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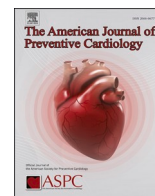Bhatia, Harpreet

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Lipoprotein(a), high-sensitivity c-reactive protein, homocysteine and cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis

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GRAPHICAL ABSTRACT

Central Illustration. The potential benefits of additional measurement of total plasma homocysteine in combination with high-sensitivity c-reactive protein and lipoprotein(a) for predicting coronary heart disease and stroke in the Multi-Ethnic Study of Atherosclerosis. Abbreviations: high-sensitivity c-reactive protein, hs-CRP; total plasma homocysteine, tHcy; lipoprotein(a), lp(a). Cox proportional hazards regression hazard ratios and 95 % confidence intervals adjusted for age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, HDL-C, total cholesterol, triglycerides (log-transformed), BMI and eGFR. Low hs-CRP <2 mg/dL; High hs-CRP ≥2 mg/dL; Low Lp(a) <50 mg/dL; High Lp(a) ≥50 mg/dL; Low tHcy <12 μmol/L; High tHcy=≥12 μmol/L.

Central Illustration The potential benefit of additional measurement of total plasma homocysteine in combination with high-sensitivity c-reactive protein and lipoprotein(a) for predicting coronary heart disease and stroke in the Multi-Ethnic Study of Atherosclerosis.
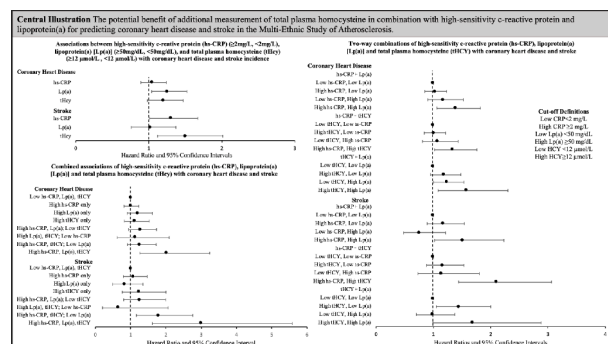
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ABSTRACT

Background and aims: Elevated lipoprotein(a) [Lp(a)], high-sensitivity C-Reactive Protein (hs-CRP), and total homocysteine (tHcy) are associated with atherosclerotic cardiovascular disease (ASCVD) risk. This study

Homocysteine
Lipoprotein(a)
Stroke
Coronary heart disease

investigated the individual and joint associations of Lp(a), hs-CRP and tHcy with coronary heart disease (CHD) and stroke.

Methods: This study was conducted in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort (2000–2017) (CHD analytic $N = 6,676$; stroke analytic $N = 6,674$ men and women). Associations between Lp(a) (<50 vs. ≥50 mg/dL), hs-CRP (<2 vs. ≥2 mg/L) and tHcy (<12 vs. ≥12 μmol/L) and CHD and stroke incidence were evaluated individually and jointly using Cox proportional hazards regression.

Results: Individually, elevated tHcy was associated with CHD and stroke incidence, Lp(a) with CHD only and hs-CRP with stroke only. In combined analyses, CHD risk was higher when multiple biomarkers were elevated [hs-CRP+Lp(a), hazard ratio (HR)=1.39, 95 % confidence interval (CI): 1.06, 1.82; hs-CRP+ tHcy, HR = 1.34, 95 % CI: 1.02, 1.75; Lp(a)+ tHcy HR = 1.58, 95 % CI: 1.08, 2.30; hs-CRP+Lp(a)+ tHcy HR = 2.02, 95 % CI: 1.26, 3.24]. Stroke risk was elevated when hs-CRP and either Lp(a) (HR = 1.51, 95 % CI: 1.02, 2.23) or tHcy (HR = 2.10, 95 % CI: 1.44, 3.06) was also high, when all three biomarkers were elevated (HR = 2.99, 95 % CI: 1.61, 5.58), or when hs-CRP and tHcy (HR = 1.79, 95 % CI: 1.16, 2.76) were both high.

Conclusions: Risk of ASCVD was highest with concomitant elevation of tHcy, hs-CRP and Lp(a). Inclusion of tHcy and consideration of biomarker combination rather than individual biomarker levels may help better identify individuals at greatest risk for ASCVD events.

1. Introduction

Despite improvements in identifying individuals at risk for atherosclerotic cardiovascular disease (ASCVD), it remains the leading cause of morbidity and mortality worldwide [1]. The integration of non-traditional risk markers, including lipoprotein(a) [Lp(a)], high-sensitivity C-reactive protein (hs-CRP) and plasma total homocysteine (tHcy) may help reduce risk for ASCVD [2]. Lp(a) and hs-CRP are established ASCVD risk markers and are currently listed as risk enhancing factors in the 2019 American Health Association (AHA)/American College of Cardiology (ACC) cardiovascular disease prevention guidelines [3]. tHcy, however, is not currently included in guidelines and research is lacking on the potential benefit of considering biomarker combinations to refine ASCVD risk surveillance.

Lp(a) may influence ASCVD risk through multiple mechanisms including atherosclerosis, thrombosis and inflammation [4,5]. Markers of systemic inflammation have also been associated with ASCVD, including hs-CRP [6–9] and tHcy [10–14]. Elevated tHcy is associated with vascular inflammation and oxidative stress secondary to reactive oxygen species accumulation [15]. It is possible that these inflammatory pathways have synergistic effects and measurements could provide additional information regarding ASCVD risk. Additionally, tHcy and Lp(a) are both associated with increased coagulation and research suggests a possible synergistic effect [16–18].

A previous study in the Multi-Ethnic Study of Atherosclerosis (MESA) found that Lp(a) was only associated with ASCVD risk in individuals with elevated hs-CRP levels [19] and a recent analysis observed that combinations of risk enhancers appeared to better identify intermediate risk individuals who would benefit from treatment [20]. To our knowledge no prior studies have evaluated tHcy in combination with hs-CRP and Lp(a) for ASCVD risk. The present study investigated relationships between Lp(a), hs-CRP, and tHcy with CHD and stroke risk among participants in the MESA prospective cohort. We hypothesized that elevated Lp(a), hs-CRP and tHcy would have additive associations with incident CHD and stroke.

2. Materials and methods

2.1. Study population

The Multi-Ethnic Study of Atherosclerosis (MESA) recruited 6814 men and women aged 45 to 84 years and free of clinically apparent cardiovascular disease, from four ethnic/racial groups (White, Black, Hispanic, Chinese) at six centers in the United States (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; St. Paul, MN) between July 2000 and August 2002 [21]. The present study utilizes baseline exposure and covariate data and outcome follow-up through 2017. Participants without baseline hs-CRP, Lp(a) or

tHcy measurements ($N = 118$), and without outcome data for CHD ($N = 22$) and stroke ($N = 24$) were excluded. Additional participants with Missing covariate data were excluded ($N = 107$). The analytic sample was 6567 for CHD and 6565 for stroke, with a median follow-up time of 15.8 years.

The study was approved by the institutional review boards for the MESA field centers and all participants provided informed consent.

2.2. Data collection

2.2.1. Biomarker measurements

Lp(a), hs-CRP and tHcy were measured using 12-hour fasting blood samples collected at the baseline study visit and stored at -70 °C using standardized procedures [21]. High-sensitivity c-reactive protein was measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring, Inc., Deerfield, IL). Plasma tHcy was measured using high performance liquid chromatography with fluorometric detection. Lp(a) mass concentrations were measured using a latex-enhanced turbidimetric immunoassay that controls for the heterogeneous sizes of apolipoprotein(a) (Health Diagnostics Laboratory, Richmond, VA; Denka Seiken, Tokyo, Japan). Coefficients of variation for measured biomarkers were <5 % (Lp(a)), 3.6 % (hs-CRP), and 3.8 % (tHcy).

