Title
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Novel Genetic Markers Associate with Atrial Fibrillation Risk in Europeans and Japanese

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Abstract

Objectives—To identify non-redundant atrial fibrillation (AF) genetic susceptibility signals and examine their cumulative relations with AF risk.

Background—AF-associated loci span broad genomic regions that may contain multiple susceptibility signals. Whether multiple signals exist at AF loci has not been systematically explored.

Methods—We performed association testing conditioned on the most significant, independently associated genetic markers at nine established AF loci using two complementary techniques in 64,683 individuals of European ancestry (3,869 incident and 3,302 prevalent AF cases). Genetic risk scores were created and tested for association with AF in Europeans and an independent sample of 11,309 individuals of Japanese ancestry (7,916 prevalent AF cases).

Results—We observed at least four distinct AF susceptibility signals on chromosome 4q25 upstream of *PITX2*, but not at the remaining eight AF loci. A multilocus score comprised of 12 genetic markers demonstrated an estimated 5-fold gradient in AF risk. We observed a similar spectrum of risk associated with these markers in Japanese. Regions containing AF signals on chromosome 4q25 displayed a greater degree of evolutionary conservation than the remainder of the locus, suggesting that they may tag regulatory elements.

Conclusions—The chromosome 4q25 AF locus is architecturally complex and harbors at least four AF susceptibility signals in individuals of European ancestry. Similar polygenic AF susceptibility exists between Europeans and Japanese. Future work is necessary to identify causal variants, determine mechanisms by which associated loci predispose to AF, and explore whether AF susceptibility signals classify individuals at risk for AF and related morbidity.

Keywords

Atrial fibrillation; atrial flutter; genetic; risk; prognosis

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Atrial fibrillation (AF) is a heritable (1-6) and morbid (7) arrhythmia. Genome-wide association studies have identified nine susceptibility regions on eight chromosomes that implicate genes encoding transcription factors involved in cardiopulmonary development, cardiac expressed ion channels, and other signaling molecules in the pathogenesis of AF (8-12).

Genetic variants associated with AF at previously reported loci extend over broad genomic distances, often spanning tens or hundreds of thousands of bases. The large span of associated variants at some AF loci raises the possibility that the loci may contain multiple independent, or at least non-redundant, susceptibility signals. A refined understanding of the architecture of association signals at the top loci may identify additional novel susceptibility signals, help characterize functional elements involved in the pathogenesis of AF, and enable stratification of individuals according to genetic risk for arrhythmia.

We sought to determine whether additional AF susceptibility signals exist within loci previously identified in genome-wide association studies of AF among participants of European ancestry within the AFGen Consortium (12). We then sought to determine whether our observations regarding AF-associated genetic variants are generalizable in an independent sample of Japanese ancestry.

Methods

Study participants

We included subjects of European ancestry from eight prospective cohort and twelve case-control study samples derived from the Age, Gene/Environment Susceptibility (AGES) Reykjavik Study, Atherosclerosis Risk in Communities (ARIC), Cleveland Clinic Lone AF GeneBank Study (CCAF), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), German Competence Network for Atrial Fibrillation / Cooperative Research in the Region of Augsburg (AFNET/KORA), Ludwigshafen Risk and Cardiovascular Health Study (LURIC), Massachusetts General Hospital Atrial Fibrillation Study (MGH), Heart and Vascular Health Study (HVH), PHArmacogenetic study of Statins in the Elderly at risk / PROspective Study of Pravastatin in the Elderly at Risk for vascular disease (PHASE/ PROSPER), Rotterdam Study (RS-I, RS-II), Study of Health in Pomerania (SHIP), and Women's Genome Health Study (WGHS). Validation of our findings was performed in the BioBank Japan case-control sample. Brief summaries of each study are provided in the **online supplement**. The Institutional Review Boards at each of the respective studies approved all of the studies. Study participants provided written informed consent to participate in genetic research.

AF ascertainment

In each study, AF was ascertained from electrocardiograms, Holter recordings, medical records, or hospital discharge diagnostic codes (ICD-8 427.92, ICD-9-CM 427.3, 427.31 or 427.32, or ICD-10 I48 in any position), as previously described (13-19). AF was considered prevalent if ascertained in case-control studies or if it was present at or prior to DNA collection in cohort studies. Incident AF was defined if it occurred after DNA collection in

participants without a history of AF. In ARIC, age at baseline was used rather than age at DNA collection for these definitions and follow-up.

