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2019-03-19

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### Repository Citation

Ko D, Benson MD, Ngo D, Yang Q, Larson MG, Wang TJ, Trinquart L, McManus DD, Lubitz SA, Ellinor PT, Vasan RS, Gerszten RE, Benjamin EJ, Lin H. (2019). Proteomics Profiling and Risk of New-Onset Atrial Fibrillation: Framingham Heart Study. Open Access Publications by UMMS Authors. <https://doi.org/10.1161/JAHA.118.010976>. Retrieved from <https://escholarship.umassmed.edu/oapubs/3793>

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# Proteomics Profiling and Risk of New-Onset Atrial Fibrillation: Framingham Heart Study

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**Background**—Prior studies relating proteomics markers to incident AF screened for limited numbers of proteins.

**Methods and Results**—We performed proteomics assays among participants from the Framingham Heart Study Offspring attending their fifth examination. Plasma protein levels ( $n=1373$ ) were measured by the SOMAscan proteomic profiling platform. We used robust inference for the Cox proportional hazards model to relate each protein level with incident AF. In addition, we examined the association between AF-related genetic loci and levels of proteins associated with AF. Our study included 1885 participants (mean age  $55\pm 10$  years, 54% women) who had proteomic profiles measured. A total of 349 participants developed AF during follow-up (mean follow-up 18.3 years). We observed that 8 proteins were significantly associated with incident AF after adjusting for age, sex, technical covariates, and correction for multiple testing ( $P<0.05/1373=3.6\times 10^{-5}$ ). After additional adjustments for clinical factors associated with AF, ADAMTS13 and N-terminal pro-B-type natriuretic peptide remained significantly associated with the risk of incident AF (hazard ratio, 0.78; 95% CI, 0.70–0.88; and 1.44; 95% CI, 1.22–1.70, respectively;  $P<3.6\times 10^{-5}$  for both). None of the 8 proteins were encoded by genes at AF-related genetic loci previously identified by genome-wide association studies.

**Conclusions**—We identified 8 proteins associated with risk of incident AF after adjustment for age and sex; 2 proteins were associated with AF after adjustment for AF risk factors. Future studies are needed to replicate our findings, identify whether the markers are mechanistically related to AF development, and whether they are clinically useful for identification of future AF risk. (*J Am Heart Assoc.* 2019;8:e010976. DOI: 10.1161/JAHA.118.010976.)

**Key Words:** atrial fibrillation • biomarker • proteomics • risk

Approximately 1 in 3 whites and 1 in 5 blacks will develop atrial fibrillation (AF) in their lifetime.<sup>1–3</sup> The risk of AF increases with advancing age, European ancestry, smoking, higher height, weight, and blood pressure, antihypertensive medication use, diabetes mellitus, and history of myocardial infarction and heart failure.<sup>4</sup> Independent of traditional clinical factors, various biomarkers have been identified in relation to the risk of incident AF including markers of myocardial

necrosis,<sup>5–7</sup> myocardial stress,<sup>7–13</sup> inflammation,<sup>7,14–16</sup> oxidative stress,<sup>17</sup> and mineral metabolism.<sup>18,19</sup> Identification of novel biomarkers may advance our understanding of disease mechanisms, enhance opportunities for risk prediction, and potentially provide new therapeutic targets for AF.

Proteomic profiling enables systematic high-throughput analysis of proteins and holds the promise to substantially accelerate novel biomarker discovery. Relatively unbiased

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Accompanying Tables S1 through S6 are available at <https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.010976>

This article was handled independently by N.A. Marc Estes, III, MD, as a guest editor.

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Received September 14, 2018; accepted February 4, 2019.

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## Clinical Perspective

### What Is New?

- In a community-based prospective cohort study, we identified novel biomarkers associated with incident atrial fibrillation after screening for 1373 proteins using proteomics profiling.
- Both ADAMTS13 and N-terminal pro-B-type natriuretic peptide remained significantly associated with incident atrial fibrillation after multivariable adjustment and Bonferroni correction.

### What Are the Clinical Implications?

- Identification of ADAMTS13 may suggest a novel pathophysiological mechanism or a risk marker for atrial fibrillation.

proteomics approaches have the advantage of allowing simultaneous screening for large numbers of proteins involved in different biological pathways. Recently, 2 longitudinal cohort studies have reported proteomic profiling and the risk of new-onset AF.<sup>13,14</sup> The first study used a proximity extension assay to screen 92 proteins in community-based cohorts of older adults in Sweden and identified 7 proteins that were associated with incident AF after adjustment for age and sex.<sup>13</sup> The second study focused on 75 inflammatory marker proteins identified from proximity extension assays, none of which were associated with new-onset AF after age and sex adjustment.<sup>14</sup> In our current study, we report the use of single-stranded DNA-based aptamers as affinity reagents to screen for 1373 proteins and identify novel biomarkers that are associated with risk of incident AF in a prospective cohort study.

## Methods

### Data Sharing

The results of proteomic assay for all 1373 proteins measured in the Framingham Heart Study are available in the database of Genotypes and Phenotypes.<sup>20</sup> Additional results and analyses not shown in the article are available from the authors upon request. The details of the commercially available aptamer-based proteomics assay are included in the article. Noncommercial study materials are available to other researchers for purposes of reproducing the results or replicating the procedure, as respective Institutional Review Board and Material Transfer Agreements permit.

### Study Samples

The Framingham Heart Study is a community-based cohort initiated in 1948. The Framingham Offspring cohort (Second Generation) was recruited in 1971. They are the offspring and

the spouses of the offspring of the Original cohort.<sup>21</sup> The present study focused on 1885 Offspring cohort participants who attended the fifth examination (1991–1995) and completed an assessment of proteomics profiling. Twenty-eight individuals with AF at baseline were excluded after proteomics profiling. All participants gave written informed consent. The study was approved by the Institutional Review Boards of Boston University Medical Center, Massachusetts General Hospital, and Beth Israel Deaconess Medical Center. All aspects of the study were performed in compliance with relevant guidelines and regulations.

### Covariate Assessments

Current smoking was defined as smoking  $\geq 1$  cigarettes per day within 1 year preceding the Framingham Heart Study visit, similar to our previously studies.<sup>1</sup> Systolic and diastolic blood pressures were measured twice with subjects in the seated position. Participants were classified as having diabetes mellitus if their blood glucose was  $\geq 200$  mg/dL or fasting glucose was  $\geq 126$  mg/dL or if they were using insulin or oral hypoglycemic medications.<sup>22</sup> A panel of 3 physicians determined myocardial infarction and heart failure based on the criteria used previously.<sup>23</sup>

### AF Ascertainment

The AF status was ascertained through multiple sources in the Framingham Heart Study. A 12-lead ECG was conducted on each participant during clinic visits scheduled every 4 to 8 years. The cardiovascular history was also solicited during surveillance interviews biennially,<sup>24,25</sup> and from hospitalizations related to cardiovascular disease and clinician visits. All ECGs, including the Framingham Heart Study ECG, and other electrocardiographic data (ie, Holter monitoring) performed for clinical reasons, were reviewed by at least 2 cardiologists to adjudicate incident AF. Participants with prevalent AF at baseline were excluded from the analysis.

### Proteomics Profiling

Details of proteomics profiling have been described previously.<sup>26,27</sup> In brief, citrate-plasma was obtained from blood samples that were collected during clinical visits and stored at  $-80^{\circ}\text{C}$ .<sup>28</sup> Protein levels in the plasma samples were measured by the SOMAscan platform, which uses single-stranded DNA-based aptamers to capture conformational protein epitopes.<sup>29</sup> The technology has been validated in a study of cardiovascular disease.<sup>26,30</sup> Samples were assayed in 2 batches ( $n=821$  and  $1092$ , respectively). The measurements were  $\log_e$  transformed and standardized to mean=0 and SD=1 in each batch separately after adjusting for age and sex. The distribution of the significant  $\log_e$ -transformed protein

concentrations is shown in Table S1. We previously published median intra-assay coefficient of correlation of 8.2% and interassay coefficient of correlation of 7.8% using the same assay in the Framingham Heart Study.<sup>26</sup> A total of 1373 proteins were assayed.

## Statistical Analyses

Baseline characteristics were described as mean±SD for continuous covariates and counts (%) for dichotomous covariates. Our primary analysis tested the association between protein level and incident AF. Cox proportional hazards regression models with clustering on pedigrees and robust sandwich estimators were used to relate each protein level to incident AF (censored at the last follow-up time or death). The analysis was adjusted for age and sex. In addition, we conducted a multivariable analysis adjusted for previously reported AF risk factors,<sup>4</sup> including smoking, height, weight, systolic blood pressure, diastolic blood pressure, antihypertensive treatment, diabetes mellitus, prevalent myocardial infarction, and prevalent heart failure. In an exploratory analysis, we performed a forward selection analysis by first adjusting for height and weight, followed by systolic and diastolic blood pressures, and finally by adjusting for the rest of covariates. Given the difference in the protein profiling batches, the 2 batches were analyzed separately, and the summary results from both batches were meta-analyzed using the inverse-variance weighting approach. Bonferroni correction was used to correct for multiple testing; we considered  $P < 0.05 / 1373 = 3.64 \times 10^{-5}$  statistically significant.