2.2.2. Covariates

At the baseline study exam, demographics, personal and medical history and lifestyle behaviors were obtained by interview and questionnaire. Biospecimen samples (12-hour fasting blood and urine) were collected and anthropometrics and blood pressure were measured by study staff. Procedures are outlined in detail elsewhere [21,22]. Hypertension was defined as blood pressure medication usage, diastolic blood pressure ≥90 mmHg or systolic blood pressure ≥140 mmHg and diabetes as taking diabetes medication or a fasting glucose >125 mg/dL.

2.2.3. Primary outcome ascertainment

Incident CHD and stroke events through 2017 were primarily self-reported at study visits or follow-up calls occurring every 9–12 months and verified by medical records, reviewed by trained personnel to confirm events. Records were reviewed by cardiologists or cardiovascular physician epidemiologists for non-neurovascular events, and neurologists for all neurovascular events. Incident CHD was defined as the first occurrence of any of the following: myocardial infarction, resuscitated cardiac arrest, CHD death, or definite angina. Definite angina was defined as symptoms of typical chest pain and physician diagnosis of angina followed by coronary artery bypass graft and percutaneous transluminal coronary angioplasty, evidence of ischemia by stress tests or resting ECG, or ≥70 % obstruction on coronary angiography. Probable angina cases were included if followed by revascularization. Incident stroke was defined as new fatal or non-fatal strokes

due to ischemic, hemorrhage, or transient ischemic attack.

2.3. Statistical analysis

Preliminary associations between Lp(a), hs-CRP, tHcy, outcomes of interest and population characteristics were evaluated using Spearman correlation, ANOVA or Wald X^2. Lp(a), hs-CRP and tHcy were evaluated categorically using standard cut-points [Lp(a): low <50 mg/dL, high ≥50 mg/dL; hs-CRP: low <2mg/L, high ≥2 mg/L; tHcy: low <12 μmol/ L, high ≥12 μmol/L) [3,12,23]. Lp(a) was additionally evaluated using the alternatively cut-point 30 mg/dL [Lp(a): low <30 mg/dL, high ≥30 mg/dL].

To evaluate joint effects, all possible combinations of two bio-markers, in addition to all three combined, were evaluated in relation to CHD and stroke incidence. In evaluating two biomarkers together, participants were stratified by high/low status and comparisons were made to individuals with low levels of both biomarkers. Categories for evaluating the combinations of all three biomarkers, again stratified by high/low status were compared to individuals with low hs-CRP, low Lp (a), and low tHcy.

Time to event for CHD and stroke was modeled for hs-CRP, Lp(a) and tHcy separately and by categories of the combined biomarkers of interest using Cox proportional hazards regression. Models were conducted (1) unadjusted; (2) age, sex, race/ethnicity; and (3) age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, high-density lipoprotein-cholesterol, total cholesterol (HDL-C), triglycerides (log-transformed), body mass index (BMI) and estimated glomerular filtration rate (eGRF). The following variables were also evaluated as covariates, but not included in the final presented models: waist circumference, low-density lipoprotein cholesterol, non-HDL-C, education, income, alcohol intake, Healthy Eating Index diet score, physical activity, aspirin use, and lipid-lowering medication use. For multivariable-adjusted models, *a priori* chosen factors there were associated with exposure and/or outcome or that changed the association between Lp(a), hs-CRP, tHcy and the outcome(s) were included in the final version of the fully adjusted model. Tests for interaction were conducted using cross-product terms (2-way for two biomarker combinations, 3-way for combination of all three). Multivariable-adjusted survival curves using the same covariates outlined previously were computed for the associations between biomarker combinations and incidence of CHD and stroke. Harrell's Concordance Statistics (c-statistics) were calculated in adjusted models with and without hs-CRP, Lp(a) and tHcy. Sensitivity analyses were conducted using ischemic stroke only. Findings were similar to those using all stroke types, so results are presented using all stroke types. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc) and statistical significance was defined as a two-tailed P value <0.05.

3. Results

There were 669 incident cases of CHD and 294 incident cases of stroke. Baseline population characteristics among individuals with 0/3, 1/3, 2/3 and 3/3 biomarkers above thresholds are presented in Table 1. Among the 6589 participants, 48.0 % (N = 3161) had elevated h-sCRP, 20.1 % (N = 1318) had elevated Lp(a), and 13.3 % (N = 874) had elevated tHcy.

Associations of individual biomarkers with CHD and stroke are presented in Table 2. Lp(a) was associated with CHD risk while tHcy was positively associated with CHD when evaluated continuously, but not categorically. Hs-CRP was not independently associated with CHD in fully adjusted models. tHcy and hs-CRP were both positively associated with stroke incidence, while Lp(a) was not associated with stroke risk. Results were similar when Lp(a) cut-point of 30 mg/dL was used (**Supplementary Table 1**).

Two-way combinations of hs-CRP, Lp(a) and tHcy and associations with CHD and stroke are presented in Table 3. Survival curves for the

Table 1
Baseline characteristics of the Multi-Ethnic Study of Atherosclerosis study population by number of biomarkers (high sensitivity c-reactive protein, lipoprotein (a) and homocysteine) above thresholds (*N* = 6676).

	0/3 Elevated	1/3 Elevated	2/3 Elevated	3/3 Elevated	P-value[c]
Overall[a]	2406 (36.5)	3270 (49.6)	809 (12.3)	107 (1.6)	—
Age[b]	60.0 (52.0, 69.0)	61.0 (53.0, 69.0)	68.0 (59.0, 76.0)	69.0 (64.0, 75.0)	<0.0001
Sex (female)[a]	1084 (45.1)	2072 (63.4)	276 (34.1)	50 (46.7)	<0.0001
Race/ Ethnicity[a]					<0.0001
White	1020 (42.5)	1118 (36.3)	311 (38.4)	22 (20.6)	
Black	415 (17.3)	1096 (33.5)	229 (28.3)	63 (58.9)	
Chinese	485 (20.2)	201 (6.2)	101 (12.5)	1 (0.9)	
Hispanic	483 (20.1)	785 (24.1)	168 (20.8)	21 (19.6)	
Hypertension[a]	838 (34.9)	1548 (47.3)	481 (59.5)	79 (74.8)	<0.0001
HTN medication[a]	697 (28.6)	1263 (38.6)	418 (51.7)	74 (69.2)	<0.0001
Diabetes[a]	215 (9.0)	448 (13.7)	137 (16.9)	24 (22.04)	<0.0001
Smoking (pack-years)[b]	0.0 (0.0, 11.0)	0.0 (0.0, 16.8)	0.75 (0.0, 21.8)	0.50 (0.0, 25.0)	<0.0001
BMI (kg/m^2)[b]	25.9 (23.3, 28.8)	29.0 (25.7, 33.0)	27.4 (24.6, 30.8)	29.7 (26.4, 32.7)	<0.0001
hs-CRP (mg/ L)[fb]	0.85 (0.50, 1.31)	3.78 (2.33, 6.77)	1.64 (0.74, 3.51)	4.43 (3.02, 8.94)	<0.0001
Lp(a) (mg/dL)[b]	11.6 (5.9, 22.5)	26.6 (9.7, 62.6)	13.4 (6.4, 28.8)	80.9 (66.7, 115.6)	<0.0001
tHcy (μmol/L)[b]	8.3 (7.1, 9.7)	8.3 (7.0, 9.6)	13.7 (12.6, 15.8)	14.4 (13.2, 17.5)	<0.0001
HDL-C (mg/ dL)[b]	49.0 (41.0, 59.0)	49.0 (41.0, 59.0)	46.0 (39.0, 57.0)	46.0 (39.0, 57.0)	0.0008
Total Cholesterol (mg/dL)[b]	189.0 (169.0, 211.0)	196.0 (174.0, 219.0)	186.0 (163.0, 210.0)	197.0 (173.0, 220.0)	<0.0001
Triglycerides (mg/dL)[b]	105.0 (74.0, 153.0)	114.0 (81.0, 165.0)	112.0 (77.0, 166.0)	112.0 (84.0, 171.0)	0.01
eGFR[b]	81.0 (71.9, 92.4)	80.7 (70.7, 92.8)	71.0 (59.3, 84.1)	64.6 (50.0, 75.4)	<0.0001

