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ORIGINAL ARTICLE

Homocysteine and Incident Atrial Fibrillation: The Atherosclerosis Risk in Communities Study and the Multi-Ethnic Study of Atherosclerosis

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Background Although many studies have investigated the association of blood homocysteine with major cardiovascular diseases such as coronary heart disease and stroke, research on its association with atrial fibrillation (AF) is scarce.

Methods We analysed data from Atherosclerosis Risk in Communities (ARIC) Study (n = 492, age 45–64 years) and Multi-Ethnic Study of Atherosclerosis (MESA) (n = 6,641, age 45–84 years).

Results During the 10,106 and 67,613 person-years of follow-up, we identified 85 and 351 AF events in ARIC and MESA, respectively. An age-, sex-, and race-adjusted model showed dose-response relations between plasma homocysteine concentrations and AF incidence in both ARIC and MESA. Further adjustments for other AF risk factors did not change the associations. In the fully adjusted model, a meta-analysis of both studies showed a significant association between homocysteine and AF [hazard ratio (95% confidence interval) per 1 unit increment in $\log_2$(homocysteine), 1.27 (1.01–1.61)]. Individuals with higher levels of all three B vitamins (vitamin B6 and B12, and folate) had a lower risk of AF, but those associations were not statistically significant. In the full ARIC cohort [n = 12,686 (2079 AF events)], there was no association between the $C_{677}T$ methylenetetrahydrofolate reductase (*MTHFR*) mutation and AF.

Conclusions In the prospective population-based ARIC and MESA cohorts, elevated homocysteine was modestly associated with an increased risk of incident AF, but the $C6_{77}T$ *MTHFR* mutation was not associated with AF risk, suggesting that homocysteine may be a novel risk marker for AF rather than a causal risk factor.

Keywords Homocysteine • Atrial fibrillation • Prospective study • General population

Introduction

Very high concentrations of homocysteine, as in homocystinuria patients, increase the risk of cardiovascular disease (CVD) [1]. Observational epidemiological and clinical studies have suggested that modestly elevated blood concentrations of homocysteine in the general population are also associated with an increased risk of CVD including coronary

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heart disease, heart failure and stroke [2–5]. On the other hand, several randomised controlled trials have suggested no protective effect on overall CVD or coronary heart disease risk through lowering plasma homocysteine levels with vitamin B supplements [6–10], but meta-analyses of clinical trials have reported that homocysteine reduction with B vitamins significantly reduced stroke occurrence [11,12]. A Mendelian randomisation analysis of published studies suggested a positive association with coronary heart disease, but unpublished studies did not, suggesting potential publication bias [13]. Thus, whether or not plasma homocysteine is a causal risk factor for CVD in the general population remains suspect. In any case, elevated levels of homocysteine can be considered at least to be a risk marker for CVD.

Atrial fibrillation (AF) is the most frequent sustained cardiac arrhythmia encountered in Western countries, and millions of individuals are expected to develop AF in the next decades [14]. Several risk factors for AF have been so far identified [15]. However, few studies have investigated the association between homocysteine and AF risk [16–18]. Cross-sectional clinical studies indicated that AF patients had elevated homocysteine levels [16,17] and decreased vitamin B6 levels compared with controls [16], whereas a prospective epidemiological study showed no significant association of AF with homocysteine [18]. Several observational clinical studies have reported the positive association of homocysteine with recurrent AF risk after ablation [19–21]. Given the close relation of homocysteine with atherosclerotic CVD and AF recurrence, homocysteine may be associated with incident AF in the general population.

Therefore, we sought to test the hypothesis that higher plasma concentrations of homocysteine are associated with increased incident AF risk independent of other AF risk factors in two US multiracial cohort studies, the Atherosclerosis Risk in Communities (ARIC) Study and the Multi-Ethnic Study of Atherosclerosis (MESA). In addition, since ARIC measured plasma B vitamins and variants of methylenetetrahydrofolate reductase (*MTHFR*), which are associated with homocysteine concentration, we also assessed the associations of these measures with AF.

