Atherosclerosis 241 (2015) 12–17

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

25-hydroxyvitamin D levels, vitamin D binding protein gene polymorphisms and incident coronary heart disease among whites and blacks: The ARIC study

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ARTICLE INFO

Article history:
Received 10 March 2015
Received in revised form
13 April 2015
Accepted 17 April 2015
Available online 25 April 2015

Keywords:
Vitamin D
Vitamin D binding protein
Coronary heart disease
Race
Epidemiology

ABSTRACT

Background: In observational studies, low 25-hydroxyvitamin D (25(OH)D) has been associated with increased risk of coronary heart disease (CHD), and this association may vary by race. Racial differences in the frequency of vitamin D binding protein (DBP) single nucleotide polymorphisms (SNPs) might account for similar bioavailable vitamin D in blacks despite lower mean 25(OH)D. We hypothesized that the associations of low 25(OH)D with CHD risk would be stronger among whites and among persons with genotypes associated with higher DBP levels.

Methods: We measured 25(OH)D by mass spectroscopy in 11,945 participants in the ARIC Study (baseline 1990–1992, mean age 57 years, 59% women, 24% black). Two DBP SNPs (rs7041; rs4588) were genotyped. We used adjusted Cox proportional hazards models to examine the association of 25(OH)D with adjudicated CHD events through December 2011.

Results: Over a median of 20 years, there were 1230 incident CHD events. Whites in the lowest quintile of 25(OH)D (<17 ng/ml) compared to the upper 4 quintiles had an increased risk of incident CHD (HR 1.28, 95% CI 1.05–1.56), but blacks did not (1.03, 0.82–1.28), after adjustment for demographics and behavioral/socioeconomic factors (p-interaction with race = 0.22). Results among whites were no longer significant after further adjustment for potential mediators of this association (i.e. diabetes, hypertension). There was no statistically significant interaction of 25(OH)D with the DBP SNPs rs4588 (p = 0.92) or rs7041 (p = 0.87) in relation to CHD risk.

Conclusions: Low 25(OH)D was associated with incident CHD in whites, but no interactions of 25(OH)D with key DBP genotypes was found.

1. Introduction

Low levels of vitamin D, as measured by serum 25-hydroxyvitamin D [25(OH)D], have been estimated to affect approximately 1 billion people worldwide [1] and are associated with increased risk for cardiovascular diseases (CVD) [2–5] and specifically for coronary heart disease (CHD) [6,7]. Suboptimal vitamin D status is thought to influence CVD risk predominantly by acting on established CVD risk factors, namely hypertension, diabetes, dyslipidemia and inflammation (i.e. mediators) [8]. Whether adequate vitamin D supplementation in those that are deficient can prevent CVD events is still unknown, and clinical trials are in progress to test this question [9].

Individuals with darker skin pigmentation are more likely to have low 25(OH)D [1,10]. However, research examining the association of low 25(OH)D with CVD outcomes in nonwhites is limited, though there is some evidence that associations may vary by race [7,10]. In a recent analysis from the Multi-Ethnic Study of Atherosclerosis (MESA), low 25(OH)D was associated with increased CHD risk among whites and Chinese but not in blacks or Hispanics [7]. Similarly, a prior analysis from the National Health and Nutrition Examination Survey (NHANES) found that low 25(OH)D was associated with stroke in whites but not blacks [10].

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http://dx.doi.org/10.1016/j.atherosclerosis.2015.04.803
0021-9150/

Bioavailable vitamin D may underlie racial differences in associations between total 25(OH)D and CVD outcomes. On average, blacks have lower levels of total 25(OH)D compared to whites [11]. However, recent work has shown that blacks and whites have similar concentrations of estimated bioavailable vitamin D because blacks have lower levels of both total 25(OH)D and vitamin D binding protein (DBP) compared to whites [11]. Approximately 85–90% of 25(OH)D circulates tightly bound to DBP, which may impair the ability of vitamin D to act on target cells [12]. The remainder, referred to as bioavailable vitamin D, circulates mostly bound to albumin, with a small proportion in the free form. There are two common single nucleotide polymorphisms (SNPs) in the DBP gene, rs7041 and rs4588, which are believed to explain ~80% of the variability in serum DBP levels [11]. Blacks are more likely than whites to have a T allele at rs7041 and a C allele at rs4588, which both result in lower levels of serum DBP.

Our objective was to examine the association between 25(OH)D and incident CHD in both blacks and whites and to characterize any interplay between 25(OH)D and DBP SNPs with incident CHD occurring over approximately 20 years of follow-up in the community-based Atherosclerosis Risk in Communities (ARIC) Study. We hypothesized that lower concentrations of 25(OH)D would be associated with greater CHD risk, and that these associations would be modified by race (stronger in whites versus blacks) and by rs7041 and rs4588 SNPs (stronger in those with either the rs7041 G allele or the rs4588 A allele).

