Genetic Interactions with Age, Sex, Body Mass Index, and Hypertension in Relation to Atrial Fibrillation: The AFGen Consortium

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SUPPLEMENTAL METHODS

Description of study cohorts

The following studies included in the current analysis have been described elsewhere: the **Age, Gene/Environment Susceptibility Study (AGES) Reykjavik study**,[1] the **Arrhythmia-Biobank-LMU** (**AFLMU**, formerly known as **AFNET**) and the **Cooperative Health Research in the Region of Augsburg (KORA)**,[1] the **Atherosclerosis Risk in Communities (ARIC) study**,[1] **Cleveland Clinic Lone Atrial Fibrillation GeneBank Study (CCAF)**,[1] the **Cardiovascular Health Study (CHS)**,[1] **Framingham Heart Study (FHS)**,[1] **Massachusetts General Hospital (MGH) AF study**,[1] the **Rotterdam Study (RS)**,[1] the **Study of Health in Pomerania (SHIP)**,[1] the **Women's Genome Health Study (WGHS)**,[1] **a**nd the **PROspective Study of Pravastatin in the Elderly at Risk (PROSPER)**.[2] Additional studies were described here:

Discovery studies

BioVU

BioVU is the Vanderbilt University Medical Center's biorepository linked to de-identified electronic health records. BioVU operations[3] and ethical oversight[4] been described elsewhere. Briefly, DNA is collected from discarded blood samples remaining after routine clinical testing at Vanderbilt outpatient clinics in Nashville, Tennessee and surrounding areas, and is linked to a de-identified version of the patient's electronic health record termed the "Synthetic Derivative." AF cases were defined as individuals who were aged >18 years, had an ICD-9 diagnosis for AF or flutter (ICD-9: 427.3, 427.31, and 427.32), or a cardiologist diagnosis of AF as identified by a natural language processing tool from the unstructured free text of the ECG impression. In all instances, patients with a history of a heart transplant were excluded (Current Procedural Terminology: 33935, 3394, and 580; ICD-9: V42.1, 996.83).[5]

LURIC

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study is an ongoing prospective study of more than 3,300 individuals of German ancestry in whom cardiovascular and metabolic phenotypes (CAD, MI, dyslipidemia, hypertension, metabolic syndrome and diabetes mellitus) have been defined or ruled out using standardized methodologies in all study participants.[184] Inclusion criteria for LURIC were: German ancestry (limitation of genetic heterogeneity), clinical stability (except for acute coronary syndromes) and availability of a coronary angiogram. Exclusion criteria were: any acute illness other than acute coronary syndromes, any chronic disease where non-cardiac disease predominated and a history of malignancy within the last five years. Genome-wide analyses using the Affymetrix 6.0 have been completed in all participants. A 10-year clinical follow-up for total and cause specific mortality has been completed.

MESA

The Multi-Ethnic Study of Atherosclerosis (MESA) is a study of the characteristics of subclinical cardiovascular disease (disease detected non-invasively before it has produced clinical signs and symptoms) and the risk factors that predict progression to clinically overt cardiovascular

disease or progression of the subclinical disease. The cohort is a diverse, population-based sample of 6,814 asymptomatic men and women aged 45-84. Approximately 38 percent of the recruited participants are white, 28 percent African-American, 22 percent Hispanic, and 12 percent Asian (predominantly of Chinese descent). Participants were recruited during 2000-2002 from 6 field centers across the U.S. (at Wake Forest University; Columbia University; Johns Hopkins University; the University of Minnesota; Northwestern University, and the University of California – Los Angeles). All underwent anthropomorphic measurement and extensive evaluation by questionnaires at baseline, followed by 4 subsequent examinations at intervals of approximately 2-4 years. Age and sex were self-reported.

ECGs were recorded in the supine position after a period of rest. MESA ECG data were collected using GE MAC 1200 electrocardiographs. Digitally collected ECGs were transferred via phone lines to the MESA ECG center (EPICARE). The ECGs were automatically processed by use of GE Marquette 12-SL software (2001 version), after visual inspection of the recordings for quality.

Current AF at baseline was an exclusion criterion. Follow-up phone calls to study participants (every 9-12 months) were used to identify all hospitalizations. Medical records, including discharge diagnoses, were obtained for each hospitalization. Incident AF was defined by International Classification of Disease codes 427.31 or 427.32 (9th revision). In addition, new diagnoses of AF were identified at follow-up by the presence of AF or atrial flutter on a study ECG at Exam 5 (approximately 10 years after baseline).

