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Lipoprotein (a) concentrations, apolipoprotein (a) phenotypes, and peripheral arterial disease in three independent cohorts

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Aims The relevance of lipoprotein(a) [Lp(a)] concentrations and low-molecular-weight (LMW) apo(a) phenotypes in peripheral arterial disease (PAD) has only been investigated by few studies. Therefore, we analysed this association in three independent cohorts and performed a Mendelian Randomization approach using instrumental variable regression.

Methods and results Lp(a) concentrations, apo(a) phenotypes, and one SNP in the *LPA* gene (rs10455872) were measured in the CAVASIC study, including 241 male patients with intermittent claudication and 246 age- and diabetes-matched controls as well as in the two population-based studies KORA F3 ($n = 3184$) and KORA F4 ($n = 3080$). In KORA F3/F4, 109/80 persons suffered from intermittent claudication, 200/144 from PAD, and 128/103 showed an ankle–brachial index (ABI) <0.9. In CAVASIC, adjusted logistic regression analyses revealed significant associations between an increase of log-Lp(a) per one standard deviation (SD) (OR = 1.28, $P = 0.02$) as well as LMW apo(a) phenotypes and symptomatic PAD (OR = 1.65, $P = 0.03$). Linear regression models with continuous ABI showed a significant association in the combined analyses of KORA F3/F4: an increase in log-Lp(a) per one SD ($\beta = -0.006$, $P = 0.005$) and the presence of LMW apo(a) phenotypes ($\beta = -0.011$, $P = 0.02$) or the minor allele of rs10455872 ($\beta = -0.016$, $P = 0.03$) were associated with a decrease in ABI in the fully adjusted linear and instrumental variable regression models.

Conclusion Analyses in three independent populations showed significant associations of Lp(a) concentrations, LMW apo(a) phenotypes, and rs10455872 with PAD. This points to a causal relationship between Lp(a) and PAD since the genetically determined apo(a) phenotypes and SNP alleles are indeed associated with PAD.

Keywords Lp(a) concentrations • Apolipoprotein(a) phenotypes • Peripheral arterial disease • Ankle-brachial index • Mendelian randomization • Causality

1. Introduction

Lipoprotein(a) [Lp(a)] consists of a low-density lipoprotein molecule that is covalently connected to the high-molecular-weight glycoprotein apolipoprotein(a) [apo(a)]. Plasma concentrations of Lp(a) are determined to a large extent by the size of the apo(a) isoforms, which are expressed by a copy number variation of the *LPA* gene, varying between 11 and >50 repeats of the kringle-IV domain.[1] The apo(a)

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size polymorphism determines between 30 and 70% of the variance of Lp(a) concentrations depending on the population investigated.[1] An inverse relationship was found between the apo(a) isoform size and Lp(a) concentrations.[2] Both, high Lp(a) concentrations and a low molecular weight (LMW) of apo(a) phenotypes are associated with cardiovascular disease (CVD).[3–6] The pathophysiological role of Lp(a) is not fully understood yet but it has been reported to possess atherogenic and prothrombotic properties (reviewed in Kronenberg and Utermann[1]). Moreover, apo(a) has a high homology with plasminogen and *in vitro* studies reported interferences with the blood clotting and fibrinolytic cascades.[1]

Peripheral arterial disease (PAD) is a common manifestation of vascular disease resulting from atherosclerotic occlusions of the lower extremities. Depending on the definition of PAD, the prevalence ranges between 4 and 14% in the general population[7] and between 15 and 30% in adults above 60 years.[8,9] Symptomatic PAD clinically presents as intermittent claudication which is defined as pain in the calf muscles that occurs when walking and is relieved by a period of rest.[7] About one-third of all PAD patients show classical symptoms of intermittent claudication while asymptomatic PAD can be assessed by measuring the ankle–brachial index (ABI).[10] Both symptomatic and asymptomatic PAD markedly increases the risk of fatal and non-fatal cardiovascular events and mortality.[8,10–12] These observations triggered the search for related risk factors to enable early diagnosis especially for asymptomatic PAD to improve possibilities for an early intervention before fatal events occur. However, the possible relevance of Lp(a) concentrations and the presence of LMW apo(a) phenotypes in PAD patients have only been addressed by a few studies so far. Therefore, we investigated the association between Lp(a) concentrations, apo(a) phenotypes, and symptomatic as well as asymptomatic PAD determined by the ABI in a diseased population and two large population-based cohorts. Moreover, since apo(a) phenotypes and the SNP rs10455872 within the *LPA* gene were determined for each of the investigated cohorts, it was possible to apply a Mendelian Randomization approach to evaluate a putative causal effect of Lp(a) on PAD which is otherwise hardly possible using conventional epidemiological methods.

