Materials and Methods

MESA is a multi-center, community-based, prospective cohort study. The full study design and methods are published elsewhere (1). Briefly, between 2000 and 2002, MESA recruited 6814 men and women, aged 45 to 84 years, from four different ethnic groups (Caucasian, African-American, Chinese-American, and Hispanic). Patients were enrolled from six geographically distinct United States communities: Forsyth County, North Carolina; New York City; Baltimore, Maryland; St. Paul, Minnesota; Chicago; and Los Angeles, California. Exclusion criteria included angina and known cardiovascular disease at baseline. All participants provided informed consent and the study was approved by the institutional review boards at all MESA field centers.

Patient Population

Participants were classified by self-report as current-smokers, former-smokers, or never-smokers. Participants with a lifetime smoking history of fewer than 100 cigarettes, and or a report of “never smoking”, were classified as never-smokers. Participants who smoked in the 30 days prior to the baseline visit were designated current-smokers. Both former-smokers and current-smokers were stratified into quartiles of pack-year history of cumulative tobacco exposure (pack-years). Where appropriate we combined both groups (former- and current-smokers) as ‘ever-smokers’ when presenting pack-year results. Cumulative pack-years were calculated using the reported average number of cigarettes smoked per day and the number of years of smoking. We excluded 18 participants without smoking information for the present analysis.

In a sub-sample of MESA participants enrolled in the MESA Lung Sub-study (3,965 of 4,484 randomly sampled MESA participants) smoking was also confirmed by cotinine levels from stored urine obtained at the baseline visit (Immulite 2000 Nicotine Metabolite Assay; Diagnostic Products Corp., Los Angeles, CA)(2). Self-reported former-smokers and never-smokers who had cotinine levels >500ng/mL were reclassified as current-smokers (N=84 [1.2%]). (3) Similarly, per MESA protocol, pack-years of cigarettes were increased by 25% among former-smokers with cotinine levels >100 ng/mL(2) (**Supplementary eTable 1**).

Cardiovascular Risk Factors

At the baseline visit (July 2000-August 2002), study participants completed self-administered questionnaires, standardized interviews, and in-person examinations of lifestyle characteristics, medical history, anthropometric measurements, and laboratory data. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Diabetes was defined as a fasting blood glucose concentration of ≥126 mg/dL or the use of insulin or oral hypoglycemic medications. Blood pressure was recorded as the mean of the last 2 of 3 seated measurements. Hypertension was defined as systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg, or the use of medications prescribed for hypertension. Total and high-density lipoprotein cholesterol were measured using the cholesterol oxidase method. Low-density lipoprotein cholesterol was calculated using the Friedewald equation. HsCRP and fibrinogen were measured using the BNII nephelometer (N-High-Sensitivity CRP and N-Antiserum to Human Fibrinogen; Dade Behring). Intra-assay coefficients of variation

(CVs) for hsCRP ranged from 2.3 to 4.4% and inter-assay CVs ranged from 2.1 to 5.7%. The intra-assay and inter-assay CVs for fibrinogen were 2.7 and 2.6%, respectively.

Cardiac CT Protocol

The full MESA scanning protocol has been previously published (4). Cardiac CT was performed at baseline at 3 MESA sites using a cardiac-gated electron-beam CT scanner (Imatron C-150XL, GE-Imatron, San Francisco, CA) and at 3 sites using a 4-slice multi-detector CT scanner. Both scanner-types produce near-identical CAC results by Agatston score (4). All images were read at the MESA CT reading center (Harbor-UCLA). While no specific action was taken based on CAC results, participants were told either that they had no CAC or that the amount was less than average, average, or greater than average and that they should discuss the results with their physicians.

Definition of Cardiovascular events

At intervals of 9–12 months, an interviewer contacted each participant or a family member about interim hospital admissions, outpatient diagnoses of CHD and CVD, and deaths. Two physicians from the MESA mortality and morbidity review committee independently classified events; in the event of disagreement, the full committee adjudicated. Hard CHD events were defined as: myocardial infarction and death from CHD. All-cause CHD events were defined as: Hard CHD events plus definite angina, probable angina resulting in revascularization, and resuscitated cardiac arrest. All-cause CVD events were defined as: All-cause CHD events plus cerebrovascular accident

(CVA, transient ischemic attack or ischemic or hemorrhagic stroke), CVA death, and other CVD death. More details of the MESA follow-up methods are available at the MESA website (http://www.mesa-nhlbi.org).

Statistical Analysis

We calculated proportions for categorical variables and either mean ± standard deviation or median ± interquartile range for continuous variables. The smoking groups were compared using analysis-of-variance, Kruskal-Wallis, or Chi-square testing, as appropriate. Incidence rates for the 3 main outcomes were compared by categories of smoking status. We also estimated Kaplan-Meier cumulative event-free survival within each smoking category.

We performed multivariable Cox-proportional hazards modeling examining the association between smoking status and events in which Model 1 was adjusted for age, sex, race, MESA site, body mass index, heart rate, hypertension status, diabetes, LDL-Cholesterol, HDL-Cholesterol, triglycerides, cholesterol lowering medications, family history of MI and education level (a measure of socioeconomic status). We then conducted a mediation analysis(5), adding hsCRP (a measure of inflammation), Fibrinogen (a measure of inflammation and thrombosis), and CAC (a measure of subclinical atherosclerosis) as continuous variables to hierarchical proportional hazards Cox-models. Specifically, we created Model 2 by adding the log(hsCRP) to Model 1, Model 3 by adding the log(CAC +1) to Model 1, Model 4 by adding Fibrinogen to Model 1, and Model 5 by adding all 3 of hsCRP, CAC, and Fibrinogen to Model 1. There was no collinearity by variance inflation factors. We also tested for statistical interaction

between smoking and events, based on ethnicity, gender, hsCRP, or CAC. For analyses comparing smoking status groups, we also conducted a sensitivity analysis further adding pack-years to the variables in Model 1.

Cox-models were also constructed to examine the association between cumulative exposure (by quartiles of pack-year history) and events in former and current-smokers. Further, we evaluated the stratified association of CAC and hsCRP (by categories) with events in each smoking group, adjusting for the variables in model 1 above. The proportional hazards assumption was visually assessed for each Cox-model. To test the robustness of our findings, all analyses were also performed based on self-reported smoking variables alone (without cotinine based reclassification). All analyses were conducted with Stata 12 (StataCorp).

REFERENCES

1. Bild DE, Bluemke DA, Burke GL et al. Multi-ethnic study of atherosclerosis: objectives and design. American journal of epidemiology 2002;156:871-81.
2. Rodriguez J, Jiang R, Johnson WC, MacKenzie BA, Smith LJ, Barr RG. The association of pipe and cigar use with cotinine levels, lung function, and airflow obstruction: a cross-sectional study. Annals of internal medicine 2010;152:201-10.
3. Pirkle JL, Flegal KM, Bernert JT, Brody DJ, Etzel RA, Maurer KR. Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA : the journal of the American Medical Association 1996;275:1233-40.
4. Carr JJ, Nelson JC, Wong ND et al. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. Radiology 2005;234:35-43.
5. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. Journal of personality and social psychology 1986;51:1173-82.