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

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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

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Submitted February 5, 2013; accepted May 23, 2013.

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0002-8703/$ - see front matter

http://dx.doi.org/10.1016/j.ahj.2013.05.024

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.

Statistical analysis

Baseline characteristics were compared using either the Student *t* test for continuous variables or the χ^2 test for categorical variables. Predictors of incident AF were assessed using Cox hazard regression analysis in women from the OS. The 4,397 participants with prevalent AF and 6,653 participants with incomplete data were excluded. Multivariate analyses of incident AF were adjusted for age, hypertension, diabetes, hyperlipidemia, heart failure, myocardial infarction, coronary artery disease, peripheral arterial disease, body mass index (BMI), smoking, HT use, and level of education. Associations were reported as hazard ratios (HR) with 95% CIs. The proportional hazards assumption was verified by visual inspection of the log-likelihood plot of developing AF over time.

Logistic regression analyses with adjustment for age, congestive heart failure (CHF), myocardial infarction, diabetes mellitus, BMI, and hypertension were used to estimate the odds ratios (ORs) between genome-wide ancestry and AF. An additive logistic regression model with the same covariates plus the first 4 principal components was also used to estimate the ORs between SNPs known to be associated with AF in European populations and AF. The African American subjects were then divided into subgroups of different proportions of European ancestry, and the ORs between rs2200733, the most statistically significant and best replicated SNP associated with AF in European cohorts, and AF within each subgroup were calculated. PLINK[32] and SAS version 9.1.3 were used to perform these analyses.

The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, US Department of Health and Human Services through contracts N01WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221. This manuscript was prepared in collaboration with investigators of the WHI and has been reviewed and/or approved by the WHI. Funding for WHI SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Marco Perez was funded by an American Heart Association Fellow to Faculty Award; Thomas J. Hoffmann, by NIH R25CA112355; and Timothy Thornton, by NIH K01CA148958 grants. The authors are solely responsible for the design and conduct of this study, all study analyses, and drafting and editing of the manuscript.

Results

African American race is protective of AF in the OS

A total of 93,676 women were enrolled in the WHI OS and followed up for an average of 9.8 years with a maximum follow-up time of 13.8 years. After exclusion of the 6,653 subjects with incomplete data and 4,397 subjects with AF at baseline, 81,555 women were available for analysis, 7,234 (8.9%) of whom were African American. Baseline characteristics of African American subjects compared with white subjects are presented in Table I. On average, African American subjects were slightly younger (62 vs 63.7 years, $P < .001$), had significantly more hypertension (63.9% vs 40.1%, $P < .001$), more diabetes (12.2% vs 2.9%), more CHD (4.6% vs 2.9%, $P < .001$), and were more likely to have an income < $20,000 (28.4% vs 12.3%, $P < .001$).

Table I. Differences in baseline characteristics between whites and African Americans in the observational cohort

Characteristic	White (n = 74321)	African American (n = 7234)	P
Age (y), mean (SD)	63.7 (7.3)	62.0 (7.3)	<.001
Income <$20,000	9172 (12.3)	2055 (28.4)	<.001
Smoking ever	37055 (49.8)	3516 (48.6)	<.001
BMI (kg/m^2), mean (SD)	27.0 (5.7)	30.7 (6.8)	<.001
Hypertension	2987 (40.1)	4623 (63.9)	<.001
Diabetes mellitus	2188 (2.9)	886 (12.2)	<.001
Hyperlipidemia	9647 (14.0)	1047 (16.6)	<.001
CHD (MI/CABG/PTCA)	2192 (2.9)	334 (4.6)	<.001
MI	1505 (2.0)	264 (3.6)	<.001
CABG/PTCA	1291 (1.7)	147 (2.0)	<.001
Stroke	865 (1.2)	216 (3.0)	<.001
CHF	426 (0.6)	131 (1.8)	<.001
Peripheral arterial disease	1253 (1.8)	209 (3.3)	<.001
Statin use at baseline	5914 (8.0)	624 (8.6)	<.001
Systolic BP, mean (SD)	126.2 (17.8)	132.2 (18.4)	<.001
Diastolic BP, mean (SD)	74.4 (9.2)	78.0 (9.8)	<.001
Heart rate, mean (SD)	69.3 (12.1)	70.5 (13.1)	<.001

Values are presented as total number (percentage of subjects) or mean (SD). *P* value represents difference from the *t* test for continuous variables and χ^2 test for categorical variables between whites and African Americans. *MI*, Myocardial infarction; *CABG*, coronary artery bypass graft surgery; *PTCA*, percutaneous transluminal coronary angioplasty; *BP*, blood pressure.