2. Methods

2.1 Study description and clinical characterization

In line with the Declaration of Helsinki, approval to the examination protocol for all three studies was given by the Ethics Committee and all participants provided written informed consent. A detailed description of the study populations can be found in the Supplementary material online. Shortly, the CAVASIC Study is a case–control study to explore the occurrence and determinants of CVD in male patients with intermittent claudication.[13,14] Patients were included consecutively when presenting with chronic intermittent claudication according to the criteria of Fontaine (PAD IIa or IIb) or a history of intermittent claudication, irrespective of former treatment procedures (bypass surgery or intervention). Overall, 249 patients and 251 age-and type-2 diabetes-matched controls were enrolled between 2002 and 2006. Lp(a) concentrations and LMW apo(a) phenotypes were measured in 241 patients and 246 controls and all subsequent data analyses included in this manuscript are based on these 487 individuals.

KORA F3 and KORA F4 are population-based follow-up studies recruited from the KORA S3 and S4 surveys and representative for the general population in Augsburg, Southern Germany. KORA F3 was carried out between 2004 and 2005 and a total of 3184 subjects participated. KORA F4 was conducted between 2006 and 2008 and a total of 3080 individuals were finally included.[15,16] Lp(a) concentrations and LMW apo(a) phenotypes were measured in 3156 participants in KORA F3 and 3061 participants in KORA F4. In the KORA studies, PAD was defined as symptomatic disease according to the Edinburgh questionnaire or asymptomatic disease (ABI$<$0.90) or both. Moreover, dichotomous outcome variables for patients presenting with ABI values $<$0.90 or with intermittent claudication were computed. A detailed description of the ABI measurement can be found in the Supplementary material online.

2.2 Lp(a) measurement, apo(a) phenotyping, and SNP genotyping

Lp(a) quantification was performed with a double-antibody ELISA and apo(a) phenotyping by SDS–agarose gel electrophoresis (for details refer Kronenberg *et al.*[17]). Apo(a) phenotypes were classified as low (LMW)- and high-molecular-weight (HMW) phenotypes.[1] The LMW group included all subjects with at least one apo(a) isoform with 11–22 KIV repeats; the HMW group included all subjects having only isoforms with $>$22 KIV repeats. If two apo(a) isoforms were detectable, we used only the smaller apo(a) isoform for categorization as discussed earlier.[18] In addition, the SNP rs10455872 within the LPA gene that has already been used in Mendelian randomization studies of Lp(a)[19] was investigated in each of the study populations (for details see Supplementary material online). The SNP was in Hardy–Weinberg Equilibrium in all three studies (all *P*-values from the exact test$>$ 0.07). In all analyses of this SNP, an additive inheritance model was assumed.

2.3 Statistical analysis

In CAVASIC, dichotomous and continuous variables were compared by χ^2 test, unpaired *t*-test or Mann–Whitney *U* tests as appropriate. To assess the causal association of Lp(a) on the respective PAD and/or ABI outcome variables, three different levels of analyses were applied (*Figure 1*): (i) association of Lp(a) concentrations on PAD/ABI; (ii) association of LMW apo(a) phenotypes and rs10455872 on PAD/ABI; and (iii) instrumental variable (IV) regression analyses. More specifically, logistic regression analyses were applied to evaluate the association between log-transformed Lp(a) concentrations, apo(a) phenotypes, rs10455872, and the dichotomous outcome variables. The odds ratios (ORs) and beta estimates correspond to an increment of one standard deviation of log-Lp(a) (SDlog-Lp(a)) in all analyses. Outcomes were PAD (presence of intermittent claudication) defined by the case–control status in the CAVASIC Study. In KORA, dichotomous outcomes were either the presence or absence of intermittent claudication or an ABI$</\geq$0.90 or both (defined as PAD). Furthermore, linear regression analyses were carried out to assess the association between log-Lp(a), LMW apo(a) phenotypes, and rs10455872 on ABI as a continuous variable. To obtain a combined estimate for KORA F3 and KORA F4, logistic and linear mixed models were applied.

Nonlinear P-Splines were used to check for linearity of Lp(a) in both linear and logistic regression analyses.[20] Since different cutpoints have been discussed for Lp(a) to dichotomize into high- and low-risk groups, optimal cutpoints were derived for all three studies by minimizing the Akaike information criterion (AIC). As a sensitivity analysis, estimates are additionally given for the most established cutpoints at 30 and 50 mg/dL. Further sensitivity analyses were performed applying different exclusion criteria: excluding individuals with prevalent CVDs and excluding individuals with ABI values above 1.30. Furthermore, Lp(a)-adjusted analyses of apo(a) phenotypes on ABI/PAD were performed.

All models shown are based on those individuals with available data on Lp(a) and apo(a) phenotypes and adjusted for age and/or sex. Extended models were additionally adjusted for a number of variables including the classical risk factors for PAD.