Genotyping

Genome-wide genotyping for array-specific SNPs was conducted in each study as previously described (12). Imputation was performed for up to 2.2 million autosomal SNPs based on the HapMap CEU panel (20). For AFNet/KORA, the SNP rs12235316_G was substituted for rs10821415_A (distance ~ 40 kilobases [kb], r^2=1) on chromosome 9q22 as this SNP was unavailable based on the study's imputation. Details regarding genotyping platforms, quality control metrics, and imputation methods are provided in **Supplemental Table 1**.

Statistical analysis

We defined AF loci *a priori* as the genomic region centered on the most significantly associated SNP from a prior meta-analysis (12) and flanked by one megabase (Mb) on either side. To determine whether multiple associated signals for AF exist beyond the top associated variant at each AF associated locus, we employed two different conditional analysis approaches.

First, we performed a ***traditional conditional analysis***, in which we iteratively repeated association testing within each genome-wide significant locus with adjustment for the most significantly associated remaining genome-wide association signal ($P<5\times10^{-8}$) at the locus in each cohort, until no further genome-wide significant SNPs remained. Since we previously identified a total of three distinct susceptibility signals at the chromosome 4q25 locus, we adjusted for the genotypes of SNPs tagging these signals (rs6817105, a perfect proxy for a previously reported signal rs2200733; rs17570669; and rs3853445) when performing association testing on chromosome 4q25 (21). At all other loci, we began the iterative association testing process by adjusting for the single most significantly associated SNP at the locus. Study-specific effect estimates were combined via meta-analyses as described below.

Second, as an alternative method of discovery, we employed an ***approximate conditional analysis*** to estimate non-redundant signals directly from the summary statistics of a prior genome-wide meta-analysis (12) using the GCTA software package (22). Linkage disequilibrium and allele frequencies were estimated from 2,058 unrelated individuals from FHS. Potential non-redundant signals identified were then tested for association with AF in each study cohort, and the study-specific effect estimates were combined by meta-analyses.

For each approach we examined study-specific associations between SNPs and AF using logistic regression for prevalent AF, and proportional hazards regression for incident AF. In FHS, we used generalized estimating equations with an independence working correlation structure in a logistic model for prevalent AF, as implemented in the geepack package in R (23) and robust variance estimators (clustering on family) in a Cox model for incident AF as implemented in the survival package in R (24) to account for potential relatedness among participants. All models were fitted assuming additive genetic effects for each SNP (i.e.,

multiplicative relative risks). Age at DNA collection (or baseline for ARIC), sex, and principal components of ancestry significantly associated with AF were included in the models.

For all analyses, study-specific regression estimates were meta-analyzed using an inverse variance weighted method. Prevalent and incident AF were meta-analyzed together as previously performed (10,12). We considered a two-sided $P<5\times10^{-8}$ to provide significant evidence for independent associations between SNPs and AF. We considered a two-sided $P\leq1\times10^{-7}$ to provide suggestive evidence of association. We calculated linkage disequilibrium metrics (r^2, D') for all AF associated variants in the same region.

Since the two conditional analysis approaches yielded similar results (see results below), when different SNPs identified from the two different approaches at a given locus were in linkage disequilibrium with one another, we selected the SNP with the smaller *P*-value for further modeling. We then fit multi-SNP models that included each of the selected non-redundant SNPs to examine adjusted SNP associations with AF.

We constructed both unweighted and weighted multimarker genetic risk scores with the selected independent AF susceptibility signals by summing the dosages of AF risk SNPs. In weighted scores, we multiplied the allele dosages for each individual by SNP-specific regression coefficient estimates derived from either the conditional analysis (for SNPs on chromosome 4q25) or a prior meta-analysis (for the remaining SNPs) (12). Unweighted scores were the sum of dosages for all SNPs included in the score.

We hypothesized that SNPs contribute additively to AF risk and therefore tested the associations between an aggregate multimarker panel of risk alleles and AF. We constructed multimarker scores for the chromosome 4q25 locus alone as well as across all AF-associated loci. We included a total of four non-redundant SNPs from chromosome 4q25 and eight from the remaining loci (see results below); therefore the total number of AF risk alleles ranged from zero to eight at the chromosome 4q25 locus and from zero to 24 across all loci. We created eight categories for multimarker scores at the chromosome 4q25 locus, reflecting each of the estimated number of risk alleles. For scores across all loci, we created 12 potential categories, each reflecting increments of two estimated AF risk alleles, in order to avoid rare and inestimable categories of risk alleles that might occur with single risk allele increments. For weighted multimarker risk scores, ten categories were selected based on cutoff values used to derive score deciles in the MGH sample (**Supplemental Table 2)**.