Further information can be found at:
http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000209.v13.p3

PREVEND

The PREVEND cohort study was founded in 1997, and is an ongoing community-based cohort study including 8592 inhabitants of the city of Groningen, The Netherlands.[6] PREVEND is investigating the natural course of microalbuminaria and its relation to renal and cardiovascular disease. Details of the protocol, AF ascertainment and covariate definitions have been described elsewhere (www.prevend.org). AF was ascertained if either atrial flutter or AF was present on a 12-lead ECG obtained at one of the three PREVEND follow-up visits, or at an outpatient visit or hospital admission in the two hospitals in the city of Groningen (University Medical Center Groningen and Martini Hospital). Systolic and diastolic blood pressures were calculated as the mean of the last two measurements of the two visits, using an automatic GE Dinamap XL Model 9300 series device. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or use of antihypertensive drugs. Use of antihypertensive drugs was based on available information from the pharmacy prescription database. Body mass index (BMI) was calculated as the ratio of weight to height squared (kg/m^2). Genotyping was performed using the Illumina CytoSNP12v2 array. Genotype calling was performed using GenomeStudio, and imputation was performed using Beagle with the HapMap release 22 CEU referent panel. Analyses were performed with Plink v1.07 and R.

Replication studies

BEAT-AF/GAPP

The Basel Atrial Fibrillation Cohort Study (BEAT-AF) is a prospective observational, multicenter cohort study. Between 2010 and 2014, 1550 patients with documented AF were enrolled across 7 centers in Switzerland. Exclusion criteria were the inability to sign informed consent and the presence of short transient forms of AF. At baseline, patients completed detailed questionnaires about personal, medical, nutritional and lifestyle factors, current AF symptoms and co-morbidities. Current medications were recorded. A resting 12-lead electrocardiogram (ECG) was recorded and all patients underwent venous blood sampling at the local study center, including DNA from leukocytes. Yearly follow-ups by mailed questionnaires and phone interviews were performed in all patients in order to collect similar information as at baseline and to obtain details about adverse events.

Controls were enrolled from the 'genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors' (GAPP) study, which is an ongoing prospective population-based cohort study among healthy adults in the Principality of Liechtenstein. Between 2010 and 2013, all inhabitants of the Principality of Liechtenstein aged between 25 and 41 years were invited and 2170 agreed to participate in the study. Main exclusion criteria were established cardiovascular disease, chronic kidney disease, diagnosed sleep apnea, a body mass index (BMI) > 35 kg/m^2, intake of antidiabetic drugs or any other severe illness. Examinations included detailed assessment of personal, medical, lifestyle and nutritional factors, standardized assessment of weight, height and waist circumference, blood pressure measurement, electrocardiography, bioimpedance analysis, blood, urinary and genetic sampling, spirometry and sleep pulse oximetry with nasal flow measurement. Follow-up examinations are scheduled

every 3-5 years. The detailed study design has previously been published (http://www.ncbi.nlm.nih.gov/pubmed/23299990)

FINCAVAS

The purpose of the Finnish Cardiovascular Study (FINCAVAS) is to construct a risk profile - using genetic, haemodynamic and electrocardiographic (ECG) markers - of individuals at high risk of cardiovascular diseases, events and deaths. All patients scheduled for an exercise stress test at Tampere University Hospital, who gave informed consent to participate, were recruited between October 2001 and December 2007. The total number of participants was 4,567. In addition to repeated measurements of heart rate and blood pressure, digital high-resolution ECG at 500 Hz was recorded continuously during the entire exercise test, including the resting and recovery phases. About 20% of the patients were examined with coronary angiography. Genetic variations known or suspected to alter cardiovascular function or pathophysiology were analyzed to elucidate the effects and interactions of these candidate genes, exercise, and commonly used cardiovascular medications.

