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Review

Lipoprotein(a) and Cardiovascular Disease

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BACKGROUND: High lipoprotein(a) concentrations present in 10%–20% of the population have long been linked to increased risk of ischemic cardiovascular disease. It is unclear whether high concentrations represent an unmet medical need. Lipoprotein(a) is currently not a target for treatment to prevent cardiovascular disease.

CONTENT: The present review summarizes evidence of causality for high lipoprotein(a) concentrations gained from large genetic epidemiologic studies and discusses measurements of lipoprotein(a) and future treatment options for high values found in an estimated >1 billion individuals worldwide.

SUMMARY: Evidence from mechanistic, observational, and genetic studies support a causal role of lipoprotein(a) in the development of cardiovascular disease, including coronary heart disease and peripheral arterial disease, as well as aortic valve stenosis, and likely also ischemic stroke. Effect sizes are most pronounced for myocardial infarction, peripheral arterial disease, and aortic valve stenosis where high lipoprotein(a) concentrations predict 2- to 3-fold increases in risk. Lipoprotein(a) measurements should be performed using well-validated assays with traceability to a recognized calibrator to ensure common cut-offs for high concentrations and risk assessment. Randomized cardiovascular outcome trials are needed to provide final evidence of causality and to assess the potential clinical benefit of novel, potent lipoprotein(a) lowering therapies.

Introduction

Despite notable advances in the prevention and treatment of cardiovascular disease (CVD), it remains a leading cause of morbidity and mortality (1, 2). This likely reflects the still high prevalence of classical risk factors such as increased cholesterol concentrations, smoking, hypertension, increased body mass index, diabetes, and physical inactivity. However, residual disease risk (i.e., risk unaccounted for by classical risk factors targeted today), also contributes, pointing to additional risk factors.

Lipoprotein(a) is a unique liver-derived lipoprotein with primarily genetically determined concentrations and large interindividual concentration variation with values ranging from <1 to >200 mg/dL in the general population (Fig. 1) (3); an estimated approximately 20% of the population have high concentrations corresponding to >42 mg/dL total mass lipoprotein(a) in a Danish general population cohort and using a lipoprotein(a) assay traceable to an international calibrator. Notably, lipoprotein(a) concentration distributions vary with race/ethnicity with individuals of African descent having on average higher concentrations than individuals of European or Asian descent (3). High lipoprotein(a) concentrations have long been linked to increased risk of ischemic CVD and, in particular, coronary heart disease (CHD) (3, 5). In the past decade, high concentrations have additionally been associated with increased risk of aortic valve stenosis (AVS) (6–8), heart failure (9), and low concentrations, paradoxically, with increased risk of type 2 diabetes (T2D) (10).

LIPOPROTEIN(A) STRUCTURE

A pathogenic pro-atherosclerotic and/or pro-thrombotic effect of lipoprotein(a) promoting ischemic CVD is consistent with its composition; a cholesterol-laden low-density lipoprotein (LDL)-like particle identified as a unique lipoprotein by the addition of one large plasminogen-like glycoprotein, apolipoprotein(a), covalently bound to apolipoprotein B (3) (Fig. 1). While plasminogen consists of 5 different kringle-shaped protein structures (I through V) and a protease region, apolipoprotein(a) consists of several plasminogen-like kringle IV structures (type 1–10), one plasminogen-like kringle V structure, and an inactive protease region (3). The number of kringle IV type 2 (KIV2) structures varies determining isoform size, which correlates inversely with hepatic production rates, likely due to prolonged intracellular processing and increased intracellular degradation of larger isoforms (3). Consequently, isoform size correlates inversely with lipoprotein(a) plasma concentrations and most individuals express 2 differently sized isoforms, with high concentrations usually found only for small isoforms while large isoforms are present at low concentrations (3).

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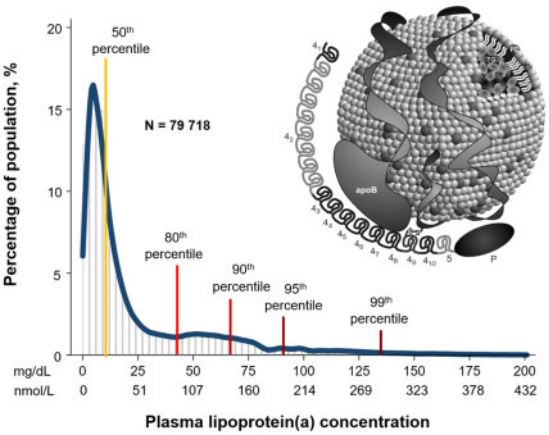

Fig. 1. Lipoprotein(a) composition and concentration distribution. Lipoprotein(a) consists of an LDL-like moiety with one plasminogen-like apolipoprotein(a) covalently bound to apolipoprotein B. Apolipoprotein(a) contains an inactive protease region and a variable number of kringle-shaped, 80–90 amino acid large, protein structures; kringle IV type 1–10 where the number of type 2 (KIV2) varies from 2 to >40, and kringle V. Plasma concentrations of lipoprotein(a) (total mass and particle number) in the Copenhagen General Population Study (n = 79 718 white individuals of Danish decent, 118 measurements >200 mg/dL not displayed). All measurement values were calibrated to fresh sample measurements by the latex-enhanced Denka Seiken (Denka Seiken, Tokyo, Japan) immunoturbidimetric assay with traceability to an international calibrator (WHO SRM 2B); the 50th and 80th percentile correspond to concentrations of 10.5 mg/dL and 41.8 mg/dL. Conversion to nmol/L was done according to the following equation based on ~13 900 individuals with measurements in both mg/dL and nmol/L (Denka Seiken Roche distributed assay using different calibrations for mg/dL and nmol/L): lipoprotein(a) nmol/L = 2.18*lipoprotein(a) mg/dL-3.83. Lipoprotein(a) illustration adapted with permission from Koschinsky et al. (4).

Lipoprotein(a) concentrations are usually reported as lipoprotein(a) total mass (i.e., mg/dL), however, with an increasing tendency to report particle number (i.e., nmol/L) and with a third rarely used option of reporting lipoprotein(a) cholesterol mass. For comparing results, it is important to note the distinction between the different measurements.

MECHANISM OF ACTION

In vitro and animal studies have implicated lipoprotein(a) in key processes in atherosclerosis, including foam cell formation, smooth muscle cell proliferation, and plaque inflammation and instability (11, 12). However, even in individuals with high lipoprotein(a) concentrations, the amount of pro-atherogenic cholesterol carried by lipoprotein(a) is usually considerably less than that carried by LDL; in the Copenhagen General Population Study (n = 50 000) the subgroup with the 5% highest lipoprotein(a) concentrations had a median LDL cholesterol level of 98 mg/dL [(2.5 mmol/L) corrected for the lipoprotein(a) cholesterol contribution) while the median lipoprotein(a) cholesterol concentration was 35 mg/dL (0.9 mmol/L) assuming a conservative estimate of 30% cholesterol mass in the lipoprotein(a) particle (13). Notably, kinetic studies have pointed to a preferential accumulation of lipoprotein(a) particles in the vessel wall, which may increase the atherogenic potential of lipoprotein(a) cholesterol vs. LDL cholesterol (14). More recently, lipoprotein(a) has also been identified as the main carrier of oxidized phospholipids considered proinflammatory and proatherogenic (15).