In order to determine whether observed associations were generalizable beyond individuals of European ancestry, we performed association testing in the independent BioBank Japan sample using the same statistical methodology described above.

We examined whether non-redundant signals at the chromosome 4q25 locus were more likely to be evolutionarily conserved than the rest of the locus by comparing sequence alignments in 44 vertebrate species. We compared the average phylogenetic conservation scores between 10 kb regions centered on each of the non-redundant signals on chromosome 4q25 to that of the rest of the 1 Mb locus using the Student's t-test (see **online supplement** for details).

In order to examine the relations between identified genetic variants and prognosis, we assessed the relations between non-redundant AF-associated SNPs and both survival and survival free of major disease or mortality based on a prior genome-wide association study of aging in individuals of European ancestry (25). Briefly, the analysis was performed by examining associations between SNPs and time to incident disease or death in 25,007 individuals aged greater than 55 years. Associations were modeled using proportional hazards regression with time since DNA collection as the time scale, with adjustment for age at DNA collection and sex. Survival free of major disease or mortality was modeled by using time to death or to the first myocardial infarction, heart failure, stroke, dementia, hip fracture, or cancer diagnosis. Participants with any of the modeled outcomes at baseline were excluded from the analysis.

Results

Characteristics of participants in the included studies are provided in **Table 1**. Overall, the analysis included a total of 64,683 individuals of European ancestry, including 3,302 individuals with prevalent AF and 3,869 individuals with incident AF.

We observed evidence for multiple genome-wide significant AF susceptibility signals on chromosome 4q25, but no independent signals beyond the first at the remaining eight AF-associated loci (**Figure 1**). With the ***traditional conditional analysis*** approach, we identified two potential signals associated with AF (rs2723288 and rs4400058) with $P<5\times10^{-8}$ after simultaneous adjustment for previously reported signals (rs6817105, rs17570669, and rs3853445). After including rs2723288, rs6817105, rs4400058, rs17570669, and rs3853445 in a model, rs6817105 ($P=1.1\times10^{-85}$) and rs4400058 ($P=2.2\times10^{-16}$) remained significantly associated with AF (**Table 2**). One of the previously reported (21) signals (rs3853445, $P=1.0\times10^{-7}$) remained suggestive of association whereas another was not associated with AF at the prespecified genome-wide significance threshold (rs17570669, $P=5.2\times10^{-3}$). Notably, rs17570669 had a low imputation quality score in two studies (HVH, CHS, Rsq=0.12, 0.18), and had imputation quality less than 0.8 in several others (SHIP, RS-I, AGES, CCAF, WGHS, AFNET, FHS), which may have affected the association signal. Nevertheless, we did not include it in subsequent multimarker analyses given the lack of genome-wide significant association that was observed in the adjusted association analyses.

In the ***approximate conditional analysis*** approach, we identified four potential signals at the chromosome 4q25 locus, tagged by SNPs rs1448818, rs6817105, rs4032974, and rs6838973 (**Table 2**). In models in which we adjusted for all four potential SNPs, rs1448818 ($P=1.6\times10^{-8}$), rs6817105 ($P=5.1\times10^{-95}$), and rs6838973 ($P=6.0\times10^{-9}$) remained significantly associated with AF. Results from the ***traditional conditional analysis*** and ***approximate conditional analysis*** were similar to one another in that the significantly associated signals were in linkage disequilibrium with one another (**Table 2** and **Supplemental Table 3**).

When different SNPs identified from the two different approaches at a given locus were in linkage disequilibrium with one another, we selected the SNP with the smallest *P*-value from either approach for further modeling (rs1448818, rs6817105, rs4400058, rs6838973).

We examined adjusted SNP associations with AF by meta-analyzing results from cohort-specific models that included all of the selected non-redundant SNPs on chromosome 4q25 alone, as well as across all loci (**Table 3**). We observed persistent genome-wide association between the selected non-redundant chromosome 4q25 SNPs and AF, but attenuation of associations at some of the other loci that was most pronounced for the chromosome 7q31 locus (rs3807989, $P=6.3\times10^{-3}$).

The identified signals on chromosome 4q25 span a 195 kb intergenic region (**Figure 2** and **Supplemental Figure 1)**. The newly identified signal tagged by rs1448818 is 135 kb centromeric of the top signal at the locus, and is located 7 kb upstream of the transcription factor *PITX2.* The signal tagged by rs4400058 is 11 kb telomeric of the top signal. Overall, the 10 kb genomic regions flanking each non-redundant SNP identified in our analysis were associated with a greater degree of phylogenetic conservation than nucleotides at the remainder of the 1 Mb locus on chromosome 4q25 (average conservation score 0.29±1.16 vs. 0.19±1.03, $P<0.001$, **Supplemental Table 4**).

