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Metabolomic Profiling in Relation to New-Onset Atrial Fibrillation (from the Framingham Heart Study)

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Previous studies have shown several metabolic biomarkers to be associated with prevalent and incident atrial fibrillation (AF), but the results have not been replicated. We investigated metabolite profiles of 2,458 European ancestry participants from the Framingham Heart Study without AF at the index examination and followed them for 10 years for new-onset AF. Amino acids, organic acids, lipids, and other plasma metabolites were profiled by liquid chromatography−tandem mass spectrometry using fasting plasma samples. We conducted Cox proportional hazard analyses for association between metabolites and new-onset AF. We performed hypothesis-generating analysis to identify novel metabolites and hypothesis-testing analysis to confirm the previously reported associations between metabolites and AF. Mean age was 55.1 ± 9.9 years, and 53% were women. Incident AF developed in 156 participants (6.3%) in 10 years of follow-up. A total of 217 metabolites were examined, consisting of 54 positively charged metabolites, 59 negatively charged metabolites, and 104 lipids. None of the 217 metabolites met our a priori specified Bonferroni corrected level of significance in the multivariate analyses. We were unable to replicate previous results demonstrating associations between metabolites that we had measured and AF. In conclusion, in our metabolomics approach, none of the metabolites we tested were significantly associated with the risk of future AF. © 2016 Elsevier Inc. All rights reserved. (Am J Cardiol 2016;118:1493−1496)

Previous metabolomics investigations have focused on identifying metabolic pathways responsible for the initiation and maintenance of the arrhythmia in patients with known atrial fibrillation (AF) or postoperative AF.[1−13] Recently, the Atherosclerotic Risk in Communities Study identified bile acids glycolithocholate sulfate and glycocholenate sulfate as markers of increased risk of new-onset AF.[14] In the present study, we aimed to identify novel metabolic markers and to confirm the association between previously reported metabolites in relation to new-onset AF.

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Methods

We studied participants from the Framingham Heart Study Offspring cohort, which was initiated in 1971. Participants (n = 5,124) underwent medical and laboratory evaluation every 4 to 8 years. Our study involved the fifth examination, consisting of 3,799 participants evaluated from 1991 to 1995. Metabolites were measured on 2,526 participants, in whom 49 were excluded because of prevalent AF and 19 because of missing covariates. Institutional Review Boards at Boston University Medical Center and Massachusetts General Hospital approved the study protocols. All participants provided written informed consent.

Fasting EDTA plasma metabolites were analyzed using targeted liquid chromatography−tandem mass spectrometry using 3 methods focusing on amino acids and amines,[15,16] organic acids,[17] and lipids.[18] Data were acquired using either an AB SCIEX 4000 QTRAP triple quadrupole mass spectrometer (positively charged polar compounds and lipids) or an AB SCIEX 5500 QTRAP triple quadrupole mass spectrometer (negatively charged polar compounds). Briefly, polar, positively charged metabolites were separated using hydrophobic interaction liquid chromatography and analyzed using multiple reaction monitoring in the positive ion mode. Polar, negatively charged compounds, including central and polar phosphorylated metabolites, were separated using a Luna NH2 column (150 × 2 mm, Luna NH2; Phenomenex, Torrance, California) and analyzed using multiple reaction monitoring in the negative ion mode. Lipids were separated on a Prosphere C4 HPLC column and underwent full scan mass spectrometer analysis in the positive ion mode. MultiQuant software version 1.2 (AB SCIEX, Concord, Ontario, Canada) was used for automated peak integration and manual review of data quality before statistical analysis. For all 3 profiling platforms, a pooled plasma sample also was run after every 20 samples, and the peak areas in samples were normalized to metabolite peak areas in the nearest pooled plasma. We have previously published that coefficient of variabilities (CVs) for ∼80% of the analytes are <20%.[15,17−19]

Physicians measured systolic and diastolic blood pressures twice in seated participants. Medications and tobacco use were ascertained by self-report. Tobacco use was defined as routine smoking of ≥1 cigarettes/day within the year before the Framingham Heart Study clinic visit. Diabetes was defined by fasting serum glucose of ≥126 mg/dl or use of insulin or oral hypoglycemic agents. Serum lipid and glucose concentrations were collected after an overnight fast. Myocardial infarction and heart failure were determined by a panel of 3 physicians who examined hospital and outpatient records of the participants, using previously reported criteria.[20]

The presence of AF was determined from participant records from the Framingham Heart Study clinic as well as both other ambulatory clinic and inpatient hospital records and Holter monitoring. Participants were diagnosed with AF if either AF or atrial flutter was noted on electrocardiogram. Cardiologists at the Framingham Heart Study confirmed the incident AF electrocardiographic diagnoses.