In plasminogen, the precursor of the fibrinolytic enzyme plasmin, the kringle-shaped protein structures facilitate binding to fibrin prior to the proteolytic cleavage and resulting fibrinolysis. In vitro and animal studies have indicated that lipoprotein(a), with similar kringle structures and an inactive protease region, inhibits fibrinolysis through competitive inhibition of plasmin activation and function, thus ultimately promoting thrombosis (11, 12). It is, however, unclear if this mechanism of competitive inhibition is active in vivo in humans, as plasminogen is generally in large molar excess of lipoprotein(a).

A physiological function of lipoprotein(a) has not been established despite the fact that lipoprotein(a) developed twice independently 40 million years apart during evolution, implying a strong positive selection pressure; lipoprotein(a) is thus found in humans, nonhuman primates, and Old World monkeys, and additionally in an aberrant form in the hedgehog (3, 16). Both the early hedgehog apo(a) variant and the later apo(a) variant found in humans arose from remodeling of plasminogen kringle domains (KIII and KIV+KV, respectively) (16). Lipoprotein(a) has been hypothesized to contribute to wound healing (17), transporting cholesterol to sites of injury for cell replenishment, and limiting bleeding via attenuated fibrinolysis. However, data to support this >30 year-old hypothesis remain scarce although a few studies have indicated that lipoprotein(a) accumulates preferentially at sites of tissue injury (14, 18, 19). A nonspecific wound healing effect of lipoprotein(a) may, however, explain an association with aortic valve disease considered the result of repeated valve injury and repair mechanisms (7). Recent in vitro studies, demonstrating osteogenic differentiation of valvular interstitial cells exposed to lipoprotein(a) and associated oxidized phospholipids, point to yet another possible

mechanism relevant for aortic valve disease, often characterized by pronounced valve calcification (20), and perhaps also for development of advanced atherosclerotic lesions. Further, the more recent findings of an association of low lipoprotein(a) concentrations with increased risk of T2D indicates a possible physiologic function of high levels, however, data in support of this hypothesis are lacking (21).

LIPOPROTEIN(A) AS A CAUSE OF CARDIOVASCULAR DISEASE

While experimental studies, such as in vitro or animal studies, may be excellent for elucidating possible pathophysiological mechanisms of action, they rarely provide firm answers as to whether the examined risk factor is truly a relevant cause of human disease. This is also the case for lipoprotein(a), where an association of high concentrations with increased risk of CVD has been observed in numerous observational studies, and where a multitude of data from experimental studies generally support a pathophysiological effect, and yet the question of whether high lipoprotein(a) concentrations represent an unmet medical need has remained unanswered for decades. To date, no randomized cardiovascular outcome trial of the effect of lowering high lipoprotein(a) concentrations has been conducted. However, in recent years, large genetic epidemiologic studies have generated renewed interest in lipoprotein(a) by providing strong genetic evidence of causal associations of high lipoprotein(a) concentrations with increased risk of CHD, AVS, heart failure, and mortality (6–9, 13, 22–25). The present review summarizes evidence of causality for high lipoprotein(a) concentrations gained from such studies and discusses measurements of lipoprotein(a) and future treatment options for high concentrations found in an estimated >1 billion individuals worldwide.

Epidemiology and Genetic Evidence of Causality

OBSERVATIONAL STUDIES

Lipoprotein(a) was first described in 1963 as a distinct beta-lipoprotein (26) and in the following years numerous studies on lipoprotein(a) were published, many of which were observational epidemiologic studies of lipoprotein(a) concentrations and CVD risk. While initial smaller cross-sectional studies generally demonstrated increased risk at high concentrations, the first large prospective studies published in the early nineties yielded null findings for atherosclerotic disease and myocardial infarction (MI) (27, 28), in retrospect likely due to the use of poor measurement methods. Thus, scientific interest in lipoprotein(a) was severely dampened, as demonstrated by the subsequent decrease in lipoprotein(a) publications. More recent large prospective general population studies (e.g., the Copenhagen City Heart Study,

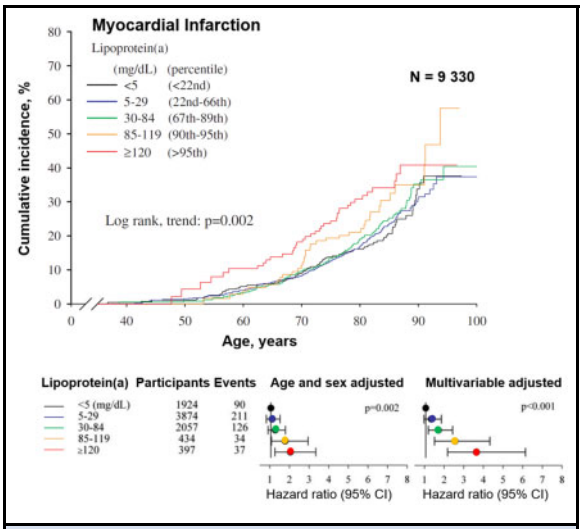

Fig. 2. Cumulative incidence and risk of myocardial infarction. Based on data from the Copenhagen City Heart Study (n = 9330 white individuals of Danish descent) with 10 years of follow-up and 498 incident cases of MI. Hazard ratios were adjusted for sex, age, and regression dilution bias and in multivariable adjusted analyses additionally for total, LDL, and HDL cholesterol, triglycerides, apolipoprotein B, body mass index, hypertension, diabetes, smoking, lipid-lowering therapy, and, for women, menopause and hormone replacement therapy. Total and LDL cholesterol, and apolipoprotein B were adjusted for the lipoprotein(a) contribution. Adapted from Kamstrup et al. (29).

Fig. 2) using fresh samples and well-validated assays have since then documented marked, independent, and stepwise increases in risk of CHD with increasing lipoprotein(a) concentrations (29). In 2009, results from prospective studies were summarized in a large individual participant data meta-analysis by the Emerging Risk Factors Collaboration documenting robust and independent, albeit moderate, increases in risk of nonfatal MI and coronary death with increasing concentrations of lipoprotein(a) (13% increased risk per 1 SD higher concentration) and also increased risk of ischemic stroke (IS) (10% increased risk per 1 SD higher concentration) (30). Importantly, while the meta-analysis primarily included studies conducted in whites, other ethnic/racial groups were represented, and no evidence of differences in risk estimates in different ethnic/racial groups was found, consistent with subsequent findings from the Atherosclerosis Risk in Communities Study where associations with cardiovascular outcomes were at least as strong in blacks with 2- to 3-fold higher median concentrations compared to whites (31). Of note, the reported risk estimates from the meta-analysis likely represent minimal estimates as early (false) negative studies

were included. Nonetheless, the meta-analysis together with the subsequent publication of two large genetic epidemiologic studies, causally implicating high lipoprotein(a) concentrations in ischemic CVD (22, 23), generated renewed interest in lipoprotein(a) as an emerging risk factor and possible new target for treatment.