## Association With AF-Related Genetic Loci

We also examined the association between AF-related genetic loci and the circulating proteins that were significantly associated with incident AF after age- and sex-adjustment, and Bonferroni correction. The analysis was restricted to the 97 genetic loci that were previously reported to associate with AF susceptibility by genome-wide association studies.<sup>31–34</sup> Linear mixed effects regression models were used to test the association between each genetic variant and protein levels, in which protein levels were treated as the dependent measures and genetic variants were treated as the predictors. The analysis was adjusted for age, sex, and the family structure in the Framingham Heart Study. We used Bonferroni correction to account for multiple testing, and the significance was claimed if the *P* value of the association was  $> 0.05 / (\text{number of proteins} \times 97 \text{ single nucleotide polymorphisms})$ .

## Results

The descriptive characteristics of the 1885 participants are provided in Table 1. The mean age of the sample was

55±10 years and 54% were women. A total of 349 participants developed new-onset AF during follow-up (mean follow-up 18.3 years).

## Association of Protein Levels With Incident AF

Table 2 reports 8 proteins that were significantly associated with incident AF after age and sex adjustment and correction for multiple testing ( $P < 3.64 \times 10^{-5}$ ). The concentration distribution of these proteins is shown in Table S1. Six were associated with decreased risk and 2 were associated with increased risk of incident AF. Neural cell adhesion molecule 1 to 120-kDa isoform had the most significant association. After further adjusting for clinical factors associated with incident AF, a disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAMTS13) protein and N-terminal pro-B-type natriuretic peptide remained significantly associated with risk of incident AF. In exploratory analyses with forward covariate selection, the remaining 6 proteins were no longer significant after adjusting for weight and height (Table S2).

Among the 349 incident AF/atrial flutter cases in our study, 39 participants had atrial flutter. We performed a sensitivity analysis by excluding the atrial flutter sample, and the association remained largely the same (Table S3).

## Association of AF-Related Genetic Variants With Protein Levels

We also examined whether any of the 8 circulating proteins that were significantly associated with incident AF were also associated with any of the reported 97 AF-related genetic

**Table 1.** Baseline Characteristics of Study Sample by Whether or Not Participants Developed Incident AF

Variable	AF Cases (n=349)	Referents (N=1536)
Age, y	61±9	54±10
Women	144 (41.3%)	872 (56.8%)
Height, cm	169±10	167±9
Weight, kg	82±17	76±16
Current smoker	52 (14.9%)	315 (20.5%)
Systolic blood pressure, mm Hg	134±20	125±18
Diastolic blood pressure, mm Hg	75±10	74±10
Antihypertensive medication use	117 (33.7%)	241 (15.8%)
Diabetes mellitus	49 (14.0%)	94 (6.1%)
Prevalent heart failure	1 (0.3%)	4 (0.3%)
Prevalent myocardial infarction	19 (5.4%)	31 (2.0%)

AF indicates atrial fibrillation. Values are n (%), or mean±SD.

**Table 2.** Protein Biomarkers Associated With Incident AF

Protein	Age and Sex Adjusted		Multivariable Adjusted*	
	HR (95% CI) <sup>†</sup>	P Value <sup>‡</sup>	HR (95% CI) <sup>†</sup>	P Value <sup>‡</sup>
NCAM-120	0.74 (0.67–0.82)	$4.29 \times 10^{-8}$	0.84 (0.74–0.95)	$5.20 \times 10^{-3}$
WFIKKN2 (WFKN2)	0.75 (0.67–0.83)	$1.58 \times 10^{-7}$	0.86 (0.76–0.96)	$1.09 \times 10^{-2}$
Ntrk3 (TrkC)	0.75 (0.68–0.84)	$6.06 \times 10^{-7}$	0.82 (0.73–0.92)	$9.90 \times 10^{-4}$
EGFR (ERBB, ERBB1)	0.75 (0.67–0.84)	$1.18 \times 10^{-6}$	0.82 (0.72–0.93)	$1.48 \times 10^{-3}$
ADAMTS13 (ATS13)	0.77 (0.69–0.86)	$2.23 \times 10^{-6}$	0.78 (0.70–0.88)	$1.75 \times 10^{-5}$
Angiopoietin-2	1.27 (1.15–1.41)	$3.09 \times 10^{-6}$	1.16 (1.04–1.31)	$1.09 \times 10^{-2}$
NT-proBNP <sup>§</sup>	1.44 (1.24–1.69)	$4.17 \times 10^{-6}$	1.44 (1.22–1.70)	$1.46 \times 10^{-5}$
BMPR1A	0.75 (0.66–0.85)	$5.93 \times 10^{-6}$	0.82 (0.72–0.93)	$2.32 \times 10^{-3}$

ADAMTS13 indicates a disintegrin and metalloproteinase with thrombospondin motifs 13; AF, atrial fibrillation; BMPR1A, bone morphogenetic protein receptor type-1A; EGFR, epidermal growth factor receptor; HR, hazard ratio; NCAM-120, neural cell adhesion molecule 1, 120 kDa isoform; NT-proBNP, N-terminal pro-brain natriuretic peptide; TrkC, tropomyosin receptor kinase C; WFKN2, WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2.

\*Covariates include smoking, height, weight, systolic blood pressure, diastolic blood pressure, antihypertensive treatment, diabetes mellitus, prevalent myocardial infarction, and prevalent heart failure.

<sup>†</sup>Hazard ratio expressed per standard deviation of the protein concentration.

<sup>‡</sup>Significance level of  $P < 0.05 / 1373 = 3.64 \times 10^{-5}$ .

<sup>§</sup>This protein was only measured in 1075 samples because of differences in SOMAscan platform between Batch 1 and Batch 2.

loci.<sup>31–33</sup> None of the 776 associations (8 proteins  $\times$  97 single nucleotide polymorphisms) reached the significance cutoff ( $P < 6.44 \times 10^{-5}$ ). The top 20 associations out of 776 are listed in Table S4. In addition, none of the 8 proteins were encoded by the AF-related genetic loci.

## Discussion

In our community-based prospective cohort study, we tested 1373 plasma proteins and observed that 8 proteins were associated with risk of incident AF after adjustment for age and sex. The currently known biological functions of the 8 proteins are described in Table S5. Both ADAMTS13 and N-terminal pro-B-type natriuretic peptide remained significantly associated with incident AF after multivariable adjustment and Bonferroni correction.

N-terminal pro-B-type natriuretic peptide, a marker of ventricular remodeling, previously has been reported to be associated with incident AF by multiple prospective population-based studies.<sup>8–13</sup> ADAMTS13 is a von Willebrand factor protease, and its deficiency is found in thrombotic thrombocytopenic purpura. Previous case-control studies have shown that lower ADAMTS13 protein level was associated with chronic and paroxysmal AF.<sup>35</sup> In addition, higher von Willebrand factor/ADAMTS13 ratio was significantly associated with chronic AF and left atrial remodeling<sup>35</sup> and higher von Willebrand factor/ADAMTS13 ratio drawn 24 hours after cardioversion was associated with higher risk of AF recurrence.<sup>36</sup> It is possible that atrial remodeling, which promotes AF, also promotes prothrombotic milieu; ADAMTS13 may be a marker of the prothrombotic environment. Alternatively, the

prothrombotic dysregulation as represented by decreased levels of ADAMTS13 may directly promote AF formation. Interestingly, prior prospective cohort studies have shown that ADAMTS13 is associated with incident myocardial infarction and ischemic stroke.<sup>37,38</sup> Whether ADAMTS13 dysregulation leads to the thrombotic events or AF was unrecognized in these studies merits further investigation.

Of the remaining 6 proteins that were significantly associated with incident AF after adjusting for age and sex, BMPR1A and angiopoietin-2 may be of particular interest in cardiovascular research. BMP ligands and receptors including BMPR1A are essential for embryonic and cardiac development, and mutations in the proteins are associated with pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia.<sup>39</sup> In addition, recent work has shown that the BMP signaling pathway plays an important role in the development of atherosclerosis and myocardial remodeling.<sup>39–41</sup> Angiopoietins are endothelial growth factors that regulate angiogenesis and vascular function. Increased levels of angiopoietin-2 have been observed in patients with myocardial infarction,<sup>42</sup> heart failure,<sup>43</sup> peripheral artery disease,<sup>44</sup> and end-stage renal disease.<sup>45</sup> The results of our study may suggest a novel downstream effect of BMPR1A and angiopoietin-2.