Abbreviations: Body mass index, BMI; high sensitivity c-reactive protein, hs-CRP; estimated glomerular filtration rate, eGFR; high-density lipoprotein cholesterol, HDL-C; homocysteine, tHcy; interquartile range, IQR; lipoprotein (a), Lp(a).
[a] n (%)
[b] Median (IQR)
[c] Wald X^2 test for categorical variables; One-way ANOVA for continuous variables.

associations are presented in Figs. 1 and 2, respectively. In general, individuals with only one elevated biomarker did not have an increased risk of CHD compared with the reference group where both biomarkers were low. However, CHD incidence was higher when two biomarkers were elevated [high hs-CRP + high Lp(a): hazard ratio (HR)=1.39, 95 % confidence interval (CI): 1.06, 1.82; high hs-CRP + tHcy: HR = 1.34, 95 % CI: 1.02, 1.76; high tHcy + Lp(a):HR = 1.58, 95 % CI: 1.08, 2.30]. Tests for interaction for the combinations of two biomarkers were not statistically significant (P>0.27). Similarly, higher stroke incidence was only observed among individuals with combined high hs-CRP + Lp(a)

Table 2

Individual associations between high sensitivity c-reactive protein (<2 mg/L, ≥2 mg/L), lipoprotein(a) (<50 mg/dL, ≥50 mg/dL), homocysteine (<12 μmol/L, ≥12 μmol/L) with coronary heart disease and stroke (though 2017) in MESA (*N* = 6676).

	Coronary Heart Disease			Stroke		
	N Cases/ N Total	Minimally Adjusted[a] HR (95 % CI)	Fully Adjusted[b] HR (95 % CI)	N Cases/ N Total	Minimally Adjusted[a] HR (95 % CI)	Fully Adjusted[b] HR (95 % CI)
hs-C-reactive protein						
hs-CRP <2 mg/L	343/3417	Reference	Reference	133/3417	Reference	Reference
hs-CRP ≥2 mg/L	316/3150	1.22 (1.05, 1.43) *P* = 0.01	1.05 (0.89, 1.24) *P* = 0.53	161/3148	1.34 (1.06, 1.69) *P* = 0.02	1.30 (1.01, 1.67) *P* = 0.04
Loghs-CRP (per unit)	669/6567	1.14 (1.07, 1.23) *P* < 0.0001	1.08 (1.00, 1.16) *P* = 0.05	294/6565	1.16 (1.05, 1.29) *P* = 0.004	1.15 (1.03, 1.29) *P* = 0.01
Lipoprotein(a)						
Lp(a) <50 mg/dL	517/5258	Reference	Reference	232/5257	Reference	Reference
Lp(a) ≥50 mg/dL	152/1309	1.25 (1.04, 1.51) *P* = 0.02	1.25 (1.04, 1.52) *P* = 0.02	62/1308	1.07 (0.80, 1.42) *P* = 0.66	1.02 (0.76, 1.37) *P* = 0.88
LogLp(a) (per unit)	669/6567	1.06 (0.99, 1.14) *P* = 0.09	1.08 (1.01, 1.17) *P* = 0.03	294/6565	1.02 (0.92, 1.14) *P* = 0.70	1.01 (0.91, 1.13) *P* = 0.81
Homocysteine						
tHcy <12 μmol/L	542/5695	Reference	Reference	229/5693	Reference	Reference
tHcy ≥12 μmol/L	127/872	1.25 (1.03, 1.51) *P* = 0.03	1.20 (0.98, 1.47) *P* = 0.08	65/872	1.44 (1.09, 1.91) *P* = 0.01	1.50 (1.12, 2.01) *P* = 0.006
LogtHcy (per unit)	669/6567	1.46 (1.13, 1.89) *P* = 0.004	1.36 (1.02, 1.80) *P* = 0.03	294/6565	1.51 (1.03, 2.12) *P* = 0.03	1.66 (1.11, 2.50) *P* = 0.01

Abbreviations: high sensitivity C-reactive protein, hs-CRP; confidence interval, CI; coronary heart disease, CHD; estimated glomerular filtration rate, eGFR; hazard ratio, HR; high-density lipoprotein cholesterol, HDL-C; homocysteine, tHcy; interquartile range, IQR; lipoprotein(a), Lp(a).

[a] Cox proportional hazards regression adjusted for age, sex, race/ethnicity adjusted.

[b] Cox proportional hazards regression adjusted for age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, HDL-C, total cholesterol, triglycerides (log-transformed), BMI and eGFR.

Table 3

Associations of two-way combinations of high sensitivity c-reactive protein, lipoprotein(a) and homocysteine with coronary heart disease and stroke (through 2017) in MESA (N=6,676)

	Coronary Heart Disease			Stroke		
	N Cases/N Total	Minimally Adjusted[a] HR (95% CI)	Fully Adjusted[b] HR (95% CI)	N Cases/N Total	Minimally Adjusted[a] HR (95% CI)	Fully Adjusted[b] HR (95% CI)
hs-CRP and Lp(a)[c]						
Low hs-CRP and Lp(a)	270/2773	Reference	Reference	111/2773	Reference	Reference
High hs-CRP only	247/2485	1.21 (1.01, 1.44) P=0.04	1.03 (0.85, 1.23) P=0.79	121/2484	1.22 (0.94, 1.58) P=0.14	1.17 (0.89, 1.54) P=0.27
High Lp(a) only	73/644	1.21 (0.93, 1.58) P=0.15	1.17 (0.90, 1.52) P=0.25	22/644	0.83 (0.53, 1.30) P=0.43	0.76 (0.48, 1.21) P=0.24
High hs-CRP, Lp(a)	79/665	1.58 (1.22, 2.06) P=0.0006 P-Interaction=0.67	1.39 (1.06, 1.82) P=0.02 P-Interaction=0.43	40/2484	1.59 (1.09, 2.32) P=0.02 P-Interaction=0.12	1.51 (1.02, 2.23) P=0.04 P-Interaction=0.07
hs-CRP and tHcy[c]						
Low hs-CRP and tHcy	282/2956	Reference	Reference	109/2956	Reference	Reference
High hs-CRP only	254/2697	1.17 (0.98, 1.39) P=0.08	1.01 (0.84, 1.21) P=0.96	118/2695	1.20 (0.92, 1.56) P=0.18	1.16 (0.88, 1.53) P=0.30
High tHcy only	61/461	1.12 (0.85, 1.48) P=0.42	1.07 (0.81, 1.43) P=0.62	24/461	1.09 (0.70, 1.70) P=0.70	1.14 (0.73, 1.80) P=0.56
High hs-CRP, HCY	72/453	1.58 (1.22, 2.05) P=0.0006 P-Interaction=0.33	1.34 (1.02, 1.76) P=0.03 P-Interaction=0.27	43/453	2.07 (1.44, 2.96) P<0.0001 P-Interaction=0.11	2.10 (1.44, 3.06) P=0.0001 P-Interaction=0.11
tHcy and Lp(a)[c]						
Low tHcy and Lp(a)	416/4534	Reference	Reference	181/4533	Reference	Reference
High tHcy only	101/724	1.23 (0.99, 1.53) P=0.07	1.18 (0.94, 1.48) P=0.15	51/724	1.40 (1.02, 1.92) P=0.04	1.45 (1.05, 2.00) P=0.03
High Lp(a) only	120/1119	1.24 (1.00, 1.51) P=0.05	1.24 (1.00, 1.53) P=0.05	46/1118	1.03 (0.74, 1.42) P=0.87	0.98 (0.70, 1.37) P=0.91
High tHcy, Lp(a)	32/190	1.62 (1.22, 2.33) P=0.01 P-Interaction=0.78	1.58 (1.08, 2.30) P=0.02 P-Interaction=0.73	16/190	1.67 (0.99, 2.80) P=0.05 P-Interaction=0.65	1.69 (0.99, 2.88) P=0.05 P-Interaction=0.60