GENETIC STUDIES AND THE CONCEPT OF MENDELIAN RANDOMIZATION

Epidemiologic studies incorporating genetic data may overcome some of the limitations of purely observational epidemiologic studies inherently prone to confounding, bias, and reverse causation preventing conclusions with regards to causality. A study design increasingly used in genetic epidemiology is the Mendelian randomization study (32) examining 3 associations; 1) the association of a putative causal risk factor with risk of disease, 2) the association of selected genotypes with said risk factor, and 3) the association of the genotypes with risk of disease. Formal statistical analysis of these associations includes instrumental variable analysis, integrating association 2 and 3. The term "Mendelian" refers to Mendel's Second Law stating that the assortment of alleles at gamete formation is independent, such that any trait inherited by the offspring is inherited independently of other traits. Thus, in a genetically homogenous population, the distribution of genotypes associated with concentrations of a putative risk factor is random with possible confounders likely evenly distributed between carriers and noncarriers of the genotype of interest permitting conclusions on causality as in a randomized clinical trial setting (Fig. 3) (33).

Although Mendelian randomization studies can provide strong genetic evidence of causality, some limitations do apply (32, 33). Pitfalls, that may lead to false negative or (less likely) false positive findings for associations of genetic variants with risk of disease, include use of underpowered studies, use of genetic variants lacking a strong association with the risk factor of interest, or use of pleiotropic genetic variants (i.e., variants associated not only with the risk factor of interest but also with other known or unknown factors). Additional pitfalls include presence of developmental compensation for deleterious genetic variation, presence of genetic confounding (e.g., if the examined genetic variants are in linkage disequilibrium with other genetic variation associated with disease), or finally, presence of population admixture where the examined genetic variants are only found in a subgroup of the study population also differing in other aspects from the remainder of the population (32, 33). However, with careful selection of well-characterized genetic variants that are strongly associated with the risk factor of interest, and the use of adequately sized studies conducted in homogenous populations, most pitfalls can be avoided minimizing risk of false negative or false positive findings.

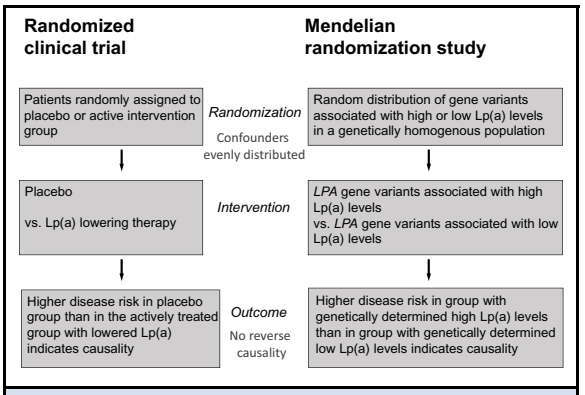

Fig. 3. Comparison of a Mendelian randomization study and a randomized clinical trial. In a randomized clinical trial, treatment lowering levels of a risk factor or placebo is randomly allocated to ensure even distributions of possible confounders between study arms. Decreased risk of disease in the actively treated arm is taken as proof that the risk factor is indeed a (treatable) cause of disease. In a Mendelian randomization study, an association of randomly distributed genotypes (associated with the risk factor) with risk of disease indicates that the risk factor is causal. Reverse causality is ruled out as disease does not affect trial allocation to treatment or placebo nor can disease alter inherited genotypes. Lp(a) = lipoprotein(a).

Lipoprotein(a) represents an excellent candidate for Mendelian randomization studies as 80%–90% of the total variation in lipoprotein(a) concentrations is genetically determined primarily by variation in the *LPA* gene coding for apolipoprotein(a) (3). Thus, while concentrations vary widely between individuals, the intraindividual variation is low with relatively stable concentrations throughout adult life (with a few exceptions, including end-stage kidney disease) (3). *LPA* variants used as genetic instruments in Mendelian randomization studies include the KIV2 copy number variant, defined by a 5.6 kb large repeat present in 2 to >40 copies per allele determining apolipoprotein(a) isoform size (3), as well as a number of single nucleotide polymorphisms (SNPs), some partly tracking the KIV2 variant (7, 23, 34).

GENETIC EVIDENCE FOR ISCHEMIC CARDIOVASCULAR DISEASE

In 2009, two large genetic epidemiologic studies were published that collectively implicated lipoprotein(a) as a causal risk factor for CHD (22, 23). First, a classic Mendelian randomization study of >40 000 individuals demonstrating increased risk of MI as a function of both increased lipoprotein(a) concentrations and low number of *LPA* KIV2 repeats, associated with increased concentrations (22). A clear stepwise increase in risk with higher lipoprotein(a) concentrations and

corresponding lower number of *LPA* KIV2 repeats was seen. Upon instrumental variable analysis, a doubling in lipoprotein(a) concentrations was associated with an approximate 20% increase in risk of MI. Second, a large case-control study including 3100 CHD cases genotyped for circa 49 000 genetic variants in 2100 candidate genes, identifying (with replication in an additional circa 4800 cases) two *LPA* SNPs (the intronic rs10455872 and the missense rs3798220), as having the strongest association with risk of CHD (23) of all SNPs tested. In a subset of participants, SNP carriers vs. noncarriers had higher lipoprotein(a) concentrations, lower numbers of *LPA* KIV2 repeats, and smaller isoform size. Combined, these 2 genetic epidemiologic studies provided strong genetic evidence of a causal association of lipoprotein(a) with CHD consistent with data from previous studies on apolipoprotein(a) phenotype and CHD risk (3) and purely genetic studies, including genome-wide association studies, identifying the *LPA* gene locus, and some studies specifically the *LPA* rs3798220 SNP, as being associated with increased risk of CHD (34–39). In follow-up studies on the Copenhagen general population cohorts, high lipoprotein(a) concentrations and corresponding *LPA* risk genotypes were associated also with cardiovascular (and all-cause) mortality and with recurrent major adverse cardiovascular events providing genetic evidence of causality also in recurrent CVD (25, 40).