To date, there have been 2 longitudinal cohort studies that have used the *proteomic approach to study protein biomarkers* associated with incident AF.<sup>13,14</sup> The Swedish cohort study<sup>13</sup> used the Olink Proseek Multiplex Cardiovascular 96  $\times$  96 kit to screen for 92 proteins. The study sample included 2 community-based samples from Uppsala, Sweden: the Prospective Investigation of the Vasculature in Uppsala

Seniors (PIVUS) and the Uppsala Longitudinal Study of Adult Men (ULSAM) cohorts. In PIVUS (mean age 70 years, 51% women), there were 148 incident AF cases among 978 participants during the median follow-up of 10 years. In ULSAM (mean age 77.5 years, 0% women), there were 123 incident AF cases among 725 participants during the median follow-up of 7.9 years. In the combined analysis of both cohorts, the study found 7 proteins to be associated with the risk of incident AF after age and sex adjustment. Two of the 7 proteins, N-terminal pro-B-type natriuretic peptide and IL-6, remained significantly associated after multivariable adjustment in the Swedish cohort study (Table S6). The second cohort study used a community-based sample from Bruneck, Italy<sup>14</sup> (mean age 58.8 years, 49% women) to screen specifically for inflammatory biomarkers using the Olink Proseek Multiplex Cardiovascular 96×96 and the Proseek Multiple Inflammation I 96×96 kits. There were 117 new AF cases among 880 participants during 20-year follow-up. The Italian study reported the results of 75 inflammatory biomarkers including fibroblast growth factor-23, IL-6, fatty acid binding protein 4, none of which were associated with AF after age and sex adjustment in their sample (Table S6). In our study, we did not find evidence of nominally or Bonferroni corrected statistically significant association for fibroblast growth factor-23 or IL-6.

In our study, we did not find evidence of a nominally or Bonferroni corrected statistically significant association for previously reported AF-related proteins from *nonproteomic immunoassays* including troponin,<sup>5–7</sup> C-reactive protein,<sup>11,12,14</sup> vascular cell adhesion molecule 1,<sup>14</sup> fibroblast growth factor-23,<sup>18,19</sup> and IL-6<sup>46</sup> (Table S6). Various factors could have contributed to the discrepancy. In the current study, we tested 1373 proteins in a modest-size cohort; modest power and accounting for multiple testing may have led to false-negative findings. Differences in the characteristics of the cohorts could be another important factor. We measured the proteins levels at a relatively young age (55±10 years versus 70 and 77 years for the 2 cohorts in the Swedish study<sup>13</sup>) and had a relatively long follow-up period (mean 18.3 years versus median 7.9 and 10 years for the 2 cohorts in the Swedish study<sup>13</sup>). Previous studies measured protein levels using targeted single protein immunoassays or proximity extension assay; it may be challenging to compare them directly with the current study because of differences in sensitivity and specificity of the different assays. Further investigation is necessary to understand the variations between different assays in capturing different protein isoforms.

Our study has several limitations. We combined atrial flutter and AF despite their distinct electrophysiological differences in disease mechanisms. We also acknowledge that we were limited in our ability to correctly classify paroxysmal versus persistent AF, and hence we did not

distinguish AF subtypes in our AF outcome. New-onset AF may have been underreported in our study because we did not have continuous ECG monitoring, and AF is often paroxysmal and asymptomatic. Protein levels were measured at a single time point, and therefore we were unable to test whether changes in protein concentrations over time are associated with development of new-onset AF. The lack of association between the protein levels and the AF-related genetic variants may be in part because of lack of power and false-negative findings because of correction for multiple testing but also because of the fact that the proteins are likely regulated by multiple genetic loci. Another possible reason may include the heterogeneous nature of AF mechanisms such as genetic predisposition to AF, atrial fibrosis, prothrombotic dysregulation, and other mechanisms.<sup>47</sup> In addition, the fact that the 8 proteins were not encoded by the AF-related genetic variants suggests that these proteins might not be causal to AF pathogenesis. For instance, they might be biomarkers for underlying AF mechanisms or subclinical AF. Future investigation such as a gene-annotation approach would be helpful to test our proteins on a genomic level and better understand the connection between genetic loci and protein concentration.<sup>48</sup>

Our study cohort was predominantly of European ancestry, and our results may not be generalizable to other races or ethnicities. In addition, as with any high-throughput “-omics” studies, batch effects may limit reproducibility of our results. Finally, although our proteomics platform is the one of the largest to date in cardiovascular research, we were only able to detect proteins that were included in the platform.

In conclusion, in our proteomics screening of 1373 proteins in a longitudinal cohort study, we found 8 proteins to be associated with the risk of incident AF after adjustment for age and sex, and 2 proteins were associated with multivariable adjustment. Further studies are needed to replicate our findings and identify the pathophysiological mechanisms underlying the reported associations and their clinical implications.

## Sources of Funding

The study was supported by NIH contracts HHSN268 201500001I and N01-HC 25195 to the Framingham Heart Study. Dr Benson is supported by the John S LaDue Memorial Fellowship at Harvard Medical School. Drs Trinquart and Lubitz are supported by American Heart Association 18SFRN34250007. Dr Lubitz is also supported by NIH grant 1R01HL139731 and a Doris Duke Charitable Foundation Clinical Scientist Development Award 2014105. Dr McManus is supported by NIH grants 5R01HL126911, 1R01HL137734, 1R01HL137794, 5R01HL135219-02, and 5UH3TR000921-04, as well as NSF grant NSF-12-512. Dr. Lin is supported in

part by the Boston University Digital Health Initiative, Boston University Alzheimer's Disease Center Pilot Grant, and the National Center for Advancing Translational Sciences, National Institutes of Health, through BU-CTSI Grant Number 1UL1TR001430. Dr Vasani is supported in part by the Evans Medical Foundation and Jay and Louis Coffman Endowment, Boston University School of Medicine. Drs Wang and Gerszten are supported by NIH grants 5R01DK081572-10, 5R01HL132320-03, and 5R01DK108159-04. Drs Benjamin and Ellinor are supported by NIH grants 1R01HL128914; 2R01HL092577; and American Heart Association 18SFRN341100825.

## Disclosures

Dr Ellinor is the PI on a grant from Bayer to the Broad Institute focused on the genetics and therapeutics of AF. Dr Lubitz receives sponsored research support from Bristol Myers Squibb, Bayer HealthCare, Biotronik, and Boehringer Ingelheim, and has consulted for Abbott, Quest Diagnostics, and Bristol Myers Squibb. Dr McManus receives sponsored research support from Bristol Myers Squibb, Pfizer, Biotronik, Boehringer Ingelheim, Apple, and has consulted for Bristol Myers Squibb, Pfizer, Samsung Electronics, FlexCon, Rose Consultants, and has inventor equity in Mobile Sense Technologies, LLC. The remaining authors have no disclosures to report.

## References

- Weng LC, Preis SR, Hulme OL, Larson MG, Choi SH, Wang B, Trinquart L, McManus DD, Staerk L, Lin H, Lunetta KL, Ellinor PT, Benjamin EJ, Lubitz SA. Genetic predisposition, clinical risk factor burden, and lifetime risk of atrial fibrillation. *Circulation*. 2018;137:1027–1038.
- Staerk L, Wang B, Preis SR, Larson MG, Lubitz SA, Ellinor PT, McManus DD, Ko D, Weng LC, Lunetta KL, Frost L, Benjamin EJ, Trinquart L. Lifetime risk of atrial fibrillation according to optimal, borderline, or elevated levels of risk factors: cohort study based on longitudinal data from the Framingham Heart Study. *BMJ*. 2018;361:k1453.
- Mou L, Norby FL, Chen LY, O'Neal WT, Lewis TT, Loehr LR, Soliman EZ, Alonso A. Lifetime risk of atrial fibrillation by race and socioeconomic status: ARIC Study (Atherosclerosis Risk in Communities). *Circ Arrhythm Electrophysiol*. 2018;11:e006350.
- Alonso A, Krijthe BP, Aspelund T, Stepas KA, Pencina MJ, Moser CB, Sinner MF, Sotoodehnia N, Fontes JD, Janssens AC, Kronmal RA, Magnani JW, Witteman JC, Chamberlain AM, Lubitz SA, Schnabel RB, Agarwal SK, McManus DD, Ellinor PT, Larson MG, Burke GL, Launer LJ, Hofman A, Levy D, Gottdiener JS, Kaab S, Couper D, Harris TB, Soliman EZ, Stricker BH, Gudnason V, Heckbert SR, Benjamin EJ. Simple risk model predicts incidence of atrial fibrillation in a racially and geographically diverse population: the CHARGE-AF consortium. *J Am Heart Assoc*. 2013;2:e000102. DOI: 10.1161/JAHA.112.000102.
- Filion KB, Agarwal SK, Ballantyne CM, Eberg M, Hoogeveen RC, Huxley RR, Loehr LR, Nambi V, Soliman EZ, Alonso A. High-sensitivity cardiac troponin T and the risk of incident atrial fibrillation: the Atherosclerosis Risk in Communities (ARIC) study. *Am Heart J*. 2015;169:31–38.e33.
- Hussein AA, Bartz TM, Gottdiener JS, Sotoodehnia N, Heckbert SR, Lloyd-Jones D, Kizer JR, Christenson R, Wazni O, deFilippi C. Serial measures of cardiac troponin T levels by a highly sensitive assay and incident atrial fibrillation in a prospective cohort of ambulatory older adults. *Heart Rhythm*. 2015;12:879–885.
- Rienstra M, Yin X, Larson MG, Fontes JD, Magnani JW, McManus DD, McCabe EL, Coglianese EE, Amponsah M, Ho JE, Januzzi Jr, Wollert KC, Fradley MG, Vasani RS, Ellinor PT, Wang TJ, Benjamin EJ. Relation between soluble ST2,

growth differentiation factor-15, and high-sensitivity troponin I and incident atrial fibrillation. *Am Heart J*. 2014;167:109–115.e102

