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Lipoprotein(a) levels and atherosclerotic plaque characteristics in the carotid artery: The Plaque at RISK (PARISK) study

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A B S T R A C T

Background and aims: Lipoprotein(a) is an independent risk factor for cardiovascular disease and recurrent ischemic stroke. Lipoprotein(a) levels are known to be associated with carotid artery stenosis, but the relation of lipoprotein(a) levels to carotid atherosclerotic plaque composition and morphology is less known. We hypothesize that higher lipoprotein(a) levels and lipoprotein(a)-related SNPs are associated with a more vulnerable carotid plaque and that this effect is sex-specific.
Methods: In 182 patients of the Plaque At RISK study we determined lipoprotein(a) concentrations, apo(a) KIV-2 repeats and *LPA* SNPs. Imaging characteristics of carotid atherosclerosis were determined by MDCTA (n = 161) and/or MRI (n = 171). Regressions analyses were used to investigate sex-stratified associations between lipoprotein(a) levels, apo(a) KIV-2 repeats, and *LPA* SNPs and imaging characteristics.
Results: Lipoprotein(a) was associated with presence of lipid-rich necrotic core (LRNC) (aOR = 1.07, 95% CI: 1.00; 1.15), thin-or-ruptured fibrous cap (TRFC) (aOR = 1.07, 95% CI: 1.01; 1.14), and degree of stenosis (β = 0.44, 95% CI: 0.00; 0.88). In women, lipoprotein(a) was associated with presence of intraplaque hemorrhage (IPH) (aOR = 1.25, 95% CI: 1.06; 1.61). In men, lipoprotein(a) was associated with degree of stenosis (β = 0.58, 95% CI: 0.04; 1.12). Rs10455872 was significantly associated with increased calcification volume (β = 1.07, 95% CI: 0.25; 1.89) and absence of plaque ulceration (aOR = 0.25, 95% CI: 0.04; 0.93). T3888P was associated with absence of LRNC (aOR = 0.36, 95% CI: 0.16; 0.78) and smaller maximum vessel wall area (β = -10.24, 95%CI: -19.03; -1.44).
Conclusions: In patients with symptomatic carotid artery stenosis, increased lipoprotein(a) levels were associated with degree of stenosis, and IPH, LRNC, and TRFC, known as vulnerable plaque characteristics, in the carotid artery. T3888P was associated with lower LRNC prevalence and smaller maximum vessel wall area. Further research in larger study populations is needed to confirm these results.

1. Introduction

Carotid artery atherosclerosis is one of the major causes of ischemic stroke. Destabilization of an atherosclerotic plaque can cause plaque rupture, leading to thrombus formation and embolization of plaque material and thrombus into distally located intracranial arteries. Rupture prone plaques, so-called vulnerable plaques, have a specific plaque composition and morphology such as presence of intraplaque hemorrhage (IPH), lipid-rich necrotic core (LRNC) and plaque ulceration [1–3].

Imaging techniques like multidetector-row computed tomographic angiography (MDCTA) and magnetic resonance imaging (MRI) can visualize and quantify atherosclerotic plaques. They can also identify vulnerable plaque features [1,4].

Various risk factors play an important role in formation and progression of atherosclerotic plaques. Besides traditional risk factors as

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hypertension, hypercholesterolemia and diabetes mellitus [5], lipoprotein(a) [Lp(a)] has now been established as a risk factor as well. Lp(a) is a low-density lipoprotein-like particle with an extra apolipoprotein(a) [apo(a)] covalently linked to its apolipoprotein B-100. Plasma Lp(a) concentrations are highly variable ranging from <0.1 mg/dL to >200 mg/dL. The plasma concentration is mostly affected by and inversely correlated with the number of kringle IV type 2 (KIV-2) repeats, determining the size of the apo(a) [6,7]. High levels of Lp(a) are an independent risk factor for cardiovascular diseases and recurrent ischemic stroke [8,9]. One of the proposed mechanisms underlying the associations between high Lp(a) levels and ischemic stroke could be carotid atherosclerosis. Previous studies suggest that Lp(a) increases cholesterol deposition in the arterial wall, foam cell formation, smooth muscle cell proliferation and generation of oxidized radicals in monocytes [9]. Lp(a) levels are also associated with stenosis in the carotid artery [10], but the relation between Lp(a) levels and carotid artery plaque composition and morphology has not been extensively investigated yet. The AIM-HIGH study reported in 2014 that type-VI carotid lesions, defined as a complex plaque with possible surface defect, IPH or mural thrombosis, were associated with higher levels of Lp(a) and larger percent wall volume [11]. In addition to these studies focusing on the degree of stenosis or overall definitions, it is also important to investigate the relation of Lp(a) on each plaque characteristic separately, since this can further unravel underlying mechanisms.