Further support for a causal association of lipoprotein(a) with risk of CHD has come from studies documenting decreased risk in carriers of loss-of-function *LPA* variants associated with low lipoprotein(a) concentrations (41, 42). In the PROCARDIS study (41) including circa 4000 cases and a similar number of controls, carriers had on average 39% lower median lipoprotein(a) concentrations and 21% decreased risk of CHD, indicating the potential therapeutic benefit of lowering lipoprotein(a) concentrations. Similarly, in a very large genetic study ($n > 100\ 000$) by Emdin et al., a gene risk score based on 4 *LPA* SNPs, strongly associated with low plasma lipoprotein(a) concentrations, predicted a 29% decrease in risk of CHD (and decreased risk of peripheral arterial disease (PAD), AVS, heart failure, and stroke) per one SD genetically lowered lipoprotein(a) concentrations (43). Finally, convincing evidence of causality has been provided by recent Mendelian randomization studies using genetic data to estimate the lipoprotein(a) lowering required to result in clinically meaningful CVD risk reductions given future therapeutic options (40, 44, 45).

Genetic epidemiologic studies have also provided evidence for causal associations of high lipoprotein(a) with atherosclerotic disease of carotid and peripheral arteries (24, 46–48). In case-control studies, Kamstrup et al. found that a doubling of genetically determined lipoprotein(a) was associated with a 12%–16% increased risk of atherosclerotic stenotic disease of coronary, carotid, and femoral arteries (24). Small apolipoprotein(a) isoforms and the minor allele of *LPA* rs10455872 have subsequently been associated with increased risk of PAD in 3 independent cohorts (47). Kamstrup et al. reported null findings for lipoprotein concentrations and number of KIV2 repeats and risk of venous thromboembolic disease (VTE) in nearly 38 000 general population participants; however, in post hoc analyses, increased risk was found for extremely high lipoprotein(a) concentrations (>95th percentile) and corresponding very low number of KIV2 repeats (24). In updated prospective analyses of the same cohorts ($n = 57\ 000$), participants with concentrations in the top 5% had a 30% increased risk of VTE, whereas instrumental variable analyses estimating risk of VTE per 50 mg/dL genetically increased concentrations based on *LPA* rs10455872 or number of KIV2 repeats yielded null findings (49). However, VTE risk associated with very low number of KIV2 repeats was not explored. In support of the initial genetic finding, a later case-control study has demonstrated lower number of *LPA* KIV2 repeats in about 500 VTE patients than in controls (50).

The abovementioned findings for atherosclerotic disease and VTE are generally consistent with results from large studies examining the associations of *LPA* SNPs with CVD(50,51); Helgadottir et al. combined 35 case-control series to detect increased risk of PAD, abdominal aortic aneurism, and IS subtype large artery atherosclerosis in minor allele carriers of *LPA* rs10455872 and/or rs3798220 associated with increased lipoprotein(a) concentrations (51). However, no association with VTE, carotid intima media thickness, IS subtype cardioembolic, or small vessel disease was found. Likewise, in the Women's Health Study ($n = 21\ 000$) no association with VTE for either of these SNPs (nor lipoprotein(a) concentrations) was found (52). Thus, large genetic epidemiologic studies have not been able to provide unequivocal evidence of causal associations of lipoprotein(a) with risk of VTE (24, 51) despite clear prothrombotic effects of lipoprotein(a) demonstrated in experimental studies (3, 11, 12), and despite positive findings in meta-analyses of observational studies (53).

Likewise, genetic epidemiologic evidence of causal associations of lipoprotein(a) with risk of IS remain relatively scarce, although Emdin et al. did find that 1 SD genetically lowered lipoprotein(a) concentration predicted 13% decreased risk of stroke (43). This contrasts with the null findings for IS and a genotype score based on *LPA* rs10455872 and rs3798220 reported in about 14 000 middle aged, primarily male, Heart Protection Study participants (54). Recently, Langsted et al., however, reported increased risk of IS for high lipoprotein(a)

concentrations and corresponding *LPA* risk genotypes in the contemporary Copenhagen General Population Study (circa 49 500 participants, 1045 incident cases) while no association was found in the historic Copenhagen City Heart Study (circa 10 500 participants, 827 incident cases), despite similar statistical power (13). In the contemporary setting, a 50 mg/dL increase in genetically determined lipoprotein(a) concentrations is associated with a 20%–27% increased risk of IS. The lack of a statistically significant association in the historic setting may reflect the inclusion of older and more high-risk (based on classical risk factors) participants, fewer of whom received statins, where the moderate effect of genetically determined lifelong high lipoprotein(a) may have been masked by the cumulative effect of lifestyle risk factors despite multivariable adjustment. A similar limitation may apply to results reported from the Heart Protection Study.

Collectively, the findings from genetic epidemiologic studies strongly support a causal role of lipoprotein(a) in CHD and PAD, and likely also IS, while evidence of causality is less strong for VTE. Consequently, results have tentatively been interpreted as lipoprotein(a) promoting atherosclerosis more than thrombosis (24), acknowledging that it is difficult to reach mechanistic conclusions based on epidemiologic findings, even when incorporating genetic information. Thus, lack of a strong prothrombotic effect of lipoprotein(a) in a venous setting does not necessarily exclude a prothrombotic effect in an arterial setting, illustrated by the fact that established risk factors for arterial thrombosis generally differ from those for venous thrombosis. Accordingly, it remains a possibility that an antifibrinolytic/prothrombotic effect of lipoprotein(a) may contribute not only to MI but also to atherosclerotic stenosis, with lipoprotein(a) here perhaps exerting also an unspecific wound healing effect in a setting of plaque rupture, healing, and stenosis (55). The hypothesis is supported by findings from the Women's Health Study (a randomized trial of low-dose aspirin) demonstrating no increased CVD risk in the treated group of minor allele carriers of *LPA* rs3798220 with high lipoprotein(a) concentrations (*P* for interaction 0.048) (56).

Genetic epidemiologic studies have also been used to test whether current risk prediction algorithms may be improved with the inclusion of information on lipoprotein(a) concentrations and/or *LPA* genotypes (57, 58). In 2012 the Emerging Risk Factors Collaboration combined data from 24 prospective studies and demonstrated only slight improvements in cardiovascular risk prediction when adding lipoprotein(a) concentrations to classical risk factors; importantly the study assessed the ability of lipoprotein(a) to improve risk prediction across the entire spectrum of lipoprotein(a) concentrations and did not assess genetic data (57). Likewise, in3 cohorts of women including the Women's Health Study and considering the entire lipoprotein(a) concentration range, only minimal improvement in prediction was found (59). However, this approach may not adequately capture the ability of a continuous risk factor with a highly skewed concentration distribution, such as lipoprotein(a), to markedly improve risk prediction for individuals in the tail part of the distribution. In the Copenhagen City Heart Study, a 10-year CHD risk prediction was subsequently found to improve substantially upon addition of information on lipoprotein(a) concentrations exclusively in individuals with values >80th percentile (58). Similarly, adding information on *LPA* risk genotypes to classical risk factors improved prediction, and a tendency towards superior risk prediction was seen when including both information on high lipoprotein(a) concentrations and *LPA* risk genotypes (58). This additive effect may point to a qualitative difference in the pathogenic potential of different lipoprotein(a) isoforms, where small isoforms may be particularly harmful beyond their association with increased concentrations, as indicated previously (60–62). Alternatively, the additive effect may result from *LPA* genotypes more accurately reflecting, compared to a single plasma measurement, lifelong lipoprotein(a) concentrations. Nonetheless, for risk prediction purposes, the simple measurement of plasma lipoprotein(a) using a well-validated assay, appears sufficient without the need for *LPA* genotyping (58), consistent with findings from the Bruneck Study where inclusion of information on lipoprotein(a) concentrations markedly improved risk prediction of both the Framingham and Reynolds risk score but with no further improvements achieved upon the addition of information on apolipoprotein(a) isoform size (63).