Materials and Methods

Study Design, Setting, and Population

ARIC recruited 15,792 mostly Caucasian or African American men and women aged 45–64 from four US communities [Washington County, Maryland; Forsyth County, North Carolina; Jackson, Mississippi (African Americans only); and suburbs of Minneapolis, Minnesota] in 1987–1989 [22]. ARIC previously measured plasma homocysteine and B vitamins, using stored samples from the baseline visit, in a nested case-cohort study of coronary heart disease cases (n = 241) from ARIC visit 1 to December 31, 1991 and also in a stratified random sample of all participants free of baseline coronary heart disease in the ARIC cohort (n = 566, of whom n = 525 had relevant plasma measurements) [23]. For the reference cohort random sample, which is the focus of this analysis, ARIC had oversampled participants with thin average carotid intima-media thickness measurements at baseline (<30th percentile) and used different sampling fractions by age, sex and race [23].

In 2000–2002, MESA enrolled 6814 men and women (2,622 Caucasians, 1893 African Americans, 1496 Hispanics, and 803 Chinese Americans), aged 45–84, without a history of clinical CVD from six US communities in Maryland, Illinois, North Carolina, California, New York, and Minnesota [24]. Of 6,814 participants, 6,794 had relevant plasma homocysteine measurements at baseline.

The institutional review boards of the collaborating institutions approved the study protocol, and each participant provided written informed consent.

Baseline Measurements

In ARIC, laboratory measurements of plasma homocysteine and B vitamins were previously described [23,25,26]. Briefly, the Oregon Regional Primate Research Center staff measured homocysteine, in duplicate, as the sum of free and bound homocystine, homocysteine, and cysteine-homocysteine mixed disulfide using high-pressure liquid chromatography and electrochemical detection based on the method of Smolin and Schneider, with minor modifications. The staff of the Oregon Regional Primate Research Center measured plasma folate and vitamin B12 by the Quantiphase II Radioassay method supplied by Bio-Rad Diagnostics Group (Hercules, CA, USA) and vitamin B6 using a radioenzymatic assay supplied by Bühlmann Laboratories AG through American Laboratory Products Co. (Salem, NH, USA). To assess laboratory reliability, ARIC included split-specimen, blinded duplicates prepared at the time of baseline blood drawing. This yielded the following Pearson coefficients: homocysteine, r = 0.95; folate, r = 0.97; vitamin B6, r = 0.90; and vitamin B12, r = 0.91. We determined the $C_{677}T$ mutation of the *MTHFR* gene using the method of Rozen and associates [27].

In MESA, plasma homocysteine concentrations were measured using a fluorescence polarisation immunoassay (IMx homocysteine assay, Axis Biochemicals ASA, Oslo, Norway) with the IMx analyzer (Abbott Diagnostics, Abbott Park, IL, USA) [28]. The analytical coefficients of variation were 3.8–5.1% [28].

We assessed other potential AF risk factors, including age, sex, race, body mass index (kg/m^2), height (cm), smoking status (current, former, or never), alcohol drinking status (current, former, or never), systolic and diastolic blood pressure (mmHg), anti-hypertension medication use, diabetes mellitus [28,29], and electrocardiogram (ECG)-based left ventricular hypertrophy [30].

Confirmation of Atrial Fibrillation

In ARIC, AF has been ascertained by ECG performed at study visits, hospitalisation discharge summaries, and death certificates [31,32]. At each study visit, a 12-lead ECG was done with the participant lying in a supine position. Electrocardiograms were transmitted electronically to the ARIC

Central ECG Reading Center for coding, interpretation and storage. ECGs automatically coded as AF or atrial flutter were visually checked and confirmed by a cardiologist [33]. Trained abstractors obtained information from all participants' hospitalisations, which were ascertained through annual phone calls and surveillance of local hospitals, including International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes for diagnoses and procedures at each hospitalisation. Atrial fibrillation was defined as the presence of ICD-9-CM codes 427.31 or 427.32. AF events associated with open cardiac surgery were not included.