Abbreviations: high sensitivity C-reactive protein, hs-CRP; confidence interval, CI; coronary heart disease, CHD; estimated glomerular filtration rate, eGFR; hazard ratio, HR; high-density lipoprotein cholesterol, HDL-C; homocysteine, tHcy; interquartile range, IQR; lipoprotein(a), Lp(a).

[a] Cox proportional hazards regression adjusted for age, sex, race/ethnicity adjusted.

[b] Cox proportional hazards regression adjusted for age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, HDL-C, total cholesterol, triglycerides (log-transformed), BMI and eGFR.

[c] Low hs-CRP <2 mg/L; High hs-CRP ≥2 mg/L; Low Lp(a) <50 mg/dL; High Lp(a) ≥50 mg/dL; Low tHcy <12 μmol/L; High tHcy=≥12 μmol/L.
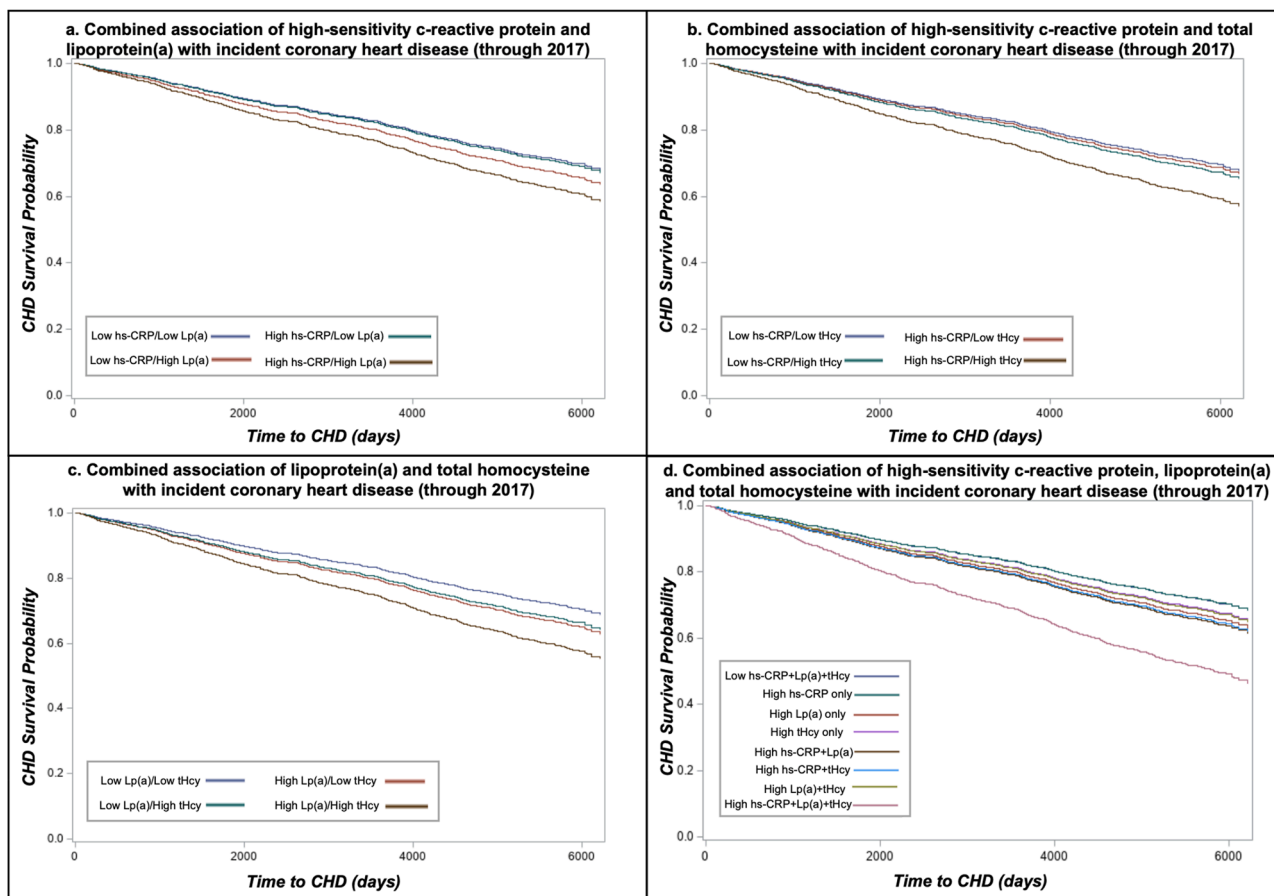
Fig. 1. Combined associations of high-sensitivity c-reactive protein, lipoprotein(a) and total homocysteine with incident coronary heart disease in the Multi-Ethnic Study of Atherosclerosis.

Abbreviations: high-sensitivity c-reactive protein, hs-CRP; coronary heart disease (CHD) total plasma homocysteine, tHcy; lipoprotein(a), LPA. Cox proportional hazards regression survival curves adjusted for age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, HDL-C, total cholesterol, triglycerides (log-transformed), BMI and eGFR. Low hs-CRP <2 mg/dL; High hs-CRP ≥2 mg/dL; Low Lp(a) <50 mg/dL; High Lp(a) ≥50 mg/dL; Low tHcy <12 μmol/L; High tHcy=≥12 μmol/L.

(HR = 1.51, 95 % CI: 1.02, 2.23, P-interaction=0.07) or combined high hs-CRP + tHcy (HR = 2.10, 95 % CI: 1.44, 3.06, P-interaction=0.11) when compared to individuals with low levels for both biomarkers. High tHcy + Lp(a) were borderline significantly associated with stroke incidence (HR = 1.69, 95 % CI: 0.99, 2.88, P-interaction=0.60), but the sample size in this group was notably smaller than the other groups (n=193). However, individuals with high tHcy only [and low Lp(a)] had increased stroke risk (HR = 1.45, 95 % CI: 1.05, 2.00). Findings were similar when using 30 mg/dL Lp(a) cut-point (**Supplementary Table 2**).

