Methods and Materials

Study Sample

The FHS offspring cohort includes individuals who have been examined approximately every four years (except for an 8-year interval between Examinations 1 and 2) since recruitment began in 1971.[1] Participants who attended the sixth FHS offspring cohort clinic examination (1995 to 1998) were eligible for this investigation (n=3522). SDF-1 levels were measured in 3359 offspring cohort participants at examination 6, which served as the baseline to investigate several cross-sectional associations and prospective outcomes described below. Participants were excluded if their log-transformed SDF-1 levels (see below) were five standard deviations from the mean.

SDF-1 measurement

Blood samples were obtained from participants after an overnight fast and immediately centrifuged and stored at -70°C until assayed. Plasma SDF-1 concentrations were measured using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN, #DSA00). The lower limit of detection is 47pg/mL, and the standard curve ranges from 156 to 10,000 pg/mL. Intra-assay and inter-assay coefficients of variation are 3.5% and 10.6%, respectively (http://www.rndsystems.com/Products/dsa00/).

Clinical outcomes

We analyzed clinical outcome events occurring after baseline and through December 2011. Our primary endpoint was incident atherosclerotic CVD, a composite endpoint consisting of new-onset myocardial infarction, coronary insufficiency (prolonged ischemic

chest pain with documented ECG changes), atherothrombotic brain infarction, or death due to coronary heart disease.[1] MI, heart failure (HF), and all-cause mortality were secondary outcomes. Subjects with pre-existing atherosclerotic CVD, MI, or HF were excluded from analyses of incident atherosclerotic CVD, MI, HF, respectively.

A panel of three physicians reviewed and adjudicated all available hospital and outpatient records with a putative diagnosis of atherosclerotic CVD or HF. The final determination of HF was based on a set of major and minor criteria as previously described.[2]

CVD risk factors

We examined the relations of SDF-1 to clinical CVD risk factors including age, sex, body mass index (BMI), waist circumference, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, glucose, BNP, smoking status, diabetes status, hypertension treatment, and statin treatment. Diabetes was defined as a fasting glucose level ≥126 mg/dL or treatment with insulin or an oral hypoglycemic drug. Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current drug treatment for high blood pressure. Current cigarette smoking was defined as smoking on average one or more cigarette per day within the past year.

LTL measurement

There were 1244 participants with LTL measured from whole-blood DNA collected upon baseline examination. LTL was derived from the mean length of the terminal restriction

fragments, measured by Southern blot analysis. Details of the method were described previously.[3] The coefficient of variation of LTL measurements was 2.4% (from duplicate DNA samples resolved on different gels).

EPC phenotypes

Fasting blood samples were collected for measurement of CD34+ from FHS offspring cohort participants who attended the eighth examination (2005-2008). Of 3021 participants who attended the eighth offspring cohort examination, 1603 underwent phenotyping for CD34+ cell frequency. Flow cytometry was utilized to analyze the expression of cell surface markers. Methods for cell phenotyping and flow cytometry have been described previously.[4]

Statistical analysis

Plasma SDF-1 levels were skewed with non-constant variance; therefore, plasma SDF-1 levels were natural log transformed (ln[SDF-1], hereafter reported as SDF-1) for analyses. Proportional hazards regression (PROC PHREG in SAS Version 9.2) was used to relate SDF-1 to incident clinical events (atherosclerotic CVD, MI, HF, and death). Three models were used. Model 1 adjusted for age and sex. Model 2 adjusted for established risk factors (for atherosclerotic CVD and MI: age, sex, systolic blood pressure, hypertension treatment, total cholesterol, HDL cholesterol, diabetes, smoking status, statin use; for heart failure: age, sex, systolic blood pressure, hypertension treatment, HDL cholesterol, BMI, diabetes, smoking status, atrial fibrillation, coronary heart disease, valvular heart disease; for death: age, sex, systolic blood pressure,

hypertension treatment, total cholesterol, HDL cholesterol, diabetes, smoking status, atherosclerotic CVD, and HF). Model 3 adjusted for risk factors (as listed above for each outcome) plus plasma levels of B-type natriuretic peptide (BNP). These covariates were selected based on their reported associations with the corresponding outcomes. For descriptive purposes, we estimated cumulative incidence curves by quartiles of ln(SDF-1) using Kaplan-Meier methods.

To relate SDF-1 to clinical CVD risk factors (age, sex, BMI, total cholesterol, HDL cholesterol, smoking status, diabetes, systolic blood pressure, and hypertension treatment), we utilized a linear mixed effect model adjusting for all covariates and accounting for family structure. The SAS (Version 9.2) procedure PROC MIXED was used to conduct this analysis. SDF-1 was the outcome and baseline characteristics were included as covariates.

To evaluate the association of SDF-1 with LTL and CD34+ frequency, SDF-1 level was used as the predictor; LTL and CD34+ cells were used as the outcomes, and adjustment was carried out for other covariates. In addition, a trend test was performed to test the association of increasing quartiles of SDF-1 with LTL and CD34+. CD34+ was natural-log transformed before analysis because of right skewed distribution. Adjustment of covariates was at two levels: 1) age and sex, and 2) all covariates (age, sex, BMI, total cholesterol, HDL cholesterol, smoking status, diabetes, systolic blood pressure, and hypertension treatment). Linear mixed effect models (PROC MIXED in SAS Version 9.2)

were used in these association analyses and family structure was accounted for in the models.

References (Methods)

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