Genome-wide association studies have identified several genetic variants that modulate plasma levels of Lp(a) [12]. The single-nucleotide polymorphisms (SNPs) rs10455872 and rs3798220 at the *LPA* locus are both strongly associated with increased levels of Lp(a) and coronary disease [13]. Although these variants were not associated with carotid intima-media thickness in previous studies, they were associated with ischemic stroke caused by large artery atherosclerosis [14]. T3888P (rs41272110) is known to be associated with lower Lp(a) concentrations [15].

Previous studies showed that both Lp(a) distribution and carotid plaque composition differ between men and women [16,17]. Men have more frequently vulnerable plaques than women [18,19]. Also, Lp(a) levels peak during late peri- and postmenopause in women [20]. We hypothesized that sex could modify the association between Lp(a) and carotid atherosclerotic disease.

The aim of this study was to investigate the association between Lp(a) levels, apo(a) KIV-2 repeats, SNPs and imaging characteristics of the atherosclerotic carotid plaque in symptomatic male and female patients with an ipsilateral mild-to-moderate carotid artery stenosis. We hypothesized that Lp(a) and its genetic determinants are associated with imaging characteristics of vulnerable plaques and that this association is different for men and women.

2. Patients and methods

2.1. Study population

Patients were selected from the Plaque At RISK (PARISK) study (clinicaltrials.gov NCT01208025). The study design of the PARISK study has been described previously [21]. This prospective multicenter cohort study investigates the hypothesis that the assessment of carotid plaque characteristics using imaging techniques improves the prediction of recurrent ischemic events in patients with a symptomatic mild-to-moderate carotid artery stenosis. All included patients had a recent (<3 months) transient ischemic attack (TIA), including amaurosis fugax, or minor stroke, in the carotid artery territory and a 30–69% ipsilateral carotid artery stenosis. Patients scheduled for a carotid endarterectomy were excluded. Additional exclusion criteria were a probable cardiac source of embolism, clotting disorder, severe comorbidity, and standard contraindications for MR imaging. Patients with a renal clearance <60 mL/min/1.73 m^2 or a documented CT contrast allergy did not undergo MDCTA. Patients with a renal clearance <30 mL/min/1.73 m^2 or a documented MRI contrast allergy did not receive MRI contrast agent. Institutional review board approval was obtained in all university hospitals and all patients gave written informed consent before inclusion. The study was performed in accordance with the principles of the Declaration of Helsinki. Between September 2010 and December 2014, 240 patients were included in the PARISK study. Of 182 patients MDCTA (n = 161) and/or MRI (n = 171) of the carotid arteries and a blood sample at baseline were available (Supplementary Fig. 1).

2.2. Cardiovascular risk factors

Hypercholesterolemia was defined as a total cholesterol of >5 mmol/L or the use of cholesterol-lowering medication at the time of the TIA or ischemic stroke. We defined hypertension as a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg during 2 episodes of at least 15 minutes of continuous non-invasive blood pressure measurement or treatment with antihypertensive medication. Diabetes mellitus was defined as a fasting serum glucose level of >6.9 mmol/L, 2-hour postload glucose level of >11.0 mmol/L, or the use of antidiabetic medication. We dichotomized smoking status at the time of the ischemic event into current smoker or current non-smoker. In addition, we recorded body mass index and medical history.

2.3. Lp(a) measurement

Blood samples were processed within 1-hour after collection and stored at −80 °C until analysis. Plasma Lp(a) concentrations were measured using a particle-enhanced immunoturbidimetric assay (Diagnostic System; DiaSys Diagnostic System, GmGH, Germany), which was largely independent of apo(a) KIV-2 repeats [22].

2.4. Apo(a) KIV-2 repeat copy number measurement

The KIV-2 repeats were determined by immunoblotting as previously described by Vongpromek et al. [23] Apo(a) phenotypes were categorized into two groups: low-molecular-weight apo(a) (≤22 KIV-2 repeats) and high-molecular-weight apo(a) (>22 KIV-2 repeats). In case of two detected apo(a) isoforms in the immunoblot, the smaller isoform was used for categorization [7].

2.5. Measurement of LPA SNPs

LPA variants rs10455872, rs3798220, and T3888P were chosen for genotyping based on previously reported associations [6,13–15]. Genotyping was performed with Taqman allelic discrimination assays designed and optimized by Applied Biosystems (Foster City, CA, USA http://store.appliedbiosystems.com). Reactions were performed on the Taqman StepOne Plus platform according to the manufacturer's instructions.

2.6. MDCTA and MRI data acquisition and analysis

We performed contrast-enhanced MDCTA and multi-sequence contrast-enhanced 3 Tesla MRI of the carotid arteries. We used a phased-array carotid surface coil and performed plaque imaging using the standardized protocol described in the study design article [21]. All imaging data were evaluated by trained readers blinded to clinical data and blood-based biomarkers.