In MESA, participants (or a proxy) were contacted every 9 to 12 months by phone and during clinic follow-up exams to identify all new hospitalisations [34]. Trained staff abstracted discharge diagnostic and procedure codes from these hospitalisations. Atrial fibrillation was defined by the same codes as in ARIC and ascertained by the MESA events detection protocol. AF events associated with open cardiac surgery were not included.

The validity of the hospital discharge codes used for ascertainment of AF in large cohort studies has been demonstrated to be adequate [31,32].

Statistical Analysis

SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA) was used for statistical analyses. All statistical tests were two-tailed and p values <0.05 were regarded as significant.

From the eligible ARIC cohort stratified random sample (n = 525), we excluded participants who had ECG evidence of prebaseline AF (n = 1) and those without baseline ECG data (n = 6). We further excluded participants whose data on covariates (n = 12) was missing and those with prevalent heart failure (n = 14). Thus, 492 participants in the cohort random sample were available for the present analysis of homocysteine and B vitamins. We excluded participants with prevalent heart failure because heart failure is associated with both elevated concentrations of homocysteine and increased risk of AF [5]. Since the $C_{677}T$ mutation of the *MTHFR* gene was examined for the whole ARIC cohort, 12,686 participants were available after identical exclusions for the analysis of this gene and AF.

In MESA, from 6,794 eligible participants, we excluded those with prebaseline AF (n = 70) and those with missing data on outcomes (n = 33) and covariates (n = 50). Thus, 6,641 participants were available from MESA for the present analysis.

We computed mean levels or percentages (weighted mean levels or percentages in ARIC) of AF risk factors at baseline according to quartiles of homocysteine. Person-years of follow-up were calculated from the baseline (1987–1989 for ARIC and 2000–2002 for MESA) to the first endpoint: AF, death, loss to follow-up, or administrative censoring at 31 December 2013 for ARIC and 2012 for MESA. Hazard ratios (HRs) of AF occurrence and their 95% confidence intervals (CIs) were calculated after adjustment for other AF risk factors using Cox proportional hazards model (in ARIC, stratified sampling weights using a weighted Cox proportional hazard models were used to account for sampling). The proportional hazards assumption in the Cox regression was tested using risk factor-by-time interactions and was not violated. Plasma homocysteine and B vitamins were modelled using quartile cutpoints or using continuous variables, with $\log_2$-transformed levels because of right-skewness. Since distributions of homocysteine concentrations were similar in ARIC and MESA, we used quartile cutpoints in ARIC as cutpoints for both studies. For the continuous model, we estimated HRs and 95% CIs associated with 1 unit increment in $\log_2$(homocysteine or B vitamins), corresponding to a doubling of homocysteine or B vitamins levels. ARIC and MESA results were meta-analysed using an inverse variance weighting method [35].

Results

Baseline Plasma Homocysteine Levels and Other Risk Factors in ARIC and MESA

Median values of each homocysteine quartile were 6.2, 8.2, 9.8 and 13.1 μmol/L in ARIC, and 6.4, 8.1, 10.0 and 13.1 μmol/L in MESA (Tables 1 and 2). In both ARIC and MESA, compared with individuals with the lowest quartile of plasma homocysteine concentrations, those with higher concentrations of plasma homocysteine were more likely to be male, use anti-hypertension medication and have left ventricular hypertrophy and they were older, taller and had higher body mass index and systolic blood pressure. ARIC participants with higher concentrations of plasma homocysteine were more likely to have the homozygous mutation of *MTHFR* (r-square adjusted for age, sex and principal components of ancestry = 0.86 for Caucasians and 0.92 for African American). In ARIC, homocysteine was modestly correlated with B vitamin concentrations (r = −0.24 to −0.20), and B vitamins were modestly correlated (r = 0.23 to 0.38) with each other.