At present, recommendations to include lipoprotein(a) measurements in CVD risk assessment in at risk subgroups have been included in the 2016 European Guidelines for the Management of Dyslipidaemias and in the 2018 American Heart Association/American College of Cardiology Guidelines on the Management of Blood Cholesterol (64, 65). These guidelines are in line with consensus panel statements from the European Atherosclerosis Society and the U.S. National Lipid Association recommending screening for increased lipoprotein(a) in individuals with intermediate to high CVD risk (66, 67). Notably, a once-in-a-lifetime lipoprotein(a) measurement, with possible confirmatory repeat measurement in those with very high concentrations, is sufficient, as concentrations are relatively stable throughout adult life; for example, in circa 8100 Copenhagen General Population Study participants, a minimal bias of 1.3 mg/dL and an r^2 value of 0.89 ($P < 0.001$) was observed when comparing by linear

regression 2 measurements performed up to 12 years apart (unpublished data).

GENETIC EVIDENCE FOR AORTIC VALVE STENOSIS

AVS, a chronic progressive disease leading to heart failure, is increasingly common in aging populations, and risk factors include hypertension, smoking, T2D, and increased cholesterol concentrations in addition to bicuspid valve morphology (68). Notably, large randomized statin trials in individuals with mild to moderate AVS have failed to show benefit, possibly as risk factors for disease initiation and progression differ (69). At present, no means to halt disease progression exists, and aortic valve replacement, costly and associated with perioperative morbidity and mortality, represents the only curative treatment (68).

While familial aggregation has been noted for both bicuspid and tricuspid valve disease, knowledge of specific genetic factors predisposing to AVS has been limited. However, in 2013 a large genome-wide association study by Thanassoulis et al. identified the *LPA* rs10455872 SNP (associated with lipoprotein(a) concentrations) as strongly associated with aortic valve calcification, considered an early phenotype for AVS (6). Further, in 2 prospective general population replication studies, minor allele carriers of the rs10455872 SNP were at increased risk of overt AVS. In a subsequent expanded analysis of the Copenhagen General Population Study (n = 77 000), high lipoprotein(a) concentrations were associated in a stepwise manner with increased risk of AVS; individuals with values above the 90th percentile had a 2- to 3-fold increased risk of AVS in multivariable adjusted analyses, as compared to individuals in the lower fifth of the concentration distribution (7). Upon instrumental variable analysis based on 3 *LPA* variants explaining 41% of the total variation in plasma lipoprotein(a) concentrations, genetically determined high lipoprotein(a) concentrations were associated with increased risk of AVS (Fig. 4). In follow-up studies using the same cohort, lipoprotein(a) associated oxidized phospholipids were implicated in the AVS association (70), and high lipoprotein(a) and associated *LPA* risk genotypes additionally associated with increased risk of heart failure, with the increase in risk primarily mediated via a history of AVS or MI, providing further evidence of causality (9). Findings for AVS have since been replicated; the prospective EPIC-Norfolk general population study demonstrated 1.8- and 4.8-fold increased risk of AVS for hetero- and homozygous minor allele rs10455872 carriers as compared to noncarriers, and 1.6-fold increased risk for individuals in the top tertile of lipoprotein(a) concentrations as compared to individuals in the lower tertile (8). Further, in a meta-analysis including a large dataset from the UK Biobank, the *LPA* rs10455872 and rs3798220 variants, both associated with high lipoprotein(a) concentrations, associated with increased risk of AVS (71). Likewise, a robust association with AVS (and ischemic CVD and heart failure) has been reported in a recent large case-control study of 143 087 Icelanders with measured and imputed lipoprotein(a) concentrations and number of KIV2 repeats (72).

Collectively, these findings provide strong genetic evidence of a causal association of lipoprotein(a) with aortic valve disease. Nonetheless, it is presently unclear whether high lipoprotein(a) concentrations contribute to early and/or late stages of disease often considered a 2-step process of inflammation, akin to atherosclerosis, followed by a fibrotic and calcific stage resulting in symptomatic stenosis of the valve (73). As current imaging modalities are not suited to detect very early stage disease, opportunities for targeted prevention of disease progression may depend on an effect on also fibrotic and calcific disease stages. Indeed, recent studies suggest a role for high lipoprotein(a) and associated oxidized phospholipids in the progression of both mild-to-moderate and more advanced disease; in 220 middle-aged patients with mild-to-moderate AVS, faster disease progression rates and increased risk of aortic valve replacement were seen in patients with high lipoprotein(a) and bound oxidized phospholipids (74), and in 145 elderly patients, high lipoprotein(a) and oxidized phospholipids associated with valve calcification and disease progression (20).

LIPOPROTEIN(A), *LPA* GENOTYPES, AND RISK OF TYPE 2 DIABETES AND MORTALITY

While the present review focuses on CVD, the association of lipoprotein(a) with risk of T2D deserves mention. In 2009, Mora et al. published the somewhat surprising finding from the Women's Health Study (n = 27 000) that low lipoprotein(a) concentrations associated with increased risk of incident T2D; lipoprotein(a) concentrations in the top 2–5 quintiles predicted ~20% decreased risk of T2D compared to first quintile concentrations (10). The finding was confirmed in cross-sectional analyses of about 9650 Copenhagen City Heart Study participants (10). Studies conducted in diverse ethnic backgrounds have since replicated the initial finding (21) and raised concerns that lipoprotein(a) lowering to prevent CVD may potentially increase risk of T2D.