2. Methods

2.1. Participants

ARIC is an ongoing community-based prospective cohort of 15,792 middle-aged adults recruited from four locations: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota, and Washington County, Maryland [13]. Participants have taken part in five main visits: 1987–1989 (Visit 1), 1990–1992 (Visit 2), 1993–1995 (Visit 3), 1996–1998 (Visit 4), and 2011–2013 (Visit 5). Participants provided written consent both for their involvement in the study and for use of their genetic data. The Institutional Review Boards from all four locations and the Coordinating Center have approved the ARIC study.

The sample for this study comprised of participants attending visit 2 (baseline for the present analysis, n = 14,348). Exclusion criteria were races other than black or white (n = 42), blacks from Minneapolis or Washington County sites (n = 49), those with prevalent CHD at visit 2 (n = 826) or missing information regarding prevalent CHD at visit 2 (n = 298), those with missing 25(OH)D levels (n = 1085), those with estimated glomerular filtration rate (eGFR) < 15 ml/min/1.73 m^2 (n = 14), and those missing any covariate information (n = 89). After all exclusions, the final sample size was 11,945 participants.

2.2. Laboratory analyses

Serum samples used for measurement of 25(OH)D_2 and 25(OH)D_3 were collected at visit 2 (1990–1992) and stored at −70 °C until 2012–2013, when measurement took place, using liquid chromatography-tandem high-sensitivity mass spectrometry (Waters Alliance e2795, Milford, Massachusetts). Using samples collected in duplicate tubes and stored, the coefficient of variation (processing plus assay variation) for 25(OH)D_2 was 20.8% and for 25(OH)D_3 was 6.9%. The Pearson correlations from these blind duplicate samples were 0.98 for 25(OH)D_2 and 0.97 for 25(OH)D_3. 25(OH)D_2 and 25(OH)D_3 were added together for total 25(OH)D concentration.

Using stored samples from visit 2, in 2012–2013, we also measured calcium, phosphorus, and parathyroid hormone (PTH) as follows: (calcium and phosphorus: Roche Modular P-Chemistry Analyzer [Roche Diagnostics, Indianapolis, Indiana], PTH: Elecsys 2010 [Roche Diagnostics, Indianapolis, Indiana]). Serum magnesium was measured by the Gindler and Heth procedure. High-sensitivity C-reactive protein (hsCRP) was measured using a latex-particle enhanced immunoturbidimetric assay kit (Roche Diagnostics) and read on the Roche Modular P Chemistry analyzer (coefficient of variation 7%). Cystatin C was also measured in 2012–2013 from stored samples collected at visit 2 using the Gentian cystatin C assay on the Roche Modular P Chemistry analyzer. Serum creatinine was measured at visit 2 using a modified kinetic Jaffé reaction. eGFR was estimated using the 2012 chronic kidney disease epidemiology collaboration equation, which incorporates both cystatin C and creatinine [14]. Fasting lipids were measured at the time of visit 2, and plasma total cholesterol and triglycerides were determined by enzymatic methods. High-density lipoprotein (HDL)-cholesterol was measured after dextran-magnesium precipitation, and the Friedewald equation was used to calculate low density lipoprotein (LDL)-cholesterol in those with triglyceride levels under 400 mg/dL.

2.3. Ascertainment of ARIC CHD

All hospitalizations and deaths in ARIC participants occurring through December 31, 2011 were identified by annual telephone follow-up call, review of hospital discharge lists, and death certificates. Data were abstracted from hospital and death records, next-of-kin interviews, and physician-completed questionnaires. CHD events, defined as definite or probable hospitalized myocardial infarctions or definite fatal CHD, were classified by a combination of computer algorithm and adjudicated physician review, using standardized ARIC criteria [15].

2.4. Genotyping

Participants' DNA samples were used to genotype the two SNPs of interest (rs7041 and rs4588), which are found in the coding region of the vitamin DBP gene. A (50 K) SNP genotyping array, ITMAT-Broad-CARE Chip, from the Broad Institute of Massachusetts Institute of Technology and Harvard was used for genotyping [16,17]. We tested whether rs7041 and rs4588 were in Hardy Weinberg Equilibrium, separately by race. There was no evidence that equilibrium was violated (Chi-square tests $p > 0.8$). We categorized the SNPs as TT versus TG versus GG for rs7041 and CC versus AC versus AA for rs4588.

2.5. Covariates

Most of the variables analyzed for this study were measured at visit 2 except for education and physical activity, which were assessed at visit 1. Medication usage, demographical, and behavioral variables were obtained through standard ARIC questionnaires administered by trained interviewers.

The main covariates included: age, race-center (Minneapolis-whites; Washington County-whites; Forsyth County-whites; Forsyth County-blacks; Jackson County-blacks), sex, education, physical activity (measured on a scale of 1–5 based on a modified Baecke Physical Activity questionnaire [18]), smoking status, body mass index (BMI), diabetes (yes/no; defined as a self-reported physician diagnosis, current diabetes medication use, fasting serum glucose ≥126 mg/dl or non-fasting glucose ≥200 mg/dl), sitting systolic and diastolic blood pressure (continuous; measured in triplicate with random-zero sphygmomanometer; used mean of