We present baseline characteristics as mean ± standard deviation for continuous covariates and counts (%) for dichotomous covariates. Each metabolite was rank

Table 1
Baseline characteristics of study sample

Variable	Total Population (n = 2,477)
Age (years)	55.1±9.9
Women	1296 (53%)
Height (cm)	168±9.3
Weight (kg)	78±16
Current smoker	459 (19%)
Systolic blood pressure (mmHg)	126±19
Diastolic blood pressure (mmHg)	75±10
Antihypertensive medication use	482 (20%)
Statin use	96 (4%)
Diabetes mellitus	169 (7%)
Prevalent heart failure	7 (0.3%)
Prevalent myocardial infarction	51 (2%)

Values are n (%), or mean ± SD.

Table 2
Age- and sex-adjusted associations of candidate metabolites with incident AF

Metabolite	Hazard Ratio (95% confidence interval)*	p Value[†]
Beta hydroxybuterate	1.07 (0.88-1.29)	0.50
Glycine	1.05 (0.87-1.26)	0.63
Phosphocreatine	0.87 (0.72-1.05)	0.15
Creatine	0.91 (0.77-1.08)	0.28
Alanine	1.16 (0.98-1.36)	0.08
Glutamine	1.01 (0.87-1.18)	0.86
Betaine	1.03 (0.87-1.22)	0.03
Glucose/fructose/galactose	1.39 (1.17-1.65)	0.0002

* Hazard ratio expressed per standard deviation of the metabolite.
[†] Significance level of p ≤0.00625 (0.05/8) for hypothesis testing.

normalized before the analysis using Blom's method.[21] For the 209 metabolites, we used the corrected p values of ≤0.00024 (0.05/209) for hypothesis generating. For the 8 metabolites previously reported in the literature to be associated with AF (β hydroxybuterate,[1] glycine,[1] phosphocreatine,[3] glucose,[1] creatine,[2] alanine,[2] glutamine,[2] betaine[2]), we used the Bonferroni corrected significance level of p ≤0.00625 (0.05/8) for hypothesis testing. We conducted Cox proportional hazard analyses for association between baseline metabolite (rank normalized values) and incident AF, adjusting for age and gender. We additionally adjusted for height, weight, systolic and diastolic blood pressures, current tobacco use, antihypertensive medication use, diabetes, myocardial infarction, heart failure, and statin use.[22] We analyzed 10-year risk of AF by censoring on death, last contact, or 10 years from examination 5 date, whichever came first. Hazard ratios (HR) are expressed per SD of the metabolites. Analyses were conducted with SAS version 9.4 software (SAS Institute, Cary, North Carolina).

Results

Baseline characteristics of the study sample are provided in Table 1. Of 2,458 participants, incident AF developed in 156 participants (6.3%) during 10 years of follow-up. A total of 217 metabolites were identified from the baseline

Table 3
Metabolites previously studied

First Author, Year Metabolite Source	Main Findings
Metabolomics studies	
Alonso, 2015[14] Human serum	Higher incident AF risk associated with higher concentrations of glycolithocholate sulfate and glycocholenate sulfate
De Souza, 2010[2] Canine atrial tissue	After 2 weeks of ventricular-tachpacing, ADP+ATP, alanine, betaine, glucose, glutamate, NAD+NADH, and taurine levels were increased and α-ketoisovalerate level was decreased
Mayer, 2008[1] Human atrial tissue	Higher concentrations of β-hydroxybutyrate and glycine in the AF group compared to the sinus rhythm group
Non-metabolomics studies	
Tamariz, 2011[5] Human serum	Higher risk of AF associated with higher uric acid concentration
Neuman, 2007[9] Human plasma	Increased oxidation of glutathione and derivatives of reactive oxygen metabolites in the AF group compared to control
Ramlawai, 2007[10] Human atrial tissue, serum	Tissue: Higher peroxide level in the AF group compared to control Serum: Higher peroxide level in the AF group at 6 hours after surgery but not at post-operative day 4
Mihm, 2001[8] Human atrial tissue	MM-CK activity was reduced and 3-nitrotyrosine concentration was increased in the AF group compared to control
Ausma, 2000[3] Goat atrial tissue	Phosphocreatine level dropped during the first 8 weeks of AF induction and then returned to normal at 16 weeks; levels of creatine, ATP, ADP, AMP, GDP, GTP, and NAD did not change significantly.
Uno, 1986[4] Canine plasma	cGMP concentration increased significantly with AF induction; cAMP concentration did not change

ADP = adenosine diphosphate; AF = atrial fibrillation; ATP = adenosine triphosphate; cAMP = cyclic (c) adenosine monophosphate (AMP); cGMP = cyclic (c) guanosine monophosphate (GMP); GDP = guanosine diphosphate; GTP = guanosine triphosphate; MM-CK = myofibrillar creatine kinase; NADH = nicotinamide adenine dinucleotide (NAD) + hydrogen (H).

samples of the entire cohort, consisting of 54 positively charged metabolites, 59 negatively charged metabolites, and 104 lipids (Supplementary Table).