We then constructed multimarker genetic risk scores comprising the genome-wide significant non-redundant SNPs at chromosome 4q25 listed in Table 3 and across all AF loci to determine the composite associations between AF risk alleles and AF. Both unweighted and weighted risk scores were significantly associated with AF (**Table 4**).

We observed a graded risk of AF that correlated with the number of inherited AF risk alleles (**Figure 3** and **Supplemental Figure 2**). The most commonly observed number of AF risk alleles across the 12 non-redundant SNPs (9-10) was observed in 25% of our sample. We observed 22% of individuals in our sample that had greater than ten estimated AF risk alleles and that carried an increased age- and sex-adjusted risk for AF, and 42% that had fewer than nine AF risk alleles and that carried a reduced risk for AF. By comparing the estimated relative risks between those carrying the greatest and lowest numbers of inherited AF risk alleles, we observed an estimated 4-fold difference in AF risk captured by SNPs at the chromosome 4q25 locus, and 5-fold when considering all independent loci.

We further sought to determine whether the observed associations were generalizable beyond individuals of European ancestry by examining AF-associated genetic variants in 11,309 independent individuals of Japanese ancestry from the BioBank Japan sample, 7,916 of whom had AF. We observed that AF risk alleles for the most-significant SNPs in Europeans at four of the nine loci associated with AF were similarly associated with AF beyond genome-wide significance thresholds in Japanese after adjustment for one another (**Table 3**). Overall, effect estimates were in the same direction for nine of the twelve tested variants in both European and Japanese samples. Results were not substantively changed in a subset in which adjustment for principal components of ancestry was possible (**Supplemental Table 5**). We further observed that multimarker genetic scores were significantly associated with AF similar to those observed in individuals of European ancestry (**Table 4**, **Figure 3** and **Supplemental Figure 2**). Among Japanese, we observed an approximately 5-fold gradient of AF risk when considering the non-redundant markers on chromosome 4q25 identified in Europeans, and 4-fold when considering all loci (**Figure 3**).

We also examined the associations between each independent AF-associated SNP with survival (n=25,007, events = 8,444) and survival free of major disease or mortality (n=16,995, events = 7,314) from prior genome-wide association studies of aging (sample characteristics provided in **Supplemental Table 6**)(25). We did not observe any significant associations between each of the AF susceptibility SNPs and either survival or survival free of major disease after adjustment for multiple hypothesis testing with 12 SNPs (**Supplemental Table 7**). The most significant association was with SNP rs3903239 at the *PRRX1* locus on chromosome 1q24 (RR for G [AF risk] allele 1.04, 95% CI 1.01-1.08, $P=6.6\times10^{-3}$ for survival; and RR 1.04, 95% CI 1.01-1.08, $P=0.02$) for survival free of major disease.

Discussion

We employed two complementary methods to systematically search for multiple AF susceptibility signals at nine genome-wide associated loci in a total of 64,683 individuals of European ancestry, in which 7,171 individuals with AF were included. Our findings demonstrate the presence of at least four distinct AF susceptibility signals in a large intergenic region on chromosome 4q25. A multiallelic risk score comprising twelve AF susceptibility signals contributed to an estimated 5-fold age- and sex- adjusted gradient of AF risk. Whereas about 22% of individuals had increased age- and sex-adjusted risk of AF relative to those with the most common number of AF risk alleles, about 42% of individuals had decreased AF risk on the basis of the number of AF risk alleles that they carried. In an independent Japanese sample, we observed nearly identical findings, suggesting that the AF-genetic risk markers identified in our analysis may be generalizable beyond populations of European ancestry.

We did not observe evidence for multiple genome-wide significant susceptibility signals at AF loci other than chromosome 4q25. Our observations extend previous reports about the relations between genetic markers and AF risk, and underscore the complex nature of the AF susceptibility locus on chromosome 4q25. Prior analyses have reported independent markers on chromosome 4q25 related to AF (8,21), or post-operative AF (26). Indeed, SNP rs4400058 is in perfect linkage with a previously reported SNP rs10033464 at chromosome 4q25 in a genome-wide association study of Icelanders (8). However, rs10033464 has not been consistently associated with AF in other analyses of individuals of European ancestry (21,26,27).