- Patton KK, Ellinor PT, Heckbert SR, Christenson RH, DeFilippi C, Gottdiener JS, Kronmal RA. N-terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation: the Cardiovascular Health Study. *Circulation*. 2009;120:1768–1774.
- Patton KK, Heckbert SR, Alonso A, Bahrami H, Lima JA, Burke G, Kronmal RA. N-terminal pro-B-type natriuretic peptide as a predictor of incident atrial fibrillation in the Multi-Ethnic Study of Atherosclerosis: the effects of age, sex and ethnicity. *Heart*. 2013;99:1832–1836.
- Kara K, Geisel MH, Mohlenkamp S, Lehmann N, Kalsch H, Bauer M, Neumann T, Dragano N, Moebus S, Jockel KH, Erbel R, Mahabadi AA. B-type natriuretic peptide for incident atrial fibrillation-The Heinz Nixdorf Recall Study. *J Cardiol*. 2015;65:453–458.
- Sinner MF, Stepas KA, Moser CB, Krijthe BP, Aspelund T, Sotoodehnia N, Fontes JD, Janssens AC, Kronmal RA, Magnani JW, Witteman JC, Chamberlain AM, Lubitz SA, Schnabel RB, Vasani RS, Wang TJ, Agarwal SK, McManus DD, Franco OH, Yin X, Larson MG, Burke GL, Launer LJ, Hofman A, Levy D, Gottdiener JS, Kaab S, Couper D, Harris TB, Astor BC, Ballantyne CM, Hoogeveen RC, Arai AE, Soliman EZ, Ellinor PT, Stricker BH, Gudnason V, Heckbert SR, Pencina MJ, Benjamin EJ, Alonso A. B-type natriuretic peptide and C-reactive protein in the prediction of atrial fibrillation risk: the CHARGE-AF Consortium of community-based cohort studies. *Europace*. 2014;16:1426–1433.
- Schnabel RB, Larson MG, Yamamoto JF, Sullivan LM, Pencina MJ, Meigs JB, Tofler GH, Selhub J, Jacques PF, Wolf PA, Magnani JW, Ellinor PT, Wang TJ, Levy D, Vasani RS, Benjamin EJ. Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. *Circulation*. 2010;121:200–207.
- Lind L, Sundstrom J, Stenemo M, Hagstrom E, Arnlov J. Discovery of new biomarkers for atrial fibrillation using a custom-made proteomics chip. *Heart*. 2017;103:377–382.
- Willeit K, Pechlaner R, Willeit P, Skroblin P, Paulweber B, Scherthaner C, Toell T, Egger G, Weger S, Oberhollenzer M, Kedenko L, Iglseder B, Bonora E, Schett G, Mayr M, Willeit J, Kiechl S. Association between vascular cell adhesion molecule 1 and atrial fibrillation. *JAMA Cardiol*. 2017;2:516–523.
- Guo Y, Lip GY, Apostolakis S. Inflammation in atrial fibrillation. *J Am Coll Cardiol*. 2012;60:2263–2270.
- Schnabel RB, Larson MG, Yamamoto JF, Kathiresan S, Rong J, Levy D, Keaney JF Jr, Wang TJ, Vasani RS, Benjamin EJ. Relation of multiple inflammatory biomarkers to incident atrial fibrillation. *Am J Cardiol*. 2009;104:92–96.
- Tahhan AS, Sandesara PB, Hayek SS, Alkhorayji A, Chivukula K, Hammadah M, Mohamed-Kelli H, O'Neal WT, Topel M, Ghasemzadeh N, Ko YA, Aida H, Gafeer M, Sperling L, Vaccarino V, Liang Y, Jones DP, Quyyumi AA. Association between oxidative stress and atrial fibrillation. *Heart Rhythm*. 2017;14:1849–1855.
- Mathew JS, Sachs MC, Katz R, Patton KK, Heckbert SR, Hoofnagle AN, Alonso A, Chonchol M, Deo R, Ix JH, Siscovick DS, Kestenbaum B, de Boer IH. Fibroblast growth factor-23 and incident atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS). *Circulation*. 2014;130:298–307.
- Alonso A, Misialek JR, Eckfeldt JH, Selvin E, Coresh J, Chen LY, Soliman EZ, Agarwal SK, Lutsey PL. Circulating fibroblast growth factor-23 and the incidence of atrial fibrillation: the Atherosclerosis Risk in Communities study. *J Am Heart Assoc*. 2014;3:e001082. DOI: 10.1161/JAHA.114.001082.
- Mailman MD, Feolo M, Jin Y, Kimura M, Tryka K, Bagoutdinov R, Hao L, Kiang A, Paschall J, Phan L, Popova N, Pretel S, Ziyabari L, Lee M, Shao Y, Wang ZY, Sirotkin K, Ward M, Kholodov M, Zbicz K, Beck J, Kimelman M, Shevelev S, Preuss D, Yaschenko E, Graeff A, Ostell J, Sherry ST. The NCBI dbGaP database of genotypes and phenotypes. *Nat Genet*. 2007;39:1181–1186.
- Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. *Am J Epidemiol*. 1979;110:281–290.
- Rutter MK, Parise H, Benjamin EJ, Levy D, Larson MG, Meigs JB, Nesto RW, Wilson PW, Vasani RS. Impact of glucose intolerance and insulin resistance on cardiac structure and function: sex-related differences in the Framingham Heart Study. *Circulation*. 2003;107:448–454.
- McKee PA, Castelli WP, McNamara PM, Kannel WB. The natural history of congestive heart failure: the Framingham study. *N Engl J Med*. 1971;285:1441–1446.
- Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. *Stroke*. 1991;22:983–988.
- Piccini JP, Hammill BG, Sinner MF, Jensen PN, Hernandez AF, Heckbert SR, Benjamin EJ, Curtis LH. Incidence and prevalence of atrial fibrillation and associated mortality among Medicare beneficiaries, 1993–2007. *Circ Cardiovasc Qual Outcomes*. 2012;5:85–93.