Malmo Diet and Cancer Study (MDCS)

MDCS is a community-based prospective epidemiologic cohort of middle-aged individuals from Southern Sweden.[7] In total, 30,447 subjects attended a baseline exam in 1991-1996, when they filled out a questionnaire and underwent anthropometric and blood pressure measurements. Hypertension was defined as self-reported use of antihypertensive medications or measured blood pressure ≥140/90 mmHg. Prevalent or incident cases of atrial fibrillation (AF), heart failure and ischemic heart disease were ascertained from nation-wide hospital registers with high validity as described previously.[8] Genome-wide genotyping of single nucleotide variants was performed using the Illumina Human Omni Express Exome BeadChip kit. Genotyping was performed in a nested case-cohort design, including a random subset of 7255 subjects with complete data and 876 cases with incident AF. Imputation was performed to genotypes in the 1000 Genomes Project phase 1 using IMPUTE.

UK Biobank

Details of genotyping, imputation, and calculation of principal components of ancestry in the UK biobank interim dataset can be found on the UK biobank website (http://www.ukbiobank.ac.uk/). Briefly, samples were genotyped either by UK BiLEVE Axiom array (UKBL) or UK Biobank Axiom array (UKBB). Both arrays include ~800,000 SNPs and more than 95% of common marker contents are similar. Imputation was phased by modified version of SHAPEIT2 and imputed by IMPUTE2, using a combined panel of UK10K haplotype and 1000G phase 3 as the reference panel. All significant variants detected in the discovery study passed quality control filters in the UK biobank data (imputation quality info ≥ 0.4, variant missing rate < 5%, individual missing rate < 10%, and variant genotype probability > 0.9 in > 90% of the individuals). Variants were then transformed to hard-called genotypes (probability threshold ≥ 0.9, minor allele frequency (MAF) ≥ 0.01, and missing rate per variant <5%). We used logistic regression to test the association between each hard-called variant and risk of AF using an additive genetic model, adjusting for baseline age, sex, array, and the first 5 principal

components of ancestry. Quality control, transformation and analyses were performed by QCTOOL and Plink v1.90b.

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Supplemental Table 1. Analysis-specific numbers of included autosomal SNPs and genomic inflation estimates by study sample.

	Age				Sex		Body mass index		Hypertension	
	≤65 years		>65 years							
Cohort	N SNPs	(λ)	N SNPs	(λ)	N SNPs	(λ)	N SNPs	(λ)	N SNPs	(λ)
Incident										
AGES	—	—	—	—	2136078	0.995	2136115	1.060	2101296	1.033
ARIC	2165687	0.987	—	—	2179076	1.004	2179099	1.169	2179081	1.012
CHS	—	—	2067494	1.045	2067674	1.027	2067674	1.121	2067674	1.021
FHS	—	—	2144443	1.004	2144443	1.005	2144443	1.061	2144439	0.994
MESA	—	—	2093768	1.024	2171564	1.039	2171941	1.061	2171884	1.033
PREVEND	—	—	—	—	1859130	1.040	1859123	1.105	1858235	1.006
PROSPER	—	—	2163841	1.014	2163841	0.998	2163841	1.004	2163841	1.006
RS	2164948	1.022	2165443	1.20	2165273	1.002	2165437	0.992	2165217	1.008
WGHS	2159805	1.023	2159805	1.001	—	—	2159805	1.032	2159805	0.995
Prevalent										
AFNET/KORA	2168049	1.017	—	—	2168048	0.988	2168003	1.028	2168050	1.030
AGES	—	—	—	—	2133548	0.998	2126844	1.033	2133550	1.060
BioVU 660	—	—	—	—	1658797	1.015	1660844	0.846	1625789	0.951
BioVU o1	—	—	1813638	1.021	1813587	1.008	1813746	0.991	1803269	0.984
CCAF	2176365	1.036	2173984	1.027	2175987	1.001	2175951	1.014	2175987	1.024
FHS	—	—	2144443	1.031	2144393	1.009	2143524	1.009	2144443	1.031
LURIC	2169011	1.005	2169011	1.003	2169007	0.999	2169003	1.014	2163105	1.022
MGH/MIGEN	2166720	1.004	—	—	2167717	0.967	—	—	—	—
RS	—	—	2164958	1.021	2165408	1.011	2165435	1.019	2165315	1.000
SHIP	—	—	—	—	2237384	1.036	2239095	1.075	2238479	1.033

Abbreviations: λ, genomic inflation factor.

Supplemental Table 2. Sixteen AF-associated loci reported in previous association studies.