In the present analysis we observed a gradient of risk that correlated with the number of AF risk alleles present. We have observed that AF risk may vary substantially between individuals with the same number of risk alleles in a subset of the present AFGen sample (21), possibly due to variable effects of particular SNPs, nonlinear interactions between specific risk alleles, or differences in other clinical or environmental AF risk factors. Few studies have explored the utility of family history (6) or genotypic information (28,29) to discriminate AF risk. Future work will be necessary to assess the best modeling strategy for incorporating genetic markers into AF risk prediction efforts, and to determine the best clinical setting in which to utilize such tools.

Genetic associations that extend beyond single ancestral groups may facilitate the identification of true biological variation underlying disease (30). Few prior analyses have examined the relations between genetic factors and AF in individuals of Japanese descent. A prior analysis from the AFGen consortium related the top SNPs identified at genome-wide susceptibility loci for AF to those in a sample of 843 individuals with AF and 3,350 without from the BioBank Japan sample, demonstrating marginal associations between the top variants at the *PRRX1*, *PITX2*, *CAV1*, and *ZFHX3* loci. In aggregate, current and prior observations provide support for a shared genetic susceptibility to AF in individuals of European and Japanese descent, despite a lower prevalence of AF among individuals of Japanese ancestry (31,32).

Our findings implicate a broad AF susceptibility locus on chromosome 4q25. The four susceptibility signals we identified span 195 kb across an intergenic region on chromosome 4q25. The identified variants are upstream of *PITX2*, a homeodomain transcription factor involved in determining right-left cardiac symmetry, specifying pulmonary venous myocardium, and suppressing formation of a default sinus node in the left atrium (33-35). The expression of the Pitx2c isoform is reduced in left atrial samples from humans with AF as compared to those without a history of AF (36). Knockout of the Pitx2c isoform in mice is associated with increased susceptibility to pacing-induced atrial arrhythmias and shortened atrial refractory periods (37), consistent with electrical reentry as a predominant mechanism of AF. Our present findings implicate genetic variation within 7 kb of *PITX2* in the pathogenesis of AF, nearly 150 kb closer to the gene than the top AF-associated signal at the locus in the AFGen sample.

Our findings also implicate regulatory elements in the pathogenesis of AF. Examination of phylogenetic conservation demonstrates that the identified AF susceptibility signals cluster around conserved noncoding regions at chromosome 4q25. Future work will be necessary to determine the functional role of these loci and the causal elements tagged by the identified AF susceptibility SNPs. The identification of individuals at high and low genetic risk of AF may enhance the power of future sequencing efforts to identify genetic variation at the chromosome 4q25 locus underlying AF.

Our manuscript extends previous knowledge about the nature of AF susceptibility regions. First, our analysis is the first to systematically assess for multiple susceptibility signals at all genome-wide significant AF susceptibility loci. Second, our results provide the strongest evidence to date that multiple susceptibility signals exist at chromosome 4q25; prior analyses did not rely on stringent genome-wide significance criterion to identify multiple signals at existing susceptibility loci. Third, our analysis is the first to implicate a distinct susceptibility region within 7 kb of *PITX2* in the pathogenesis of AF, whereas prior data had not distinguished this region from the peak association signal about 150 kb upstream of the gene. Fourth, our analysis extends previous observations by demonstrating that the additive effects of genetic variants tagging AF susceptibility signals in Europeans, particularly at chromosome 4q25, transcend ancestry and associate similarly with AF risk in Japanese.

Limitations

Our study must be interpreted in the context of the study design. Specifically, our analysis included only individuals of European and Japanese ancestry, and therefore it is not clear whether our findings are generalizable to individuals of other ancestral backgrounds. Second, we cannot exclude the possibility that our stringent significance threshold excluded other true independent susceptibility signals with heterogeneous associations across cohorts. Indeed, we previously identified a genetic variant on chromosome 4q25 (rs17570669) that was independently associated with AF in prior work and which was nominally associated with AF in the present analysis, but not at our predefined genome-wide significance threshold. Whether this is due to poor imputation of the SNP genotypes, heterogeneity in the association across study samples, or true absence of association when considering other more significantly associated SNPs is not clear. Third, as with any SNP-based genetic association study, the discovered SNPs are likely proxies for causal functional elements underlying AF rather than the causal variants themselves. Fourth, we assumed that the risk of AF associated with each allele is multiplicative and that the effects for each SNP contribute to AF risk additively. Our analysis does not address the potential for interactions between SNPs, or between SNPs and environmental factors, which may associate with AF risk.