To investigate a putative causal effect of Lp(a) on PAD, IV regression analysis was performed using LMW apo(a) phenotypes and the SNP rs10455872

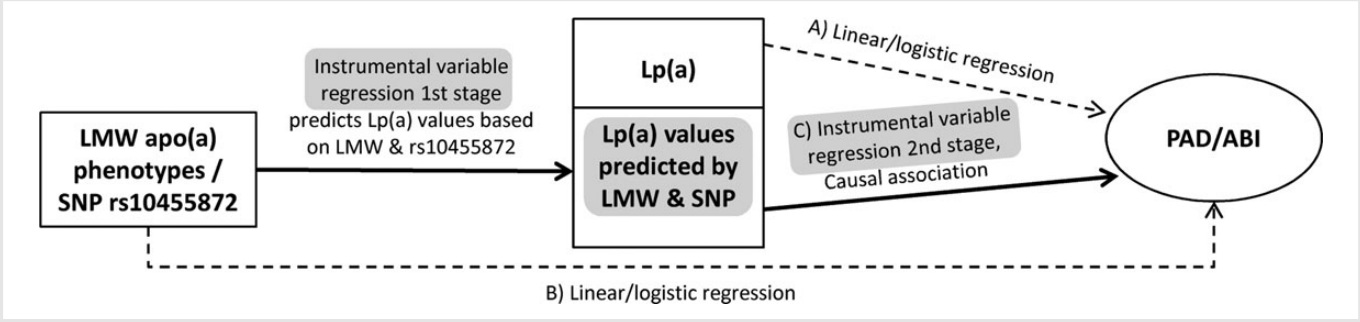

Figure 1 Chart illustrating the Mendelian Randomization approach based on different levels of analyses: regression of Lp(a) on PAD/ABI yielding the observed estimate (*A*); regression of LMW apo(a) phenotypes or SNP rs10455872 on PAD/ABI (*B*), and IV regression analyses (in grey boxes), which is a two-stage least-squares method, predicting Lp(a) levels from LMW apo(a) phenotypes and SNP rs10455872 (first stage IV regression) and regressing these predicted values on PAD/ABI (*C*).

Table 1 General and clinical characteristics of controls and patients with PAD of the CAVASIC study and the two population-based studies KORA F3 and F4

	Controls ($n = 246$)	PAD ($n = 241$)	*P*-value[a]	KORA F3 ($n = 3157$)	KORA F4 ($n = 3061$)
Age (years)	56 ± 9	58 ± 6	0.87	57 ± 13	56 ± 13
Sex (male), *n* (%)	246 (100)	241 (100)	–	1545 (48.5)	1478 (48.3)
Diabetes mellitus, *n* (%)	41 (16.7)	38 (15.8)	0.79	258 (8.1)	215 (7)
Current smoking, *n* (%)	29 (11.8)	124 (52.1)	<0.001	551 (17.5)	547 (17.9)
Hypertension, *n* (%)	146 (59.6)	208 (86.3)	<0.001	1589 (50.2)	1175 (38.3)
Cardiovascular disease, *n* (%)	18 (7.3)	76 (31.5)	<0.001	308 (9.7)	265 (8.7)
Ankle–brachial index	1.07 ± 0.12 (1.00/1.08/1.15)	0.72 ± 0.23 (0.54/0.70/0.89)	<0.001	1.13 ± 0.15 (1.05/1.13/1.20)	1.15 ± 0.16[b] (1.08/1.16/1.23) b
Intermittent claudication, *n* (%)	0 (0)	241 (100)	–	109 (3.4)	80 (2.6)
Peripheral arterial disease, *n* (%)	0 (0)	241 (100)	–	200 (6.4)	144 (8.0)[b]
Ankle–brachial index <0.9, *n* (%)	14 (6)	178 (74)	<0.001	128 (4.1)	103 (5.7)[b]
eGFR (mL/min/1.73m^2)	76.7 ± 12.2	81.4 ± 16.4	<0.001	77.4 ± 17.2	78.5 ± 17.3
C-reactive protein (mg/dL)	0.26 ± 0.33 (0.10/0.13/0.28)	0.63 ± 1.05 (0.22/0.42/0.71)	<0.001	–	–
Total cholesterol (mg/dL)	207.7 ± 35.0	204.8 ± 40.9	0.40	218.2 ± 39.8	215.9 ± 39.4
LDL-cholesterol (mg/dL)	135.6 ± 33.0	132.9 ± 37.0	0.39	127.9 ± 32.5	135.9 ± 34.8
HDL-cholesterol (mg/dL)	59.4 ± 16.2	49.2 ± 13.5	<0.001	58.8 ± 17.2	55.9 ± 14.4
Triglycerides (mg/dL)	132 ± 79 (79/131/156)	172 ± 123 (95/135/213)	<0.001	164 ± 125 c (88/135/201)	124 ± 89[c] (72/104/150)
Lp(a), (mg/dL)	19.5 ± 23.1 (4.2/10.1/28.5)	28.7 ± 31.9 (4.8/13.8/49.5)	0.006	22.0 ± 26.0 (4.9/11.1/28.5)	21.7 ± 25.6 (5.2/11.7/30.2)
LMW apo(a) phenotypes, *n* (%)	64 (26.0)	86 (35.7)	0.02	726 (23)	746 (24.4)
Minor allele frequency of rs10455872, %	5.6	8.4	0.14	5.2	6.0

