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Lipoprotein(a) is associated with large artery atherosclerosis stroke aetiology and stroke recurrence among patients below the age of 60 years: results from the BIOSIGNAL study

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Aims Lipoprotein(a) [Lp(a)] is a recognized causal risk factor for atherosclerotic cardiovascular disease but its role for acute ischaemic stroke (AIS) is controversial. In this study, we evaluated the association of Lp(a) with large artery atherosclerosis (LAA) stroke and risk of recurrent cerebrovascular events in AIS patients.

Methods and results For this analysis of the prospective, observational, multicentre BIOSIGNAL cohort study we measured Lp(a) levels in plasma samples of 1733 primarily Caucasian (98.6%) AIS patients, collected within 24 h after symptom onset. Primary outcomes were LAA stroke aetiology and recurrent cerebrovascular events (ischaemic stroke or transient ischaemic attack) within 1 year. We showed that Lp(a) levels are independently associated with LAA stroke aetiology [adjusted odds ratio 1.48, 95% confidence interval (CI) 1.14-1.90, per unit $\log_{10}$Lp(a) increase] and identified age as a potent effect modifier ($P_{interaction}$ =0.031) of this association. The adjusted odds ratio for LAA stroke in patients aged <60 years was 3.64 (95% CI 1.76–7.52) per unit $\log_{10}$Lp(a) increase and 4.04 (95% CI 1.73–9.43) using the established cut-off ≥100 nmol/l. For 152 recurrent cerebrovascular events, we did not find a significant association in the whole cohort. However, Lp(a) levels ≥100 nmol/l were associated with an increased risk for recurrent events among patients who were either <60 years [adjusted hazard ratio (HR) 2.40, 95% CI 1.05–5.47], had evident LAA stroke aetiology (adjusted HR 2.18, 95% CI 1.08–4.40), or had no known atrial fibrillation (adjusted HR 1.60, 95% CI 1.03–2.48).

Conclusion Elevated Lp(a) was independently associated with LAA stroke aetiology and risk of recurrent cerebrovascular events among primarily Caucasian individuals aged <60 years or with evident arteriosclerotic disease.

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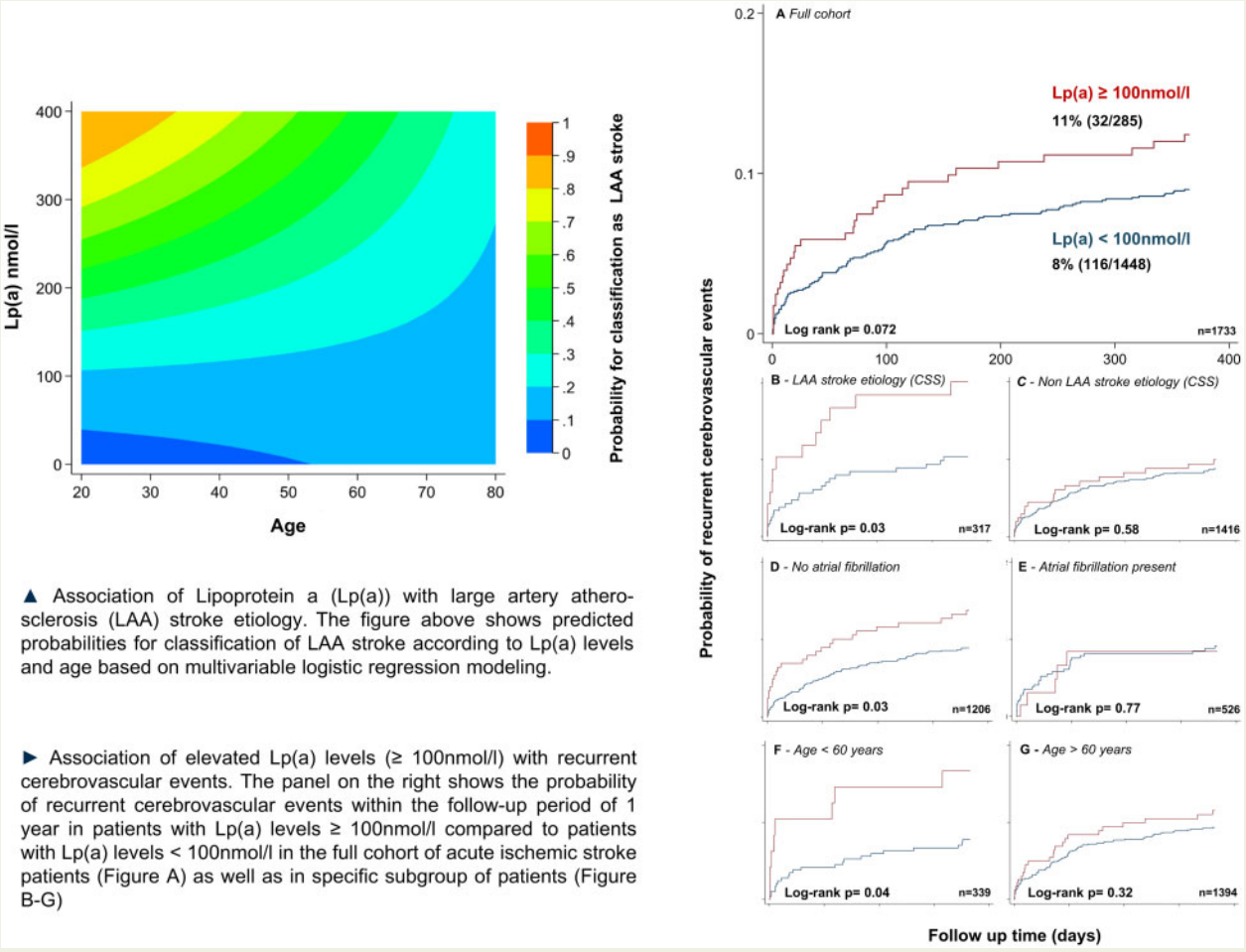
Graphical Abstract



▲ Association of Lipoprotein a (Lp(a)) with large artery atherosclerosis (LAA) stroke etiology. The figure above shows predicted probabilities for classification of LAA stroke according to Lp(a) levels and age based on multivariable logistic regression modeling.