Conclusions

In conclusion, we systematically examined nine genome-wide significant AF susceptibility loci for additional independent signals. We identified at least four distinct signals on chromosome 4q25 upstream of *PITX2*, which implicate an arrhythmia susceptibility region at this locus that spans about 195 kb. In aggregate, the number of genetic risk markers for AF correlated with a marked gradient of AF risk in both samples of European and Japanese descent, and identified individuals both at increased as well as at decreased risk of AF relative to those with the most common number of risk markers. Our observations underscore the biological complexity of the chromosome 4q25 locus and importance of the region in AF pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AF	atrial fibrillation
Kb	kilobase
Mb	megabase
SNP	Single nucleotide polymorphism

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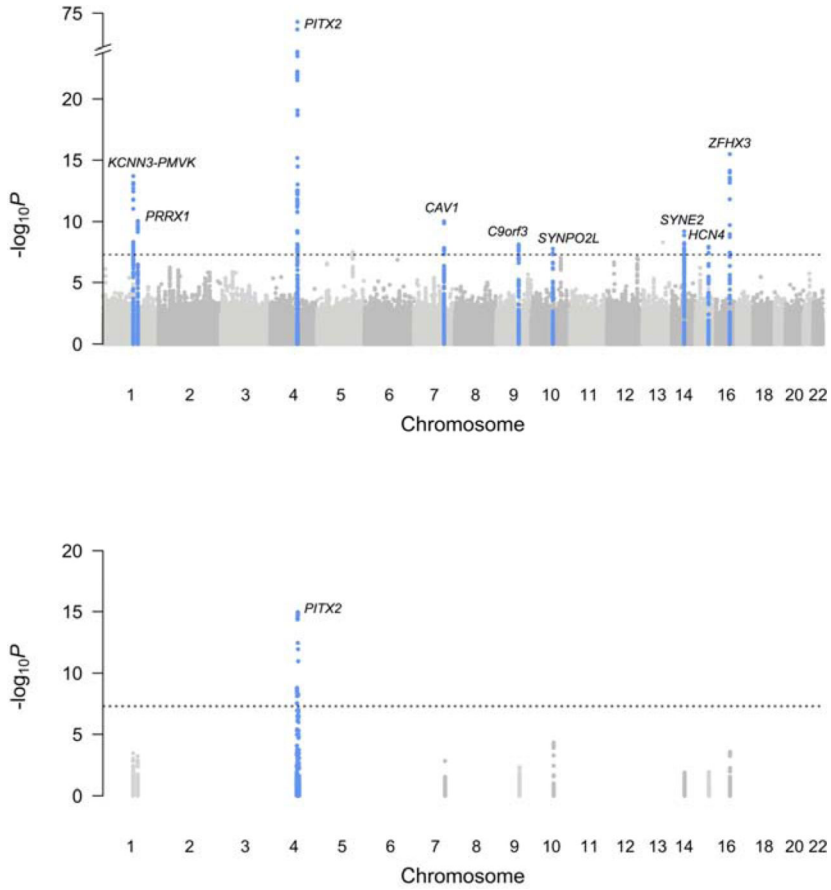

Figure 1. Genome-wide and conditional associations between genetic variants and atrial fibrillation

Associations between genetic variants and atrial fibrillation are displayed (A) across the genome in marginal association analyses as previously reported (12) and (B) at each genome-wide significant susceptibility locus after adjustment for the genotype of the most significantly associated SNP at that locus.