Genetic epidemiologic studies have subsequently tested the hypothesis that low lipoprotein(a) concentrations cause increased risk of T2D. In the combined Copenhagen studies (n = 78 000), low lipoprotein(a) concentrations (first quintile with median concentrations of 3 mg/dL) and corresponding high number of KIV2 repeats associated with 25% and 16% increased risk of T2D strongly indicating a causal association

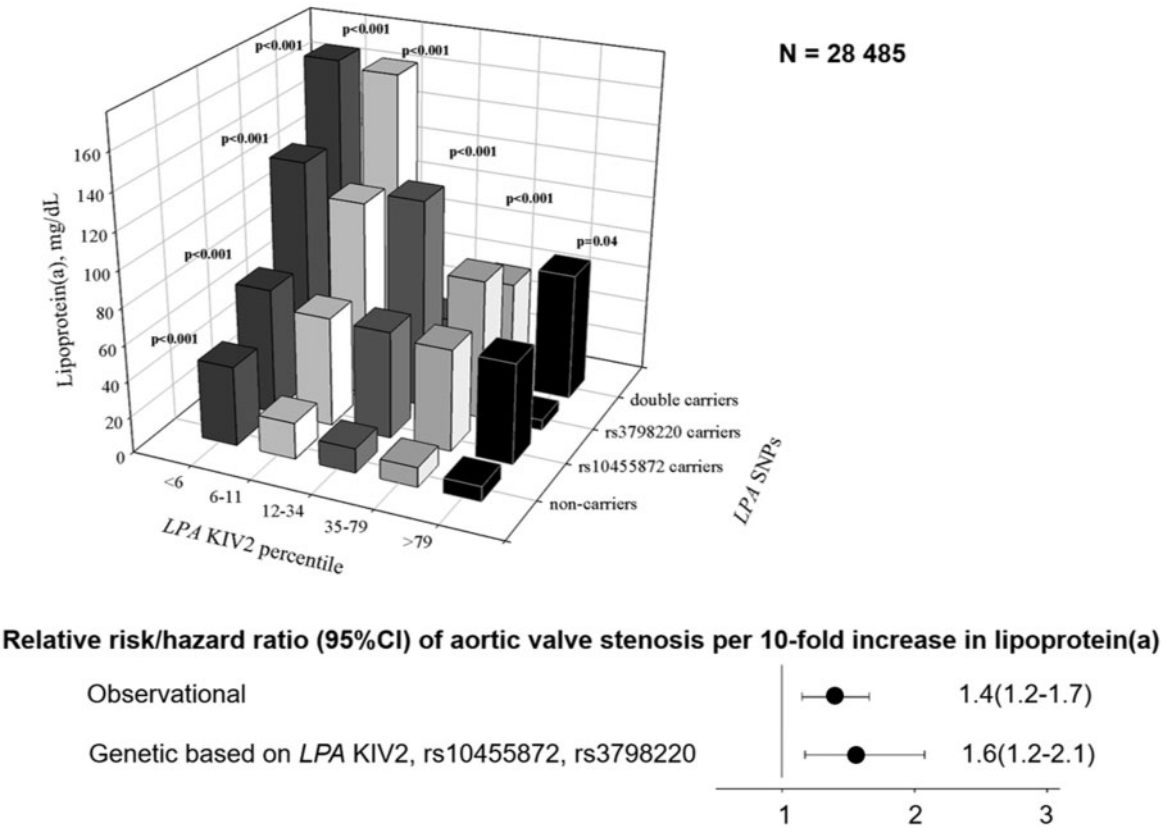

Fig. 4. Plasma lipoprotein(a) concentration as a function of *LPA* genotype and risk of aortic valve stenosis for a 10-fold increase in lipoprotein(a). In the Copenhagen General Population study (n = 28 485 white individuals of Danish descent), the *LPA* rs10455872 genotype explained 28%, the rs3798220 genotype 5%, and the KIV2 genotype (displayed as percentiles of sum of repeats on both alleles) 24%, and combined the genotypes explained 41% of the total variation in plasma lipoprotein(a) concentrations. The figure depicts the expected inverse association of KIV2 repeats with plasma lipoprotein(a) concentrations; unexpected low median lipoprotein(a) concentrations for KIV-2 percentiles 12–34 in SNP minor allele double carriers (n = 61) is likely due to chance. *P*-values denote test for trend of lipoprotein(a) concentrations across genotypes. Risk estimates were adjusted for age and sex and genetic estimates derived from instrumental variable analysis of individual participant data. Reproduced with permission from Kamstrup et al. (7).

(75). However, no association was found for *LPA* rs10455872 despite similar statistical power in terms of effect on lipoprotein(a) concentrations, tentatively interpreted as it is not the low lipoprotein(a) concentrations per se, but rather large isoform size causing increased risk of T2D through yet unknown mechanisms. The genetic findings for both *LPA* KIV2 and rs10455872 have since been replicated (21), although not consistently (72), and the interpretation remains controversial. Thus, it will likely require both mechanistic studies and clinical trial data to provide firm answers as to whether low lipoprotein(a) concentrations per se increases risk of T2D or not. Notably, increased risk of T2D is only seen for very low concentrations of lipoprotein(a) (<10 mg/dL) and reduction of high values to median concentrations will likely be enough to reduce risk of CVD avoiding potentially increased risk of T2D in treated individuals. In support of an over-all beneficial effect of low concentrations, large genetic epidemiologic studies have reported increased risk of cardiovascular and all-cause mortality in individuals with high concentrations compared to individuals with low concentrations (25, 76). In the Copenhagen studies (n = 119 000), a 50 mg/dL increase in lipoprotein(a) concentrations was associated with a 5% increase in risk of all-cause mortality, consistent with the 10% increase in risk found for a 50 mg/dL increase in genetically determined lipoprotein(a) through *LPA* KIV2.