► Association of elevated Lp(a) levels (≥ 100nmol/l) with recurrent cerebrovascular events. The panel on the right shows the probability of recurrent cerebrovascular events within the follow-up period of 1 year in patients with Lp(a) levels ≥ 100nmol/l compared to patients with Lp(a) levels < 100nmol/l in the full cohort of acute ischemic stroke patients (Figure A) as well as in specific subgroup of patients (Figure B-G)

Keywords Lipoprotein(a) • Acute ischaemic stroke • Large artery atherosclerosis

Introduction

Despite a decreasing incidence of acute ischaemic stroke (AIS) in most developed countries, the absolute numbers of stroke survivors and the lifetime risk for stroke are increasing globally.[1] Based on population-based studies up to 25% of all strokes are recurrent strokes and they are more likely to be disabling or fatal than first ever strokes.[2] Optimal secondary prevention depends on the level of evidence for a specific underlying aetiology, which remains undetermined in about 30% of cases.[3] Especially in younger stroke patients, the underlying aetiology is often undetermined partly because recognized vascular risk factors have not yet developed but also because so far unknown risk factors may play a more prominent role. This outlines the importance to search for and evaluate these unknown risk factors especially in the younger stroke population. Blood markers currently not routinely measured may be an important adjunct to routine diagnostic work-up with regard to aetiological classification and risk estimation.[4]

Lp(a) is a lipoprotein composed of apolipoprotein B-100 and apolipoprotein(a). Its structural similarities to low-density lipoprotein (LDL) and plasminogen contribute to its atherogenic and thrombogenic properties.[5,6] First described in 1963,[7] scientific interest was reignited in the past decades after several lines of evidence indicated that Lp(a) is a causal risk factor of atherosclerotic cardiovascular disease.[5,8,9] A large number of studies have suggested an association between elevated Lp(a) and the promotion of cardiovascular disease in the setting of primary[10,11] as well as secondary prevention,[12–15] backed up by a recent meta-analysis.[16] Genetic studies have strengthened the evidence for a causal relationship of Lp(a) with cardiovascular events.[17,18] Furthermore, by some estimates, up to 90% of the variation in plasma Lp(a) concentration between individuals is attributed to genetic variance in the apo(a) gene.[19]

Despite the growing body of evidence supporting a causal association of Lp(a) with atherosclerotic cardiovascular disease, the relationship between Lp(a) and ischaemic stroke remains conflicting. While incident ischaemic stroke was included as an outcome measure in cardiovascular risk cohorts and population-based studies,[4,18,20] only few studies examined the association of Lp(a) with stroke recurrence.[21,22] Evidence on the association of Lp(a) with underlying stroke aetiology is even more scarce[23,24] despite the fact that overall risk of stroke recurrence differs widely between aetiological subgroups.[3]

We therefore aimed to validate the association of Lp(a) with LAA stroke aetiology through a prospective multicentre cohort study within a predominantly Caucasian population. In addition, we investigated the association of Lp(a) with the risk of recurrent cerebrovascular events [ischaemic stroke or transient ischaemic attack (TIA)] within 1 year in all AIS patients, as well as in subgroups stratified by age and stroke aetiology.

Methods

Study design and participants

The BIOSIGNAL (Biomarker Signature of Stroke Aetiology) study (ClinicalTrials.gov NCT02274727) is a prospective, observational, multicentre, inception cohort study to evaluate selected blood biomarkers in patients with acute ischaemic stroke measured within 24 h from symptom onset. From October 2014 through October 2017, a total of 1759 adult patients admitted with AIS were enrolled at nine European stroke centres. Ischaemic stroke was defined according to the World Health Organization criteria as an acute focal neurological deficit lasting longer than 24 h and not attributed to another definite cause, or, if symptoms lasted less than 24 h, cranial imaging [computed tomography (CT) or magnetic resonance imaging (MRI)] must have confirmed a new ischaemic infarct. Patients with haemorrhagic stroke, TIA lacking tissue-based confirmation of ischaemia, patients discharged with a diagnosis other than ischaemic stroke (i.e. stroke mimics) or patients who refused informed consent were excluded. The BIOSIGNAL study was approved by local ethics committees and conducted according to the principles expressed in the Declaration of Helsinki. All patients or their welfare guardians provided written informed consent. The de-identified data supporting the findings of this study are available from the corresponding author on reasonable request.

Procedures and outcomes

On admission, all participants received CT and/or MRI. Demographic variables, vital signs, vascular risk factors and stroke severity by the the National Institute of Health Stroke Scale (NIHSS) were collected by neurovascular fellows. Comorbidities were assessed by the Charlson Comorbidity Index modified for stroke patients (CCI) by patient self-reported medical history and available discharge reports.[25] Per protocol, participants received standard of care aetiological work-up including 12-lead electrocardiography (ECG), >24 h continuous ECG monitoring, transthoracic or transoesophageal echocardiography, neurovascular ultrasound and/or CT or magnetic resonance angiography. Stroke aetiology was determined according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) classification.[26] Additionally, the Causative Classification System (CCS), a web-based version of the SSS-TOAST classification,[27] was used to reduce intra- and inter-rater variability and minimize potential bias arising from misclassification of stroke aetiology.

Patients were followed either during an outpatient visit or with a structured telephone interview. Functional outcome was measured using the modified Rankin Scale (mRS).[28] Predefined outcomes were LAA stroke assessed by TOAST and SSS-TOAST classification on hospital discharge as well as time to recurrent ischaemic cerebrovascular event (AIS or TIA) within 1 year after the index stroke. Specific definitions of outcomes are provided in the supplemental material.