Data are *n* (%) or mean ± SD and (25th, median 75th percentile) where appropriate.
[a]Comparison between cases and controls of the CAVASIC study were performed by χ^2 test, Fisher's exact test, unpaired *t*-test or Mann–Whitney *U* test as appropriate.
[b]ABI values in the KORA F4 study were only measured in 1796 individuals aged 52–81 years.
[c]Blood samples in KORA were collected in non-fasting state (KORA F3) or after an overnight fasting period (KORA F4).

as the IVs on log-Lp(a). A two-stage least-squares regression method was applied that regresses the LMW- and SNP-predicted log-Lp(a) values on the respective outcome variables (see Supplementary material online for details). To exclude a pleiotropic effect of LMW apo(a) phenotypes, we excluded upfront an association between LMW apo(a) phenotypes and classical risk factors or markers for PAD (data not shown).

Statistical analyses were performed using SPSS version 18.0, R version 2.15.2, and STATA version 11.

3. Results

3.1 Symptomatic PAD in the CAVASIC case–control study

Table 1 gives the characteristics of the CAVASIC Study including 241 patients with symptomatic PAD and 246 age- and T2DM-matched controls. As expected, patients with PAD were more often smokers or

hypertensive, more frequently had CVD and a lower ABI. Mean Lp(a) concentrations were significantly higher in patients than in controls (28.7 ± 31.9 vs. 19.5 ± 23.1 mg/dL, $P = 0.006$) and the frequency of LMW apo(a) phenotypes was higher in patients than in controls (35.7 vs. 26.0%, $P = 0.02$).

Table 2 shows the results from the logistic regression analyses. Since nonlinear splines showed a linear relationship between SDlog-Lp(a) and PAD (Supplementary material online, *Figure S3*), we performed analyses with log-Lp(a) as a continuous variable. When data were only adjusted for age, the OR for SDlog-Lp(a), LMW apo(a) phenotypes, and rs10455872 was 1.30 (95%CI 1.09–1.56, $P = 0.005$),1.60 (95%CI 1.08–2.36, $P = 0.019$), and 1.62 (95%CI 0.97–2.17, $P = 0.06$), respectively. The associations remained stable or were only slightly attenuated after further adjustment for current smoking and eGFR: OR = 1.28 (95%CI 1.04–1.57, $P = 0.02$) for SDlog-Lp(a), OR = 1.65 (95%CI 1.07–2.57, $P = 0.03$) for LMW apo(a) phenotypes, and OR = 1.50 (95%CI 0.83–2.71, $P = 0.12$) for rs10455872. After further adjustment for hypertension, the association for SDlog-Lp(a) with PAD was unchanged (OR = 1.30; 95%CI 1.05–1.61; $P = 0.02$), and was attenuated for LMW apo(a) phenotypes (OR = 1.42; 95%CI 0.90–2.25; $P = 0.14$) and the SNP (OR = 1.27; 95%CI 0.68–2.38; $P = 0.45$). Excluding patients and controls with prevalent CVD did not change the estimates of SDlog-Lp(a) or LMW in any of the models, but increased the OR for rs10455872 (*Table 2*).

The IV regression obtained significant causal ORs from Lp(a) on PAD (Model 1 and Model 2) that were even higher than the respective observed regression estimates (*Table 2*). First-stage regression from LMW apo(a) phenotypes and the SNP rs10455872 on Lp(a) yielded a F-Statistic (2df) of 128, verifying that the LMW apo(a) phenotype in combination with the *LPA* SNP are suitable instruments for Lp(a). LMW phenotypes alone explained 33.7% of Lp(a) variance, and the rs10455872 about 15%. In combination, 35% of Lp(a) variance were explained.

3.2 Symptomatic and asymptomatic PAD in the population-based KORA studies

In a next step, we investigated whether findings in the diseased population of the CAVASIC study can be confirmed in the two population-based studies of KORA. KORA F3 and F4 included 3157 and 3061 participants, respectively. Of these, 200 individuals in F3 and 144 individuals in F4 were diagnosed with symptomatic and/or asymptomatic PAD. Intermittent claudication according to the Edinburgh questionnaire was present in 109 participants of KORA F3 and 80 participants of KORA F4 (see Characteristics in *Table 1*). ABI measurements were available for 3143 participants in KORA F3 and an ABI <0.90 was detected in 128 individuals (4.1%). In KORA F4, ABI was measured only in 1796 study participants who were older than 51 years. An ABI <0.9 was detected in 103 of these participants (5.7%).