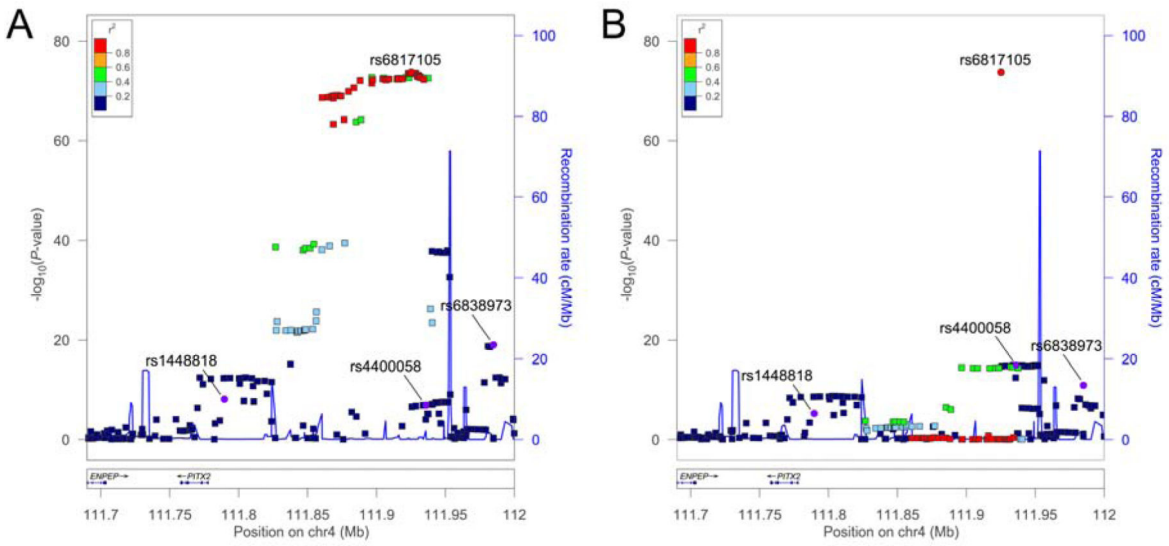

Figure 2. Regional association between variants on chromosome 4q25 and atrial fibrillation after adjustment for the top SNP at the locus in the AFGen sample

Associations between SNPs and atrial fibrillation at the chromosome 4q25 locus (A) before and (B) after adjustment for the genotype of the top SNP (rs6817105) are displayed. Additional distinct susceptibility signals discovered in this analysis are represented by purple circles and are labeled. The strength of linkage disequilibrium between genetic variants in relation to rs6817105 is indicated by the color gradient as denoted in the legend. The region displayed is limited to a 310 kb segment containing the associated non-redundant signals. Linkage data and recombination rates are derived from the HapMap phase II CEU panel.

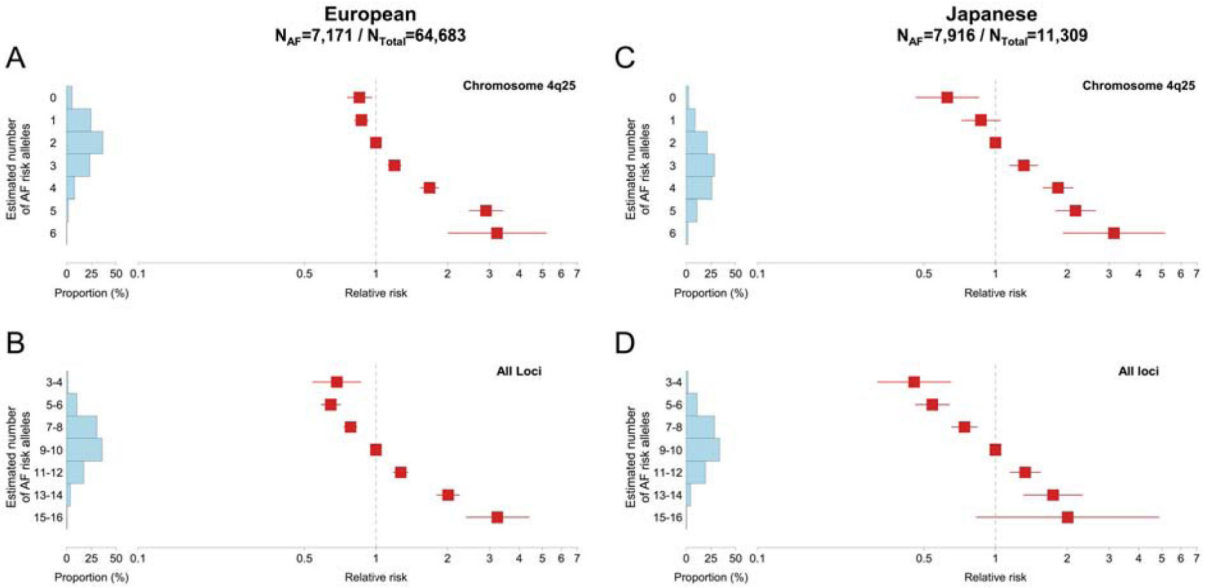

Figure 3. Graded relative risk of atrial fibrillation stratified by the number of susceptibility alleles in Europeans and Japanese

The risk of atrial fibrillation is plotted according to the unweighted number of estimated distinct atrial fibrillation risk alleles, relative to that among individuals with the most common number of estimated risk alleles for (A) chromosome 4q25, and (B) all genome-wide significant atrial fibrillation susceptibility loci in individuals of European ancestry from AFGen. Replicated associations in the BioBank Japan sample are displayed in (C) and (D), respectively. The distribution of risk alleles in the sample is displayed in the bar graph to the left of the risk plots. The AF risk alleles are listed in Table 3.

Table 1

Participating study characteristics.

