African American race but not genome-wide ancestry is negatively associated with atrial fibrillation among postmenopausal women in the Women's Health Initiative

Marco V. Perez, MD,[a] Thomas J. Hoffmann, PhD,[b] Hua Tang, PhD,[c] Timothy Thornton, PhD,[d] Marcia L. Stefanick, PhD, FAHA,[e] Joseph C. Larson, MS,[f] Charles Kooperberg, PhD,[f] Alex P. Reiner, MD, MPH,[g] Bette Caan, DrPH,[h] Carlos Iribarren, MD, MPH, PhD,[h] and Neil Risch, PhD[b,h] ***Stanford, San Francisco, Palo Alto, and Oakland CA; and Seattle, WA***

Background Atrial fibrillation (AF) is the most common arrhythmia in women and is associated with higher rates of stroke and death. Rates of AF are lower in African American subjects compared with European Americans, suggesting European ancestry could contribute to AF risk.

Methods The Women's Health Initiative (WHI) Observational Study (OS) followed up 93,676 women since the mid 1990s for various cardiovascular outcomes including AF. Multivariate Cox hazard regression analysis was used to measure the association between African American race and incident AF. A total of 8,119 African American women from the WHI randomized clinical trials and OS were genotyped on the Affymetrix Human SNP Array 6.0. Genome-wide ancestry and previously reported single nucleotide polymorphisms associated with AF in European cohorts were tested for association with AF using multivariate logistic regression analyses.

Results Self-reported African American race was associated with lower rates of AF (hazard ratio 0.43, 95% CI 0.32-0.60) in the OS, independent of demographic and clinical risk factors. In the genotyped cohort, there were 558 women with AF. By contrast, genome-wide European ancestry was not associated with AF. None of the single nucleotide polymorphisms previously associated with AF in European populations, including rs2200733, were associated with AF in the WHI African American cohort.

Conclusion African American race is significantly and inversely correlated with AF in postmenopausal women. The etiology of this association remains unclear and may be related to unidentified environmental differences. Larger studies are necessary to identify genetic determinants of AF in African Americans. (Am Heart J 2013;166:566-572.e1.)

Atrial fibrillation (AF) is the most common cardiac arrhythmia, affecting >2.2 million people in the United States,[1] a number expected to rise to 5.6 million by 2050 as the population ages.[2,3] Atrial fibrillation is associated with significant morbidity, accounting for 75,000 strokes each year[4,5] and is independently associated with a 1.5- to 1.9-fold increased risk of death.[6,7] Several genes, primarily encoding for components of ion channels, have been linked to the familial forms of early-onset, lone AF.[8,9] Population-based studies of AF in European cohorts have identified several single nucleotide polymorphisms (SNPs) associated with AF.[10-14] However, there have been no common variants associated with AF in African Americans with genome-wide statistical significance.

Genetic heterogeneity exists across different ethnicities and is manifested by differences in allele frequencies and genetic substructures.[15-18] The incidence of AF was lower in African Americans compared with whites in the Cardiovascular Health Care Study (CHS), independent of socioeconomic and other risk factors.[19] Similar differences have been reported in the ATRIA study,[2] the

From the [a]Division of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, [b]Institute for Human Genetics and Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, [c]Department of Genetics, Stanford University, Palo Alto, CA, [d]Department of Biostatistics, University of Washington, Seattle, WA, [e]Stanford Prevention Research Center, Department of Medicine, Stanford University, Stanford, CA, [f]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, and [g]University of Washington, School of Public Health, Seattle, WA, and [h]Kaiser Permanente Division of Research, Oakland, CA.

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Reprint requests: Marco V. Perez, MD, Stanford University, Cardiac Electrophysiology and Arrhythmia Service, 300 Pasteur Drive #H2146, Stanford, CA 94305-5233.
E-mail: mvperez@stanford.edu

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ARIC study,[20] and male veteran populations.[21] Global European ancestry was associated with incident AF in the African American cohorts from ARIC and CHS,[22] suggesting that some genetic risk variants of AF may occur at different allele frequencies between the European and African ancestral populations. However, subsequent analysis of 3 European loci predictive of AF did not find an association between European ancestry and AF in African Americans.[23]

The Women's Health Initiative (WHI) included 3 population-based randomized clinical trials (CT) and a large observational study (OS) cohort designed to assess the impact of various markers on common diseases.[24-26] The genotyping of >8,000 African Americans from WHI represents the largest genotyping effort that has been made in non-Europeans. We sought to measure differences in rates of AF between African Americans and whites, to measure associations between AF and common genomic variants previously associated with AF in European populations, and to replicate the association between genome-wide European ancestry and AF in the African American women from the WHI.