Figure 2 shows the skewed distribution of Lp(a) concentration in a combined analysis from KORA F3 und F4 with in total 6218 individuals (*Figure 2A*). When Lp(a) concentration was analysed stratified by groups of KIV repeat numbers (*Figure 2B*), we observed a major difference of Lp(a) concentrations between LMW and HMW apo(a) phenotype groups with about 5- to 10-fold higher median concentrations in individuals with LMW than those with HMW apo(a) phenotype groups. This strongly justifies the stratification in these two phenotype groups for the further analyses. Median concentrations of Lp(a) also varied strongly between genotype groups of SNP rs10455872: 9.9 mg/dL for the AA, 50.3 mg/dL for the GA, and 90.9 mg/dL for the GG genotype.

In KORA F3, Lp(a) concentrations and the frequency of LMW apo(a) phenotypes were slightly but non-significantly higher in participants with compared with those without PAD (26.1 ± 29.6 vs. 21.7 ± 25.8 mg/dL, $P = 0.07$; 27.6 vs. 23.0%, $P = 0.14$). However, in KORA F4, neither Lp(a) concentrations nor the frequency of LMW apo(a) phenotypes differed significantly between participants with and without PAD (Supplementary material online, *Table S1*). Similar observations in the two cohorts were

Table 2 Logistic regression analysis on the association of SDlog-Lp(a) and LMW apo(a) phenotypes with PAD in the CAVASIC study

	SDlog-Lp(a)			IVs: LMW apo(a) phenotype/SNP rs10455872 (additive coding)				IV regression based on LMW apo(a) phenotype and SNP rs10455872		
	OR	(95%CI)	*P*-value		OR	(95%CI)	*P*-value	OR	(95%CI)	*P*-value
Model 1: adjusted for age										
All patients and controls	1.30	(1.09, 1.56)	0.005	LMW	1.60	(1.08, 2.36)	0.02	1.47	(1.08, 1.99)	0.01
				SNP	1.62	(0.97, 2.71)	0.06			
Excluding CVDs	1.34	(1.08, 1.65)	0.007	LMW	1.60	(1.03, 2.48)	0.04	1.48	(1.05, 2.10)	0.03
				SNP	1.80	(1.03, 3.18)	0.04			
Model 2: adjusted for age, current smoking, and eGFR										
All patients and controls	1.28	(1.04, 1.57)	0.02	LMW	1.65	(1.07, 2.57)	0.03	1.48	(1.05, 2.09)	0.03
				SNP	1.50	(0.83, 2.71)	0.12			
Excluding CVDs	1.37	(1.07, 1.75)	0.01	LMW	1.74	(1.05, 2.90)	0.03	1.56	(1.04, 2.32)	0.03
				SNP	1.86	(0.95, 3.64)	0.07			
Model 3: adjusted for age, current smoking, eGFR, and hypertension										
All patients and controls	1.30	(1.05, 1.61)	0.02	LMW	1.42	(0.90, 2.25)	0.14	1.30	(0.94, 2.01)	0.14
				SNP	1.27	(0.68, 2.38)	0.45			
Excluding CVDs	1.40	(1.08, 1.80)	0.01	LMW	1.56	(0.92, 2.63)	0.10	1.41	(0.94, 2.12)	0.09
				SNP	1.67	(0.82, 3.39)	0.16			

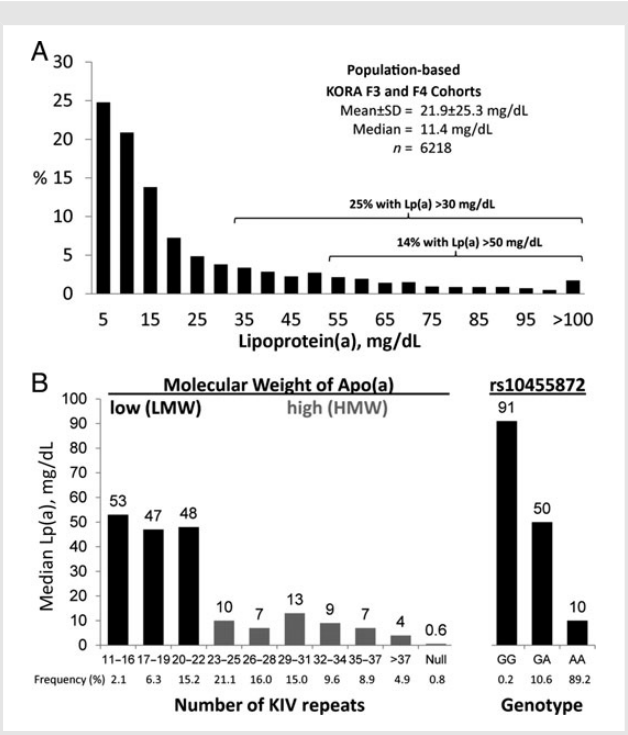

Figure 2 (*A*) Distribution of Lp(a) concentration in 6218 individuals from the two population-based studies KORA F3 and F4. (*B*) Median Lp(a) concentrations in various groups of subjects stratified by the number of KIV repeats and genotypes of SNP rs10455872; 11–22 KIV repeats are considered as low molecular weight (LMW) or small isoforms and those with >22 KIV repeats are considered as high molecular weight (HMW) or large apo(a) isoforms.