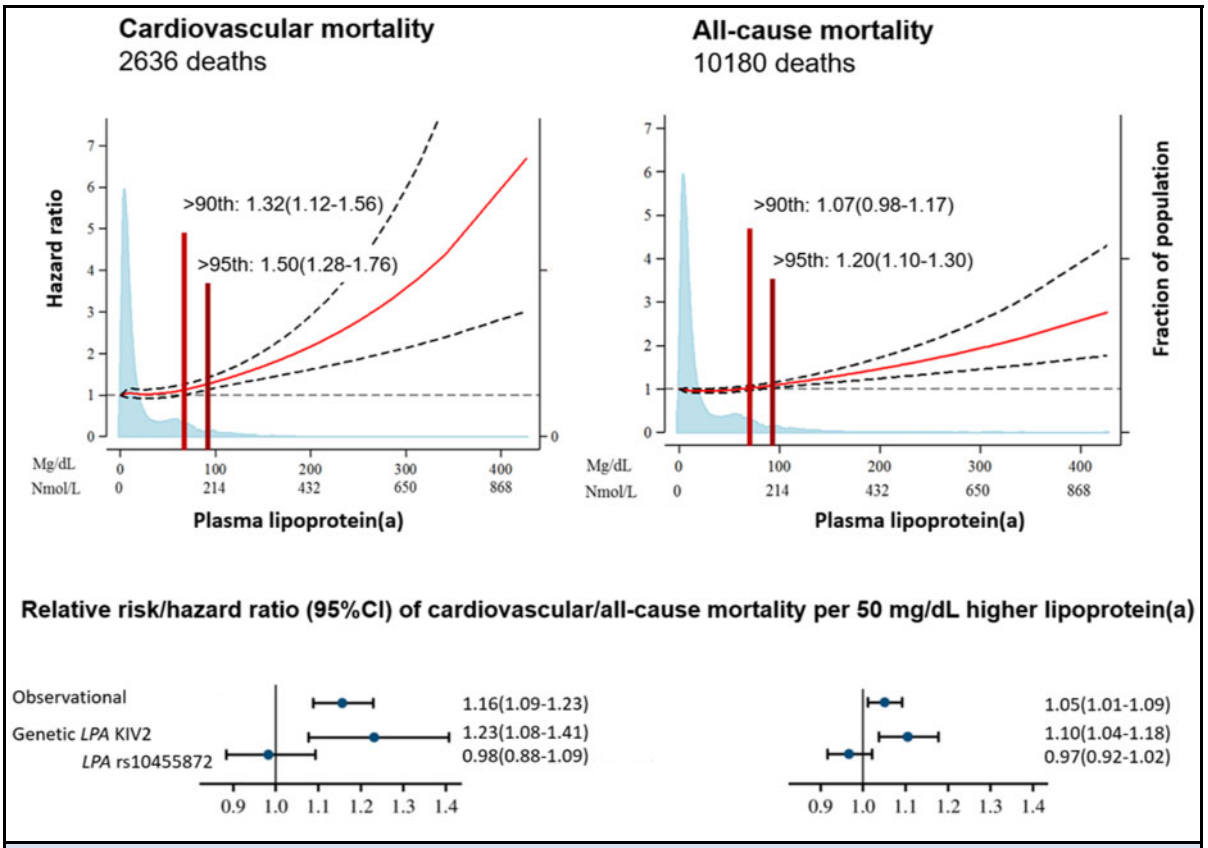

Fig. 5. Risk of cardiovascular and all-cause mortality as a function of lipoprotein(a) levels. Based on data from 119 000 white participants in the Copenhagen General Population Study and the Copenhagen City Heart Study. Solid lines are multivariable-adjusted hazard ratios, and dashed lines indicate 95% confidence intervals derived from restricted cubic spline regression with 4 knots (chosen based on best fit). The blue shade indicates the underlying lipoprotein(a) concentration distribution in the examined individuals. The graphs are truncated at the 1st and 99th percentiles. Cox regressions were adjusted for age, sex, smoking status, body mass index, hypertension, menopausal status (women only), hormone replacement therapy (women only), and lipoprotein(a) corrected LDL cholesterol. Adapted with permission from Langsted et al (25).

However, no increased risk was found for *LPA* rs10455872(24), suggesting that small isoforms may be particularly harmful beyond their association with increased concentrations (Fig. 5).

LIPOPROTEIN(A) MEASUREMENTS

Epidemiologic studies have reported highly variable median lipoprotein(a) concentrations and the often substantial differences in reported median concentrations may, in addition to established race/ethnicity population differences, be the result of using different assays. Lipoprotein(a) concentrations have historically been reported primarily as total mass concentrations (e.g., mg/dL) and using a variety of immunoassays (i.e., assays based on antigen-antibody interaction), most often immunoturbidimetric (77). At present, an increasing number of assays, including immunoturbidimetric and enzyme linked immunosorbent assays report particle number (i.e., nmol/L). Reporting of lipoprotein(a) cholesterol represents a third rarely used option relying on methodologies (e.g., electrophoresis or high-performance liquid chromatography) not suitable for high-throughput analysis. Regardless of assay methodology, a common problem has been the lack of traceability to a common calibrator or reference material with an assigned lipoprotein(a) target value preferably reported as particle number due to the variable lipoprotein(a) composition and size. Traceability to a common calibrator is a prerequisite for comparing measurements from different assays and thus for establishing common cut-points for increased risk. Indeed, at a mean lipoprotein(a) concentration of 57 mg/dL (total mass), standard deviations of 13 mg/dL have been reported when using 17 different immunoturbidimetric assays lacking

common calibration to measure lipoprotein(a) concentrations (78). Additionally, use of long-term frozen vs. fresh samples may contribute to observed differences in reported mean concentrations in comparable populations; in the Copenhagen General Population Study, the 80th percentile of the concentration distribution corresponded to a value of 41 mg/dL using fresh samples and 33 mg/dL using samples stored for on average 7 years at -80° Celsius (unpublished data). Finally, some assays are particularly prone to apolipoprotein(a) isoform size-dependent measurement bias, which may also affect reported lipoprotein(a) concentrations.

Apolipoprotein(a) isoform-dependent measurement bias may occur with assays using polyclonal antibodies directed at apolipoprotein(a) to determine lipoprotein(a) concentrations (77). Such assays rely on the assumption that isoform size does not substantially affect the degree of antibody binding and signal strength. However, considerable isoform-dependent measurement bias (see Fig. 6 for principle) of >100% positive and >50% negative bias has been reported, which may in epidemiologic studies, bias risk estimates toward the null (77, 79). Thus, assays using monoclonal antibodies directed at nonvariable apolipoprotein(a) domains are in theory preferable, however, not as easily developed nor implemented in high-throughput laboratories. Consequently, most commercially available assays use polyclonal antibodies and take measures to minimize isoform-dependent bias (e.g., using latex-particle-enhanced immunoassays), where complexes of cross-bound antibody-coated large latex particles minimize apolipoprotein(a) size-dependent differences in signal strength associated with the apolipoprotein(a) antigen-antibody binding itself, or the use of calibrators containing large apolipoprotein(a) isoforms for lower concentration calibration points and small isoforms for higher concentration calibration points. Of note, the latter strategy may lead to inaccurate measurements in individuals not expressing the expected inverse association of lipoprotein(a) concentrations with apolipoprotein(a) isoform size. The clinical consequences will, however, likely be minimal as presumably few individuals will cross a future decision limit for initiating lipoprotein(a) therapy and decisions to initiate therapy will be based on other CVD risk factors as well.

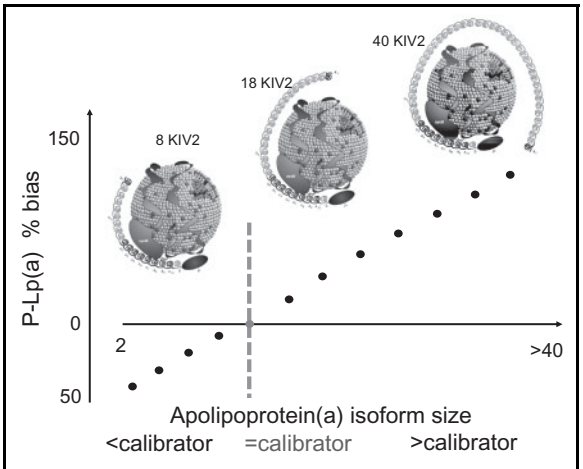

Fig. 6. Illustration of the principle of apolipoprotein(a) isoform-dependent measurement bias in a lipoprotein(a) assay using antibodies binding KIV2 repeats. Lipoprotein(a) concentrations are overestimated in patient samples containing isoforms larger than the assay calibrator (used to assign concentrations to assay signal values) and underestimated in samples containing small isoforms. Presence of isoform-dependent bias may bias risk estimates towards the null given the inverse association of apolipoprotein(a) isoform size with concentrations; high concentrations (associated with small isoforms) present in individuals at increased risk of CVD are underestimated while low concentrations (associated with large isoforms) present in individuals at low risk are overestimated. Lp(a) = lipoprotein(a).