Results for combinations of all three biomarkers in relation to incidence of CHD and stroke are presented in Table 4 with survival curves in Figs. 1d and Fig. 2d. When evaluated as the number of biomarkers over the corresponding clinical thresholds, incidence of both CHD (HR = 1.99, 95 % CI: 1.24, 3.20) and stroke (HR = 2.95, 95 % CI: 1.58, 5.49) were highest when all three biomarkers were above clinical cut-points and hazard ratios increased and associations strengthened which each additional elevated. There was a significant increased risk of CHD only in individuals with combined high Lp(a), hs-CRP and tHcy (HR = 2.02, 95 % CI: 1.26, 3.24, P-interaction=0.58). Similarly, individuals classified as high for all three biomarkers were at an increased risk for stroke (HR = 2.99, 95 % CI: 1.61, 5.58, P-interaction=0.34). However, individuals with both high hs-CRP and tHcy but low Lp(a) also had a statistically significantly higher incidence of stroke relative to individuals with the combination of low hs-CRP, tHcy and Lp(a) (HR = 1.79, 95 % CI: 1.16, 2.76). Results using Lp(a) cut-point of 30 mg/dL are

presented on **Supplementary Table 3** and are similar to findings using 50 mg/dL). C-statistics for models with and without hs-CRP, Lp(a) and tHcy were similar [CHD models c-statistic range: 0.7274 (no biomarkers) to 0.7294 (all three biomarkers); stroke models c-statistic range: 0.7329 (no biomarkers and Lp(a) only) to 0.7374 (all three biomarkers) **Supplementary Table 4**.

4. Discussion

The results of this study suggest it may be beneficial to consider biomarker combinations and to include tHCY measurements in assessing ASCVD risk. When considering combinations of two biomarkers, significantly higher CHD risk was only seen when both biomarkers were concomitantly elevated, and not with elevation of single biomarkers. Similarly, elevated stroke risk was only seen with elevated hs-CRP in combination with elevated Lp(a) or tHcy; only tHcy alone conferred significant stroke risk individually. In three-way analyses, CHD risk was elevated only when all three biomarkers were at or above clinical cut-points; stroke incidence was increased only when all three biomarkers were elevated, or when both hs-CRP and tHcy were elevated. Additionally, both CHD and stroke risk appeared highest when all three biomarkers were above clinical thresholds, and associations increased with each additional biomarker over the corresponding threshold.

Lp(a) [5,24–27], hs-CRP [6–9] and tHcy [10–14] have all been shown to be associated with ASCVD risk. Two prior studies, one involving primary and the other secondary prevention of ASCVD have
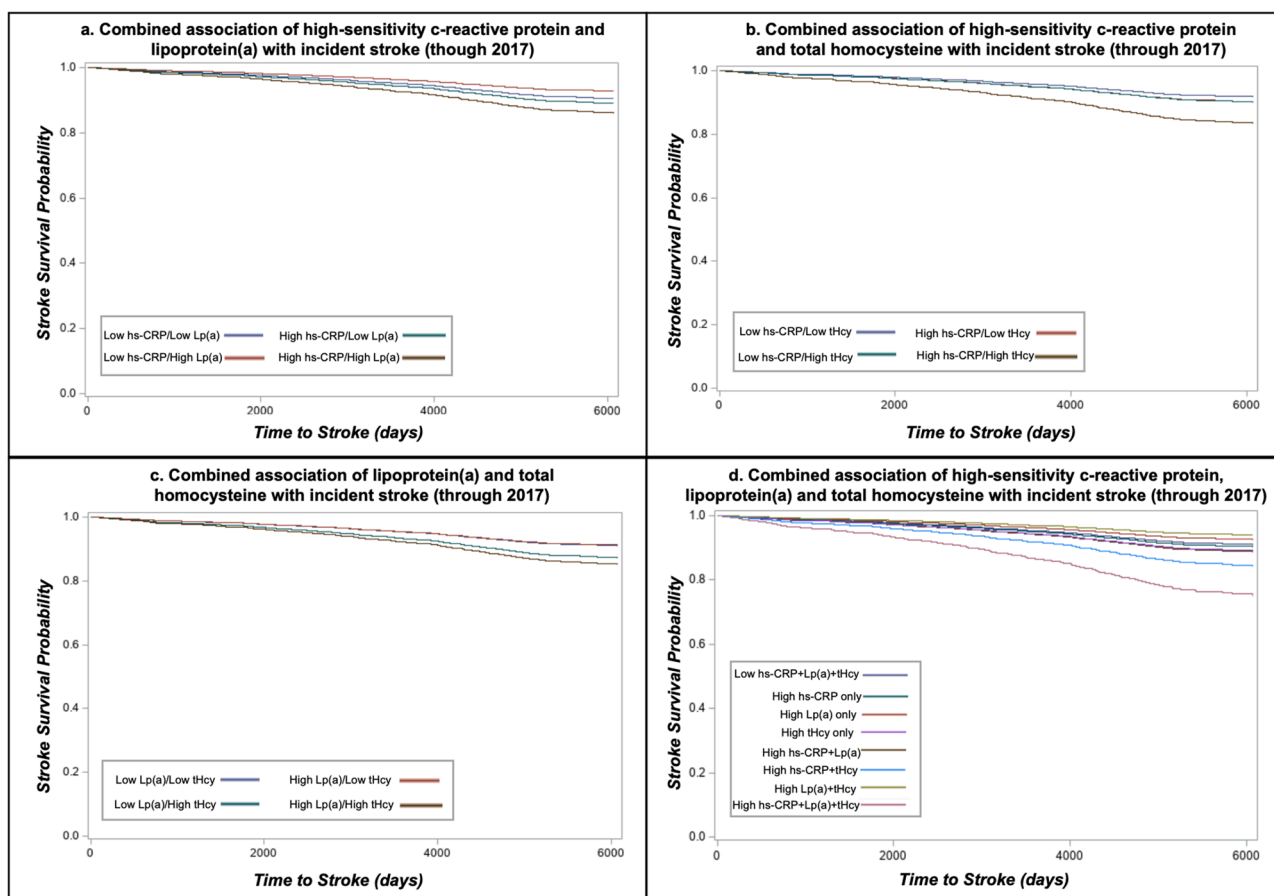
Fig. 2. Combined associations of high-sensitivity c-reactive protein, lipoprotein(a) and total homocysteine with incident stroke in the Multi-Ethnic Study of Atherosclerosis.
Abbreviations: high-sensitivity c-reactive protein, hs-CRP; total plasma homocysteine, tHcy; lipoprotein(a), LPA. Cox proportional hazards regression survival curves adjusted for age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, HDL-C, total cholesterol, triglycerides (log-transformed), BMI and eGFR. Low hs-CRP <2 mg/dL; High hs-CRP $\geq$2 mg/dL; Low Lp(a) <50 mg/dL; High Lp(a) $\geq$50 mg/dL; Low tHcy <12 μmol/L; High tHcy=$\geq$12 μmol/L.