Associations of Plasma Homocysteine With Risk of Atrial Fibrillation in ARIC and MESA

During the 10,106 person-years of follow-up for the 492 ARIC cohort random participants, we identified 85 incident AF events (Table 3). During the 67,613 person-years of follow-up for the 6,641 MESA participants, we identified 351 incident AF events. The age-, sex-, and race-adjusted model (Model 1) showed dose-response relations between plasma homocysteine concentrations and AF incidence in both ARIC and MESA. Further adjustments for other AF risk factors (Model 2 and 3) attenuated the associations. In Model 3, compared to individuals in the lowest quartile of homocysteine, those in the highest quartile had 2.94 and 1.22 times the risk of incident AF in ARIC and MESA, respectively. Using the cutpoints in MESA instead of those in ARIC produced

Table 1 **Baseline Characteristics of Participants According to Plasma Homocysteine Quartiles (n = 492), ARIC, 1987–1989.**

	Plasma homocysteine concentration (median, range μmol/L)			
	Q1: 6.2 (3.5–7.1)	Q2: 8.2 (7.2–9.0)	Q3: 9.8 (9.1–11.4)	Q4: 13.1 (11.5–45.3)
Participants, n	123	122	125	122
Age, y	52.2 ± 0.5	55.1 ± 0.6	54.2 ± 0.7	54.1 ± 0.7
Female, %	76.7	58.1	43.3	25.5
African American, %	18.5	34.8	15.1	18.7
Current smoker, %	27.0	9.2	21.3	29.5
Current drinker, %	55.0	54.9	64.4	60.9
Body mass index, kg/m^2	26.0 ± 0.6	27.6 ± 0.7	27.0 ± 0.5	27.2 ± 0.7
Height, cm	165.2 ± 0.7	167.6 ± 1.1	169.3 ± 1.0	172.8 ± 1.2
Systolic blood pressure, mmHg	136.7 ± 2.0	143.1 ± 2.3	137.9 ± 2.1	139.8 ± 2.0
Diastolic blood pressure, mmHg	92.1 ± 1.2	94.0 ± 1.5	91.6 ± 1.4	94.6 ± 1.7
Anti-hypertension medication use, %	14.3	20.4	20.2	21.3
Diabetes, %	8.4	5.7	8.9	7.8
Left ventricular hypertrophy, %	0	0.1	3.4	2.5
Plasma vitamin B6, pmol/L	66.0 ± 8.1	65.1 ± 15.1	48.7 ± 7.6	27.2 ± 2.7
Plasma vitamin B12, pmol/L	370.8 ± 21.0	328.6 ± 16.1	283.0 ± 12.8	238.0 ± 13.4
Plasma folic acid, pmol/L	14.7 ± 1.5	8.7 ± 0.9	7.8 ± 1.0	5.8 ± 0.5
Methylenetetrahydrofolate reductase mutation (homozygous)	5.8	9.5	12.0	22.8

Values are mean ± standard error for continuous variables and % for categorical variables.

Table 2 **Baseline Characteristics of Participants According to Plasma Homocysteine Quartiles (n = 6,631), MESA, 2000–2002.**

	Plasma homocysteine concentration (median, range μmol/L)			
	Q1: 6.4 (3.2–7.1)	Q2: 8.1 (7.2–9.0)	Q3: 10.0 (9.1–11.4)	Q4: 13.1 (11.5–118.0)
Participants, n	1,511	2,248	1,753	1,129
Age, y	57.6 ± 0.3	61.2 ± 0.2	63.9 ± 0.2	66.6 ± 0.3
Female, %	74.7	53.8	43.0	36.4
African American, %	37.9	38.4	40.1	35.9
Current smoker, %	11.4	12.6	13.5	15.6
Current drinker, %	54.3	57.1	57.4	51.6
Body mass index, kg/m^2	27.9 ± 0.1	28.4 ± 0.1	28.5 ± 0.1	28.4 ± 0.2
Height, cm	163.5 ± 0.3	166.4 ± 0.2	167.7 ± 0.2	168.0 ± 0.3
Systolic blood pressure, mmHg	121.3 ± 0.5	125.4 ± 0.4	128.6 ± 0.5	132.2 ± 0.6
Diastolic blood pressure, mmHg	70.0 ± 0.3	71.7 ± 0.2	72.8 ± 0.2	73.6 ± 0.3
Anti-hypertension medication use, %	25.9	32.6	40.8	53.3
Diabetes, %	11.0	12.5	15.7	17.4
Left ventricular hypertrophy, %	1.4	2.3	2.5	3.4