In our Cox proportional analysis for association between previously reported baseline metabolites and incident AF, only fructose, glucose, and/or galactose met our a priori specified Bonferroni corrected level of significance when adjusted for age and gender (Table 2). None of the metabolites met our corrected level of significance with additional adjustments.

None of the 217 metabolites met our a priori specified Bonferroni corrected level of significance with multivariate adjustments (Supplementary Table).

Given our sample size (n = 2,458) and number of participants with incident AF, there was 80% of power to replicate previously reported metabolites with HR ≥1.37 at $\alpha = 0.00625$ level; there was 80% of power to discover metabolites with HR ≥1.49 at $\alpha = 0.00024$ level.

Discussion

In our longitudinal analysis of participants of the Framingham Heart Study, we found no plasma metabolites to be associated with the risk of future AF at our a priori specified level of significance. Both metabolomics and nonmetabolomics studies have examined associations between biomarkers and the risk of AF (Table 3). Recently, the community-based Atherosclerotic Risk in Communities Study reported associations between serum metabolites identified through nontargeted metabolomics approach and the risk of new-onset AF.[14] In their analysis, bile acids, glycolithocholate sulfate, and glycocholenate sulfate, were

significantly associated with the risk of new-onset AF after multivariate adjustments.[14] Our targeted liquid chromatography–tandem mass spectrometry platform did not detect either of the metabolites; it detected bile salts, glycocholate, and glycodeoxycholate, which were not significantly associated with the risk of new-onset AF. Before the Atherosclerotic Risk in Communities Study, Mayr et al.[1] identified several metabolites using human atrial tissues as potential markers of increased risk of AF after cardiac surgery, and De Souza et al.[2] found various metabolites using canine atrial tissues as markers of increased risk of heart failure–induced AF (Table 3). Our metabolomics profiling did not confirm the results of the 3 studies.

Additional nonmetabolomics studies have focused on specific metabolites and demonstrated significant variation by AF status in the circulating and tissue concentrations of several metabolites in both animals and humans (Table 3).[3–5,8–10] The molecules studied include phosphocreatine, cyclic guanosine monophosphate, uric acid, 3-nitrotyrosine, myofibrillar creatine kinase, glutathione, and peroxide. Phosphocreatine was detected in our study but was not significantly associated with the risk of new-onset AF.

Several reasons may explain inconsistency between our results compared to the previous reports. First, in our liquid chromatography–tandem mass spectrometry approach, we may have missed metabolites outside the targeted platform.[23] Second, use of a strict threshold for corrected p values may have masked subtle associations.[23] Finally, the participants, tissues, and study designs were heterogeneous. Our study examined participants of European ancestry free of AF at baseline. The studies by Mayr et al. and De Souza

et al. were both cross-sectional in design. The Atherosclerotic Risk in Communities Study analyzed African-Americans free of AF at baseline. The study by Mayr et al. examined the risk of postoperative AF among the patients undergoing cardiac surgery. De Souza et al. used an animal model to investigate heart failure—induced AF.

There are several limitations to our study. First, metabolite profiles may be tissue specific; sampling from the plasma may have failed to detect metabolite associations in other samples such as serum or at the atrial tissue level.[23] Second, we may have underestimated new-onset AF because AF is frequently clinically unrecognized. Third, the CVs of some of the metabolites may have led to a substantial misclassification which may have biased the results toward the null. Fourth, we may have had modest power to detect small effect sizes. Fifth, our study predominantly included middle-aged to older subjects of European ancestry, which may not generalize to other ethnicities or age groups. There is some evidence that metabolomics patterns differ by race.[24,25] Finally, our AF population includes all types of AF, and we may have missed association between the metabolites with specific AF subtypes such as atrial flutter, paroxysmal, persistent, or permanent AF.

Disclosures

Dr. Ellinor is a principal investigator on a grant from Bayer HealthCare to the Broad Institute. All other authors have nothing to disclose.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.amjcard.2016.08.010.

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