Table II. Multivariate analysis of correlations with incident AF in the WHI observational cohort

Variable	HR	CI
African American race	0.43	0.32-0.60
Age (5 y)	1.70	1.60-1.75
Diabetes	1.76	1.54-2.01
Coronary disease	1.85	1.62-2.12
CHF	2.13	1.67-2.71
Peripheral arterial disease	1.48	1.25-1.75
BMI (5 kg/m^2)	1.15	1.12-1.18
HT	1.03	0.96-1.11
Smoking	1.19	1.11-1.27
Higher education	0.85	0.78-0.93

The full multivariate model included each variable listed as a covariate. The HRs represent values derived from this multivariate model.

Over an average of 9.8 years, 3,754 (4.6%) of all included participants from the OS developed new-onset AF. Cox hazard regression analysis revealed that African American race was protective of incident AF (HR 0.43, 95% CI 0.32-0.60) after adjustment for age, diabetes, CHD, CHF, peripheral arterial disease, BMI, use of HT, smoking, and higher education (Table II).

Genotyped cohort analyses

The baseline characteristics of African American women in the genotyped cohort with AF compared

Table III. Differences in baseline characteristics between subjects with and without AF among African Americans genotyped in the SHARe cohort

Characteristic	AF (n = 558)	No AF (n = 7561)	P
Age (y), mean (SD)	63.4 (7.1)	61.5 (7.0)	<.001
BMI (kg/m^2), mean (SD)	31.3 (6.4)	31.2 (6.2)	.79
Diabetes	102 (18.3)	1035 (13.7)	.003
Hypertension	360 (67.2)	3986 (54.6)	<.001
CHD (MI/CABG/PTCA)	103 (18.5)	508 (6.7)	<.001
MI	61 (11.0)	228 (3.0)	<.001
CABG/PTCA	61 (10.9)	364 (4.8)	<.001
CHF	37 (6.6)	111 (1.5)	<.001
Smoking (ever)	295 (53.3)	3893 (52.1)	.56
Stroke	65 (11.6)	339 (4.5)	<.001

Values are presented as total number (percentage of subjects) or mean (SD). *P* value represents difference from the *t* test for continuous variables and χ^2 test for categorical variables between whites and African Americans.

with those without AF are presented in Table III. The African American women with AF were slightly older (63.4 vs 61.5 years, $P < .001$), had more diabetes (18.3% vs 13.2%, $P = .003$), more hypertension (67.2% vs 54.6%, $P < .001$), more CHD (18.5% vs 6.7%, $P < .001$), and more CHF (6.6% vs 1.5%, $P < .001$).

Of the 8,119 African American women who consented to genotyping and who passed quality control measures, 423 women had AF at baseline, and 123 developed AF over an average follow-up period of 9.8 years. We performed a logistic regression analysis and found that genome-wide European ancestry (OR 0.922, $P = .79$) is not significantly associated with AF after adjustment for age, CHF, myocardial infarction, BMI, and hypertension (Table IV).

Replication of previously reported SNPs

Single nucleotide polymorphisms that had previously been reported as associated with AF in European cohorts (Table V) were tested for association with AF in the WHI SHARe cohort. All SNPs were directly genotyped on the array. An additive logistic regression model adjusted for age, diabetes, hypertension, heart failure, CHD, the first 4 principal components, and BMI was used. After Bonferroni adjusting for the 13 SNPs that were tested, none of these SNPs was significantly associated with AF in this African American cohort at the target $P < .0038$ (Table V). Additional sensitivity analyses using a logistic regression model adjusted only for age and the first 4 principal components also revealed no significant associations with AF (data not shown). To further explore some of the loci previously associated with AF in European cohorts, regional Manhattan plots with a 1 megabase window centered around several SNPs associated with AF in European cohorts were plotted (Supplementary Figure 1). There were no SNPs within these regions that approached genome-wide significant association with AF.