Locus	SNP	Closest gene	Minor allele frequency (%)	Relative risk (95% CI)	*P* Value	Reference
1q21	rs6666258	*KCNN3*	29.9	1.18 (1.13-1.23)	2.0×10^{-14}	[1]
1q24	rs3903239	*PRRX1*	44.7	1.14 (1.10-1.18)	9.1×10^{-11}	[1]
3p25	rs4642101	*CAND2*	35	1.10 (1.06-1.14)	9.8×10^{-9}	[2]
4q25	rs1448818	*PITX2*	25	1.12 (1.08-1.17)	1.6×10^{-8}	[9]
4q25	rs6817105	*PITX2*	13.1	1.64 (1.55-1.73)	1.8×10^{-74}	[1]
4q25	rs4400058	*PITX2*	9	1.28 (1.21-1.36)	2.2×10^{-16}	[9]
4q25	rs6838973	*PITX2*	44	1.11 (1.08-1.15)	6.0×10^{-9}	[9]
6q22	rs13216675	*GJA1*	31	1.10 (1.06-1.14)	2.2×10^{-8}	[2]
7q31	rs3807989	*CAV1*	40.4	0.88 (0.84-0.91)	9.6×10^{-11}	[1]
9q22	rs10821415	*C9orf3*	42.4	1.13 (1.08-1.18)	7.9×10^{-9}	[1]
10q22	rs10824026	*SYNPO2L*	15.8	0.85 (0.81-0.90)	1.7×10^{-8}	[1]
10q24	rs12415501	*NEURL*	16	1.18 (1.13-1.23)	6.5×10^{-16}	[2]
12q24	rs10507248	*TBX5*	27	1.12 (1.08-1.16)	5.7×10^{-11}	[2]
14q23	rs1152591	*SYNE2*	47.6	1.13 (1.09-1.18)	6.2×10^{-10}	[1]
15q24	rs7164883	*HCN4*	16.0	1.16 (1.10-1.22)	1.3×10^{-8}	[1]
16q22	rs2106261	*ZFHX3*	17.6	1.24 (1.17-1.30)	3.2×10^{-16}	[1]

Supplemental Table 3. Details of study populations.

Discovery Cohorts					
Cohort	**Country**	**Study design**	**AF diagnosis**	**Hypertension Definition**	**Reference**
AFNET/KORA	Germany	Case-Control	ECG	>140mmHg, or diastolic blood pressure >90mmHg, or use of antihypertensive medication for this purpose	10,11
AGES	Iceland	Cohort	ICD-10: I48	Hypertension, either self reported from qustionnaire, derived from physiological measurements (systolic blood pressure, diastolic blood pressure), or use of hypertension medications	12
ARIC	United States	Cohort	ECG at study visits, hospital discharge codes or death certificates (ICD-9 code 427.31 or 427.32, or ICD-10 code I48).	SBP>=140, DBP>=90 or use of antihypertensive meds	13,14
BioVU	United States	Cohort: hospital-based study (Biobank)	>18 years and an ICD-9 diagnosis for AF or flutter (ICD-9: 427.3, 427.31, and 427.32), or a cardiologist diagnosis of AF as identified by a natural language processing tool from the unstructured free text of the ECG impression. In all instances, patients with a history of a heart transplant were excluded (Current Procedural Terminology: 33935, 3394, and 580; ICD-9: V42.1, 996.83)	Hypertension was identified from the EHR using a multimodal algorithm using blood pressures, billing codes, medications, and text mentions of hypertension.	3,5,15
CHS	United States	Cohort	Annual CHS ECG or by ICD-9 code on a hospital discharge.	SBP>=140, DBP>=90 or use of antihypertensive meds for treatment of high blood pressure	16
CCFS	United States	Case-control	ECG or medical report	SBP>=140, DBP>=90 or history of hypertension	1
FHS	United States	Cohort	Cardiovascular hospital and outside records and ECG at all FHS examinations	SBP>=140, DBP>=90 or use of antihypertensive meds	17,18
LURIC	Germany	Cohort: hospital-based study	ECG or medical report	SBP>=140, DBP>=90 or history of hypertension	19