MDCTA images were reviewed using dedicated 3D analysis software (Syngo.via; Siemens, Erlangen, Germany). The most severe stenosis in the symptomatic internal carotid artery and carotid bifurcation was measured according to the ECST (European Carotid Surgery Trial) criteria, perpendicular to the central lumen line [24]. In addition, we assessed the presence of plaque ulceration. We defined plaque ulceration as an extension of contrast material of >1 mm into the atherosclerotic plaque on at least 2 orthogonal slices [25,26]. Finally, a custom-made

plug-in for the software ImageJ (National Institutes of Health, Bethesda, Maryland) was used to quantify calcifications in the symptomatic artery within 3 cm proximal and distal to the carotid bifurcation. We used a threshold of 600 Hounsfield units to differentiate calcifications from contrast material. A detailed description of the measurements is provided elsewhere [27].

MR images were evaluated with dedicated vessel wall analysis software (VesselMASS; Leiden University Medical Center, Leiden, the Netherlands). Information of five different MRI sequences was used [21]. MR images were automatically registered by delineating the lumen and outer vessel wall of the symptomatic carotid artery. Registration was manually corrected if needed. Plaque components (LRNC and IPH) were manually segmented in the ipsilateral carotid artery. The ipsilateral artery was based on both clinical symptoms and brain imaging. Fifteen transverse adjoining slices of 2 mm each covering the entire ipsilateral plaque were annotated. Further details about the plaque measurements have been described previously [28].

2.7. Statistical analysis

If normally distributed, continuous variables are presented as mean ± standard deviation, otherwise as median [interquartile range]. Pearson χ^2 test, Fisher exact test, Student's t-test, Wilcoxon rank test and Mann-Whitney *U* test were used to analyse differences in continuous and categorical data. Dichotomous variables are presented as number (%). Lp(a) concentration was analysed as continuous variable and in quartiles. Because our cohort included symptomatic patients and does not represent the general population, we made Lp(a) quartiles based on the distribution in our patient cohort. Calcification volume was log-transformed since its skewed distribution. We added 1.0 mm^3 to the non-transformed values to deal with patients with a calcification volume of zero. IPH and LRNC volumes were not normally distributed as well, but could not be transformed to a normal distribution. We performed pre-defined sex-specific analyses.

To analyse the association between Lp(a) quartiles and plaque characteristics Jonckheere's trend test and Cochran-Armitage trend test were used (Fig. 1, pathway B). Linear regression was used to analyse the association between continuous Lp(a) levels and degree of stenosis, calcification volume and maximum vessel wall area. IPH and LRNC volumes could not be analysed by linear regressions since their non-normal distribution. Logistic regression was used for analysing the association between Lp(a) and the presence of IPH, LRNC, plaque ulceration, TRFC, and calcifications. All linear and logistic regression analyses were adjusted for age and sex. Model 2 was additionally adjusted for hypertension, LDL cholesterol, statin use, diabetes mellitus, current smoking, and history of cardiovascular disease, known as factors influencing carotid atherosclerosis and Lp(a). We adjusted in model 2 also for maximum vessel wall area, because this reflects the plaque burden which is highly correlated with presence of vulnerable plaque components [18]. Additionally, we calculated R^2 for the linear regression models and Nagelkerke's pseudo-R^2 for the logistic regression models in order to quantify the explained variance in outcome by the models (Supplementary Table 4). We performed sensitivity analyses for differences between participating sites and for Lp(a)-corrected measurements of LDL cholesterol. Lp(a)-corrected LDL cholesterol levels were calculated by subtracting estimated Lp(a) cholesterol from the measured LDL cholesterol. Lp(a) cholesterol was estimated as 20%, 25%, 30% and 45% of total Lp(a) mass. We used this range to take into account that the proportion of Lp(a) cholesterol content of total Lp(a) mass varies among individual patients [29,30].

Linear and logistic regressions were performed to analyse the association between KIV-2 repeats and imaging characteristics.

For the analyses of the relation between the *LPA* gene SNPs and imaging characteristics we used linear and logistic regressions (Fig. 1, pathway C). To explore the effect of the SNPs on plaque characteristics that cannot be explained by the effect of Lp(a) (Fig. 1, pathway A-B), we adjusted for age and sex in Model 1 and additionally for Lp(a) in Model 2.

All statistical analyses were conducted using R statistical software (version 3.4.4; R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Baseline characteristics

Baseline characteristics of the 182 patients are shown in Table 1. Fig. 2 shows an illustration of the included imaging characteristics. Mean age of the patients was 68 ± 9 years, 136 (75%) were men and 173 (95%) were Caucasian. Median Lp(a) plasma level was 19.9 [7.7–62.9] mg/dL and 34% of the patients showed apo(a) KIV-2 repeats ≤22. Two isoforms were detectable in the plasma of 57% of the patients. No differences between men and women in the distribution of Lp(a), apo(a) KIV-2 repeats and *LPA* gene SNPs were observed. In men, a higher prevalence of IPH (47 *vs.* 20%, $p = 0.001$), LRNC (73 *vs.* 39%, $p < 0.001$) and TRFC (44 *vs.* 20%, $p = 0.004$) was found than in women. Maximum vessel wall area, IPH and LRNC volumes were also larger in men (Supplementary Fig. 2).