Table IV. Correlates of AF in the African Americans from the SHARe cohort

Variable	OR	P
European Ancestry	0.922	.79
Age (1 y)	1.03	<.0001
CHF	2.30	<.0001
Myocardial infarction	2.89	<.0001
BMI (1 kg/m^2)	1.001	.70
Hypertension	1.40	.0002

The multivariate model was adjusted for age, CHF, myocardial infarction, BMI, the first 4 principal components, and hypertension. The ORs represent values derived from this multivariate logistic regression model.

To assess whether background European ancestry might influence the association between rs2200733 and AF in African Americans, we divided the African Americans into subgroups of genome-wide European ancestry. The distribution of European ancestry was heavily right-ward skewed with a median of 17.2%, a total range between 0% and 83% and approximately three quarters of the participants having <30% European genome-wide ancestry. Although the association between AF and rs2200733 is higher in subjects with <10% European ancestry (OR 1.184, 95% CI 0.91-1.54) compared with other subgroups, there is some overlap in the 95% CIs among these ORs (Figure).

Discussion

A lower rate of AF has been consistently reported in African Americans compared with whites across several cohorts.[2,19-21] Our finding that African American race is protective of incident AF in the WHI observational cohort is consistent with these reports. Disparities in socioeconomic status and lack of exposure to the health care system leading to underreporting of AF in African Americans have been implicated for these differences. However, the relationship between race and AF persisted after adjusting for a wide range of socioeconomic and other risk factors.

Ethnic differences in disease rates found in multivariate analyses imply that there are (1) unmeasured environmental exposures accounting for these disparities, (2) genomic factors driving different rates of disease, or (3) a combination of these 2 factors. The CARe investigators reported an association between European ancestry and incident AF in both the ARIC and CHS African American cohorts.[22] Despite our larger cohort, we were not able to establish a similar association with European ancestry (Table IV). A similar analysis that focused on 3 established loci associated with AF in Europeans found no relationship between European ancestry and AF in African Americans.[23]

There are several possible explanations for the disparate findings in these studies. One plausible explanation is that our cohort consisted entirely of

Table V. Association between AF and SNPs known to be associated with AF in European populations

Variant	Locus	Plausible gene	Minor allele	Case MAF	Control MAF	OR	*P*
rs2200733	4q25	*PITX2*	T	0.210	0.215	0.946	.49
rs6843082	4q25	*PITX2*	G	0.303	0.301	1.019	.79
rs6817105	4q25	*PITX2*	C	0.228	0.240	1.073	.36
rs2106261	16q22	*ZFHX3*	T	0.260	0.253	1.012	.87
rs13376333	1q21	*KCNN3*	T	0.291	0.287	1.028	.69
rs6666258	1q21	*KCNN3*	C	0.376	0.371	0.963	.59
rs3903239	1q24	*PRRX1*	G	0.227	0.207	0.871	.09
rs2040862	5q31	*WNT8A*	T	0.058	0.075	1.323	.04
rs3807989	7q31	*CAV1*	G	0.362	0.361	0.996	.95
rs10821415	9q22	*C9orf3*	A	0.256	0.235	0.889	.11
rs10824026	10q22	*SYNPO2L*	A	0.393	0.380	1.046	.50
rs1152591	14q23	*SYNE2*	A	0.163	0.143	1.164	.09
rs7164883	15q24	*HCN4*	G	0.352	0.317	0.861	.03