26. Ngo D, Sinha S, Shen D, Kuhn EW, Keyes MJ, Shi X, Benson MD, O'Sullivan JF, Keshishian H, Farrell LA, Fifer MA, Vasan RS, Sabatine MS, Larson MG, Carr SA, Wang TJ, Gerszten RE. Aptamer-based proteomic profiling reveals novel candidate biomarkers and pathways in cardiovascular disease. *Circulation*. 2016;134:270–285.
27. Hathout Y, Brody E, Clemens PR, Cripe L, DeLisle RK, Furlong P, Gordish-Dressman H, Hache L, Henricson E, Hoffman EP, Kobayashi YM, Lorts A, Mah JK, McDonald C, Mehler B, Nelson S, Nikrad M, Singer B, Steele F, Sterling D, Sweeney HL, Williams S, Gold L. Large-scale serum protein biomarker discovery in Duchenne muscular dystrophy. *Proc Natl Acad Sci USA*. 2015;112:7153–7158.
28. Benson MD, Yang Q, Ngo D, Zhu Y, Shen D, Farrell LA, Sinha S, Keyes MJ, Vasan RS, Larson MG, Smith JG, Wang TJ, Gerszten RE. Genetic architecture of the cardiovascular risk proteome. *Circulation*. 2018;137:1158–1172.
29. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, Carter J, Dalby AB, Eaton BE, Fitzwater T, Flather D, Forbes A, Foreman T, Fowler C, Gawande B, Goss M, Gunn M, Gupta S, Halladay D, Heil J, Heilig J, Hicke B, Husar G, Janjic N, Jarvis T, Jennings S, Katilius E, Keeney TR, Kim N, Koch TH, Kraemer S, Kroiss L, Le N, Levine D, Lindsey W, Lollo B, Mayfield W, Mehan M, Mehler R, Nelson SK, Nelson M, Nieuwlandt D, Nikrad M, Ochsner U, Ostroff RM, Otis M, Parker T, Pietrasiewicz S, Resnicow DJ, Rohloff J, Sanders G, Sattin S, Schneider D, Singer B, Stanton M, Sterkel A, Stewart A, Stratford S, Vaught JD, Vrkljan M, Walker JJ, Watrobka M, Waugh S, Weiss A, Wilcox SK, Wolfson A, Wolk SK, Zhang C, Zichi D. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One*. 2010;5:e15004.
30. Ganz P, Heidecker B, Hveem K, Jonasson C, Kato S, Segal MR, Sterling DG, Williams SA. Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. *JAMA*. 2016;315:2532–2541.
31. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Muller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dorr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagener DR, Magnani JW, Wakili R, Clauss S, Rotter J, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Volker U, Volzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE, Witteman JC, Kao WH, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjogren M, Newman AB, Liu Y, Gollob MH, Melander O, Tanaka T, Stricker BH, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kaab S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet*. 2012;44:670–675.
32. Sinner MF, Tucker NR, Lunetta KL, Ozaki K, Smith JG, Trompet S, Bis JC, Lin H, Chung MK, Nielsen JB, Lubitz SA, Krijthe BP, Magnani JW, Ye J, Gollob MH, Tsunoda T, Muller-Nurasyid M, Lichtner P, Peters A, Dolmatova E, Kubo M, Smith JD, Psaty BM, Smith NL, Jukema JW, Chasman DI, Albert CM, Ebana Y, Furukawa T, Macfarlane PW, Harris TB, Darbar D, Dorr M, Holst AG, Svendsen JH, Hofman A, Uitterlinden AG, Gudnason V, Isobe M, Malik R, Dichgans M, Rosand J, Van Wagener DR, Consortium M, Consortium AF, Benjamin EJ, Milan DJ, Melander O, Heckbert SR, Ford I, Liu Y, Barnard J, Olesen MS, Stricker BH, Tanaka T, Kaab S, Ellinor PT. Integrating genetic, transcriptional, and functional analyses to identify 5 novel genes for atrial fibrillation. *Circulation*. 2014;130:1225–1235.
33. Christophersen IE, Rienstra M, Roselli C, Yin X, Geelhoed B, Barnard J, Lin H, Arking DE, Smith AV, Albert CM, Chaffin M, Tucker NR, Li M, Klarin D, Bihlmeyer NA, Low SK, Weeke PE, Muller-Nurasyid M, Smith JG, Brody JA, Niemeijer MN, Dorr M, Trompet S, Huffman J, Gustafsson S, Schurmann C, Kleber ME, Lyytikainen LP, Seppala I, Malik R, Horimoto A, Perez M, Sinisalo J, Aeschbacher S, Theriault S, Yao J, Radmanesh F, Weiss S, Teumer A, Choi SH, Weng LC, Clauss S, Deo R, Rader DJ, Shah SH, Sun A, Hopewell JC, Debette S, Chauhan G, Yang Q, Worrall BB, Pare G, Kamatani Y, Hagemeyer YP, Verweij N, Siland JE, Kubo M, Smith JD, Van Wagener DR, Bis JC, Perz S, Psaty BM, Ridker PM, Magnani JW, Harris TB, Launer LJ, Shoemaker MB, Padmanabhan S, Haessler J, Bartz TM, Waldenberger M, Lichtner P, Arendt M, Krieger JE, Kahonen M, Risch L, Mansur AJ, Peters A, Smith BH, Lind L, Scott SA, Lu Y, Bottinger EB, Hernesniemi J, Lindgren CM, Wong JA, Huang J, Eskola M, Morris AP, Ford I, Reiner AP, Delgado G, Chen LY, Chen YI, Sandhu RK, Li M, Boerwinkle E, Eisele L, Lannfelt L, Rost N, Anderson CD, Taylor DM, Campbell A, Magnusson PK, Porteous D, Hocking LJ, Vlachopoulou E, Pedersen NL, Nikus K, Orho-Melander M, Hamsten A, Heeringa J, Denny JC, Kriebel J, Darbar D, Newton-Cheh C, Shaffer C, Macfarlane PW, Heilmann-Heimbach S, Almgren P, Huang PL, Sotoodehnia N, Soliman EZ, Uitterlinden AG, Hofman A, Franco OH, Volker U, Jockel KH, Sinner MF, Lin HJ, Guo X; ISGC MCot, Neurology Working Group of the CC, Dichgans M, Ingelsson E, Kooperberg C, Melander O, Loos R, Laurikka J, Conen D, Rosand J, van der Harst P, Lokki ML, Kathiresan S, Pereira A, Jukema JW, Hayward C, Rotter J, Marz W, Lehtimäki T, Stricker BH, Chung MK, Felix SB, Gudnason V, Alonso A, Roden DM, Kaab S, Chasman DI, Heckbert SR, Benjamin EJ, Tanaka T, Lunetta KL, Lubitz SA, Ellinor PT; Consortium AF. Large-scale analyses of common and rare variants identify 12 new loci associated with atrial fibrillation. *Nat Genet*. 2017;49:946–952.
34. Roselli C, Chaffin MD, Weng LC, Aeschbacher S, Ahlberg G, Albert CM, Almgren P, Alonso A, Anderson CD, Aragam KG, Arking DE, Barnard J, Bartz TM, Benjamin EJ, Bihlmeyer NA, Bis JC, Bloom HL, Boerwinkle E, Bottinger EB, Brody JA, Calkins H, Campbell A, Cappola TP, Carlquist J, Chasman DI, Chen LY, Chen YI, Choi EK, Choi SH, Christophersen IE, Chung MK, Cole JW, Conen D, Cook J, Crijns HJ, Cutler MJ, Damrauer SM, Daniels BR, Darbar D, Delgado G, Denny JC, Dichgans M, Dorr M, Dudink EA, Dudley SC, Esa N, Eskola M, Fatkin D, Felix SB, Ford I, Franco OH, Geelhoed B, Grewal RP, Gudnason V, Guo X, Gupta N, Gustafsson S, Gutmann R, Hamsten A, Harris TB, Hayward C, Heckbert SR, Hernesniemi J, Hocking LJ, Hofman A, Horimoto A, Huang J, Huang PL, Huffman J, Ingelsson E, Ipek EG, Ito K, Jimenez-Conde J, Johnson R, Jukema JW, Kaab S, Kahonen M, Kamatani Y, Kane JP, Kastrati A, Kathiresan S, Katschnig-Winter P, Kavousi M, Kessler T, Kietselaer BL, Kirchhof P, Kleber ME, Knight S, Krieger JE, Kubo M, Launer LJ, Laurikka J, Lehtimäki T, Leineweber K, Lemaitre RN, Li M, Lim HE, Lin HJ, Lin H, Lind L, Lindgren CM, Lokki ML, London B, Loos R, Low SK, Lu Y, Lyytikäinen LP, Macfarlane PW, Magnusson PK, Mahajan A, Malik R, Mansur AJ, Marcus GM, Margolin A, Margulies KB, Marz W, McManus DD, Melander O, Mohanty S, Montgomery JA, Morley MP, Morris AP, Muller-Nurasyid M, Natale A, Nazarian S, Neumann B, Newton-Cheh C, Niemeijer MN, Nikus K, Nilsson P, Noordam R, Oellers H, Olesen MS, Orho-Melander M, Padmanabhan S, Pak HN, Pare G, Pedersen NL, Pera J, Pereira A, Porteous D, Psaty BM, Pulit SL, Pullinger CR, Rader DJ, Refsgaard L, Ribases M, Ridker PM, Rienstra M, Risch L, Roden DM, Rosand J, Rosenberg MA, Rost N, Rotter J, Saba S, Sandhu RK, Schnabel RB, Schramm K, Schunkert H, Schurmann C, Scott SA, Seppala I, Shaffer C, Shah S, Shalaby AA, Shim J, Shoemaker MB, Siland JE, Sinisalo J, Sinner MF, Slowik A, Smith AV, Smith BH, Smith JG, Smith JD, Smith NL, Soliman EZ, Sotoodehnia N, Stricker BH, Sun A, Sun H, Svendsen JH, Tanaka T, Tanriverdi K, Taylor KD, Teder-Laving M, Teumer A, Theriault S, Trompet S, Tucker NR, Tveit A, Uitterlinden AG, Van Der Harst P, Van Gelder IC, Van Wagener DR, Verweij N, Vlachopoulou E, Volker U, Wang B, Weeke PE, Weijs B, Weiss R, Weiss S, Wells QS, Wiggins KL, Wong JA, Woo D, Worrall BB, Yang PS, Yao J, Yoneda ZT, Zeller T, Zeng L, Lubitz SA, Lunetta KL, Ellinor PT. Multi-ethnic genome-wide association study for atrial fibrillation. *Nat Genet*. 2018;50:1225–1233.
35. Uemura T, Kaikita K, Yamabe H, Soejima K, Matsukawa M, Fuchigami S, Tanaka Y, Morihisa K, Enomoto K, Sumida H, Sugiyama S, Ogawa H. Changes in plasma von Willebrand factor and ADAMTS13 levels associated with left atrial remodeling in atrial fibrillation. *Thromb Res*. 2009;124:28–32.
36. Freynhofer MK, Bruno V, Jarai R, Gruber S, Hochtl T, Brozovic I, Farhan S, Wojta J, Huber K. Levels of von Willebrand factor and ADAMTS13 determine clinical outcome after cardioversion for atrial fibrillation. *Thromb Haemost*. 2011;105:435–443.
37. Sonneveld MA, Kavousi M, Ikram MA, Hofman A, Rueda Ochoa OL, Turecek PL, Franco OH, Leebeek FW, de Maat MP. Low ADAMTS-13 activity and the risk of coronary heart disease—a prospective cohort study: the Rotterdam Study. *J Thromb Haemost*. 2016;14:2114–2120.
38. Sonneveld MAH, de Maat MPM, Portegies MLP, Kavousi M, Hofman A, Turecek PL, Rottensteiner H, Schefflinger F, Koudstaal PJ, Ikram MA, Leebeek FW. Low ADAMTS13 activity is associated with an increased risk of ischemic stroke. *Blood*. 2015;126:2739–2746.
39. Morrell NW, Bloch DB, ten Dijke P, Goumans MJ, Hata A, Smith J, Yu PB, Bloch KD. Targeting BMP signalling in cardiovascular disease and anaemia. *Nat Rev Cardiol*. 2016;13:106–120.
40. Pachori AS, Custer L, Hansen D, Clapp S, Kempa E, Klingensmith J. Bone morphogenetic protein 4 mediates myocardial ischemic injury through JNK-dependent signaling pathway. *J Mol Cell Cardiol*. 2010;48:1255–1265.
41. Sun B, Huo R, Sheng Y, Li Y, Xie X, Chen C, Liu HB, Li N, Li CB, Guo WT, Zhu JX, Yang BF, Dong DL. Bone morphogenetic protein-4 mediates cardiac hypertrophy, apoptosis, and fibrosis in experimentally pathological cardiac hypertrophy. *Hypertension*. 2013;61:352–360.
42. Patel JV, Lim HS, Varughese GI, Hughes EA, Lip GY. Angiotensin-2 levels as a biomarker of cardiovascular risk in patients with hypertension. *Ann Med*. 2008;40:215–222.
43. Chong AY, Caine GJ, Freestone B, Blann AD, Lip GY. Plasma angiotensin-1, angiotensin-2, and angiotensin receptor tie-2 levels in congestive heart failure. *J Am Coll Cardiol*. 2004;43:423–428.
44. Findley CM, Mitchell RG, Duscha BD, Annex BH, Kontos CD. Plasma levels of soluble Tie2 and vascular endothelial growth factor distinguish critical limb ischemia from intermittent claudication in patients with peripheral arterial disease. *J Am Coll Cardiol*. 2008;52:387–393.
45. Shroff RC, Price KL, Kolatsi-Joannou M, Todd AF, Wells D, Deanfield J, Johnson RJ, Rees L, Woolf AS, Long DA. Circulating angiotensin-2 is a marker for early cardiovascular disease in children on chronic dialysis. *PLoS One*. 2013;8:e56273.