made for intermittent claudication and for ABI <0.90 (Supplementary material online, *Table S2–S3*). Results from the logistic regression analyses revealed no significant influence of SDlog-Lp(a) on these dichotomized phenotypes in both studies and only a borderline association with LMW apo(a) phenotypes in KORA F3 ($P \sim 0.09$). The ORs for the SNP were consistent with the causal assumption in both studies, but not significant (*Table 3* for KORA F3 and KORA F4 combined and Supplementary material online, *Table S4* for additional individual study results).

Since dichotomizing a variable leads to a loss of valuable information, we also performed linear regression analyses on continuous ABI to capture the whole spectrum of ABI values and to improve statistical power. The results from linear regression and linear mixed models are provided in *Table 3* for combined results of KORA F3 and KORA F4 and in Supplementary material online, *Table S5* for individual study results. Significant associations between increasing SDlog-Lp(a) concentrations, LMW apo(a) phenotype, and rs10455872 and lower ABI values were found for KORA F3. The estimates for KORA F4 were similar but did not reach statistical significance due to the smaller sample size. The combined analysis of both cohorts revealed significant results. The magnitude of these associations remained stable in the fully adjusted model (SDlog-Lp(a) $P = 0.005$; LMW apo(a) phenotypes $P = 0.02$, rs10455872 $P = 0.03$) (*Table 3*). Causal effects derived from the IV regression from SDlog-Lp(a) on ABI (*Figure 1A*) were all even stronger than the respective observed regression estimates. They were significant in all adjusted models in the combined analysis of KORAF3/F4 (fully adjusted model: $P = 0.02$) (*Table 3*). These results were similar when the grouping into LMW/HMW apo(a) phenotypes were performed based on the most strongly expressed apo(a) isoform in the western blot and/or when the apo(a) isoform was analysed in a continuous manner based on the number of K-IV repeats (see Supplementary material online, *Table S6*).

In KORA F3 and F4, 28–29% of the Lp(a) concentration are explained by the combination of rs10455872 and the LMW apo(a) phenotype, of which ~26.5% can be explained by LMW alone. The SNP alone without considering apo(a) phenotypes explains 13.3% in KORA F4 and 17.7% in KORA F3.

3.3 Sensitivity analyses and search for the optimal Lp(a) threshold

For sensitivity analysis in the KORA studies (fully adjusted models), the Lp(a) levels were dichotomized, applying a threshold value of 30 mg/dL as well as the recently suggested threshold value of 50 mg/dL.[21] We observed significant associations with decreasing continuous ABI values in KORA F3 for Lp(a) >30 mg/dL ($\beta = -0.016$; $P = 0.01$) and Lp(a) >50 mg/dL ($\beta = -0.028$; $P = 0.0002$). No significant association was observed for Lp(a) >30 mg/dL for KORA F4 ($\beta = -0.009$, $P = 0.28$) while the analysis with a threshold of 50 mg/dL was significant ($\beta = -0.023$; $P = 0.03$). In the CAVASIC study, the same trend could be observed with a higher OR for the threshold of 50 mg/dL (OR = 2.657, $P = 0.001$) compared with 30 mg/dL (see Supplementary material online, *Table S7* for detailed results for all dichotomized analyses). These results suggest that the higher threshold of 50 mg/dL seems to be more relevant for PAD than 30 mg/dL. To further support this hypothesis, optimal cutpoints were derived for all three studies applying the AIC criterion. This analysis resulted in an optimal Lp(a) threshold of 54 mg/dL in the linear regression on ABI in KORA F3, 69 mg/dL in KORA F4, and 53 mg/dL in the logistic regression on PAD in the CAVASIC study. However, nonlinear P-Splines do not contradict the linear assumption in general (Supplementary material online, *Figure S1–S3*).

Further sensitivity analyses were performed applying different exclusion criteria: when we excluded individuals with prevalent CVDs, we observed similar results with slightly attenuated estimates. When we excluded individuals with ABI values above 1.30 estimates were slightly weakened as well.

Additional analysis were performed adjusting the association of LMW apo(a) phenotypes on ABI in the KORA studies or PAD in CAVASIC for SDlog-Lp(a). As expected, all estimates and ORs were attenuated and *P*-values were not significant any more (Supplementary material online, *Tables S8* and *S9*).

Finally, we analysed a possible sex interaction in both KORA studies and could not find any evidence of an interaction. *P*-values for interaction for the main analyses were 0.88 and 0.42 for KORA F3 and F4, respectively.