Biomarker measurement

Blood was drawn within 24 hours of symptom onset during the first routine blood sampling in EDTA containing plastic tubes. Samples were immediately centrifuged at 3000*g* at 4°C for 20 min, aliquoted and frozen at -80°C until the time of analysis. Lp(a) levels were assessed in batches of thawed plasma with the Roche Tina-quant Lipoprotein(a) second generation assay on Cobas c702 platform (Roche Diagnostics, Mannheim, Germany) in the Institute of Clinical Chemistry of the University Hospital of Zurich, Switzerland as the central laboratory. Plasma levels are expressed in nanomol per liter (nmol/L). The intra-assay and inter-assay coefficient of variation, defined as the ratio of the standard deviation to the mean, was <5%, respectively. The manufacturer defined the lower limits of quantification (LoQ) and detection (LoD) with 20 and 7 nmol/L, respectively. Lp(a) levels below LoD (22.8%) were measured twice for confirmation and were assigned a value of LoD divided by the square root of two as recommend previously.[29] For measurements below LoQ (53.9%), we used the apparent concentrations recorded by the assay as suggested for epidemiological studies.[30] By a method comparison with another immunoturbidimetric assay using Denka Seiken antibodies and calibrators traceable to the WHO/IFCC reference SRM 2B material, we found the Roche Tinaquant assay to measure lower levels of Lp(a) at very low concentrations, which has been reported earlier.[31] However, both at concentrations ≤12 nmol/L and >12 nmol/L, the two measurements showed very good correlations ($r = 0.942$ and $r = 0.976$, respectively, Passing Bablock).

Statistical analysis

Discrete variables are summarized as counts (percentages) and continuous variables as medians [interquartile ranges (IQR)]. Common logarithmic transformation (base 10) was performed to transform to normality for skewed distributions. For two-group comparisons, Fisher's exact test and Mann–Whitney U-test were performed, while for multigroup comparisons the Kruskal–Wallis test with appropriate post-hoc testing was used. To investigate the association of Lp(a) (as continuous as well as dichotomized value) with LAA stroke aetiology, binary logistic regression models were constructed to calculate odds ratios (OR) and 95% confidence intervals (95% CI). Multivariable models were built by identifying statistically relevant covariates in univariate logistic regression with a cut-off P-value of <0.1. Demographic variables (age, sex) and established vascular risk factors (arterial hypertension, diabetes mellitus, dyslipidaemia, active smoking, positive family history for cardiovascular events, history of stroke and body mass index) were included regardless of their significance. Backward elimination was then used for the selection of the final model, with $P < 0.05$ considered significant. To assess the discriminatory ability of Lp(a), area under the receiver operating characteristic curve (AUROC) was calculated for the models with and without Lp(a) and compared using the likelihood-ratio test. To test for model optimism we additionally applied 10-fold cross-validation to obtain mean AUROCs with bias corrected 95% CIs. In addition, continuous net reclassification index (NRI) was calculated. To account for inter-rater variability in regard to aetiological classification, we validated the model with the CCS by using the SSS-TOAST classification. Interaction analyses were performed to assess effect modification of Lp(a) by demographic and vascular risk factors including the use of lipid-lowering drugs prior to the index stroke.

Table 1 Baseline characteristics stratified by large artery atherosclerosis stroke according to the TOAST and CCS classification

Characteristics	Total	LAA stroke (TOAST)	Non-LAA stroke (TOAST)	P-value	LAA stroke (CCS)	Non- LAA stroke (CCS)	P-value
No. (%)	1759 (100)	258 (14.7)	1500 (85.3)		324 (18)	1435 (82)	
Demographic data							
Age, median (IQR)	74.4 (64-82)	73.3 (65-81)	74.6 (63-83)	0.6	74 (65-81)	75 (63-83)	0.4
Female sex, *n*(%)	736 (41.8)	79 (30.6)	656 (43.7)	**<0.001**	107 (33)	629 (44)	**< 0.001**
Caucasian ethnicity, *n*(%)	1711 (98.6)	250 (98)	1460 (98.7)	0.4	317 (98)	1394 (98)	0.4
Medical history							
BMI, kg/m^2, median (IQR) *	25.5 (23-28)	25.3 (24-28)	25.6 (23-28)	0.8	25 (24-28)	26 (23-28)	0.6
Hypertension, *n*(%)	1281 (72.8)	202 (78)	1078 (72)	**<0.05**	256 (79)	1025 (71)	**<0.01**
Dyslipidaemia, *n*(%)	1356 (77)	222 (86)	1133 (75)	**<0.001**	275 (85)	1081 (75)	**<0.001**
Diabetes mellitus, *n*(%)	313 (18)	52 (20)	261 (17)	0.3	64 (20)	249 (17)	0.3
Smoking, *n*(%)	394 (23)	80 (31)	314 (21)	**<0.001**	96 (30)	298 (12)	**<0.01**
History of stroke, *n*(%)	303 (17)	51 (20)	252 (17)	0.3	61 (18)	242 (17)	0.4
Family history of cardiovascular events, *n*(%) +	215 (13)	24 (10)	190 (14)	0.2	33 (11)	182 (14)	0.3
Heart failure, *n*(%)	111 (6.3)	7 (3)	104 (7)	**<0.01**	6 (2)	105 (7)	**<0.001**
Coronary heart disease, *n*(%)	359 (20)	66 (26)	292 (20)	**<0.05**	84 (26)	257 (19)	**<0.01**
Atrial fibrillation *n*(%)	323 (18)	11 (4)	312 (21)	**<0.001**	14 (4)	309 (22)	**<0.001**
Peripheral arterial disease, *n*(%)	219 (13)	35 (14)	183 (12)	0.5	51 (16)	168 (12)	0.1
Alcohol abuse, *n*(%)	102 (6)	22 (9)	79 (5)	0.1	28 (9)	74 (5)	**<0.05**
Renal disease, *n*(%)	259 (15)	34 (13)	225 (15)	0.5	38 (12)	221 (15)	0.1
Liver disease, *n*(%)	30 (2)	2 (1)	28 (2)	0.3	4 (1)	26 (2)	0.6
Charlson Comorbidity Index, median (IQR)	1 (0-2)	1 (0-2)	0 (0-2)	0.5	1 (0 - 2)	0 (0–2)	0.3
Stroke severity							
NIHSS, median (IQR)	5 (2-11)	6 (2-11)	5 (2-11)	0.2	6.5 (3–12)	5 (2–11)	**<0.05**
Acute Treatment							
IV thrombolysis, *n*(%)	706 (40)	105 (41)	601 (40)	0.9	142 (44)	564 (39)	0.2
Mechanical thrombectomy, *n*(%)	334 (19)	78 (30)	256 (17)	**<0.001**	91 (28)	243 (17)	**< 0.001**
Medication on admission							
Antiplatelets, *n*(%)	666 (38)	114 (44)	552 (36)	**<0.05**	148 (45)	518 (36)	**<0.01**
Anticoagulation, *n*(%)	243 (14)	14 (5)	228 (15)	**<0.001**	18 (6)	225 (16)	**<0.001**
Antihypertensive drugs, *n*(%)	1050 (60)	167 (65)	882 (59)	0.1	216 (67)	834 (58)	**<0.01**
Lipid-lowering drugs, *n*(%)	556 (32)	91 (35)	465 (31)	0.2	111 (34)	445 (31)	0.3
Laboratory values, median (IQR)							
Lipoprotein(a), nmol/l	17 (8-54)	21 (8-94)	16 (7-50)	**0.02**	21 (8-85)	16 (7-50)	**0.04**
LDL-cholesterol, mmol/l &	2.7 (2.0-3.4)	3.0 (2.1-3.7)	2.7 (2.0-3.3)	**<0.01**	2.9 (2.1-36)	2.7 (2-3.3)	**< 0.001**
HDL-cholesterol, mmol/l %	1.3 (1.1-1.6)	1.2 (1.0-1.6)	1.3 (1.1-1.7)	**0.02**	1.3 (1 -1.6)	1.3 (1-1.7)	0.05
CRP, mg/l	3.4 (1.3-9.7)	3.5 (1.3-9.7)	3.4 (1.3-9.7)	0.8	3.5 (1.3- 10)	3.4 (1.3-10)	0.6
Creatinine, µmol/l	83 (69-99)	86 (72-103)	82 (68-98)	**0.02**	84 (72-102)	83 (68-98)	0.1