46. Wu N, Xu B, Xiang Y, Wu L, Zhang Y, Ma X, Tong S, Shu M, Song Z, Li Y, Zhong L. Association of inflammatory factors with occurrence and recurrence of atrial fibrillation: a meta-analysis. *Int J Cardiol.* 2013;169:62–72.
47. Fabritz L, Guasch E, Antoniades C, Bardinet I, Benninger G, Betts TR, Brand E, Breithardt G, Bucklar-Suchankova G, Camm AJ, Cartlidge D, Casadei B, Chua WW, Crijns HJ, Deeks J, Hatem S, Hidden-Lucet F, Kaab S, Maniadakis N, Martin S, Mont L, Reinecke H, Sinner MF, Schotten U, Southwood T, Stoll M, Vardas P, Wakili R, West A, Ziegler A, Kirchhof P. Expert consensus document: defining the major health modifiers causing atrial fibrillation: a roadmap to underpin personalized prevention and treatment. *Nat Rev Cardiol.* 2016;13:230–237.
48. Li MX, Gui HS, Kwan JS, Sham PC. GATES: a rapid and powerful gene-based association test using extended Simes procedure. *Am J Hum Genet.* 2011;88:283–293.

# Supplemental Material

**Table S1. Distribution of the significant log<sub>e</sub>-transformed protein concentration among samples.**

Protein	Batch 1 <sup>†</sup>		Batch 2 <sup>†</sup>	
	AF cases	Referents	AF cases	Referents
NCAM-120	8.30±0.20	8.30±0.20	9.19±0.21	9.25±0.20
WFKN2	7.79±0.28	7.82±0.26	8.33±0.28	8.43±0.29
TrkC	7.35±0.21	7.39±0.22	7.77±0.20	7.83±0.23
ERBB1	9.83±0.16	9.90±0.14	10.14±0.15	10.21±0.15
ADAMTS13	7.97±0.20	8.05±0.23	8.25±0.26	8.38±0.25
Angiopoietin-2	6.24±0.26	6.17±0.24	4.60±0.29	4.56±0.24
NT-proBNP <sup>§</sup>			8.37±0.78	8.06±0.73
BMPR1A	5.86±0.29	5.92±0.35	6.27±0.28	6.35±0.26

ADAMTS13: a disintegrin and metalloproteinase with thrombospondin motifs 13; BMPR1A: bone morphogenetic protein receptor type-1A; NCAM-120: Neural cell adhesion molecule 1, 120 kDa isoform; NT-proBNP: N-terminal pro-brain natriuretic peptide; SBP: systolic blood pressure; DBP: diastolic blood pressure; TrkC: tropomyosin receptor kinase C; WFKN2: WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2

<sup>†</sup>Data are expressed as mean ± SD

<sup>§</sup>This protein was only measured in Batch 2 samples due to differences in SOMAscan platform between Batch 1 and Batch 2.

**Table S2.** Stepwise forward selection analysis for the 8 proteins significantly associated incident AF after age and sex adjustment.

Protein	Adjusted for age and sex		Adjusted for age and sex, weight, height		Adjusted for age and sex, weight, height, SBP, DBP		Multivariable-adjusted model*	
	HR (95% CI) <sup>†</sup>	p value <sup>‡</sup>	HR (95% CI) <sup>†</sup>	p value <sup>‡</sup>	HR (95% CI) <sup>†</sup>	p value <sup>‡</sup>	HR (95% CI) <sup>†</sup>	p value <sup>‡</sup>
NCAM-120	0.74 (0.67-0.82)	4.29x10 <sup>-8</sup>	0.8 (0.71-0.89)	1.30x10 <sup>-4</sup>	0.81 (0.72-0.91)	4.83x10 <sup>-4</sup>	0.84 (0.74-0.95)	5.20x10 <sup>-3</sup>
WFKN2	0.75 (0.67-0.83)	1.58x10 <sup>-7</sup>	0.81 (0.72-0.91)	3.27x10 <sup>-4</sup>	0.83 (0.74-0.93)	1.62x10 <sup>-3</sup>	0.86 (0.76-0.96)	1.09x10 <sup>-2</sup>
TrkC	0.75 (0.68-0.84)	6.06x10 <sup>-7</sup>	0.8 (0.72-0.9)	2.19x10 <sup>-4</sup>	0.81 (0.72-0.91)	3.81x10 <sup>-4</sup>	0.82 (0.73-0.92)	9.90x10 <sup>-4</sup>
ERBB1	0.75 (0.67-0.84)	1.18x10 <sup>-6</sup>	0.78 (0.7-0.88)	6.88x10 <sup>-5</sup>	0.79 (0.7-0.89)	1.34x10 <sup>-4</sup>	0.82 (0.72-0.93)	1.48x10 <sup>-3</sup>
ADAMTS13	0.77 (0.69-0.86)	2.23x10 <sup>-6</sup>	0.77 (0.69-0.85)	1.70x10 <sup>-6</sup>	0.78 (0.7-0.87)	9.18x10 <sup>-6</sup>	0.78 (0.7-0.88)	1.75x10 <sup>-5</sup>
Angiopietin-2	1.27 (1.15-1.41)	3.09x10 <sup>-6</sup>	1.23 (1.11-1.37)	1.29x10 <sup>-4</sup>	1.2 (1.07-1.34)	1.12x10 <sup>-3</sup>	1.16 (1.04-1.31)	1.09x10 <sup>-2</sup>
NT-proBNP <sup>§</sup>	1.44 (1.24-1.69)	4.17x10 <sup>-6</sup>	1.53 (1.3-1.79)	1.51x10 <sup>-7</sup>	1.51 (1.29-1.78)	5.40x10 <sup>-7</sup>	1.44 (1.22-1.7)	1.46x10 <sup>-5</sup>
BMPR1A	0.75 (0.66-0.85)	5.93x10 <sup>-6</sup>	0.79 (0.7-0.9)	2.44x10 <sup>-4</sup>	0.8 (0.71-0.91)	7.01x10 <sup>-4</sup>	0.82 (0.72-0.93)	2.32x10 <sup>-3</sup>

ADAMTS13: a disintegrin and metalloproteinase with thrombospondin motifs 13; BMPR1A: bone morphogenetic protein receptor type-1A; NCAM-120: Neural cell adhesion molecule 1, 120 kDa isoform; NT-proBNP: N-terminal pro-brain natriuretic peptide; SBP: systolic blood pressure; DBP: diastolic blood pressure; TrkC: tropomyosin receptor kinase C; WFKN2: WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2

\*Covariates include smoking, height, weight, systolic blood pressure, diastolic blood pressure, antihypertensive treatment, diabetes mellitus, prevalent myocardial infarction, and prevalent heart failure

<sup>†</sup>Hazard ratio expressed per standard deviation of the protein concentration

<sup>‡</sup>Significance level of  $p < 0.05/1373 = 3.64 \times 10^{-5}$

<sup>§</sup>This protein was only measured in 1075 samples due to differences in SOMAscan platform between Batch 1 and Batch 2.