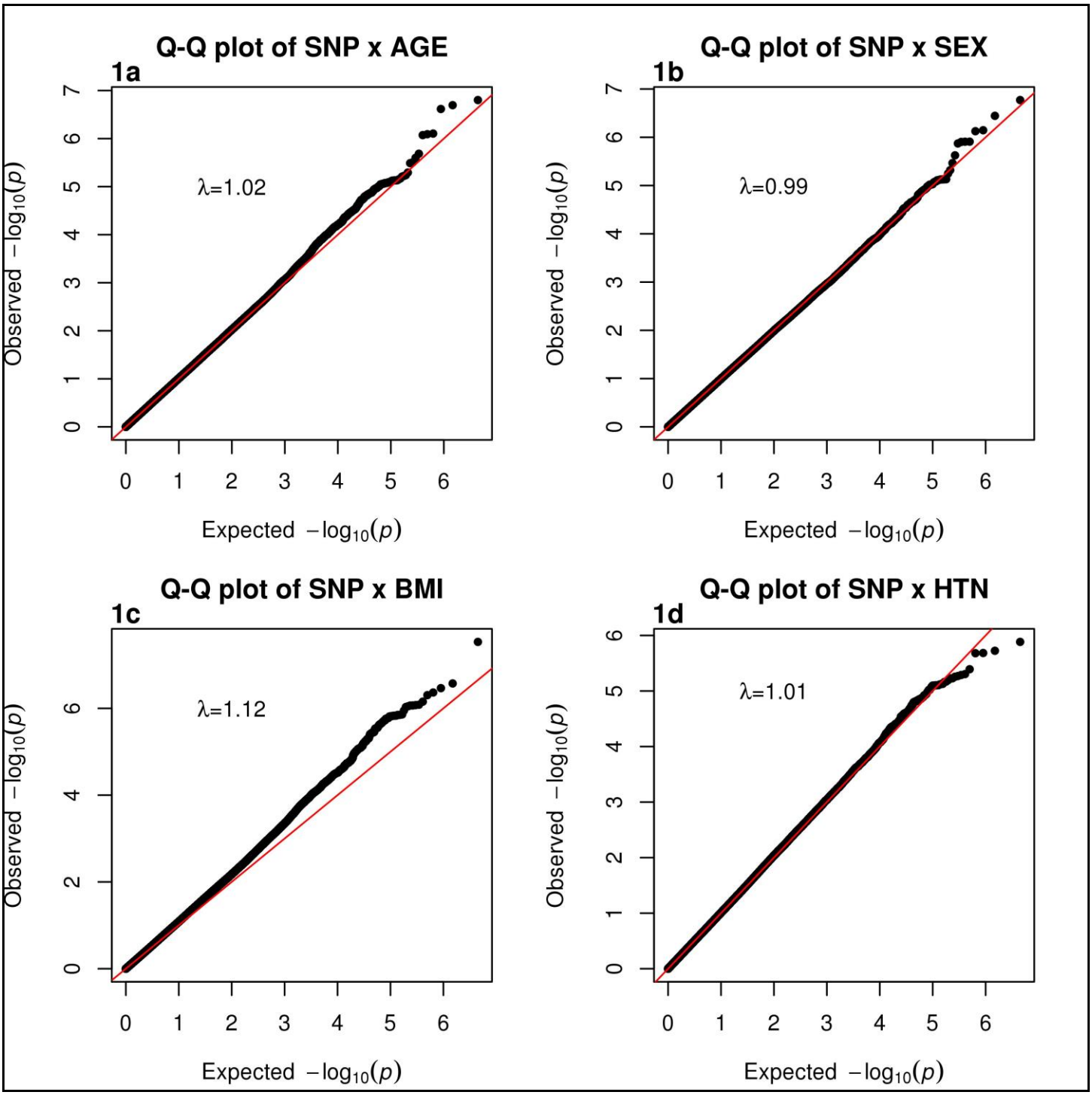
MESA	United States	Cohort	All interim hospital admissions, cardiovascular outpatient diagnoses, and deaths. More information is included in the cohort description.	Self-reported treatment for hypertension or a systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg	20,21
MGH/MIGEN	United States	Cohort: hospital-based study	ECG	-	22,23
PREVEND	The Netherlands	Cohort	ECG	Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or use of antihypertensive drugs	6,24
PROSPER	Ireland, Scotland, and the Netherlands	Randomized, double-blind, placebo-controlled trial	ECG	systolic blood pressure was ≥ 140 mm Hg or the diastolic blood pressure was ≥ 90 mm Hg	25
Rotterdam Study	The Netherlands	Cohort	Study visit ECG, hospital discharge information, and general practitioner diagnoses.	SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or antihypertensive medication	26,27
SHIP	Germany	Cohort	Study visit ECG or tele-ecg	SBP ≥ 140 mmHg and/or DBP ≥ 140 mmHg or antihypertensive medication	28
WGHS	United States	Cohort	ECG or medical report	History of hypertension ≥ 140/90, defined as MD diagnosis at enrollment or run-in OR SBP ≥ 140 OR DBP ≥ 90	29-31
Replication Cohorts					
Cohort	**Country**	**Study design**	**AF diagnosis**	**Hypertension Definition**	**Reference**
Beat-AF/ GAPP	Switzerland / Liechtenstein	Case-control	ECG	≥140/90 mmHg or use of BP lowering drugs	32
FINCAVAS	Finland	Cohort: hospital-based study	ECG, hospital discharge information	≥140/90 mmHg or use of BP lowering drugs	33
MDCS	Sweden	Case-control	National register	≥140/90	7
UK Biobank	United Kingdom	Cohort: Biobank	Verbal interview, diagnosis codes, or procedure codes	On anti-hypertensive medication at enrollment	