LAA, large artery atherosclerosis; TOAST, Trial of Org 10172 in Acute Stroke Treatment classification system; CCS, Causative Classification System; IQR, interquartile range; BMI, body mass index; NIHSS, National Institute of Health Stroke Scale; IV, intravenous; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein; MI, myocardial infarction. **Statistics**: values are median (IQR) or counts (percentages). Testing performed using the Mann–Whitney U-Test for continuous variables and Fisher's exact test for binary variables. **Missing Values**: * 8.3% missing values; + 7.6% missing values; & 6.6% missing values; % 5.2% missing values. All other variables had <3% of values missing. Percentages and IQR are based on available data.
Bold text indicates a statistically significant difference with a *P*-value less than 0.05.

In the final model, we further included Lp(a) as a dichotomized variable with a cut-off of 100 nmol/L as commonly suggested in the literature.[32,33]

To determine the association of Lp(a) with recurrent cerebrovascular events, Cox proportional-hazards regression analysis was performed utilizing the established cut-off of 100 nmol/L. Only previous stroke was associated with recurrent events in univariate Cox regression; however, models were adjusted also for established demographic and vascular risk factors (sex, age, arterial hypertension, diabetes mellitus, dyslipidaemia, active smoking and history of stroke). We estimated hazard ratios (HR) and their 95% CI. Subgroup analysis was performed for patients below 60 years of age and for LAA stroke aetiology according to the CCS as well as for subjects without known or newly diagnosed atrial fibrillation. Resulting Kaplan–Meier failure estimates were compared with the log-rank test. Additional subgroup analysis was performed with Lp(a)

Table 2 Multivariable logistic regression and ROC analyses for large artery atherosclerosis stroke with and without Lp(a)

	Large artery atherosclerosis according to the TOAST classification system					
	Model with Lp(a)			Model without Lp(a)		
Predictors	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Log Lp(a)	1.48	1.14–1.90	**<0.01**	–	–	–
Age	1.02	1.01–1.04	**<0.001**	1.02	1.01–1.03	**<0.001**
Female sex	0.55	0.41–0.74	**<0.001**	0.57	0.42–0.77	**<0.001**
Dyslipidaemia	1.72	1.18–2.52	**<0.01**	1.79	1.23–2.62	**<0.01**
Smoking	1.65	1.20–2.27	**<0.01**	1.61	1.17–2.21	**<0.01**
History of AF	0.16	0.08–0.29	**<0.001**	0.16	0.09–0.30	**<0.001**
	Large artery atherosclerosis according to the CCS classification system					
	Model with Lp(a)			**Model without Lp(a)**		
Predictors	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Log Lp(a)	1.38	1.10–1.75	**<0.01**			–
Age	1.03	1.02–1.04	**<0.001**	1.03	1.02–1.04	**<0.001**
Female sex	0.59	0.45–0.77	**<0.001**	0.60	0.46–0.79	**<0.001**
Dyslipidaemia	1.64	1.17–2.31	**<0.01**	1.70	1.21–2.38	**<0.01**
Smoking	1.63	1.21–2.20	**<0.01**	1.60	1.19–2.15	**<0.01**
History of AF	0.14	0.08–0.25	**<0.01**	0.15	0.08–0.25	**<0.001**

	ROC analysis					
	AUC (TOAST)	**95% CI**	**Likelihood ratio test (*P*-value)[a]**	**AUC (CCS)**	**95% CI**	**Likelihood ratio test (*P*-value)[a]**
Lp(a)	0.55	0.51–0.59	–	0.54	0.50–0.57	–
Model without Lp(a)[b]	0.68	0.65–0.71	–	0.69	0.66–0.72	–
Model with Lp(a)	0.69	0.66–0.72	**<0.01**	0.69	0.66–0.72	**<0.01**

ROC, receiver operating characteristic; LAA, large artery atherosclerosis; TOAST, Trial of Org 10172 in Acute Stroke Treatment classification system; CCS, Causative Classification System; Lp(a), Lipoprotein(a); AF, atrial fibrillation.
[a]To compare the AUC of nested (without Lp(a)) vs whole model (with Lp(a)).
[b]Model contained age, sex, dyslipidaemia, smoking and history of atrial fibrillation.
Bold text indicates a statistically significant difference with a *P*-value less than 0.05.

quartiles instead of dichotomized values. $P<0.05$ were considered statistically significant. Data analysis was performed using STATA version 15 (StataCorp LLC, College Station, TX, USA).