**Table S3. Association of protein biomarkers with incident AF after excluding atrial flutter (n=39).**

<b>Protein</b>	<b>HR (95% CI)<sup>†</sup></b>	<b>p value</b>
NCAM-120	0.73 (0.65-0.82)	6.44x10 <sup>-8</sup>
WFKN2	0.76 (0.68-0.86)	4.04x10 <sup>-6</sup>
TrkC	0.75 (0.67-0.84)	1.06x10 <sup>-6</sup>
ERBB1	0.74 (0.66-0.84)	1.46x10 <sup>-6</sup>
ADAMTS13	0.76 (0.68-0.86)	4.99x10 <sup>-6</sup>
Angiopoietin-2	1.29 (1.17-1.44)	8.57x10 <sup>-7</sup>
NT-proBNP <sup>§</sup>	1.51 (1.28-1.79)	1.47x10 <sup>-6</sup>
BMPR1A	0.75 (0.66-0.86)	2.38x10 <sup>-5</sup>

ADAMTS13: a disintegrin and metalloproteinase with thrombospondin motifs 13; BMPR1A: bone morphogenetic protein receptor type-1A; NCAM-120: Neural cell adhesion molecule 1, 120 kDa isoform; NT-proBNP: N-terminal pro-brain natriuretic peptide; SBP: systolic blood pressure; DBP: diastolic blood pressure; TrkC: tropomyosin receptor kinase C; WFKN2: WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2

<sup>†</sup>Hazard ratio expressed per standard deviation of the protein concentration

**Table S4. Top 20 associations out of 776 (8 proteins \* 97 SNPs) between the 8 proteins identified in the current study and the 97 previously reported AF-genetic loci.** None of these associations reached the pre-defined statistical significance threshold ( $P < 6.44 \times 10^{-5}$ ).

SNP	Locus	Protein name	HR (95% CI)	P-value
rs7508	8p22	ADAMTS13	0.89 (0.83-0.96)	$3.37 \times 10^{-3}$
rs60212594	10q22.2	ADAMTS13	1.13 (1.03-1.24)	$9.58 \times 10^{-3}$
rs62483627	7q22.3	ADAMTS13	1.11 (1.02-1.20)	$1.28 \times 10^{-2}$
rs60212594	10q22.2	Angiopoietin-2	0.86 (0.78-0.94)	$1.52 \times 10^{-3}$
rs11001667	10q22.3	BMPR1A	0.87 (0.79-0.96)	$4.54 \times 10^{-3}$
rs242557	17q21.31	BMPR1A	1.11 (1.02-1.20)	$1.04 \times 10^{-2}$
rs72700114	1q24.2	BMPR1A	1.19 (1.04-1.36)	$1.29 \times 10^{-2}$
rs60212594	10q22.2	BMPR1A	1.12 (1.02-1.24)	$1.64 \times 10^{-2}$
rs12298484	12q24.31	ERBB1	1.12 (1.05-1.20)	$4.73 \times 10^{-4}$
rs7508	8p22	ERBB1	0.92 (0.86-0.98)	$1.66 \times 10^{-2}$
rs7508	8p22	NCAM-120	0.90 (0.84-0.97)	$7.10 \times 10^{-3}$
rs7978685	12q13.3	NT-proBNP	1.15 (1.05-1.27)	$3.55 \times 10^{-3}$
rs6546620	2p23.3	NT-proBNP	1.15 (1.02-1.29)	$1.88 \times 10^{-2}$
rs7789146	7q36.1	TrkC	1.12 (1.03-1.22)	$1.17 \times 10^{-2}$
rs10760361	9q33.3	TrkC	0.91 (0.85-0.98)	$1.28 \times 10^{-2}$
rs13191450	6q22.31	TrkC	1.09 (1.02-1.17)	$1.60 \times 10^{-2}$
rs60212594	10q22.2	TrkC	1.12 (1.02-1.24)	$1.79 \times 10^{-2}$
rs7508	8p22	TrkC	0.87 (0.80-0.94)	$2.37 \times 10^{-4}$
rs949078	11q24.1	WFKN2	0.91 (0.84-0.98)	$1.50 \times 10^{-2}$
rs10760361	9q33.3	WFKN2	0.92 (0.85-0.98)	$1.60 \times 10^{-2}$

ADAMTS13: a disintegrin and metalloproteinase with thrombospondin motifs 13; BMPR1A: bone morphogenetic protein receptor type-1A; NCAM-120: Neural cell adhesion molecule 1, 120 kDa isoform; NT-proBNP: N-terminal pro-brain natriuretic peptide; TrkC: tropomyosin receptor kinase C; WFKN2: WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2

\*Significance level of  $p < 0.05 / (8 \text{ proteins} * 97 \text{ SNPs}) = 6.44 \times 10^{-5}$ . None of them reached the significance cutoff.

**Table S5. Biological functions of the 8 proteins associated with the risk of incident AF<sup>1,2</sup>**

Protein	Function
NCAM-120	Immunoglobulin-like glycoprotein. Activates fibroblast growth factor receptor and induces neurite outgrowth. Over-expression in neuroblastoma cells.
WFKN2 (WFIKKN2)	Multivalent protease-inhibitor. Inhibits growth differentiation factor and myostatin.
TrkC (Ntrk3)	One of tropomyosin receptor kinases that bind to neurotrophin-3, which in turn induces growth and differentiation of neuronal cells. Mutations in the gene associated with medulloblastoma, neuroblastoma, breast cancer, and other cancers.
EGFR (ERBB1)	Transmembrane glycoprotein kinase that acts as a receptor for epidermal growth factor. Mutations in the gene associated with different types of cancers.
ADAMTS13 (ATS13)	Multivalent protein that cleaves von Willebrand Factor. Mutations in the gene are associated with thrombotic thrombocytopenic purpura.
Angiopoietin-2	Inhibits angiopoietin-1 and endothelial TEK tyrosine kinase, thereby regulating angiogenesis and endothelial function.
NT-proBNP	Secreted by ventricular myocardium upon myocardial stretching and causes natriuresis, diuresis, vasodilation, and inhibition of the renin-angiotensin-aldosterone system.
BMPR1A	Transmembrane serine/threonine kinase receptor that binds to the members of the TGF- $\beta$ superfamily. Mutations in the gene are associated with pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia.

ADAMTS13: a disintegrin and metalloproteinase with thrombospondin motifs 13; BMPR1A: bone morphogenetic protein receptor type-1A; CI: confidence interval; EGFR: epidermal growth factor receptor; HR: hazard ratio; NCAM-120: Neural cell adhesion molecule 1, 120 kDa isoform; NT-proBNP: N-terminal pro-brain natriuretic peptide; TrkC: tropomyosin receptor kinase C; WFKN2: WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2