3.2. Association between Lp(a) and plaque characteristics

The associations between elevated Lp(a) levels and plaque characteristics are presented in Supplementary Table 1. Table 2 shows the regression models to determine the association between Lp(a) levels and plaque characteristics. After adjustments for age, sex and additional cardiovascular risk factors, a 10 mg/dL increase of Lp(a) concentration was significantly associated with presence of LRNC (odds ratio (OR) = 1.07 [95% confidence interval 1.00; 1.15]), TRFC (OR = 1.07 [1.01; 1.14]) and degree of stenosis (β = 0.44 [0.00; 0.88]) in all patients. When we stratified the analyses for sex, we found that in women a 10 mg/dL increase of Lp(a) concentration was associated with presence of IPH (OR = 1.25 [1.06; 1.61]) and in men with degree of stenosis (β = 0.58 [0.04; 1.12]). Sensitivity analyses for Lp(a)-corrected LDL cholesterol (Supplementary Table 2) showed that the assumed contribution of Lp(a) cholesterol content of total Lp(a) mass influenced the results.

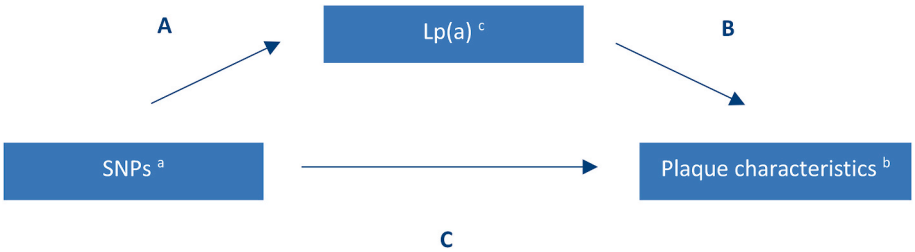

Fig. 1. Relations between Lp(a), SNPs and plaque characteristics.
[a] *LPA* gene polymorphisms rs10455872, rs3798220, and T3888P. [b] The assessed vulnerable plaque characteristics in this study are presence of intraplaque hemorrhage, lipid-rich necrotic core, calcifications, plaque ulceration, thin-or-ruptured fibrous cap, degree of stenosis, and maximum vessel wall area. [c] We analysed Lp(a) levels both continuously and in quartiles. Pathway B is assessed (1) by the Jonckheere's trend test and Cochran-Armitage trend test for Lp(a) quartiles (supplemental material), and (2) by linear and logistic regression analyses using Lp(a) levels as continuous variable. Pathway C is assessed by linear and logistic regression analyses.

Table 1
Baseline characteristics stratified by sex.

	All $n = 182$	Male $n = 136$	Female $n = 46$
Clinical characteristics			
Age	68 ± 9	68 ± 9	68 ± 9
Current smoking	39 (22%)	26 (20%)	13 (28%)
Body mass index (kg/m^2)	26.3 [24.3–29.1]	26.4 [24.6–28.9]	25.0 [22.5–30.1]
Hypertension	129 (71%)	96 (71%)	33 (72%)
Hypercholesterolemia	141 (81%)	105 (81%)	36 (80%)
Diabetes Mellitus	45 (25%)	37 (28%)	8 (17%)
History of cardiovascular disease	102 (56%)	81 (60%)	21 (46%)
Statin use	93 (51%)	76 (56%)	17 (37%)
Classification event			
Stroke	82 (45%)	63 (46%)	19 (41%)
Transient ischemic attack [amaurosis fugax]	100 (55%) [23 (13%)]	73 (54%) [15 (11%)]	27 (59%) [8 (17%)]
Blood measurements			
Lipoprotein(a) concentration (mg/dL)	19.9 [7.7–62.9]	18.9 [6.3–61.8]	24.4 [12.1–66.8]
Apo(a) kringle IV type 2 repeats ≤22	59 (34%)	45 (34%)	14 (31%)
rs10455872 carrier frequency of variant	24 (13%)	18 (14%)	6 (13%)
rs3798220 carrier frequency of variant	8 (5%)	6 (5%)	2 (5%)
T3888P carrier frequency of variant	40 (22%)	25 (18%)	15 (33%)
LDL cholesterol (mmol/L)	2.0 ± 0.5	2.0 ± 0.5	2.1 ± 0.5
Imaging characteristics (symptomatic artery)			
MDCTA			
Degree of stenosis (ECST)	54 ± 16	55 ± 16	52 ± 16
Calcification presence	146 (91%)	110 (92%)	36 (88%)
Calcification volume (mm^3)	27.9 [5.1–80.7]	25.1 [5.8–85.2]	36.8 [2.8–73.4]
Plaque ulceration presence	43 (27%)	36 (30%)	7 (17%)
MRI			
Intraplaque hemorrhage presence	68 (40%)	59 (47%)	9 (20%)
Intraplaque hemorrhage volume (mm^3)	0.0 [0.0–57.8]	0.0 [0.0–92.1]	0.0 [0.0–0.0]
Lipid-rich necrotic core presence	109 (64%)	91 (73%)	18 (39%)
Lipid-rich necrotic core volume (mm^3)	26.3 [0.0–150.8]	0.0 [0.0–196.5]	0.0 [0.0–46.4]
Thin-or-ruptured fibrous cap presence	64 (37%)	55 (44%)	9 (20%)
Maximum vessel wall area (mm^2)	78.1 ± 26.6	83.8 ± 27.5	62.7 ± 16.1