Supplemental Table 4. Details regarding study genotyping and data analysis.

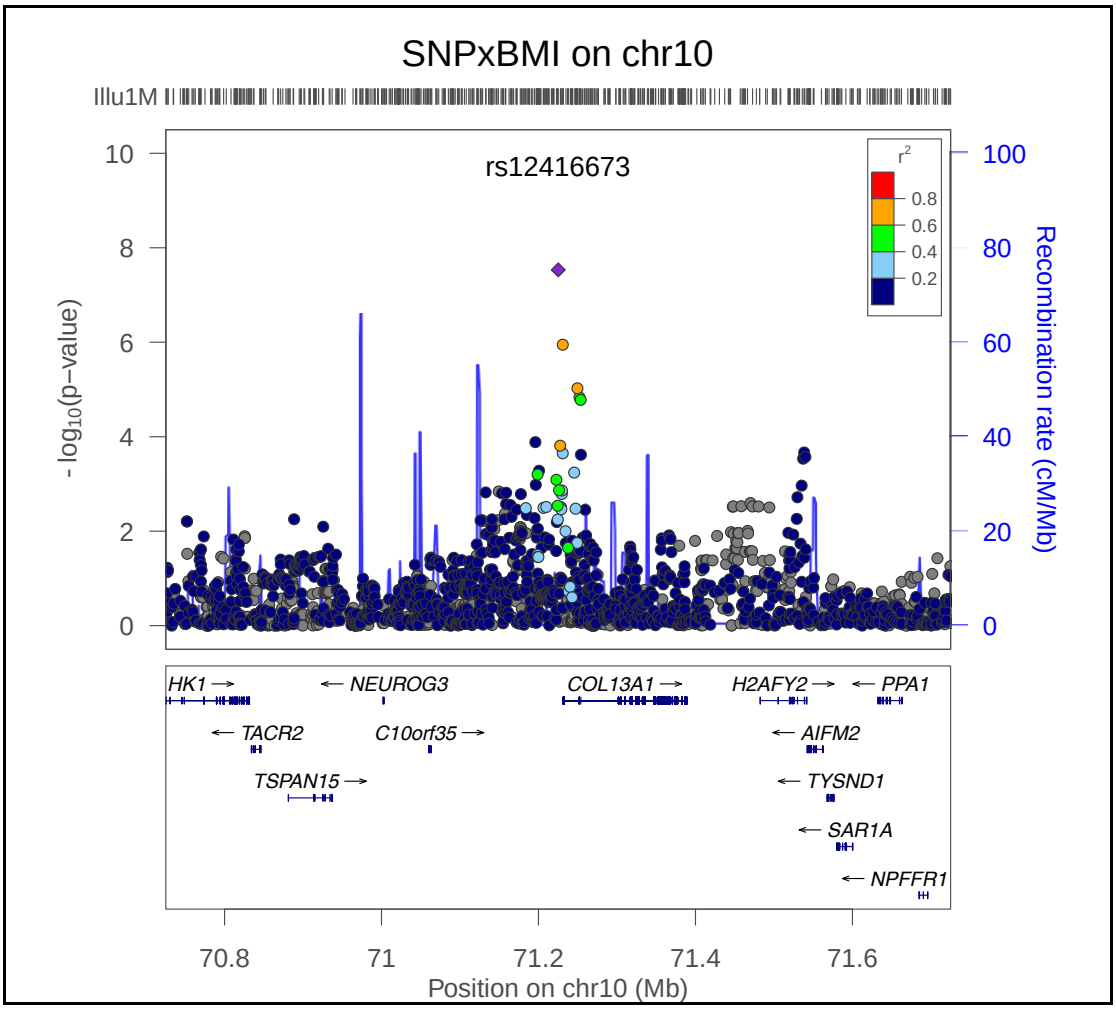
Discovery Cohorts							
Study	**Genotyping platform**	**Imputation software**	**Imputation Backbone / NCBI Build**	**Statistical software**	**GWAS Statistical Analysis**	**Covariates**	**Robust variance estimator (Y/N)**
AFNET/KORA	Published data[9]			ProbABEL, R	Logistic regression	Age, sex, 4 PCs	Y
AGES	Published data[9]			ProbABEL, R	Inc: Cox proportional hazard regression; Pre: Logistic regression	Age, sex	Y
ARIC	Published data[9]			ProbABEL, R	Cox proportional hazard regression	Age, sex, center	N
BioVU	Human660W-Quad BeadChip / HumanOmni1-Quad BeadChip	SHAPE-IT/IMPUTE 2	1000G Phase 1 integrated v3 panel /b37	PLINK	Logistic regression	Age, sex, 2PCs	N
CHS	Published data[9]			R	Cox proportional hazard regression	Age, sex, clinic	N
CCFS	Published data[9]			ProbABEL, R	Logistic regression	Age, sex, 3 PCs + CAD	Y
FHS	Published data[9]			R	Inc: Cox proportional hazard regression; Pre: GEE	Age, sex	Y
LURIC	Published data[9]			ProbABEL	Logistic regression	Age, sex, 3 PCs	Y
MESA	Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA)	IMPUTE2	1,000 Genomes Phase I integrated variant set (NCBI build 37 / hg19)	ProbABEL, R	Logistic regression	Age, sex, sites, 2PCs	Y
MGH/MIGEN	Published data[9]			R	Logistic regression	Age, sex, 6 PCs	N(age), Y(sex)
PREVEND	Illumina CytoNP12 v2	SHAPE-IT/IMPUTE 2	1000G Phase 1 integrated v3 panel/b37	ProbABEL, R	Logistic regression	Age,sex, PC1-5	N