Results

Baseline cohort characteristics

A total of 1759 patients with an AIS were consecutively enrolled in the study. Blood samples were available in 1733 patients (98.5%). Median Lp(a) levels were 17 nmol/l (IQR 8–54), 285 (16.5%) participants had elevated Lp(a) levels (≥100 nmol/l). A total of 258 (14.7%) strokes were classified as LAA strokes. This number increased to 324 (18.4%) with the use of the CCS. Table 1 displays patient baseline characteristics stratified by LAA stroke aetiology according to the TOAST classification system and the CCS [for distribution of Lp(a) according to other stroke aetiologies see Table S1 of the supplementary material]. Median age of the cohort was 74 (IQR 64–82) years; 342 (19.4%) participants were <60 years; 736 (42%) were female, 1711 (98.6%) were Caucasian. Among all patients admitted within 24 h to the contributing tertiary stroke centres, 706 patients (40%) received intravenous thrombolysis and 334 patients (19%) received mechanical thrombectomy. Overall, 1407 patients (80%) completed 1-year follow-up, 338 patients (19.2%) died and 14 participants (0.8%) were lost to follow-up within 1 year. Table S2 of the supplementary material summarizes information on patients who died within the follow-up period.

Association of lipoprotein(a) with large artery atherosclerosis

Median Lp(a) levels were higher in patients with LAA stroke aetiology. In the multivariable logistic regression model containing age, sex, dyslipidaemia, smoking status and known atrial fibrillation, $\log_{10}$Lp(a) was independently associated with LAA stroke aetiology [OR 1.48, 95% CI 1.14–1.90, $P<0.01$, per unit $\log_{10}$Lp(a) increase]. Adding Lp(a) to the multivariable regression model slightly increased

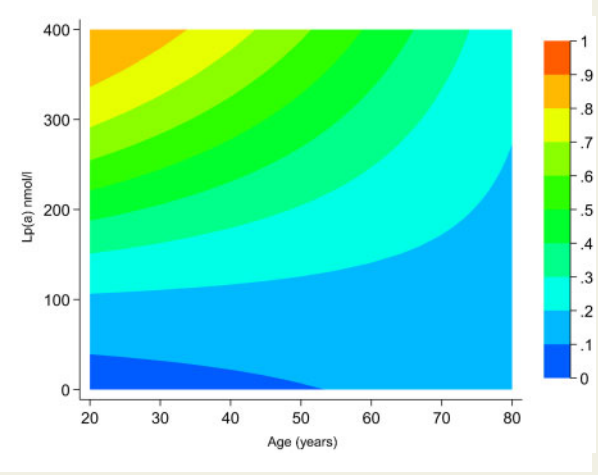


Figure 1 Contour plot of predicted probabilities for classification of large artery atherosclerosis stroke aetiology. Figure 1 shows the interaction of Lp(a) levels (nmol/L) and age in regard to the predicted probabilities (colour scale) for the classification of large artery atherosclerosis (LAA) stroke aetiology according to the TOAST criteria. The contour plot illustrates that in younger patients Lp(a) levels have greater discriminatory value in regard to the classification of LAA stroke compared to older patients. This could be explained by the fact that in younger patients traditional 5risk factors have not yet developed, thus genetically predetermined risk factors as Lp(a) levels have more clinical impact.

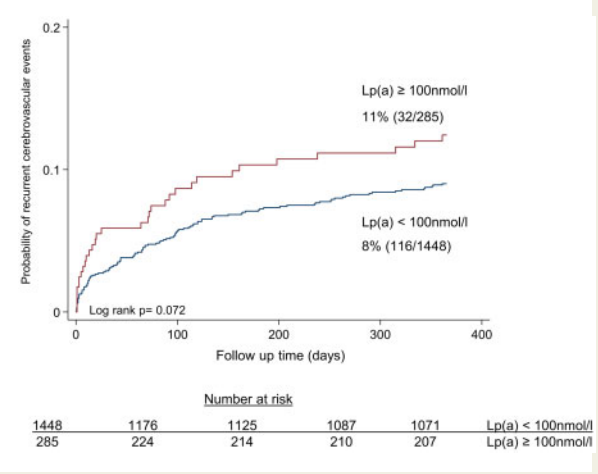


Figure 2 Kaplan–Meier failure estimates for recurrent cerebrovascular events. Kaplan–Maier failure estimates for recurrent cerebrovascular events (ischaemic stroke or transient ischaemic attack, n = 148) during a 12-month follow-up for Lp(a) levels below and above 100 nmol/L. In four patients with recurrent cerebrovascular events Lp(a) levels were missing. Lp(a), Lipoprotein(a)

the discriminatory accuracy from an AUC of 0.68 (95% CI 0.65–0.71) to 0.69 (95% CI 0.66–0.72, $P < 0.01$) (Table 2). The optimism corrected mean AUC after 10-fold cross-validation was 0.69 (bias-corrected 95% CI 0.65–0.72) supporting similar out-of-sample prediction. Among those patients with a LAA stroke, by measuring Lp(a) a net of 48% was correctly moved toward this category, while 52% were falsely moved to lower probabilities. Among those with a non-LAA stroke, by measuring Lp(a) a net of 61% patients were correctly moved to lower probabilities, while 40% were falsely moved toward the LAA group although they had no LAA stroke. The resulting NRI was 0.18 ($P < 0.01$), revealing significant but modest incremental discriminatory value. Moreover, exclusion of Lp(a) levels below LoD as well as imputing LoQ/$\sqrt{2}$ for values below the LoQ resulted in similar estimates (data not shown). Interaction analysis revealed a significant interaction between Lp(a) and age in the multivariable model ($P = 0.031$ for the interaction term). Figure 1 shows a contour plot of predicted probabilities for continuous Lp(a) values. The adjusted OR in patients aged <60 years was 3.64 [95% CI 1.76–7.52, $P < 0.001$, per unit $\log_{10}$ Lp(a) increase], in patients aged ≥60 years the association was no longer significant with an OR of 1.26 (95% CI 0.96–1.64, $P = 0.1$). For the previously suggested cut-off of 100 nmol/L, which was also a very close proximity to the optimal discriminative cut-off of 94 nmol/L (83rd percentile of the cohort) derived from the analysed cohort itself by using Youden's J statistics, the adjusted OR were 1.77 (95% CI 1.26–2.47, $P < 0.01$) in the whole cohort, 4.04 (95% CI 1.73–9.43, $P < 0.01$) in patients <60 years and 1.48 (95% CI 1.02–2.13, $P = 0.04$) in patients aged ≥60 years. There was no significant interaction between Lp(a) and LAA stroke aetiology mediated by other covariates of the model or the presence of lipid-lowering therapy prior to the index event. The predictive value of Lp(a) remained unaltered if the SSS-TOAST classification system was used for outcome classification (OR 1.38, 95% CI 1.10–1.75, $P < 0.01$) (Table 2).