In summary, preferably fresh samples in combination with well-validated lipoprotein(a) assays should be used (i.e., assays with documented acceptable precision and linearity, and with traceability to an internationally recognized calibrator, for example, the WHO SRM 2B primary reference material) ensuring common cut-offs for high concentrations. For assays lacking traceability, reporting of percentile cut points for the population concentration distribution, in addition to absolute measurement values, may provide some degree of comparability with other assays. Additionally, minimal apolipoprotein(a) isoform-dependent measurement bias should be documented by the assay provider. For reporting of lipoprotein(a) concentrations, there is consensus that particle number (e.g., nmol/L) is preferable to the traditionally used total mass (mg/dL) (66, 77). However, all assays based on polyclonal antibodies are to some degree affected by apolipoprotein(a) isoform-dependent measurement bias and may only approximate particle number. Thus, some argue that transitioning to nmol/L should await commercially available, high-throughput assays not prone to isoform dependent bias.

Treatment Options in High Lipoprotein(a)

Genetic epidemiologic studies have provided a strong rationale for trials of lipoprotein(a)-lowering therapy in individuals with high concentrations. However, to date no randomized cardiovascular outcome trial targeting high lipoprotein(a) has been conducted. Expected benefits include decreased risk of atherosclerotic CVD including MI and PAD, decreased risk of AVS, and decreased cardiovascular and all-cause mortality. Results

from genetic epidemiologic studies indicate that a lowering of 50 to 100 mg/dL (105 to 215 nmol/L) will be required to translate into clinical benefit (40, 44, 45). Of note, the high estimate of 100 mg/dL arose from a combined analysis of 5 different studies, including studies using lipoprotein(a) assays not traceable to an internationally accepted calibrator and with very high reported median lipoprotein(a) concentrations of up to 56 mg/dL vs. median concentrations of 14 mg/dL in studies using well-validated lipoprotein(a) assays (44). Nonetheless, substantial lipoprotein(a) lowering (i.e.,50 mg/dL) is likely required to demonstrate clinical benefit in the short term suitable for a randomized clinical trial.

At present there are no approved pharmacologic therapies that specifically target high lipoprotein(a) concentrations. While statins have no lipoprotein(a) lowering effect, and post hoc analyses of clinical trial data even indicate a slight lipoprotein(a) increasing effect (80), niacin, mipomersen, and PCSK9 inhibitors, all approved for dyslipidemia treatment, have demonstrated robust albeit modest (i.e., 20%–30%) lipoprotein(a) lowering effects on top of LDL cholesterol lowering effects (81–83). However, effect sizes of 20%–30% will likely not translate into substantial clinical benefit in individuals with high lipoprotein(a) concentrations and CVD. Additionally, the PCSK9 effect may be even less pronounced (<15%) in individuals with very high lipoprotein(a) concentrations (84). Further, both niacin and mipomersen are associated with side effects, and niacin has failed to show clinical benefit in large outcome trials, while mipomersen, an anti-sense oligonucleotide (ASO) targeting apolipoprotein B 100 expression, is only approved for treatment in homozygous familial hypercholesterolemia due to hepatotoxicity (81, 82). Finally, aspirin, mainly prescribed for antithrombotic purposes, has additionally been associated with modest lipoprotein(a) lowering effects (20%) in small studies, but is currently not recommended for treatment of high lipoprotein(a) concentrations (85). In Germany, individuals with high lipoprotein(a) concentrations and progressive CVD despite optimal lipid lowering therapy, are offered frequent apheresis therapy reducing lipoprotein(a) (and LDL cholesterol) concentrations and CVD rates in treated individuals (86). However, the treatment is costly, time-consuming, and difficult to test in a randomized clinical trial setting, and thus not widely implemented elsewhere.

Promising pharmacologic treatment options targeting high lipoprotein(a) concentrations are, however, on the horizon. ASOs targeting hepatic *LPA* messenger RNA, substantially reducing apolipoprotein(a) production, have successfully concluded phase 2 trials, with phase 3 cardiovascular outcome trials planned; in individuals with CVD and lipoprotein(a) concentrations >60 mg/dL, dose-dependent mean percent reductions of up to 70%–80% in lipoprotein(a) concentrations were seen with bi-monthly or monthly injections with acceptable safety profiles and no hepatotoxicity (87). Regardless, until the conclusion of outcome trials, treatment options for high lipoprotein(a) concentrations remain limited and preventive measures should focus on reducing all other modifiable CVD risk factors including lifestyle factors (despite little effect on lipoprotein(a) concentrations) and high LDL cholesterol concentrations, supported by studies demonstrating decreased risk of CVD in statin treated individuals with high lipoprotein(a) (88). Of note, very low LDL cholesterol concentrations are not achievable in individuals with high lipoprotein(a) concentrations, as lipoprotein(a) cholesterol is co-measured with LDL cholesterol, be it calculated, based on total and high-density lipoprotein cholesterol measurements, or directly measured.

Summary

Available evidence from mechanistic, observational, and genetic studies supports a causal role of lipoprotein(a) in the development of CVD, including CHD and PAD, as well as AVS, and likely also IS. While lipoprotein(a) concentration distributions differ between different racial/ethnic groups and most observational and genetic studies have been conducted in primarily white populations, there is currently no evidence to suggest that findings are not applicable across racial/ethnic groups. Effect sizes are most pronounced for CHD, PAD, and AVS where high lipoprotein(a) concentrations found in 15%–20% of the population predict up to 2- to 3-fold increases in risk. Lipoprotein(a) measurements should be performed using well-validated assays with traceability to a recognized calibrator to ensure common cut-offs for high concentrations and risk assessment. Randomized cardiovascular outcome trials are needed to provide final evidence of causality and assess the clinical benefit of novel, potent lipoprotein(a) lowering therapies.

Nonstandard Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease; AVS, aortic valve stenosis; T2D, type 2 diabetes; LDL, low density lipoprotein; KIV2, kringle IV type 2; MI, myocardial infarction; IS, ischemic stroke; SNP, single nucleotide polymorphism; PAD, peripheral arterial disease; VTE, venous thromboembolic disease; ASO, anti-sense oligonucleotide

Human genes: *LPA* lipoprotein(a).

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ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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