actual molecular mass of the given Lp(a) molecule needs to be known and the sizes and the ratio of the two apo(a) isoforms (in most individuals) have to be considered. Since this is in practice not feasible, we propose to use a factor of 3.17 (1 mg/mL of Lp(a) ~3.17 nmol/L) which has been obtained during the work of the Lp(a) standardization group.[95]

Is there an additional benefit of measuring the number of kringle four repeats?

As outlined above, the size polymorphism of apo(a) is significantly related to the individual plasma Lp(a) concentration. This implies that the plasma concentration might suffice to calculate cardiovascular risk. There are, however, reports that the size of the apo(a) isoform might be independently related to CVD and stroke. Erqou *et al.*[80] recently published the results of a meta-analysis including 40 studies and found that individuals with smaller isoforms have approximately two-fold risk to suffer from ischaemic stroke. Given the fact that the analysis of apo(a) isoforms is not a high-throughput procedure and that the role of Lp(a) as a risk factor for stroke is currently under debate, we believe that a well-standardized quantitative Lp(a) assay without measuring the isoform size will be adequate for the time being.

Synopsis (authors' recommendations)

We recommend to measure Lp(a) in patients with premature CVD and premature stroke, in particular, but not exclusively in whom other risk factors fail to explain the presence of vascular disease. We also recommend Lp(a) measurement in patients who fall into an intermediate risk group when classical risk algorithms are used such as the Framingham risk score, the PROCAM risk score, the ESC Heart Score, or the Australian and New Zealand risk calculator, because patients should be re-stratified into a higher risk category if Lp(a) is elevated >50 mg/dL, which in turn should ultimately lead to more intensive management of treatable risk factors, especially LDL cholesterol. Other group of patients which in our opinion will benefit from Lp(a) measurement are patients with recurrent or rapidly progressive vascular disease, despite being on lipid-lowering medication, patients with FH or other forms of genetic dyslipidaemias, with low HDL-C, with genetic defects related to haemostasis and homocysteine, DM and with auto-immune diseases. The European consensus statement published in 2010[1] recommends to measure Lp(a) in patients with a 10-year risk of fatal CVD 3% and more. Particular attention should be paid to haemodialysis patients and patients with renal disease since under these conditions Lp(a) is two- to three-fold elevated. If Lp(a) is found to be elevated in these patients groups, the most important next step is to treat traditional modifiable risk factors such as LDL cholesterol, hypertension, smoking, diabetes, and obesity intensively. Niacin and LDL apheresis if available can be used to lower Lp(a) in selected patients, but it has to be considered that clinical evidence for this approach is still limited to date.

Conclusion

Lp(a) remains one of the most atherogenic lipoproteins and one of the strongest genetic risk factors for vascular diseases. It can refine risk assessment in intermediate- and high-risk patients. While niacin and LDL apheresis are currently the only therapies to reduce Lp(a), promising new drugs and therapeutic concepts are currently being tested.

Conflict of interest: G.M.K. received an investigator-initiated study grant from MSD. He is employed in Medical University of Graz, Austria. He has submitted a grant application dealing with the transcriptional regulation of APOA from Australian Research Foundation. K.M.K. is employed in Master Hospital University of QLD, Brisbane, which offers check-up and treatment of dyslipoproteinaemic patients. He was paid for lectures including service on speakers bureaus from MSD, Astra Zaneka (lectures for practitioners). W.M. is employed in Synlab Services GmbH, which has applied for patents in CVD risk assessment. LURIC, a non-profit organization, offers Lp(a) testing.

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