Continuous values are expressed as mean ± standard deviation or as median [interquartile range]. Categorical variables are presented as numbers (%). Apo(a) = apolipoprotein(a), MDCTA = multidetector-row computed tomographic angiography, ECST = European Carotid Surgery Trial, MRI = magnetic resonance imaging.

Sensitivity analyses for participating centers did not show significant differences (data not shown).

3.3. Association between apo(a) KIV-2 repeats and plaque characteristics

Lp(a) levels were significantly inversely correlated with KIV-2 repeats (median Lp(a) 57.5 mg/dL for KIV-2 repeats ≤22, and 18.3 mg/dL for KIV-2 repeats >22, $p = 0.004$). We found no significant associations between apo(a) KIV-2 repeats and plaque characteristics.

3.4. Association between SNPs and plaque characteristics

The regression models to determine the association between rs10455872 and T3888P and imaging characteristics are shown in Table 3. Supplementary Table 3 shows the descriptive analyses of these associations. Because the frequency of rs3798220 was very rare in our population (n = 8, 5%), we have not further analysed the associations between rs3798220 and plaque characteristics. The Lp(a) increasing SNP rs10455872 was significantly associated with increasing Lp(a) levels (β = 1.58 [1.03; 2.13]). No differences in Lp(a) levels between carriers and non-carriers of the minor T3888P allele were observed. Rs10455872 was significantly associated with lower prevalence of plaque ulceration (OR = 0.25 [0.04; 0.93]), and T3888P with lower prevalence of LRNC (OR = 0.36 [0.16; 0.78]) and a smaller maximum vessel wall area (β = −10.24 [-19.03;-1.44]). Additional adjustments for Lp(a) (Model 2) resulted in stronger associations and also in a significant association between rs10455872 and calcification volume (β = 1.07 [0.25; 1.89]).

4. Discussion

Increased plasma Lp(a) concentration is a known risk factor for the development of cardiovascular disease and is associated with carotid artery stenosis [31–33]. In this study including patients with recent TIA or minor ischemic stroke, we identified novel associations between Lp(a) concentrations and imaging characteristics of the carotid atherosclerotic plaque. In women, elevated plasma Lp(a) levels were associated with higher prevalence of IPH. In men, elevated Lp(a) levels were associated with a higher degree of stenosis. In the pooled data of both sexes, elevated Lp(a) levels were associated with higher prevalence of LRNC and TRFC, and higher degree of stenosis. The association between Lp(a) concentration and these vulnerable plaque characteristics supports the hypothesis that Lp(a) has a role in the process of atherosclerosis. We found higher median Lp(a) plasma levels than in the general population which could be explained by the fact that our cohort consisted of symptomatic patients with therefore a higher cardiovascular risk [34].

Surprisingly, we found no association between Lp(a) and maximum vessel wall area which reflects the total plaque burden. An explanation could be that Lp(a) exerts its influence on atherosclerosis in particular via plaque composition. We hypothesize that Lp(a) has marginal or no influence on the size of the atherosclerotic plaque, but affects especially