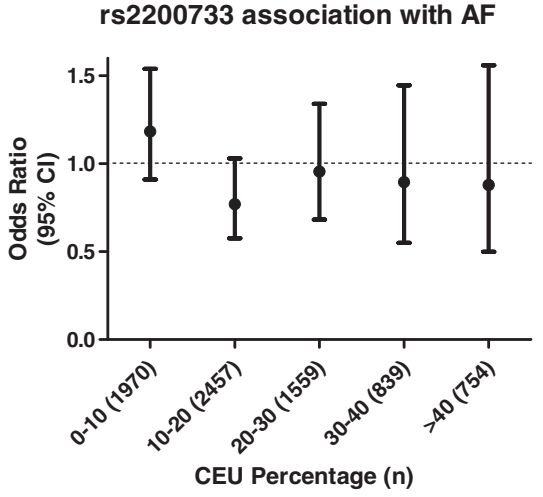
An additive logistic regression model adjusted for age, diabetes, hypertension, heart failure, CHD, principal components 1 to 4, and BMI was used to test the association between each SNP and AF. *Case MAF*, minor allele frequency in subjects with AF; *Control MAF*, minor allele frequency in subjects without AF.

women, whereas the CARe cohorts included men. An interaction between sex and AF as well as African ancestry could help explain the different findings as there are significant differences in rates of AF between men and women.[2] Another possibility is that the underlying distribution of European ancestry might be significantly different between the populations due to geographic differences in recruitment.

We were also unable to replicate the association between the SNPs that have previously been correlated with AF in European cohorts after adjusting for the multiple SNPs tested. Minor allele frequencies and linkage disequilibrium with causative variants can be substantially different between populations.[15-18] Analysis from the CARe project identified a novel SNP (rs4611994) in the *PITX2* locus associated with AF in African Americans, but only marginal significant association with the index European SNP.[33] This further suggests that the causative variant in the *PITX2* locus is linked differently in the 2 populations. Fine mapping of genomic loci in larger cohorts of African Americans may need to be performed to identify potentially causative variants. We tested the hypothesis that background genome-wide ancestry could help explain the lack of association between these established SNPs and AF but did not find a significant difference in ORs among groups of African Americans with different European ancestry makeup. However, the degree of European ancestry in our cohort was very low (74% had a European ancestry percentage of <30%). It is possible that the association between these SNPs and AF would only be seen when European ancestry is above a higher threshold. Because sex is strongly associated with AF, another possible explanation for our lack of replication includes the fact that our cohort consisted only of women.

There are a few limitations to this analysis that are worth noting. Because classification of AF partially relied on self-report of prevalent and incident AF, there is a potential for misclassification. Others have relied on self-report of this condition.[19] Most (89%) patients with AF are able to accurately report their history of AF.[34] The bias that may be introduced by misclassification is likely nondifferential, although it is possible that African Americans with more European ancestry are less likely to self-report AF. Prevalent and incident AF were combined and used in a single logistic regression model for the genomic studies; however, associations between genomic markers and incident or prevalent AF have been homogeneous.[12] Nevertheless, on sensitivity analyses, we did not find significant genotypic associations with incident AF.

Figure



Association between rs2200733 and AF by subgroups of European ancestry. Odds ratios were calculated with a logistic regression analysis adjusting for age, diabetes, heart failure, hypertension, BMI, and myocardial infarction.

In summary, we found that African American race is strongly and independently associated with a reduced risk of AF in postmenopausal women. Despite previous reports of an association between European ancestry and AF,[22] we could not replicate this finding. We also found that SNPs previously known to associate with AF in European cohorts did not replicate in our African American cohort. Fine mapping of these loci in African Americans may help further narrow down the genetic loci associated with AF. More thorough environmental characterization and studies of ancestry informative markers in African American populations will need to be performed to help identify the factors that account for the differences in rates of AF between different ethnicities. Meta-analyses combining several large African American cohorts are ongoing to identify novel variants associated with AF in this understudied population.

Acknowledgements

A full listing of WHI investigators can be found at http://whiscience.org/publications/WHI_investigators_longlist. We thank the WHI investigators, staff, and study participants for their outstanding dedication and commitment.

Disclosures

None.