Association of lipoprotein(a) with recurrent cerebrovascular events

During the 1-year follow-up, 116 ischaemic strokes and 36 TIAs occurred. In total 152 (8.64%) of the cohort experienced one of the predefined outcomes (AIS or TIA). Subjects with a recurrent cerebrovascular event were more likely to have had a previous stroke before the index stroke. Table S3 of the supplementary material displays patient baseline characteristics stratified by the occurrence of an outcome event. Among patients with Lp(a) levels <100 nmol/L, 8% experienced a recurrent cerebrovascular event, whereas in patients with Lp(a) levels ≥100 nmol/L, 11% experienced a recurrent cerebrovascular event. In the multivariable Cox regression model of the whole cohort (containing age, sex, dyslipidaemia, smoking status, hypertension, diabetes and history of stoke), there was no significant association between dichotomized Lp(a) levels and the occurrence of a recurrent event (HR 1.40, 95% CI 0.94–2.07, $P = 0.09$). To investigate if the association may be more prominent in young patients, we performed further analysis with the previous established cut-off of 60 years. In patients younger than 60 years, we found a pronounced positive association with recurrent cerebrovascular events (adjusted HR 2.4, 95% CI 1.05–5.47, $P = 0.04$) independent of aetiological classification whereas in patients aged over 60 years again no significant association was found (adjusted HR 1.23, 95% CI 0.78–1.92, $P = 0.37$). Further, among all participants with LAA stoke aetiology (according to the CCS classification), there was an association with recurrent cerebrovascular events (adjusted HR 2.18, 95% CI 1.08–4.40, $P = 0.03$) compared with patients with non-LAA strokes, i.e. TOAST

Table 3 Univariate and multivariable Cox proportional hazard regression in regard to recurrent cerebrovascular events for Lp(a) levels ≥100nmol/L

	Univariate analysis		Adjusted for age and sex[a]		Adjusted for vascular risk factors[a]	
Stratification	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Overall, *n* = 1732	1.42 (0.97–2.11)	0.07	1.43 (0.97–2.12)	0.07	1.40 (0.94–2.07)	0.09
LAA stroke (CCS), *n* = 317	2.09 (1.06–4.12)	**0.03**	2.06 (1.03–4.12)	**0.04**	2.18 (1.08–4.40)	**0.03**
Non LAA stroke (CCS), *n* = 1416	1.15 (0.70–1.88)	0.58	1.15 (0.70–1.89)	0.57	1.14 (0.69–1.86)	0.62
AF not present, *n* = 1206	1.63 (1.05–2.52)	**0.03**	1.63 (1.06–2.52)	**0.03**	1.60 (1.03–2.48)	**0.04**
AF present, *n* = 526	0.87 (0.34–2.23)	0.77	0.90 (0.35–2.31)	0.82	0.89 (0.35–2.28)	0.81
Aged <60, *n* = 339	2.32 (1.03–5.22)	**0.04**	2.41 (1.07–5.43)	**0.03**	2.40 (1.05–5.47)	**0.04**
Aged ≥60, *n* = 1394	1.26 (0.80–1.97)	0.32	1.26 (0.80–1.97)	0.32	1.23 (0.78–1.92)	0.37

Lp(a), Lipoprotein(a); LAA, large artery atherosclerosis according to the Causative Classification System (CCS); AF, atrial fibrillation. [a] In subgroup analysis for participants </≥ 60 years age was excluded from multivariable models. Adjustment for vascular risk factors included dyslipidaemia, hypertension, smoking status, diabetes and previous stroke. Bold text indicates a statistically significant difference with a *P*-value less than 0.05.

2–5 (adjusted HR 1.14, 95% CI 0.69–1.86, $P = 0.62$). When excluding all patients with known or newly diagnosed atrial fibrillation from the analysis, we were also able to confirm a significant association with recurrent cerebrovascular events (adjusted HR 1.60, 95% CI 1.03–2.48, $P = 0.04$)), whereas in patients with known or newly diagnosed atrial fibrillation there was no significant association (adjusted HR 0.89, 95% CI 0.35–2.28, $P = 0.81$). Additional interaction analyses within these subgroups did not reveal sex as a significant effect modifier. Results of univariate and multivariable Cox regression are shown in Table 3. Figures 2 and 3 show Kaplan–Meier estimates including subgroup analyses. Kaplan–Meier estimates according to Lp(a) quartiles are presented in Supplementary Figure S1.

Discussion

In this prospective, multicentre AIS cohort we found Lp(a) to be independently associated with LAA stroke aetiology as well as recurrent stroke in patients younger than 60 years. When Lp(a) was added to the best diagnostic models of stroke aetiology, we found a moderate but significant improvement of the model performance. Notably, the significance of the observed association remained unaltered after validation with the CCS, a method that has previously been shown to considerably reduce inter-rater variability.[34] Even though, agreement between CCS and TOAST classification is only moderate,[35] which is also reflected in the difference in frequencies we observed. Further, no significant interaction between Lp(a) and prior lipid-lowering therapy was noticed.[16] Previous studies on the association of Lp(a) with stroke aetiology, although being scarce, have reported comparable results. In a comparable sized Asian cohort, high levels of Lp(a) were associated with LAA stroke and atherosclerotic burden.[23] Smaller retrospective studies mainly in Caucasian cohorts made similar findings but only two of them have used the TOAST classification system.[24,36]

Interestingly, our data identified age as a prominent effect modifier, with a stronger association between Lp(a) and LAA among younger patients. This could be explained by the fact that in younger stroke patients traditional risk factors have not yet developed[37] (it takes time before lifestyle and environmental factors cumulate) and Lp(a)—which is considered a lifelong, still non-modifiable and largely genetically predetermined risk factor—has more clinical impact. For stratified analysis, we used an age cut-off of 60 years reflecting the 20th percentile of our cohort; however, there is currently no consensus on an age cut-off to define stroke in younger patients. Most authors choose an arbitrary cut-off between 55 and 65 years based on pathophysiological or epidemiological considerations.

