METHODS AND MATERIALS

Study Population

The design of the MESA study has been described previously [1], and information about the MESA protocol is available at www.mesa-nhlbi.org. Briefly, 6,814 men and women between the ages of 45 and 84 years without clinical evidence of cardiovascular disease were recruited from 6 communities in the U.S. Institutional Review Board approval was obtained at all MESA sites, and all participants gave informed consent.

The current study excluded participants who were taking lipid-lowering medication at baseline (visit 1) and those with missing covariates. Participants in the MESA 1000 population (a random subcohort of 1,000 MESA individuals who were selected for more extensive study) were also excluded due to limited supply of specimens. The remaining study population contains 4,593 individuals of the following races/ethnicities: Black (n=1,323), Caucasian (n=1,677), Chinese-American (n=548), and Hispanic (n=1044). All study participants gave informed consent and were followed for a median follow-up period of 8.5 years. Age, race/ethnicity, sex, education, baseline measurements including hypertension (on hypertension medication or with systolic blood pressure > 140 mmHg), diabetes (treated or untreated diabetes mellitus as determined by 2003 American Diabetes Association fasting criteria algorithm), and smoking status (former and current) were recorded. All related laboratory measurements and imaging data were obtained at baseline as well.

Laboratory measurement

Fasting plasma triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-C) concentrations were measured as described previously [2]. Low density lipoprotein-cholesterol (LDL-C) was calculated based on the Friedewald formula in participants with

triglycerides <400 mg/dL. The calculated LDL-C includes the cholesterol contained in Lp(a) particles. To account for this overlap in the LDL-C covariate with the Lp(a) exposure variable, we subtracted Lp(a)-cholesterol (measured by gradient gel electrophoresis at Health Diagnostics Laboratory Inc., Richmond, VA) from LDL-C to get the non-Lp(a) LDL-C. Lp(a) mass concentration was measured with a latex-enhanced turbidimetric immunoassay (Denka Seiken, Tokyo, Japan). The active reagent (R2) contains a suspension of latex particles coated with anti-Lp(a) antibodies. Following incubation with serum, agglutination is detected by a change in absorbance at a wavelength of 700 nm, which is proportional to the mass, based on a five-level calibration. This assay uses an analytical approach that circumvents the problems in measuring Lp(a) [4] by using 1) multiple calibrators that control for varying apo(a) sizes (187 to >662 kDa) among individuals; and 2) isoform-insensitive antibodies that are not directed to the repeating element within apo(a), Kringle 4 type 2.

Incident Coronary Heart Disease

Incident CHD was defined as the first occurrence of any of the following over a median 8.5 year follow up period: myocardial infarction (n=101), resuscitated cardiac arrest (n=17), CHD death (n=45), or definite angina (n=109). Definite angina was defined as symptoms of typical chest pain and physician diagnosis of angina *followed by* coronary artery bypass grafting or percutaneous coronary intervention (PTCA), evidence of ischemia by stress tests or resting ECG, or ≥70% obstruction on coronary angiography. In addition, there were 51 cases of 'probable angina,' but only nine were included as CHD cases in the present analysis. Probable angina cases that were included showed symptoms of typical chest or atypical symptoms and physician diagnosis of angina followed by coronary artery bypass grafting. Probable angina cases followed by PTCA were excluded (n=4) when obstruction did not reach 70%. An

additional 14 individuals that did not experience angina and underwent PTCA without evidence of obstruction ≥70% were also excluded.

Statistical Model

Statistical analyses were conducted using Stata (version 12.1, Stata Corp, College Station, TX) and R [3]. Baseline characteristics were presented as means (SD) for continuous variables and frequencies (%) for categorical variables. Missing data were excluded when calculating frequencies. Cox regression was used to test for association between Lp(a) and the primary outcomes of CHD, adjusting for age, sex, education, smoking, hypertension medication, systolic blood pressure, diabetes, race, and the standard lipid measures including HDL-C, non-Lp(a) LDL-C, and (log-transformed) triglycerides. Lp(a) level was first treated as a continuous, log-transformed variable; subsequent analysis treated Lp(a) as a categorical variable dichotomized by 30 mg/dL or 50 mg/dL cut points. The proportional hazards assumption was examined using Schoenfeld residuals. We further carried out subgroup analysis for each ethnic group, using the same statistical model.

REFERENCES

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