**Table S6. Protein biomarkers associated with incident AF in prospective cohort studies.**

Protein <sup>REF</sup>	Type of assay	Study sample	HR (95% CI)	p-value	HR (95% CI)	p-value
			Age-, sex-, ± race-adjusted		Multivariable-adjusted	
NT-proBNP <sup>3</sup>	PEA	PIVUS, ULSAM	1.57 (1.41-1.76)	<0.0001*	1.57 (1.39-1.76)	<0.0001*
NT-proBNP	Aptamer-based	FHS	1.44 (1.24-1.69)	<0.00001 <sup>†</sup>	1.44 (1.22-1.70)	0.000015 <sup>†</sup>
NT-proBNP/BNP <sup>4</sup>	Immunoassay <sup>§</sup>	CHARGE-US	NR	NR	1.66 (1.56-1.76)	<0.0001
FGF-23 <sup>3</sup>	PEA	PIVUS, ULSAM	1.26 (1.14-1.4)	<0.0001*	1.18 (1.06-1.32)	0.0025
FGF-23 <sup>5</sup>	PEA	Bruneck, Italy	1.04 (0.80-1.36)	0.75	NR	NR
FGF-23	Aptamer-based	FHS	0.95 (0.85-1.06)	0.052	0.93 (0.83-1.04)	0.18
FGF-23 <sup>6</sup>	Immunoassay	ARIC	1.31 (1.19-1.45)	NR	1.07 (0.96-1.18)	NR
FGF-23 <sup>7</sup>	Immunoassay	MESA	1.79 (1.45-2.21) <sup>  </sup>	<0.001	1.41 (1.13-1.76) <sup>  </sup>	0.003
FGF-23 <sup>7</sup>	Immunoassay	CHS	1.31 (1.07-1.60) <sup>  </sup>	0.010	1.29 (1.05-1.60) <sup>  </sup>	0.018
IL-6 <sup>3</sup>	PEA	PIVUS, ULSAM	1.26 (1.13-1.4)	<0.0001*	1.25 (1.11-1.4)	0.0001*
IL-6 <sup>5</sup>	PEA	Bruneck, Italy	1.19 (0.98-1.45)	0.08	NR	NR
IL-6	Aptamer-based	FHS	0.99 (0.89-1.10)	0.85	0.96 (0.86-1.07)	0.44
IL-6 <sup>8</sup>	Immunoassay	FHS	NR	NR	1.08 (0.91-1.29)	0.36
FABP4 <sup>3</sup>	PEA	PIVUS, ULSAM	1.32 (1.16-1.5)	<0.0001*	1.22 (1.05-1.43)	0.012
FABP4 <sup>5</sup>	PEA	Bruneck, Italy	1.41 (1.07-1.87)	0.02	NR	NR
GDF-15 <sup>3</sup>	PEA	PIVUS, ULSAM	1.27 (1.13-1.44)	0.0001*	1.25 (1.09-1.44)	0.0018
GDF-15 <sup>9</sup>	Immunoassay	FHS	1.31 (1.14-1.49)	<0.0001	1.15 (0.99-1.32)	0.061
TIM-1 <sup>3</sup>	PEA	PIVUS, ULSAM	1.26 (1.12-1.43)	0.0002*	1.23 (1.07-1.4)	0.0028
AM <sup>3</sup>	PEA	PIVUS, ULSAM	1.27 (1.11-1.44)	0.0004*	1.22 (1.06-1.4)	0.0044
ST2 <sup>3</sup>	PEA	PIVUS, ULSAM	1.05 (0.92-1.2)	0.44	1.08 (0.94-1.24)	0.27
ST2 <sup>5</sup>	PEA	Bruneck, Italy	1.19 (0.94-1.50)	0.14	NR	NR
ST2 <sup>9</sup>	Immunoassay	FHS	1.06 (0.92-1.22)	0.39	1.02 (0.89-1.17)	0.76
TnI	Aptamer-based	FHS	1.04 (0.93-1.16)	0.51	1.02 (0.91-1.14)	0.078
hsTnI <sup>9</sup>	Immunoassay	FHS	1.25 (1.13-1.37)	<0.0001	1.18 (1.07-1.32)	0.002
hsTnT <sup>10</sup>	Immunoassay	ARIC	1.43 (1.37-1.51)	<0.0001	1.32 (1.25-1.39)	<0.0001
hsTnT <sup>11</sup>	Immunoassay	CHS	1.67 (1.52-1.83)	NR	1.45 (1.32-1.59)	NR
CRP	Aptamer-based	FHS	1.10 (0.98-1.25)	0.11	0.94 (0.82-1.08)	0.38
CRP <sup>4</sup>	Immunoassay	CHARGE-US	NR	NR	1.18 (1.11-1.25)	<0.0001
hsCRP <sup>5</sup>	Immunoassay	Bruneck, Italy	0.94 (0.77-1.15)	0.56	NR	NR
CRP <sup>12</sup>	Immunoassay	FHS	NR	NR	1.25 (1.07-1.46)	0.004
VCAM-1	Aptamer-based	FHS	1.06 (0.94-1.19)	0.34	0.99 (0.87-1.11)	0.82
VCAM-1 <sup>5</sup>	Immunoassay	Bruneck, Italy	1.35 (1.12-1.63)	0.001	NR	NR

\*Significance level of p-value <0.000588 (Bonferroni correction)

<sup>†</sup>Significance level of p-value <0.000036 (Bonferroni correction)

<sup>§</sup>"Immunoassay" refers to standard immunoassay of a single protein with significance level of p-value < 0.05

<sup>||</sup>HR per doubling of the protein level



ARIC: The Atherosclerosis Risk in Communities Study; AM: adrenomedullin; CHARGE-US: Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium, which includes multiple international cohorts. CHARGE-US includes the US cohorts – FHS, ARIC, CHS; CHS: Cardiovascular Health Study; CI: confidence interval; CRP: C-reactive protein; IL-6: interleukin-6; FABP4: fatty acid binding protein 4; FGF-23: fibroblast growth factor 23; FHS: Framingham Heart Study; GDF-15: growth differentiation factor 15; HR: hazard ratio; hsTnI/T: high-sensitive troponin I/T; MESA: Multi-Ethnic Study of Atherosclerosis; NR: not reported; NT-proBNP: N-terminal pro-brain natriuretic peptide; PEA: proximity extension assay; PIVUS: The Prospective Investigation of the Vasculature in Uppsala Seniors study; TIM-1: T-cell immunoglobulin and mucin domain 1; ULSAM: Uppsala Longitudinal Study of Adult Men; VCAM-1: vascular cell adhesion molecule 1;

## Supplemental References:

1. The UniProt Consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Research*. 2017;45:D158-D169.
2. O'Leary NA, Wright MW, Brister JR, Ciuffo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, DiCuccio M, Kitts P, Murphy TD and Pruitt KD. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*. 2016;44:D733-745.
3. Lind L, Sundstrom J, Stenemo M, Hagstrom E and Arnlov J. Discovery of new biomarkers for atrial fibrillation using a custom-made proteomics chip. *Heart*. 2017;103:377-382.
4. Sinner MF, Stepas KA, Moser CB, Krijthe BP, Aspelund T, Sotoodehnia N, Fontes JD, Janssens AC, Kronmal RA, Magnani JW, Witteman JC, Chamberlain AM, Lubitz SA, Schnabel RB, Vasani RS, Wang TJ, Agarwal SK, McManus DD, Franco OH, Yin X, Larson MG, Burke GL, Launer LJ, Hofman A, Levy D, Gottesdiener JS, Kaab S, Couper D, Harris TB, Astor BC, Ballantyne CM, Hoogeveen RC, Arai AE, Soliman EZ, Ellinor PT, Stricker BH, Gudnason V, Heckbert SR, Pencina MJ, Benjamin EJ and Alonso A. B-type natriuretic peptide and C-reactive protein in the prediction of atrial fibrillation risk: the CHARGE-AF Consortium of community-based cohort studies. *Europace*. 2014;16:1426-1433.
5. Willeit K, Pechlaner R, Willeit P, Skrobilin P, Paulweber B, Scherthaner C, Toell T, Egger G, Weger S, Oberhollenzer M, Kedenko L, Iglseder B, Bonora E, Schett G, Mayr M, Willeit J and Kiechl S. Association Between Vascular Cell Adhesion Molecule 1 and Atrial Fibrillation. *JAMA Cardiol*. 2017;2:516-523.
6. Alonso A, Misialek JR, Eckfeldt JH, Selvin E, Coresh J, Chen LY, Soliman EZ, Agarwal SK and Lutsey PL. Circulating fibroblast growth factor-23 and the incidence of atrial fibrillation: the Atherosclerosis Risk in Communities study. *J Am Heart Assoc*. 2014;3:e001082.
7. Mathew JS, Sachs MC, Katz R, Patton KK, Heckbert SR, Hoofnagle AN, Alonso A, Chonchol M, Deo R, Ix JH, Siscovick DS, Kestenbaum B and de Boer IH. Fibroblast growth factor-23 and incident atrial fibrillation: the Multi-Ethnic Study of Atherosclerosis (MESA) and the Cardiovascular Health Study (CHS). *Circulation*. 2014;130:298-307.
8. Schnabel RB, Larson MG, Yamamoto JF, Kathiresan S, Rong J, Levy D, Keane JF, Jr., Wang TJ, Vasani RS and Benjamin EJ. Relation of multiple inflammatory biomarkers to incident atrial fibrillation. *Am J Cardiol*. 2009;104:92-96.
9. Rienstra M, Yin X, Larson MG, Fontes JD, Magnani JW, McManus DD, McCabe EL, Coglianese EE, Amponsah M, Ho JE, Januzzi JL, Jr., Wollert KC, Fradley MG, Vasani RS, Ellinor PT, Wang TJ and Benjamin EJ. Relation between soluble ST2, growth differentiation factor-15, and high-sensitivity troponin I and incident atrial fibrillation. *Am Heart J*. 2014;167:109-115.e102.
10. Filion KB, Agarwal SK, Ballantyne CM, Eberg M, Hoogeveen RC, Huxley RR, Loehr LR, Nambi V, Soliman EZ and Alonso A. High-sensitivity cardiac troponin T and the risk of incident atrial fibrillation: the Atherosclerosis Risk in Communities (ARIC) study. *Am Heart J*. 2015;169:31-38.e33.
11. Hussein AA, Bartz TM, Gottesdiener JS, Sotoodehnia N, Heckbert SR, Lloyd-Jones D, Kizer JR, Christenson R, Wazni O and deFilippi C. Serial measures of cardiac troponin T levels by a highly sensitive assay and incident atrial fibrillation in a prospective cohort of ambulatory older adults. *Heart Rhythm*. 2015;12:879-885.
12. Schnabel RB, Larson MG, Yamamoto JF, Sullivan LM, Pencina MJ, Meigs JB, Tofler GH, Selhub J, Jacques PF, Wolf PA, Magnani JW, Ellinor PT, Wang TJ, Levy D, Vasani RS and Benjamin EJ. Relations of biomarkers of distinct pathophysiological pathways and atrial fibrillation incidence in the community. *Circulation*. 2010;121:200-207.