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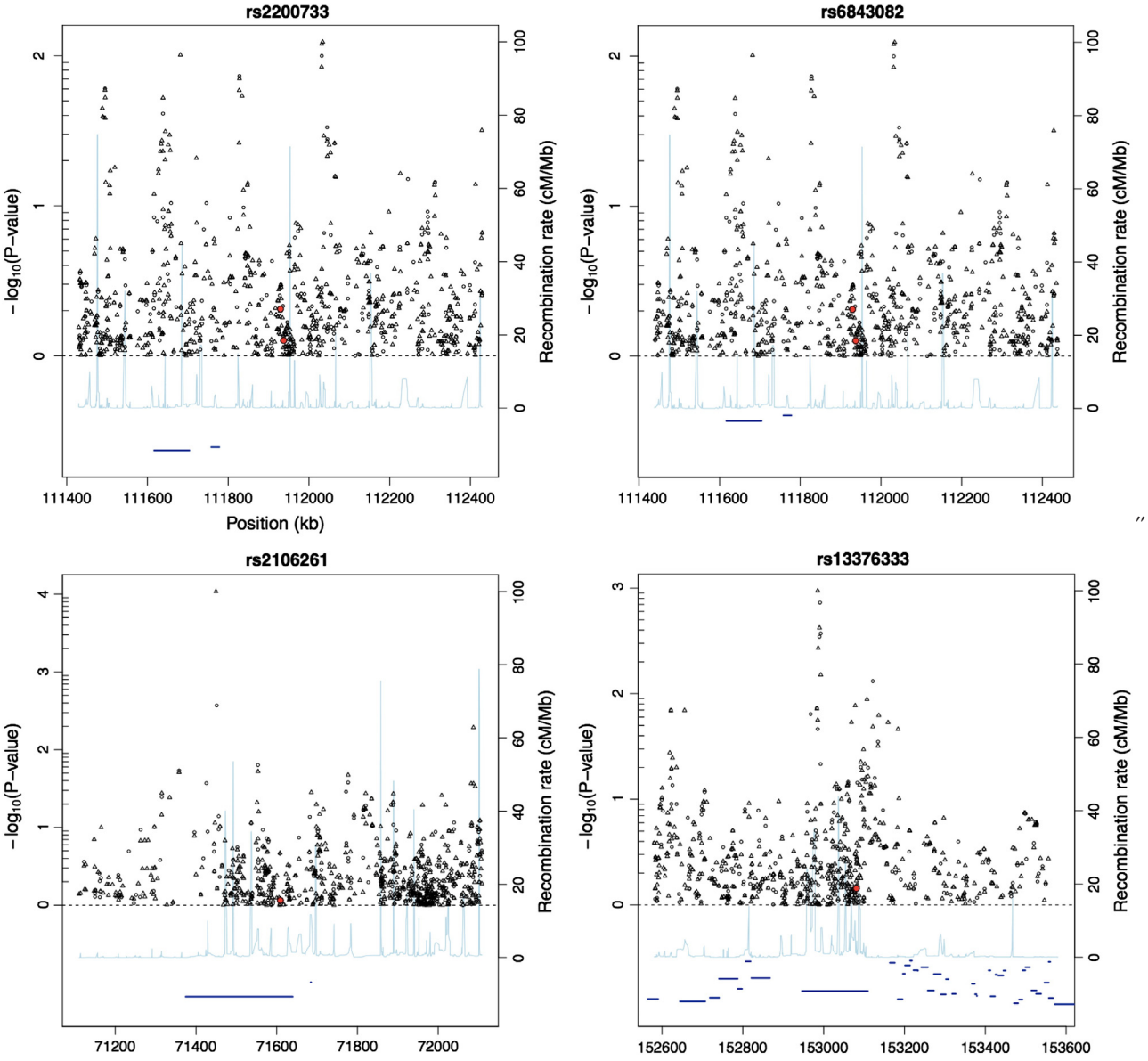
Appendix

Supplementary Figure 1



Regional Manhattan plots showing the significance of association AF for loci previously associated with AF in European cohorts. Single nucleotide polymorphisms within ±0.5 megabase from the European SNPs are plotted on the x axis according to their chromosomal position, against association with AF on the y axis (shown as $-\log_{10}P$).