PROSPER	Published data[9]			ProbABEL, R	Inc: Cox proportional hazard regression	Age, sex, PC 1-4	N
Rotterdam Study	Published data[9]			ProbABEL, R	Inc: Cox proportional hazard regression; Pre: Logistic regression	Age, sex	N
SHIP	Published data[9]			ProbABEL	Logistic regression	age, sex	Y
WGHS	Published data[9]			ProbABEL	Cox PH regression (incident AF)	age, eigenvector E2 for HXHTN interaction only	N(age), Y(sex, bmi)
Replication Cohorts							
Study	**Genotyping platform**	**Imputation software**	**Imputation Backbone / NCBI Build**	**Statistical software**	**GWAS Statistical Analysis**	**Covariates**	**Robust variance estimator (Y/N)**
Beat-AF/GAPP	Illumina HumanCoreExome	SHAPEIT v2.r790 + IMPUTE v.2.3.2	Build 37	R	Logistic regression	Sex, 10 PCs	N
FINCAVAS	Illumina Metabochip and Human CoreExome	SHAPEIT v2, IMPUTE v2.3.0	1000G Phase 1 integrated v3 panel /b37	R	Logistic regression	Age, sex and 10 PCs	N
MDCS	Illumina Human Omni Express Exome BeadChip	IMPUTE	NCBI Build 37	R	Cox proportional hazard regression	Age, sex and 10 PCs	N
UK Biobank	UK Bileveor UK Biobank Axiom Array	IMPUTE	Combined 1000G and UK10K / Hg37	R	Logistic regression	Age, sex, and first 5 PCs	N

Supplemental Figure 1a-1d. Quantile-quantile plots for each genetic interaction analysis on chromosomes 1-22.



Supplemental Figure 2. Regional association plot for genome-wide significant SNP by BMI interaction on chromosome 10.



Genetic Interactions and Atrial Fibrillation
Supplemental Material

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