Values are mean ± standard error for continuous variables and % for categorical variables.

identical results. Log_2(homocysteine), modelled continuously, was also associated positively with AF, though not statistically significantly, with HR (95% CI) per 1 unit increment = 1.52 (0.84–2.75) in ARIC and 1.24 (0.96–1.59) in MESA. A meta-analysis of both studies (Model 3 covariates) showed a significant association between log_2(homocysteine) and AF risk [HR (95% CI) per 1 unit increment, 1.27 (1.01–1.61)]. Quartiles of homocysteine were positively associated with AF in meta-analysis, but this association was not significant. No significant interactions were observed between

Table 3 Hazard Ratios and 95% Confidence Intervals for Incident Atrial Fibrillation According to Plasma Homocysteine Level, ARIC (n = 492), 1987–2013 and MESA (n = 6631), 2000–2012.

		Quartiles of plasma homocysteine concentration (μmol/L)			
	Log_2(homocysteine)	Q1 (≤7.1)	Q2: (7.2–9.0)	Q3: (9.1–11.4)	Q4: (≥11.5)
ARIC					
Participants, n	492	123	122	125	122
Person-years	10,106	2,749	2,571	2,528	2,258
Cases	85	14	17	24	30
Model 1	1.54 (0.86–2.75)	1	1.16 (0.45–3.03)	1.48 (0.58–3.79)	2.54 (0.99–6.48)
Model 2	1.44 (0.75–2.77)	1	1.05 (0.36–3.03)	1.45 (0.50–4.25)	2.76 (1.01–7.55)
Model 3	1.52 (0.84–2.75)	1	1.29 (0.37–4.51)	1.91 (0.66–5.52)	2.94 (1.07–8.10)
MESA					
Participants, n	6,641	1,511	2,248	1,753	1,129
Person-years	67,613	16,227	23,223	17,523	10,639
Cases	351	43	101	117	90
Model 1	1.39 (1.09–1.76)	1	1.17 (0.81–1.68)	1.40 (0.97–2.01)	1.49 (1.01–2.19)
Model 2	1.26 (0.98–1.62)	1	1.10 (0.76–1.57)	1.26 (0.88–1.81)	1.29 (0.87–1.90)
Model 3	1.24 (0.96–1.59)	1	1.04 (0.72–1.50)	1.33 (0.92–1.91)	1.22 (0.82–1.80)
Combined					
Model 3	1.27 (1.01–1.61)	1	1.06 (0.74–1.50)	1.38 (0.98–1.95)	1.37 (0.95–1.96)
P for heterogeneity	0.53		0.75	0.53	0.11

Model 1: Adjusted for age, sex, race, field centre.
Model 2: Adjusted for Model 1 + body mass index, height, systolic and diastolic blood pressure, anti-hypertension medication, diabetes mellitus, smoking status, drinking status, and, left ventricular hypertrophy.
Model 3: Adjusted for Model 2 + time-varying coronary heart disease and heart failure.

plasma homocysteine and study (ARIC vs. MESA) in relation to AF risk, suggesting similar associations for both studies.

Association between $C_{677}T$ *MTHFR* Mutation and Risk of Atrial Fibrillation in ARIC

The $C6_{77}T$ *MTHFR* mutation was not associated with AF incidence in either African Americans or Caucasian in either crude or adjusted models (Table 4).