Methods

Study population and design

The WHI studies consisted of randomized CT, which assigned 68,132 women to active or placebo hormone therapy (HT),[25,26] dietary modification or control,[27] and/or calcium/vitamin D supplementation or placebo[28] with specific outcomes of common diseases of aging in women, and also an OS, which collected data on biological and lifestyle factors and health outcomes. Postmenopausal women between the ages of 50 and 79 years were recruited at 40 US clinical centers. Women with a survival of <3 years, diagnosis of cancer within the past 10 years, except nonmelanomatous skin cancer, or who were determined to be unlikely to adhere to the study protocol were excluded. Women with systolic blood pressures of 200 mm Hg or diastolic blood pressures of 105 mm Hg were temporarily excluded until blood pressure was better controlled. A total of 68,132 women were enrolled in at least one of the randomized trials, and 93,676 women were enrolled in the OS.

Details of the questionnaires used, physical measurements, blood collection, and quality assurance have been described.[24-26,29] Baseline 12-lead electrocardiograms and blood pressure measurements were made at the initial clinic visit. Medical conditions, such as coronary heart disease (CHD), heart failure, and diabetes mellitus, were determined by self-report. African American race was identified by self-report. Women were followed up with annual clinic visits where standardized interviews probed for development of AF or other medical conditions, symptoms, potential outcomes, and hospitalizations. The HT trials were stopped in 2002 (Estrogen + Progestin) and 2004 (Estrogen only) or completed in 2005 (Dietary Modification and Ca/D trials), but follow-up is ongoing for both the CT and OS cohorts. Women in the randomized and observational studies were followed up for an average of 5 and 9.8 years, respectively.

Baseline AF was determined by the initial questionnaire, which probed for self-reported AF with the specific question, "Has a doctor ever told you that you had heart problems, problems with your blood circulation or blood clots?" with "Atrial fibrillation (a type of irregular heart beat)" as an option or by presence of AF on the baseline 12-lead electrocardiogram. *Baseline hypertension* was defined as elevated systolic (≥140 mm Hg) or diastolic (≥90 mm Hg) blood pressure at the initial clinic visit or self-report of taking medications for hypertension. Women were followed up with a medical history update questionnaire at years 3 to 8, which specifically probed for self-reported AF and hospitalizations. Discharge diagnosis codes for AF were obtained from these hospitalizations and were available for the Cox regression analysis of incident AF in the OS, but not for the genomic analyses.

Genotyping

Women of self-reported African American race from the CT and OS were invited for genetic testing. The subjects who consented to genome-wide scanning (SHARe cohort) underwent genotyping with the Affymetrix Genome-Wide Human SNP Array 6.0 containing 906,000 SNPs. The samples underwent initial quality control at the sample level, including inability to genotype, abnormal sex chromosomes, relatedness, and low call rates. Additional quality control measurements were made at the SNP level assessing for Hardy-Weinberg Equilibrium (goodness-of-fit χ^2 >10), call rates <90%, monomorphic SNPs, and minor allele frequencies <1%. The data sets used for the analyses described in this manuscript were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap.

Genome-wide ancestry estimates

Principal Component Analysis was performed using Eigenstrat[30] at 178,101 markers that were in common between our samples and the reference panels. Individual ancestry proportions were determined using the frappe software with 656,852 autosomal markers.[31] For the frappe analysis, the number of ancestral populations was set to 4 with the HapMap CEU and YRI samples included as fixed groups for European and African ancestry, respectively, and the HGDP samples from the Americas and East Asia were included as surrogates for Native American and East Asian ancestry, respectively. The first principal component is nearly perfectly correlated (correlation of 0.997) with the frappe-estimated African ancestry proportions for the self-reported African Americans in WHI-SHARe. Based on the frappe estimates, we identified 56 subjects who self-identified as African American, but who have an estimated African ancestry proportion that is <10%. We also flagged 1 participant with questionable estimates of both ancestry and relatedness. Because we cannot exclude the possibility of either a sample mishandling or a data entry error, we flagged these 57 samples for exclusion. There were a number of other subjects who identified as African American but appeared admixed with Native American or Asian ancestry or who identified as Hispanic but appeared admixed with Asian ancestry, who are considered in our analyses. For the genotype association analyses described below, we have adjusted our analyses for the first 4 principal components.