4. Discussion

In the present study, we investigated the association between Lp(a) concentrations, LMW apo(a) phenotypes, the rs10455872 SNP in the *LPA* gene, and various outcome variables related to PAD in three independent studies. The main findings are: (i) a significant association of SDlog-Lp(a) concentrations, LMW apo(a) phenotypes, and rs10455872 with symptomatic intermittent claudication was found in a case–control study. An association with peripheral atherosclerosis phenotypes was

Table 3 Results of logistic and linear mixed regression models and IV regression for SDlog-Lp(a) and LMW apo(a) phenotypes in KORA F3 and F4 combined

Number of individuals[a] all (for dichotomized variables: those with endpoint = 'yes')		SDlog-Lp(a)			IVs: LMW apo(a) phenotype/SNP rs10455872 (additive coding)				IV regression based on LMW apo(a) phenotype and SNP rs10455872		
		OR	(95%CI)	*P*-value		OR	(95%CI)	*P*-value	OR	(95%CI)	*P*-value
Mixed logistic regression[b] on PAD, intermittent claudication and ABI<0.90											
PAD[c]	4905 (339)	1.05	(0.93,1.17)	0.43	LMW	1.13	(0.87, 1.46)	0.37	1.10	(0.88, 1.36)	0.40
					SNP	1.27	(0.89, 1.81)	0.19			
Intermittent claudication	6208 (187)	1.08	(0.93,1.25)	0.33	LMW	1.19	(0.85, 1.67)	0.30	1.07	(0.80, 1.42)	0.65
					SNP	1.10	(0.68, 1.80)	0.69			
ABI < 0.9	4914 (227)	1.06	(0.92,1.21)	0.42	LMW	1.18	(0.86, 1.61)	0.30	1.15	(0.89, 1.49)	0.28
					SNP	1.36	(0.89, 2.07)	0.16			
		beta	(95%CI)	*P*-value		beta	(95%CI)	*P*-value	beta	(95%CI)	*P*-value
Mixed linear regression on continuous ABI											
Model 1[d]	4914	−0.007	(−0.011, −0.002)	0.002	LMW	−0.013	(−0.023, −0.003)	0.01	−0.011	(−0.019, −0.003)	0.009
					SNP	−0.014	(−0.028, 0.000)	0.05			
Model 2[e]	4724	−0.006	(-0.010, −0.002)	0.005	LMW	−0.011	(−0.021, −0.001)	0.02	−0.010	(−0.018, −0.002)	0.02
					SNP	−0.016	(−0.030, −0.002)	0.03			

[a]Number of individuals are based on those with available data on Lp(a) and apo(a) phenotypes.
[b]Logistic mixed models are adjusted for age and sex.
[c]PAD defined as either ABI<0.9 or intermittent claudication or both.
[d]Model 1: adjusted for age and sex.
[e]Model 2: adjusted for age, sex, diabetes, current smoking, eGFR, and hypertension.

confirmed in the population-based cohort studies KORA F3 and F4 only when the analysis was done on a continuous scale for the ABI; (ii) the analysis in more than 6000 subjects from the general population demonstrated a clear threshold effect in Lp(a) concentrations between small and large apo(a) phenotype groups. This strongly justifies the stratification in these two isoform groups which are the basis for (iii) the final IV regression analysis which strongly supported causality between Lp(a) concentrations and PAD phenotypes; (iv) the search for the optimal cut-off that predicts PAD phenotypes revealed that rather higher Lp(a) concentrations above 50 mg/dL might be better suitable for these PAD phenotypes.

While numerous studies have addressed the relation between Lp(a) and coronary artery disease (CAD),[3–6] only a limited number of studies have evaluated the association with PAD[22–32] and only three case–control studies have considered apo(a) phenotypes.[22–24] The main characteristics and the results of these studies can be found in Supplementary material online, *Table S10*. In summary, most of them described an association between Lp(a) concentrations and PAD defined by a broad range of criteria. This association was especially strong in case–control studies including our CAVASIC Study which typically considered patients with symptomatic PAD. Previous prospective, population-based studies provided a less clear picture and not all of them described an association of Lp(a) concentrations with outcomes (Supplementary material online, *Table S10*). In the present population-based studies of KORA F3 and F4, we observed significant associations with PAD only when the entire spectrum of ABI values without dichotomization and therefore more power was used for the data analysis.

The study design might explain differences between observations in clinical samples and general population samples. The former, such as the CAVASIC Study, consist solely of patients with a pronounced symptomatic phenotype while in the general population studies, such as KORA, a much smaller number of participants are diagnosed with symptomatic PAD according to the Edinburgh questionnaire and they usually show less severe phenotypes. Furthermore, the latter cover a wider age range including more individuals that are younger and have the potential to develop symptomatic PAD in the future and thereby dilute the expected associations. Therefore, in the KORA cohorts, the continuous ABI might be more relevant as it reflects not only symptomatic but also asymptomatic PAD and is probably a surrogate of the condition of the peripheral vascular system. Moreover, the strict and commonly used cut-off value of an ABI <0.90 for the definition of PAD has recently been questioned since individuals with ABI values between 0.90 and 1.09 already showed a higher incidence of mobility loss over a period of 5 years compared with persons with ABI values above 1.09.[33] Additionally, an inverse linear relationship between continuous ABI and cardiovascular outcomes has been reported showing that ABI is predictive for incident myocardial infarctions as well as cardiovascular and total mortality.[10] This supports that ABI measured on a continuous scale may provide relevant information on the disease severity at values also slightly above 0.90.