Associations Between Plasma B Vitamins and Risk of Atrial Fibrillation in ARIC

Individuals with higher levels of all three B vitamins had a lower risk of AF, in both models using quartiles or log_2-transformed B vitamins, but those associations were not statistically significant (Table 5).

Discussion

In this investigation comprising two population-based prospective cohort studies in the US, elevated homocysteine was associated with increased risk of AF independent of several established AF risk factors, but the $C6_{77}T$ *MTHFR* mutation, which appeared strongly associated with higher homocysteine concentrations, was not associated with AF risk. Individuals with higher levels of B vitamins correspondingly had a lower risk of AF, but those associations were not statistically significant. Although a previous study suggested plasma homocysteine levels were increased in patients with AF [16,17], to the best of our knowledge, this is the first prospective study reporting a positive association between plasma homocysteine and incident AF.

Atrial fibrillation is the result of electrical and structural atrial remodelling [36,37]. Hyperhomocysteinaemia has been suggested to cause extracellular matrix remodelling by activating the extracellular signal regulated kinase–matrix metalloproteinase-9 signalling axis in several cell types [38] and altering synthesis of collagen type I, which is a major component of the atrial interstitium, through the extracellular signal regulated kinase pathway [39,40]. It has also been reported that hyperhomocysteinaemia may cause atrial electrical remodelling by inhibiting potassium channels in atrial myocytes [41]. Thus, it seems plausible that elevated homocysteine may increase the risk of AF. However, the $C6_{77}T$ *MTHFR* mutation was not associated with AF risk, suggesting that elevated homocysteine might be a risk marker for AF rather than a causal risk factor.

Only one previous population-based prospective study has investigated the association between plasma homocysteine level and AF risk [18]. Individuals with elevated

Table 4 Hazard Ratios and 95% Confidence Intervals for Incident Atrial Fibrillation According to Methylenetetrahydrofolate Reductase Mutation Presence, Stratified by Race (3,052 African Americans and 9,634 Caucasians), ARIC, 1987–2013.

	Methylenetetrahydrofolate reductase risk alleles			
African American	**GG**	**GA**	**AA**	**P for trend**
Number at risk	2386	621	45	
Person-years	49,617	13,006	978	
Atrial fibrillation, cases	271	74	9	
Model 1	1	1.04 (0.80–1.35)	1.70 (0.88–3.31)	0.311
Model 2	1	1.07 (0.82–1.38)	1.72 (0.88–3.37)	0.240
	Methylenetetrahydrofolate reductase risk alleles			
Caucasians	**GG**	**GA**	**AA**	**P for trend**
Number at risk	4,314	4,212	1,108	
Person-years	92,172	89,302	23,304	
Atrial fibrillation, cases	783	756	186	
Model 1	1	1.00 (0.90–1.10)	0.99 (0.85–1.11)	0.621
Model 2	1	1.02 (0.92–1.13)	0.93 (0.79–1.09)	0.612

Model 1: Crude model.
Model 2: Adjusted for age, sex and principal components of ancestry.

Table 5 Hazard Ratios and 95% Confidence Intervals for Incident Atrial Fibrillation According to Plasma B Vitamins Levels among the Cohort Random Sample (n = 492), ARIC, 1987–2013.