evaluated Lp(a) and hs-CRP in combination. A secondary analysis in the prevention trial ACCELERATE demonstrated that hs-CRP may modulate Lp(a)-associated CVD risk [12]. More recently, a study of MESA participants not on statins demonstrated Lp(a) was associated with greater risk of ASCVD only when hs-CRP was >2 mg/L [19]. While Lp(a) was individually associated with CHD and hs-CRP and tHcy were individual associated with stroke when evaluated separately, these biomarkers were not individually associated with CHD when considering multiple risk factors. This may be due to smaller numbers in those categories, or the potential residual effect of the other coinciding risk factors. Our study aligns with and expands on these results by demonstrating that when considering combinations of biomarkers, the CHD risk associated with Lp(a) was only present when an individual also had elevated hs-CRP and/or tHcy. Similarly, the association between stroke risk and hs-CRP was only present when Lp(a) and/or tHcy was concomitantly elevated. In contrast, tHcy was associated with stroke risk without elevation in Lp(a), but when considering hs-CRP measurements was only associated with increased risk when hs-CRP was also elevated. The combination of elevated tHcy and Lp(a) was only of borderline significance in its association with stroke which may be due to the limited numbers of cases and participants with this biomarker combination. However, Lp(a) was not independently associated with stroke risk in this study, and these results suggest a more complicated relationship, possibly owing to the more heterogeneous nature of stroke. Using the lower clinical threshold of Lp(a) <30 mg/dL did not change study findings overall and tended to weaken associations, which may be due to

the inconsistent associations observed with ASCVD risk when using a threshold of 30 mg/dL in MESA [28,29]. While risk was highest when all three biomarkers were elevated, benefits of additional measurement for risk prediction remain unclear. C-statistics suggested limited additional predictive value in study models with the additional of hs-CRP, Lp(a) and tHcy, however, future studies are needed to further explore the potential benefits of adding these biomarkers to study models as study sample size likely limited out ability to detect incremental, but still meaningful improvements in risk stratification [30,31].

In our study, the finding that elevated tHcy significantly enhances risk for CHD or stroke when combined with either hs-CRP and/or Lp(a) represents a novel finding. Numerous studies have demonstrated that systemic inflammation plays an important role as a CVD risk factor [32]. While hs-CRP is the most validated biomarker of inflammation, Lp(a) and tHcy are also known to play important roles in inflammation [12, 33]. The current study demonstrated that in addition to hs-CRP, tHcy also plays a role in modulating the Lp(a)-associated ASCVD risk. Additionally, research suggests Lp(a) and tHcy are both associated with increased coagulation and appear to have a synergistic effect [16–18].

Homocysteine is not currently recognized as a risk-enhancing factor by the AHA/ACC guidelines. Although homocysteine has been shown to be a risk factor for ASCVD since the mid-1990s, interest in this biomarker as a CHD risk factor has diminished in the ensuing decades due in part to the failure of homocysteine-lowering intervention trials [17]. However, many of these trials had short treatment and follow up periods ($\leq$ 2 years), and/or enlisted participants with high CHD risk, and

Table 4

Combined associations of high sensitivity c-reactive protein (<2 mg/dL, ≥2 mg/dL), lipoprotein(a) (<50 mg/dL, ≥50 mg/dL) and homocysteine (<12 μmol/L, ≥12 μmol/L) with coronary heart disease and stroke (though 2017) in MESA (N = 6676).

	Coronary Heart Disease			Stroke		
	N Cases/ N Total	Minimally Adjusted[a] HR (95 % CI)	Fully Adjusted[b, d] HR (95 % CI)	N Cases/ N Total	Minimally Adjusted[a] HR (95 % CI)	Fully Adjusted[b, d] HR (95 % CI)
Overall[c]						
0:3 Elevated	220/2396	Reference	Reference	90/2396	Reference	Reference
1:3 Elevated	316/3257	1.23 (1.03, 1.48) $P = 0.02$	1.07 (0.89, 1.29) $P = 0.44$	137/3255	1.12 (0.85, 1.46) $P = 0.43$	1.04 (0.79, 1.38) $P = 0.77$
2:3 Elevated	112/808	1.30 (1.03, 1.64) $P = 0.03$	1.18 (0.93, 1.49) $P = 0.18$	54/808	1.38 (0.98, 1.94) $P = 0.07$	1.40 (0.98, 1.98) $P = 0.06$
3:3 Elevated	21/106	2.38 (1.51, 3.76) $P = 0.0002$	1.99 (1.24, 3.20) $P = 0.005$	13/106	2.98 (1.64, 5.39) $P = 0.0003$	2.95 (1.58, 5.49) $P = 0.007$
Individual Combinations[c]						
Low hs-CRP, Lp(a), tHCY	220/2369	Reference	Reference	90/2396	Reference	Reference
High hs-CRP only	196/2138	1.18 (0.97, 1.44) $P = 0.09$	1.00 (0.81, 1.23) $P = 0.98$	91/2137	1.13 (0.84, 1.52) $P = 0.42$	1.08 (0.79, 1.47) $P = 0.62$
High Lp(a) only	62/560	1.27 (0.95, 1.68) $P = 0.11$	1.21 (0.90, 1.61) $P = 0.21$	19/560	0.90 (0.56, 1.46) $P = 0.67$	0.82 (0.49, 1.35) $P = 0.43$
High tHcy only	50/377	1.18 (0.87, 1.61) $P = 0.29$	1.12 (0.82, 1.53) $P = 0.48$	21/377	1.18 (0.73, 1.90) $P = 0.50$	1.23 (0.76, 2.00) $P = 0.40$
High hs-CRP, Lp(a); Low tHcy	58/559	1.45 (1.08, 1.96) $P = 0.01$	1.28 (0.94, 1.73) $P = 0.12$	27/558	1.34 (0.86, 2.08) $P = 0.19$	1.26 (0.80, 1.99) $P = 0.31$
High Lp(a), tHcy; Low hs-CRP	11/84	1.16 (0.63, 2.14) $P = 0.62$	1.13 (0.62, 2.09) $P = 0.69$	3/84	0.65 (0.20, 2.05) $P = 0.46$	0.65 (0.20, 2.06) $P = 0.46$
High hs-CRP, tHcy; Low Lp(a)	51/347	1.49 (1.10, 2.02) $P = 0.01$	1.25 (0.91, 1.72) $P = 0.16$	30/347	1.78 (1.17, 2.70) $P = 0.007$	1.79 (1.16, 2.76) $P = 0.008$
High hs-CRP, Lp(a), tHcy	21/106	2.40 (1.52, 3.80) $P < 0.0001$	2.02 (1.26, 3.24) $P = 0.004$	13/106	2.99 (1.65, 5.41) $P = 0.0003$	2.99 (1.61, 5.58) $P = 0.0006$

Abbreviations: high sensitivity C-reactive protein, hs-CRP; confidence interval, CI; coronary heart disease, CHD; estimated glomerular filtration rate, eGFR; hazard ratio, HR; high-density lipoprotein cholesterol, HDL-C; homocysteine, tHcy; interquartile range, IQR; lipoprotein(a), Lp(a).

[a] Cox proportional hazards regression adjusted for age, sex, race/ethnicity adjusted.

[b] Cox proportional hazards regression adjusted for age, sex, race/ethnicity, hypertension, hypertension medication usage, diabetes, pack-years smoking, HDL-C, total cholesterol, triglycerides (log-transformed), BMI and eGFR.

[c] Low hs-CR$P < 2$ mg/L; High hs-CRP ≥2 mg/L; Low Lp(a) <50 mg/dL; High Lp(a) ≥50 mg/dL; Low tHcy <12 μmol/L; High tHcy=≥12 μmol/L.