We found very strong associations with ABI when Lp(a) was dichotomized at 50 mg/dL. Optimal data-driven thresholds were found to be at 54 or even 69 mg/dL in the KORA studies and 53 mg/dL for symptomatic PAD in CAVASIC. This is in line with similar observations on Lp(a) levels and stroke in a recent meta-analysis that found Lp(a) only significantly associated with stroke when levels were above 50 mg/dL.[4] Therefore, it seems that the effect of Lp(a) is stronger on the coronary arteries than on cerebrovascular or lower extremity vessels.

The two most important aspects of our analysis were based on the investigation of the genetically determined apo(a) phenotypes in the investigated cohorts which allowed to extrapolate on causal aspects. First, it is the largest study so far that analysed the apo(a) isoforms expressed in plasma and not on DNA level. This is of utmost importance since in about half to one-third of individuals only one isoform is expressed in plasma although two different alleles are present at the DNA level.[34] With regard to risk prediction, it might be questioned why one should be interested in an allele that is not expressed at all in plasma when the site of pathogenic action of Lp(a) is expected to be in the vascular system.[35] The second aspect is that the availability of the apo(a) phenotypes allowed us to apply the commonly used Mendelian Randomization approach to support causality. In addition, the SNP rs10455872 in the *LPA* gene has been determined and included in the analyses. Since this SNP is rather rare (MAF$\sim$5–7%) and tags less than half of the short isoforms,[35] it explains a rather small amount of Lp(a) variance in addition to LMW apo(a) phenotypes. The Mendelian Randomization approach is based on the fact that it is randomly determined which of the two isoforms and/or alleles of rs10455872 from the father as well as from the mother will be transmitted to the child at the time of conception. Since the transmitted alleles are of lifelong persistence, these alleles determine to a certain extent also whether a person is exposed to high Lp(a) levels and therefore to a high atherosclerosis risk. Thus, the association between apo(a) isoforms and/or alleles of the SNP and PAD phenotypes is less likely to be influenced by reverse causation or confounding. Reverse causation would mean that PAD influences the polymorphisms, which can practically be excluded. Confounding would mean that, e.g. a lifestyle factor such as smoking is associated with the disease (which is often the case) as well as with the polymorphisms (which is less probable).[36] Therefore, this method is well appropriate to underline a causal relationship between Lp(a) and PAD/ABI which is hardly possible with conventional epidemiological observation studies. We applied and extended this Mendelian Randomization approach for PAD using an IV regression technique to assess effects of Lp(a) levels which are predicted by LMW apo(a) phenotypes and rs10455872 genotypes. Therefore, these estimates can be interpreted as the causal effects of Lp(a) on PAD/ABI. Our findings indicated that these causal effects are even higher than those that could be observed in the ordinary regression models from Lp(a) on the respective outcomes. Only three case–control studies so far[22–24] investigated the association of LMW apo(a) phenotypes with PAD of various definitions. In two of these, a significant association was observed.[23,24] Few other studies analysed SNPs in the *LPA* gene region and also observed a significant association with PAD phenotypes (Supplementary material online, *Table S10*).[37–39] However, none of these studies did apply IV regression to formally estimate causal effects.

4.1 Strength and limitations of the study

A strength of our study is that apo(a) phenotypes were determined in all samples of the CAVASIC study and the two KORA studies in one laboratory. It is therefore by far the largest study up to now which analysed the apo(a) phenotypes by western blot. The use of the apo(a) phenotype in the data analysis allows the application of a Mendelian Randomization approach including an IV regression technique which supports the causal relationship between Lp(a) concentrations and PAD.

The analyses are limited by the cross-sectional study design of CAVASIC and KORA, which precludes an assessment of the predictive properties for Lp(a) concentrations and LMW apo(a) phenotypes over time and in studies of cross-sectional design a survival bias cannot be entirely excluded. Finally, we might lack statistical power for the PAD analysis in the KORA studies that included individuals with a wide age range

and probably a considerable number of subjects who are still too young to show symptomatic PAD. These subjects might have diluted the associations.

5. Conclusions

Our analyses in three independent populations point to a significant association between Lp(a) concentrations, LMW apo(a) phenotypes, and one SNP within the *LPA* gene with symptomatic and asymptomatic PAD. This association is probably of causal nature since the genetically determined apo(a) phenotypes and the investigated SNP that influence the Lp(a) concentrations to a large extent are indeed associated with these PAD phenotypes.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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