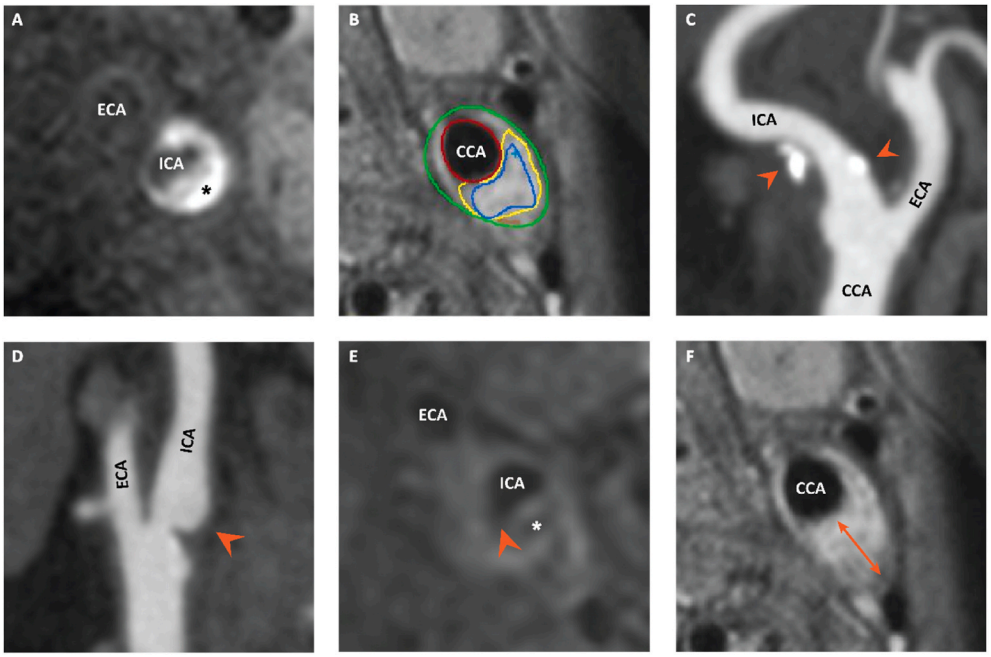

Fig. 2. Images of carotid plaque characteristics.
(A) Axial view of a 3D T1-weighted fast spoiled gradient echo MR image showing a high-intense signal (asterisk) indicative of intraplaque hemorrhage. (B) Axial view of a carotid plaque depicted with 3D T1-weighted pre-contrast quadruple inversion recovery MR, which is delineated to measure different plaque components. The part surrounded with a yellow line is lipid-rich necrotic core. (C) CTA image showing several calcifications (arrow heads) in a carotid plaque of the ICA. (D) CTA image with contrast material reaching into a carotid plaque (arrow head) which indicates plaque ulceration. (E) Post-contrast T1-weighted fast spin echo MR image which shows an interrupted signal (arrow head) between the lipid-rich necrotic core (asterisk) and the lumen of the ICA. (F) The orange arrow in this 3D T1-weighted pre-contrast quadruple inversion recovery MR image represents the maximum vessel wall area of this carotid plaque. CCA = common carotid artery, ECA = external carotid artery, ICA = internal carotid artery. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the composition of the plaque. To investigate this hypothesis further research is needed, both in larger study populations and in studies including healthy participants. This also enables us to investigate the role of Lp(a) in developing atherosclerotic disease in asymptomatic participants.

Previous studies revealed sex differences in the association between Lp (a) and carotid atherosclerosis. Baldo et al. [16] showed that serum Lp(a) levels were higher in females than in males, especially in females with unstable plaques *ex vivo*. Schreiner et al. [17] reported sex differences in the association of Lp(a) levels with carotid intimal-medial far-wall

Table 2
Logistic and linear regression models of Lp(a) levels and imaging characteristics.

		Model 1	Model 2
Intraplaque hemorrhage presence (OR)		1.03 [0.97; 1.09]	1.02 [0.96; 1.08]
	♂	0.99 [0.93; 1.06]	0.98 [0.90; 1.05]
	♀	1.15 [1.03; 1.30]	1.25 [1.06; 1.61]
Lipid-rich necrotic core presence (OR)		1.05 [0.99; 1.13]	1.07 [1.00; 1.15]
	♂	1.02 [0.95; 1.11]	1.04 [0.96; 1.14]
	♀	1.11 [1.01; 1.26]	1.08 [0.96; 1.25]
Calcification presence (OR)		1.04 [0.96; 1.16]	1.06 [0.94; 1.25]
	♂	1.05 [0.95; 1.22]	1.11 [0.93; 1.56]
	♀	1.01 [0.88; 1.22]	NA (infinite)
Calcification volume (β)		−0.02 [-0.06; 0.02]	−0.01 [-0.06; 0.03]
	♂	−0.01 [-0.06; 0.04]	0.00 [-0.05; 0.06]
	♀	−0.05 [-0.11; 0.01]	−0.05 [-0.12; 0.01]
Degree of stenosis (ECST) (β)		0.47 [0.07; 0.87]	0.44 [0.00; 0.88]
	♂	0.57 [0.09; 1.04]	0.58 [0.04; 1.12]
	♀	0.25 [-0.55; 1.04]	−0.10 [-0.94; 0.75]
Plaque ulceration presence (OR)		1.00 [0.94; 1.06]	1.00 [0.93; 1.07]
	♂	1.03 [0.96; 1.09]	1.03 [0.96; 1.12]
	♀	0.85 [0.56; 1.04]	0.85 [0.54; 1.12]
Thin-or-ruptured fibrous cap presence (OR)		1.06 [1.00; 1.12]	1.07 [1.01; 1.14]
	♂	1.04 [0.98; 1.11]	1.06 [0.99; 1.13]
	♀	1.10 [0.99; 1.22]	1.14 [0.97; 1.38]
Maximum vessel wall area (β)		0.19 [-0.42; 0.80]	0.19 [-0.45; 0.83]
	♂	0.15 [-0.65; 0.96]	0.01 [-0.86; 0.88]
	♀	0.26 [-0.48; 1.00]	0.66 [-0.07; 1.39]