In the overall cohort, we did not find any significant association of Lp(a) with recurrent ischaemic events if dichotomized by the conventional risk threshold of 100 nmol/L. This result is in line with a study of Lange *et al.* showing an increased risk for a combined cardiovascular outcome but not for stroke and TIA alone in AIS patients with high levels of Lp(a) dichotomized by a cut-off of 30 mg/dL (≈ 72 nmol/L).[21] In contrast, an association with recurrent stroke within 90 days by the same cut-off was shown recently in a primarily Asian cohort.[22]

In further subgroup analysis, however, we identified significantly increased risk for recurrent cerebrovascular event in patients with LAA according to the CCS. Also, after exclusion of patients with known or newly diagnosed atrial fibrillation, elevated Lp(a) was significantly associated with recurrent stroke in the remaining cohort. These findings support the assumption that Lp(a) differentially interacts with the aetiology of stroke. It appears of pathophysiological relevance in atherosclerotic vascular disease leading to stroke but not or less so in cardioembolic stroke. This finding is in line with data from the ARIC (Atherosclerosis Risk in Communities) study which examined 14 221 individuals free of cardiovascular disease and investigated the risk of incident ischaemic stroke. Lp(a) above the 80th percentile increased the adjusted risk for non-lacunar stroke (rate ratio 1.42, 95% CI 1.10–1.84), but not for cardioembolic or lacunar stroke.[38] A subsequent analysis revealed that Lp(a) levels >50 mg/dL (≈ 120 nmol/L) increase the relative risk of stroke among individuals without atrial fibrillation, but not among patients with atrial fibrillation,[39] reinforcing a pathophysiological role of Lp(a) for atherothrombotic stroke.

This hypothesis is also supported by the subgroup analysis in patients below the age of 60 years since competing risks are less frequent in younger patients who are on the other hand more prone to

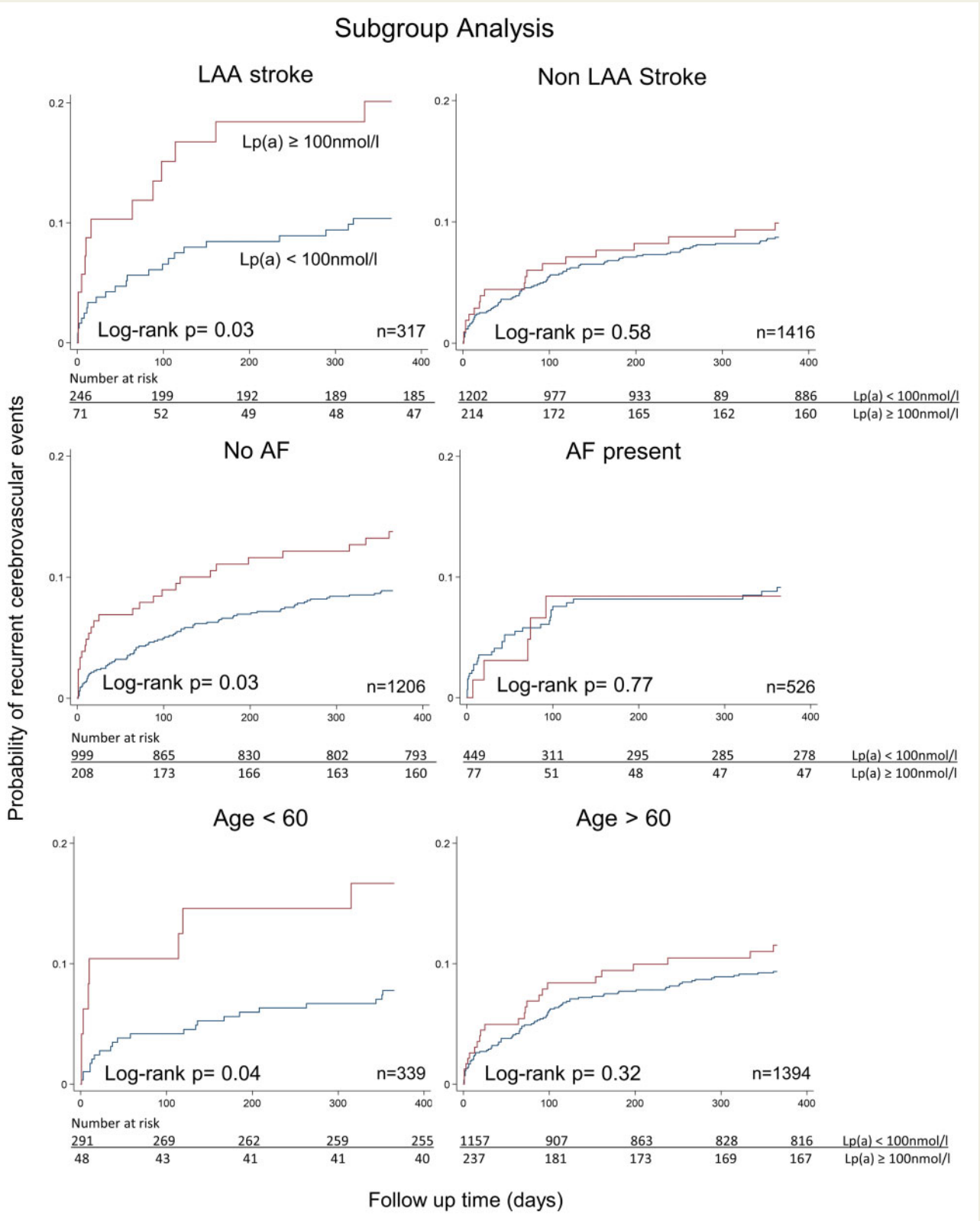


Figure 3 Kaplan–Meier failure estimates for recurrent cerebrovascular events in subgroup analyses. Kaplan–Meier failure estimates for recurrent cerebrovascular events (ischaemic stroke or transient ischaemic attack) according to subgroups during a 12-month follow-up for Lp(a) levels below and above 100 nmol/L. Lp(a), Lipoprotein(a); LAA, large artery atherosclerosis according to the causative Classification System; AF, atrial fibrillation.