[d] Interaction p-values from fully-adjusted Cox proportional hazards regression cross-product term: CHD hs-CRP category*Lp(a) category*tHcy category $P = 0.58$; stroke hs-CRP category*Lp(a) category*tHcy category $P = 0.34$.

therefore the impact of long-term homocysteine-lowering on CHD risk in low to intermediate risk individuals has not been determined. In support of tHcy risk-enhancing role, a recent publication from our group demonstrated that elevated tHcy was associated with increased risk of prevalent and incident coronary artery and descending thoracic aorta calcification and calcification progression in the MESA cohort [12].

Several recent publications have demonstrated the important role played by tHcy as a risk factor for stroke [34–37]. Research suggests lowering tHcy concentrations may have a greater impact on reducing stroke incidence, compared with myocardial infarction [38,39]. The MTHFR C677T genotype, which is associated with higher tHcy levels is associated with small vessel disease, which is more strongly associated with stroke than CHD and hyperhomocysteinemia has been shown to be more strongly associated with risk of stroke compared to CHD [38,39]. In view of the decreased focus on tHcy in recent years as a risk factor/risk enhancer, more studies are needed to determine whether measurement of tHcy, either individually or in combination with hs-CRP and Lp(a), may offer value in assessing CHD and stroke risk in the routine care and risk assessment of patients for primary prevention of CHD and stroke.

Our study has implications for future clinical care as well as avenues for future investigation. In addition to their usefulness for assessing overall risk to CHD and stroke, all three biomarkers may be potential therapeutic targets and pathways to address residual ASCVD risk. Plasma total homocysteine levels can be lowered with B vitamins including folic acid [40,41] and has been shown to lower risk of stroke [36,42,43]. Systemic inflammation, reflected in hs-CRP levels, can be effectively lowered with lifestyle interventions [44] and medications, including statins [45] and other lipid lowering therapy, such as bempedoic acid therapy [46]. Drugs that lower inflammation such as

canakinumab and colchicine have been demonstrated to improve cardiovascular outcomes in patients with established ASCVD [47,48]. While effective therapy for lowering Lp(a) is currently not available, anti-sense oligonucleotide therapy has shown promise in lowering Lp(a) [49,50]. Pelacarsen was shown to lower Lp(a) by more than 80 % in the phase II HORIZON trial with Phase III currently ongoing [51]. Additionally, all three biomarkers are currently widely available as FDA-approved assays on high-throughput testing platforms in clinical laboratories at a relatively low cost (<\$20 each), and thus there would be minimal logistical or practical barriers to incorporating these biomarkers into routine CVD risk assessment.

While this study has important strengths, including the availability of all three biomarkers for evaluating combined associations and rigorous data collection and follow-up procedures, it also has some important limitations. While MESA is a relatively large study, there were small groups of participants in certain categories of combined biomarkers, particularly in the stroke analyses which has a smaller number of events than CHD, which may have limited our ability to detect a true association. It may also have limited our ability to detect statistically significant interactions which require greater statistical power. An additional limitation is that biomarkers were only measured at baseline and repeated measures were not available. While tHcy and Lp(a) have been demonstrated to be relatively stable in prior studies, hs-CRP can vary substantially across repeated measures which could not be accounted for in the present study [52–54].

5. Conclusions

In this study, CHD and stroke risk were highest only when Lp(a), hs-CRP and tHCY were all elevated, suggesting the additional measurement

of tHCY, and consideration of combinations of multiple ASCVD risk biomarkers, may help refine identification of individuals are highest risk for CHD and stroke. Future research should consider whether adding biomarker combinations to risk prediction assessments may be beneficial for improving stratifying CHD and stroke risk.

CRediT authorship contribution statement

Sarah O. Nomura: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Harpreet S. Bhatia:** Writing – original draft, Writing – review & editing. **Parveen K. Garg:** Writing – original draft, Writing – review & editing. **Amy B. Karger:** Writing – original draft, Writing – review & editing. **Weihua Guan:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Jing Cao:** Writing – original draft, Writing – review & editing. **Michael D. Shapiro:** Methodology, Writing – original draft, Writing – review & editing. **Michael Y. Tsai:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajpc.2024.100903.

References

[1] Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, et al. Heart disease and stroke statistics-2019 update: a report from the american heart association. Circulation. 2019;139(10):e56–528.

[2] Ridker PM. How common is residual inflammatory risk? Circ Res 2017;120(4): 617–9.

[3] Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: executive summary: a report of the american college of cardiology/american heart association task force on clinical practice guidelines. J Am Coll Cardiol 2019;74 (10):1376–414.

[4] Kronenberg F, Mora S, Stroes ESG, Ference BA, Arsenault BJ, Berglund L, et al. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. Eur Heart J 2022;43(39): 3925–46.

[5] Tsimikas S, Hall JL. Lipoprotein(a) as a potential causal genetic risk factor of cardiovascular disease: a rationale for increased efforts to understand its pathophysiology and develop targeted therapies. J Am Coll Cardiol 2012;60(8): 716–21.

[6] Cao JJ, Arnold AM, Manolio TA, Polak JF, Psaty BM, Hirsch CH, et al. Association of carotid artery intima-media thickness, plaques, and C-reactive protein with future cardiovascular disease and all-cause mortality: the Cardiovascular Health Study. Circulation 2007;116(1):32–8.

[7] Puri R, Nissen SE, Arsenault BJ, St John J, Riesmeyer JS, Ruotolo G, et al. Effect of c-reactive protein on lipoprotein(a)-associated cardiovascular risk in optimally treated patients with high-risk vascular disease: a prespecified secondary analysis of the accelerate trial. Jama Cardiol 2020;5(10):1136–43.

[8] Ridker PM. From C-reactive protein to interleukin-6 to interleukin-1: moving upstream to identify novel targets for atheroprotection. Circ Res 2016;118(1): 145–56.

[9] Wong ND, Budoff MJ, Ferdinand K, Graham IM, Michos ED, Reddy T, et al. Atherosclerotic cardiovascular disease risk assessment: an american society for preventive Cardiology clinical practice statement. Am J Prev Cardiol 2022;10: 100335.

[10] Bostom AG, Rosenberg IH, Silbershatz H, Jacques PF, Selhub J, D'Agostino RB, et al. Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: the framingham study. Ann Intern Med 1999;131(5):352–5.

[11] Bostom AG, Silbershatz H, Rosenberg IH, Selhub J, D'Agostino RB, Wolf PA, et al. Nonfasting plasma total homocysteine levels and all-cause and cardiovascular disease mortality in elderly Framingham men and women. Arch Intern Med 1999; 159(10):1077–80.

[12] Karger AB, Steffen BT, Nomura SO, Guan W, Garg PK, Szklo M, et al. Association between homocysteine and vascular calcification incidence, prevalence, and progression in the MESA cohort. J Am Heart Assoc 2020;9(3):e013934.

[13] Shai I, Stampfer MJ, Ma J, Manson JE, Hankinson SE, Cannuscio C, et al. Homocysteine as a risk factor for coronary heart diseases and its association with inflammatory biomarkers, lipids and dietary factors. Atherosclerosis 2004;177(2): 375–81.

[14] Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA 1992;268(7):877–81.

[15] Fu Y, Wang X, Kong W. Hyperhomocysteinaemia and vascular injury: advances in mechanisms and drug targets. Br J Pharmacol 2018;175(8):1173–89.

[16] Coppola A, Davi G, De Stefano V, Mancini FP, Cerbone AM, Di Minno G. Homocysteine, coagulation, platelet function, and thrombosis. Semin Thromb Hemost. 2000;26(3):243–54.