Values are presented as odds ratio (OR) [95% confidence interval (CI)] for logistic and as β [95% CI] for linear regressions per 10 mg/dL increase of lipoprotein(a) levels. Calcification volume is ln-transformed. Model 1: adjusted for age, and for sex when not stratified for sex. Model 2: adjusted for age, hypertension, measured LDL cholesterol, statin use, diabetes mellitus, current smoking, history of cardiovascular disease, and maximum vessel wall area, and for sex when not stratified for sex. ECST = European Carotid Surgery Trial.

Table 3
Logistic and linear regression models of SNPs and imaging characteristics.

	Model 1	Model 2
Intraplaque hemorrhage presence (OR)		
rs10455872	1.47 [0.54; 3.98]	1.11 [0.34; 3.55]
T3888P	0.56 [0.23; 1.29]	0.50 [0.20; 1.16]
Lipid-rich necrotic core presence (OR)		
rs10455872	1.22 [0.45; 3.57]	0.69 [0.20; 2.36]
T3888P	0.36 [0.16; 0.78]	0.29 [0.12; 0.65]
Calcification presence (OR)		
rs10455872	NA (infinite)	NA (infinite)
T3888P	0.59 [0.17; 2.19]	0.54 [0.15; 2.08]
Calcification volume (β)		
rs10455872	0.69 [-0.04; 1.43]	1.07 [0.25; 1.89]
T3888P	−0.35 [-0.97; 0.27]	−0.32 [-0.95; 0.31]
Degree of stenosis (ECST) (β)		
rs10455872	6.88 [-0.55; 14.30]	3.70 [-4.61; 12.01]
T3888P	0.77 [-5.52; 7.06]	−0.23 [-6.50; 6.03]
Plaque ulceration presence (OR)		
rs10455872	0.25 [0.04; 0.93]	0.17 [0.02; 0.73]
T3888P	2.00 [0.84; 4.67]	2.00 [0.84; 4.73]
Thin-or-ruptured fibrous cap presence (OR)		
rs10455872	1.34 [0.50; 3.48]	0.69 [0.21; 2.16]
T3888P	0.58 [0.25; 1.29]	0.47 [0.19; 1.09]
Maximum vessel wall area (β)		
rs10455872	−3.35 [-14.61; 7.90]	−7.43 [-20.53; 5.67]
T3888P	−10.24 [-19.03;-1.44]	−11.08 [-19.99;-2.17]
Lipoprotein(a) (β)		
rs10455872	1.58 [1.03; 2.13]	–
T3888P	0.66 [0.17; 1.14]	–

Values are presented as odds ratio (OR) [95% confidence interval (CI)] for logistic and as β [95% CI] for linear regressions. Calcification volume and lipoprotein(a) are ln-transformed. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex and lipoprotein(a). ECST = European Carotid Surgery Trial.

thickness and suggested that the effects of menopause and the resultant increase in cardiovascular risk factors possibly underlie these differences. Our study could extend our knowledge about sex differences in the association between Lp(a) and vulnerable plaque characteristics, as we have analysed more plaque characteristics than previous studies and as we used a multi-imaging modality approach. Women have in general a more favorable plaque composition than men. However, we have to be cautious to make firm conclusions about the sex-specific Lp(a) analyses, because of the small sample size in the stratified groups and the rare prevalence of some plaque characteristics. In the current study, increasing Lp(a) levels in women were associated with IPH, known as a vulnerable plaque component, compared to men. This could suggest that for women high Lp(a) levels are a stronger risk factor for developing severe carotid atherosclerosis than for men. In line with our findings, other studies have reported a higher Lp(a)-related increased risk for cardiovascular disease in women than in men [35,36]. To elucidate this hypotheses further, studies with a larger population for both men and women are needed.

Another finding in our study is that the assumed proportion of Lp(a) cholesterol content of total Lp(a) mass (ranging from 20 to 45%) did influence the results of the regression analyses. We found that according to the assumed proportion of Lp(a) cholesterol, estimates of the associations between Lp(a) and plaque characteristics were slightly changing especially for presence of IPH and LRNC, calcification volume and degree of stenosis in women. However, the confidence intervals became also wider and the sample size of women was relatively small in our cohort. We recommend to further investigate the effect of Lp(a)-corrected LDL cholesterol and the corresponding Lp(a) cholesterol content on atherosclerosis to understand underlying pathophysiological mechanisms.

