MATERIAL AND METHODS

Study Population

The Northern Manhattan Study (NOMAS) is a community-based cohort and sampling details have been published previously [1]. Briefly, eligible participants were: stroke-free, greater than 40 years-old (55 and older beginning in 1998), and residents of Northern Manhattan for at least three months in a household with a telephone. Audits and Surveys, Inc., performed random digit dialing using dual frame sampling (telephone response rate was 91%) and participants were invited to enroll with an in-person interview and neurological assessment (enrollment response rate was 75%). The overall participation rate was 69%, and a total of 3,298 subjects were enrolled between 1993 and 2001. All participants signed written informed consent and the IRBs of Columbia University Medical Center and the University of Miami approved the study.

Baseline evaluation

Trained research assistants collected data through interviews in English or Spanish, depending on the language spoken at home. Study physicians did physical examinations. Race and ethnicity were determined based on self-identification using questions modeled after the US census [1]. Standardized questions were adapted from the Behavioral Risk Factor Surveillance System by the Centers for Disease Control regarding hypertension, diabetes, smoking, and cardiac conditions [2].

Processing of blood samples and assays

Baseline fasting blood specimens were spun immediately at 4°C and stored at –70 °C until processing. Plasma C-terminal FGF23 concentrations were measured blinded and in duplicate by 2nd generation ELISA with previously published coefficients of variation (Immutopics Int., San Clemente, CA)[3]. Serum phosphate, intact (1-84) parathyroid hormone (PTH) were measured in duplicate on a Roche Cobas 6000 analyzer (Roche Diagnostics, Indianapolis, IN). PTH was measured by electrochemiluminescence. Serum creatinine was measured using the kinetic alkaline picrate assay (Jaffé reaction). Laboratory methods and coefficients of variation are published [4].

Carotid Ultrasound Assessment

Carotid ultrasounds were done starting in 2000 at enrollment, or during follow-up. Presence of carotid plaque and Total Carotid Plaque Area (TCPA) were assessed by high-resolution B-mode ultrasound using standardized protocols with strong validity and reliability [5, 6]. Carotid plaque was defined as a focal wall thickening or protrusion in the lumen >50% of the surrounding wall thickness [6]. Carotid plaque area (mm^2) was measured using automated computerized edge-detection software (M'Ath, Paris, France) [7]. Among those with plaque, echodensity was expressed as gray scale median (GSM).

Statistical Analysis

We evaluated the association of FGF23, continuously (natural log transformed) and by quintiles, with carotid plaque presence, TCPA (cube root transformed), and GSM using logistic and linear regression models, adjusting for age, sex, race/ethnicity, estimated glomerular filtration rate (eGFR), body mass index, smoking, alcohol use, blood pressure, fasting glucose, lipids, and hypertension, diabetes, and dyslipidemia medication use. We also adjusted for time between blood collection and carotid ultrasound. We estimated glomerular filtration rate (eGFR) using the MDRD formula as: GFR = 186.3 * (sCr) − 1.154 * age − 0.203 * (0.742 if female) * (1.21 if black) [8]. We also did sensitivity analyses to see if FGF23 associated with carotid plaque in those without evidence of reduced eGFR (eGFR >59 mL/min/1.73 m^2) or after excluding those with possible primary hyperparathyroidism, defined as PTH >65 pg/mL and serum calcium >10.3 mg/dL; n=4). All tests were two-tailed and a P value <0.05 was considered significant. Analyses were done using SAS version 9.3 (SAS Institute, Cary, N.C.).

References

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