genetically determined risk factors. A recent meta-analysis in non-AIS cohorts has shown similar findings in regard to an age difference for incident stroke and adverse cardiovascular events.[4,40]

Currently, lipoprotein apheresis is the only established medical treatment targeting elevated Lp(a) levels. The effects of novel PCSK9 inhibitors in lowering Lp(a) are only moderate but promising.[41,42] A recent phase II trial investigating a hepatocyte-directed antisense oligonucleotide to specifically reduce Lp(a) levels showed encouraging results in individuals with established cardiovascular disease[43]; however, it has not been proven that lowering Lp(a) will reduce the risk for major cardiovascular events. A phase III trial (NCT04023552) currently enrolling participants with Lp(a) levels ≥70 mg/dL (≈ 168 nmol/L) will evaluate the impact of Lp(a) reduction on major cardiovascular events.

This study has limitations. Of note, evidence indicates that Lp(a) may act as an acute phase reactant, thus measurements within the acute phase might be altered. However, Lp(a) levels increase relatively slow and little compared to other acute phase proteins reaching a maximum concentration after several days.[44] Thus, possible alterations within the first 24 h are likely to be small. In addition, although limited, evidence indicates that Lp(a) levels remain stable in stroke patients up to 4 weeks.[45] Of note, we observed a high number of Lp(a) measurements below the lower LoQ of the Roche Tinaquant assay. Although a method comparison with a different assay showed good correlations between the two measurements, the Roche assay has previously been shown to measure lower levels of Lp(a) at high molecular weight Lp(a) isoforms, which are associated with low Lp(a) concentrations. At clinical relevant concentrations, however, measurements are in a similar range with other Lp(a) assays.[31] Additional sensitivity analysis with imputed values below the LoQ as well as analysis with dichotomized Lp(a) levels revealed similar results, thus bias introduced by non-random distribution of low Lp(a) levels seemed unlikely. Further, the distribution of stroke subtypes in our cohort slightly differed from population-based studies due to a low number of small vessel disease strokes and a larger number of cardioembolic strokes.[3] Possible explanations include referral bias, because patients with lacunar stroke tend to have less severe clinical features and are less likely to receive emergency intervention and therefore may not be referred to tertiary care centres. Additionally, there may be a higher detection rate of cardioembolic sources due to extensive cardiac workup in this study. Additionally, the number of participants that received acute re-canalisation treatment is quite high; however, we only included patients within the first 24 h after symptom onset that are more likely to receive interventions and enrolling centres were mainly high-volume stroke centres with 24 h intervention services. Furthermore, we chose a cut-off of 100 nmol/L (~40 mg/dL) for dichotomizing Lp(a) levels reflecting the 80th percentile in the Caucasian population.[33] However, different cut-offs exist in the literature when Lp(a) is measured in nmol/L,[46] the results from this study support a cut-off of 100 nmol/L as it is recommended by the National Lipid Association guidelines.[32] Of note, the conventional 100 nmol/L cut-off is close to the optimal discriminative cut-off derived from the Youden analysis of ROC curves in our cohort study, thus supporting the used cut-off. Due to the study design, we cannot rule out the presence of index-event bias. However, because of the general congruence between risk factors for the index and recurrent events, this will generally tend to bias results toward the null hypothesis, so that our results may rather underestimate the true effect.[47] With interaction analysis we could not find any evidence for effect modification by sex. However, we need to mention that the sample size prevents any firm conclusions. Therefore, larger studies are needed to confirm that Lp(a) has equal predictive value in men and women. Finally, we chose a combined outcome consisting of stroke and TIA; however, there are remaining uncertainties considering the diagnosis of TIAs inherent in the disease definition. Besides these limitations, our dataset reflects, to the best of our knowledge, the largest existing stroke cohort assessing the association of Lp(a) with stroke aetiology and risk for recurrent events in Caucasian AIS patients.

In summary, our data support the role of Lp(a) in promoting atheromathosis in AIS patients and suggest Lp(a) as a moderate and independent risk factor for stroke recurrence in patients with atherosclerotic stroke aetiology or younger than 60 years. Based on our findings, patients with evident LAA and younger patients might be considered as a target population for future novel Lp(a)-lowering therapies.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: M.A. reports grants from the Kurt und Senta Hermann Stiftung during the conduct of the study. M.A. reports personal fees from Bayer, personal fees from BMS, personal fees from Covidien, personal fees from Medtronic, personal fees from Amgen, personal fees from Daiichi Sankyo, personal fees from Nestle Health Science, personal fees from Boehringer Ingelheim, outside the submitted work. C.F. reports personal fees from Boehringer Ingelheim, personal fees from Prediction Bioscience, outside the submitted work; In addition, C.F. has a patent Use of GFAP for identification of intracerebral Haemorrhage US20150247867 licensed to Banyan Biomarkers. A.L. receives personal fees from AMGEN and personal fees from Bayer, outside the submitted work. A.vE. reports grants from Swiss National Science Foundation, grants from Systems X program, grants from Swiss Heart Foundation, grants from European Commission, personal fees from Amgen, personal fees from Sanofi Aventis, outside the submitted work. L.B. reports grants from Swiss National Science Foundation, grants from Swiss Heart Foundation, grants from University of Basel, during the conduct of the study; grants from Swiss Heart Foundation, grants and non-financial support from AstraZeneca, personal fees from Amgen, personal fees and non-financial support from Bayer, personal fees from Bristol-Myers Squibb, personal fees from Claret Medical, outside the submitted work. M.K. reports grants from Swiss National Science Foundation, grants and non-financial support from Spital Pool, non-financial support from ROCHE, grants from EMDO Foundation, grants from Swiss Heart Foundation, during the conduct of the study. M.M. reports grants from Swiss Heart Foundation, outside the submitted work. U.F. reports grants from Medtronic, other from Medtronic, other from Stryker, other from CSL Behring, outside the submitted work.

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