Our data showed that T3888P was significantly associated with lower volumes and absence of LRNC, and a smaller maximum vessel wall area. So, T3888P seems to be related to smaller and more stable plaques. In addition, this study showed a significant association between rs10455872 and calcification volume and absence of plaque ulceration. The association between rs10455872 and a lower prevalence of plaque ulceration feels counterintuitive, as Lp(a) was not associated with less plaque ulcerations. As this could be related to the relatively small sample size of our study, further investigation of this association in larger study populations is needed. After adjustments for Lp(a) the strength of the associations between the SNPs and plaque characteristics increased. In case of a mediating effect of Lp(a) a decrease would have happened. So, the increasing associations indicate that rs10455872 and T3888P seems to have pleiotropic genetic effects and probably have effects via other pathways than only via Lp(a). This could also be an explanation for the negative association between rs10455872 and plaque ulceration.

We did not find statistically significant associations between the number of apo(a) KIV-2 repeats and plaque characteristics, despite the strong association between Lp(a) and apo(a) KIV-2 repeat numbers. Although the low-molecular-weight apo(a) phenotype is known to be associated with cardiovascular disease, several previous studies also found no association between KIV-2 repeat numbers and atherosclerosis [37,38]. A population-wide prospective study showed that low-molecular-weight apo(a) phenotypes were associated with advanced carotid atherosclerosis, but not with early carotid atherosclerosis [10]. So, it might be that KIV-2 repeat numbers affect especially plaque composition in patients with severe carotid atherosclerosis.

In the current study, we focused on the relation between Lp(a) and plaque characteristics. From a clinical perspective, it is important to relate Lp(a) also to clinical outcomes. Despite conflicting results in the past, recently, a large general population study showed that Lp(a) is causally related to ischemic stroke [39]. One of the potential mechanisms is that high Lp(a) levels directly contribute to atherosclerotic disease which is supported by our results.

4.1. Strengths and limitations

The strength of our study is that we used two imaging modalities to characterize the vulnerability of the carotid atherosclerotic plaque. To the best of our knowledge, this is the first study that investigates the association between Lp(a), KIV-2 repeats, and Lp(a) related SNPs and vulnerable plaque characteristics *in vivo*.

This study also has several limitations. Firstly, our sample size was relatively small. This could have led to a lack of power to prove relationships between Lp(a) and some plaque characteristics, especially in the sex-specific analyses. A second limitation is that a selection bias could have confounded the results, since a renal clearance of <30 or <60 mL/min/1.73 m^2 was a contraindication for contrast-enhanced MRI and MDCTA scanning, respectively. We do not expect a major influence on the results as a reduced eGFR is related to both elevated Lp(a) levels and more severe carotid atherosclerosis [40,41]. Thirdly, the prevalence of ulcerations could be underestimated in this study due to calcifications which could mask ulcerations. Since we only have included patients with a mild-to-moderate stenosis in this study, and plaques with a more severe stenosis are often more severely calcified [25], we expect that this underestimation is small. Finally, based on this study we cannot make causal inferences because of the cross-sectional design. However, by analyzing SNPs we overcome this issue and we could draw conclusions about the relationships between Lp(a) related SNPs and plaque characteristics. Because some Lp(a) related SNPs had a rare occurrence and our sample size was relatively small, we must be careful by making firm conclusions about the relationship between these SNPs and plaque characteristics. Further analyses in larger study populations are necessary to confirm the findings of this study, which enables us to include more plaques with vulnerable characteristics and to investigate the relationship between Lp(a) and carotid atherosclerosis also in the general population.

4.2. Conclusions

In patients with symptomatic carotid artery stenosis, increased plasma Lp(a) levels were associated with degree of stenosis, and IPH, LRNC, and TRFC, known as vulnerable atherosclerotic plaque characteristics, in the carotid artery. SNPs in the *LPA* gene were also associated with carotid atherosclerosis: T3888P was associated with lower LRNC prevalence and smaller maximum vessel wall area. Further research in larger study populations is needed to confirm our results, to further investigate sex-specific associations between Lp(a) and carotid atherosclerosis, and to investigate the relationship between Lp(a) and the evolution of vulnerable plaque characteristics.

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Author contributions

M.J.A.P. Daemen, D.H.K. van Dam-Nolen, J. Hendrikse, M.E. Kooi, P.J. Koudstaal, A. van der Lugt, M.T. Mulder, P.J. Nederkoorn, J.E. Roeters van Lennep and A.F.W. van der Steen have designed the study. G.A.J.C. Crombag, D.H.K. van Dam-Nolen, A.C. van Dijk and C. Lucci have performed the data collection and image analyses. F. Kronenberg, F. Leijten, M.T. Mulder and L. van der Zee-van Vark have performed the Lp(a) related measurements. D.H.K. van Dam-Nolen, A. van der Lugt, M.T. Mulder and J.E. Roeters van Lennep have analysed the data. D.H.K. van Dam-Nolen has written the manuscript. All authors have interpreted and discussed the results, critically revised the manuscript, have given their final approval of this manuscript, and have agreed to be accountable for all aspects of this work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2021.06.004.

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