[17] Klein JH, Hegele RA, Hackam DG, Koschinsky ML, Huff MW, Spence JD. Lipoprotein(a) is associated differentially with carotid stenosis, occlusion, and total plaque area. Arterioscler Thromb Vasc Biol 2008;28(10):1851–6.

[18] Spence JD, Koschinsky M. Mechanisms of lipoprotein(a) pathogenicity: prothrombotic, proatherosclerotic, or both? Arterioscler Thromb Vasc Biol 2012;32 (7):1550–1.

[19] Zhang W, Speiser JL, Ye F, Tsai MY, Cainzos-Achirica M, Nasir K, et al. High-sensitivity c-reactive protein modifies the cardiovascular risk of lipoprotein(a): multi-ethnic study of atherosclerosis. J Am Coll Cardiol 2021;78(11):1083–94.

[20] Akintoye E, Afonso L, Bengaluru Jayanna M, Bao W, Briasoulis A, Robinson J. Prognostic utility of risk enhancers and coronary artery calcium score recommended in the 2018 ACC/AHA multisociety cholesterol treatment guidelines over the pooled cohort equation: insights from 3 large prospective cohorts. J Am Heart Assoc 2021;10(12):e019589.

[21] Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol 2002;156 (9):871–81.

[22] Tsai MY, Johnson C, Kao WH, Sharrett AR, Arends VL, Kronmal R, et al. Cholesteryl ester transfer protein genetic polymorphisms, HDL cholesterol, and subclinical cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2008;200(2):359–67.

[23] Rasmussen K, Moller J, Lyngbak M, Pedersen AM, Dybkjaer L. Age- and gender-specific reference intervals for total homocysteine and methylmalonic acid in plasma before and after vitamin supplementation. Clin Chem 1996;42(4):630–6.

[24] Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, Heath SC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med 2009;361(26):2518–28.

[25] Emerging Risk Factors C, Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 2009;302(4):412–23.

[26] Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 2009; 301(22):2331–9.

[27] Willeit P, Ridker PM, Nestel PJ, Simes J, Tonkin AM, Pedersen TR, et al. Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials. Lancet 2018; 392(10155):1311–20.

[28] Guan W, Cao J, Steffen BT, Post WS, Stein JH, Tattersall MC, et al. Race is a key variable in assigning lipoprotein(a) cutoff values for coronary heart disease risk assessment: the multi-ethnic study of atherosclerosis. Arterioscler Thromb Vasc Biol 2015;35(4):996–1001.

[29] Steffen BT, Thanassoulis G, Duprez D, Stein JH, Karger AB, Tattersall MC, et al. Race-based differences in Lipoprotein(a)-associated risk of carotid atherosclerosis. Arterioscler Thromb Vasc Biol 2019;39(3):523–9.

[30] Dhiman P, Ma J, Qi C, Bullock G, Sergeant JC, Riley RD, et al. Sample size requirements are not being considered in studies developing prediction models for binary outcomes: a systematic review. BMC Med Res Method 2023;23(1):188.

[31] Pencina MJ, D'Agostino RB. Sr. Evaluating discrimination of risk prediction models: the C statistic. JAMA 2015;314(10):1063–4.

[32] Dhindsa DS, Sandesara PB, Shapiro MD, Wong ND. The evolving understanding and approach to residual cardiovascular risk management. Front Cardiovasc Med 2020;7:88.

[33] Dangas G, Mehran R, Harpel PC, Sharma SK, Marcovina SM, Dube G, et al. Lipoprotein(a) and inflammation in human coronary atheroma: association with the severity of clinical presentation. J Am Coll Cardiol 1998;32(7):2035–42.

[34] Iso H, Moriyama Y, Sato S, Kitamura A, Tanigawa T, Yamagishi K, et al. Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. Circulation 2004;109(22):2766–72.

[35] Zhang T, Jiang Y, Zhang S, Tie T, Cheng Y, Su X, et al. The association between homocysteine and ischemic stroke subtypes in Chinese: A meta-analysis. Medicine (Baltimore) 2020;99(12):e19467.

[36] Zhao M, Wang X, He M, Qin X, Tang G, Huo Y, et al. Homocysteine and stroke risk: modifying effect of methylenetetrahydrofolate reductase C677T polymorphism and folic acid intervention. Stroke. 2017;48(5):1183–90.

[37] Larsson SC, Traylor M, Markus HS. Homocysteine and small vessel stroke: a mendelian randomization analysis. Ann Neurol 2019;85(4):495–501.

[38] Rutten-Jacobs LC, Traylor M, Adib-Samii P, Thijs V, Sudlow C, Rothwell PM, et al. Association of MTHFR C677T genotype with ischemic stroke is confined to cerebral small vessel disease subtype. Stroke. 2016;47(3):646–51.

[39] Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? Lancet Neurol 2007;6(9):830–8.

[40] Collaboration HLT. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration. BMJ 1998;316(7135):894–8.

[41] Wald DS, Bishop L, Wald NJ, Law M, Hennessy E, Weir D, et al. Randomized trial of folic acid supplementation and serum homocysteine levels. Arch Intern Med 2001;161(5):695–700.

[42] Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. JAMA 2015;313(13):1325–35.

[43] Marti-Carvajal AJ, Sola I, Lathyris D, Dayer M. Homocysteine-lowering interventions for preventing cardiovascular events. Cochrane Database Syst Rev 2017;8(8):CD006612.

[44] Kuczmarski MF, Mason MA, Allegro D, Zonderman AB, Evans MK. Diet quality is inversely associated with C-reactive protein levels in urban, low-income African-American and white adults. J. Acad. Nutrit. Dietetics 2013;113(12):1620–31.

[45] Ridker PM, Danielson E, Fonseca FAH, Genest J, Gotto AM, Kastelein JJP, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359(21):2195–207.

[46] Ballantyne CM, Laufs U, Ray KK, Leiter LA, Bays HE, Goldberg AC, et al. Bempedoic acid plus ezetimibe fixed-dose combination in patients with hypercholesterolemia and high CVD risk treated with maximally tolerated statin therapy. Eur J Prev Cardiol 2020;27(6):593–603.

[47] Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ, et al. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. Lancet 2018;391(10118):319–28.

[48] Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. N Engl J Med 2019;381 (26):2497–505.

[49] Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, Tardif JC, Baum SJ, Steinhagen-Thiessen E, et al. Lipoprotein(a) reduction in persons with cardiovascular disease. N Engl J Med 2020;382(3):244–55.

[50] Viney NJ, van Capelleveen JC, Geary RS, Xia S, Tami JA, Yu RZ, et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. Lancet 2016;388(10057):2239–53.

[51] Tsimikas S, Moriarty PM, Stroes ES. Emerging RNA therapeutics to lower blood levels of Lp(a): JACC focus seminar 2/4. J Am Coll Cardiol 2021;77(12):1576–89.

[52] Garg UC, Zheng ZJ, Folsom AR, Moyer YS, Tsai MY, McGovern P, et al. Short-term and long-term variability of plasma homocysteine measurement. Clin Chem 1997; 43(1):141–5.

[53] Trinder M, Paruchuri K, Haidermota S, Bernardo R, Zekavat SM, Gilliland T, et al. Repeat measures of Lipoprotein(a) molar concentration and cardiovascular risk. J Am Coll Cardiol 2022;79(7):617–28.

[54] Bogaty P, Dagenais GR, Joseph L, Boyer L, Leblanc A, Belisle P, et al. Time variability of C-reactive protein: implications for clinical risk stratification. PLoS One 2013;8(4):e60759.