		Quartiles of plasma vitamin B6 concentration (median, range pmol/L)			
	Log_2(vitamin B6)	**14.3 (0.1–20.2)**	**25.7 (20.3–34.0)**	**43.0 (34.1–59.7)**	**89.4 (60.4–480.0)**
Person-years	10,106	2,343	2,419	2,829	2,515
Cases	85	23	22	17	23
Model 1	0.89 (0.77–1.03)	1	0.69 (0.31–1.54)	0.64 (0.27–1.50)	0.53 (0.24–1.21)
Model 2	0.88 (0.74–1.05)	1	0.72 (0.33–1.58)	0.70 (0.23–2.14)	0.49 (0.20–1.20)
Model 3	0.91 (0.77–1.09)	1	1.05 (0.45–2.44)	0.81 (0.32–2.03)	0.49 (0.17–1.38)
		Quartiles of plasma vitamin B12 concentration (median, range pmol/L)			
	Log_2(vitamin B12)	**162.0 (40.0–200.0)**	**235.0 (201.0–280.0)**	**323 (281.0–367.0)**	**449 (368.0–1217.0)**
Person-years	10,106	2,562	2,515	2,493	2,535
Cases	88	25	28	14	18
Model 1	0.70 (0.46–1.07)	1	0.84 (0.37–1.88)	0.60 (0.25–1.47)	0.55 (0.24–1.28)
Model 2	0.77 (0.50–1.17)	1	0.89 (0.37–2.12)	0.59 (0.24–1.47)	0.58 (0.25–1.33)
Model 3	0.75 (0.42–1.34)	1	0.55 (0.22–1.39)	0.52 (0.20–1.37)	0.70 (0.28–1.76)
		Quartiles of plasma folate concentration (median, range pmol/L)			
	Log_2(folate)	**1.4 (0.3–3.2)**	**5.3 (3.3–7.1)**	**9.3 (7.2–12.9)**	**18.4 (13.2–60.1)**
Person-years	10,106	2,590	2,402	2,563	2,551
Cases	88	23	26	21	15
Model 1	0.85 (0.69–1.06)	1	0.65 (0.28–1.48)	0.69 (0.29–1.67)	0.43 (0.19–0.99)
Model 2	0.89 (0.72–1.11)	1	0.85 (0.33–2.16)	0.97 (0.39–2.43)	0.45 (0.19–1.09)
Model 3	0.90 (0.69–1.18)	1	1.03 (0.34–3.14)	1.45 (0.55–3.85)	0.41 (0.12–1.43)

Model 1: Adjusted for age, sex, race, field center.
Model 2: Adjusted for Model 1 + body mass index, height, systolic and diastolic blood pressure, anti-hypertension medication, diabetes mellitus, smoking status, drinking status, and, left ventricular hypertrophy.
Model 3: Adjusted for Model 2 + time-varying coronary heart disease and heart failure.

homocysteine had modestly increased risk of AF, but the association was not statistically significant [HR per log-transformed 1-SD (95%CI): 1.08 (0.94–1.24)] [18]. In our study, each cohort showed a similar positive association, but the meta-analysis showed a significant association only when homocysteine was modelled as a continuous variable. Thus, the association between homocysteine and AF risk seems modest compared with well-documented AF biomarkers, such as B-type natriuretic peptide and C-reactive protein [18].

Although the association between B vitamins and AF risk was not significant, in part probably because of low statistical power, those with higher levels of B vitamins tended to have a lower risk of AF. This association might be expected given that B vitamins reduce homocysteine levels [42]. In addition, low vitamin B6 levels have been associated with increased levels of C-reactive protein independent of homocysteine levels [43], and vascular inflammation may be associated with increased risk of AF [44]. Further studies with more power will be needed to confirm or refute any association between B vitamins and AF risk.

Some limitations of our study need to be mentioned. Firstly, as already noted, statistical power to detect a moderate association between B vitamins and AF risk was limited. Secondly, we had only a single measurement of homocysteine and B vitamins; changes in these biomarkers during follow-up could have led to their misclassification, which would likely bias the observed HRs toward the null. Thirdly, the possibility of confounding of the observed associations by unmeasured AF risk factors that might be associated with homocysteine cannot be negated, although the potential bias is likely small given our detailed adjustment for many known confounding variables. Lastly, we could not capture paroxysmal AF events. Thus, we might have underestimated the number of AF events.

In conclusion, in the prospective population-based ARIC and MESA cohorts, elevated homocysteine was modestly associated with an increased risk of incident AF, but the $C6_{77}T$ *MTHFR* mutation was not associated with AF risk, suggesting that homocysteine may be a novel risk marker for AF rather than a causal risk factor.

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