Study sample	Participants, N	AF, N	Age*, mean ± SD	Age*, range	Male, N (%)	Hypertension, N (%)
European						
Incident AF						
AGES	2,718	158	76.5 ± 5.5	66-95	1,154 (39.0)	2,595 (87.7)
ARIC	8,890	802	54.3 ± 5.7	44-66	4,181 (47.0)	2,376 (26.7)
CHS	3,204	764	72.2 ± 5.3	65-98	1,242 (38.8)	1,678 (52.4)
FHS	4,062	310	64.7 ± 12.6	31-101	1,771 (43.6)	2,001 (49.3)
PROSPER	5,244	505	75.3 ± 3.4	69-83	2,525 (48.1)	3,257 (62.1)
RS-I	5,665	542	69.1 ± 9.0	55-99	2,282 (40.3)	1,866 (32.9)
RS-II	1,739	65	64.8 ± 7.9	55-95	795 (45.7)	600 (34.5)
WGHS	20,843	723	54.1 ± 7.0	43-89	0	5,022 (24.1)
Prevalent AF	**Sample**	**N**				
AFNET / KORA	Cases	468	51.8 ± 7.2	29-74	236 (50.4)	252 (53.8)
	Controls	438	56.2 ± 7.1	45-69	219 (50.0)	185 (42.2)
AGES	Cases	241	78.5 ± 5.9	67-95	88 (55.7)	143 (90.5)
	Controls	2,718	76.1 ± 5.4	66-94	70 (36.1)	2,002 (78.2)
CCAF	Cases	496	58.8 ± 10.7	20-84	375 (75.6)	269 (54.2)
	Controls	2,971	28.5 ± 22.2	0-87	1,124 (37.8)	–
HVH	Cases	95	59.5 ± 6.5	40-68	28 (29.5)	50 (52.6)
	Controls	193	59.5 ± 6.0	40-69	106 (54.9)	153 (79.3)
CHS	Cases	67	76.3 ± 5.8	66-90	38 (56.7)	35 (52.2)
	Controls	3,204	72.2 ± 5.3	65-98	1,242 (38.8)	1,678 (52.4)
FHS	Cases	253	76.9 ± 9.9	45-97	151 (59.7)	180 (71.1)
	Controls	4,151	64.7 ± 12.6	31-101	1,807 (43.5)	2,036 (49.1)
LURIC	Cases	361	66.4 ± 9.2	32-88	258 (71.7)	269 (74.5)
	Controls	2,598	62.2 ± 10.7	17-92	1,819 (70.0)	1,885 (72.6)
MGH / MIGEN	Cases	366	53.4 ± 10.5	21-77	295 (80.6)	85.8 (22.7)
	Controls	911	47.9 ± 8.8	18-83	485 (53.2)	–
RS-I	Cases	309	76.2 ± 8.7	56-98	145 (46.9)	131 (42.4)
	Controls	5,665	69.1 ± 9.0	55-99	2,282 (40.3)	1,866 (32.9)

Study sample	Participants, N	AF, N	Age*, mean ± SD	Age*, range	Male, N (%)	Hypertension, N (%)
RS-II	Cases	66	73.9 ± 9.5	56-95	35 (53.0)	35 (53.0)
	Controls	1,739	64.8 ± 7.9	55-95	795 (45.7)	600 (34.5)
SHIP	Cases	107	65.1 ± 11.5	21-81	69 (64.5)	59 (55.1)
	Controls	1,816	50.7 ± 14.9	21-81	906 (49.9)	437 (24.1)
WGHS	Cases	473	56.2 ± 7.8	45-85	0	152 (32.2)
	Controls	20,843	54.1 ± 7.0	43-89	0	5,022 (24.1)
Japanese						
Prevalent AF						
BioBank Japan	Cases	7,916	68.2 ± 10.3	19-100	5,545 (70.1)	5,655 (71.4)
	Controls	3,393	51.6 ± 16.6	3-96	1,853 (54.6)	1,160 (34.2)

*Age at DNA collection (or baseline for ARIC)

Table 2

Results of conditional analysis at the chromosome 4q25 locus in the AFGen consortium.

				Marginal association*		Conditional association	
Traditional Conditional Analysis							
SNP	**AF risk / referent allele**	**Chr 4q25 HG19 Position**	**AF risk allele frequency**	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
rs2723288†	A / C	111581402	0.30	1.16 (1.11-1.21)	5.3×10^{-12}	1.06 (1.03-1.09)	1.4×10^{-4}
rs6817105//	C / T	111705768	0.14	1.64 (1.55-1.73)	1.8×10^{-74}	1.62 (1.54-1.70)	**1.1×10^{-85}**
rs4400058‡	A / G	111716673	0.09	1.18 (1.11-1.26)	1.1×10^{-7}	1.28 (1.21-1.36)	**2.2×10^{-16}**
rs17570669	A / T	111736882	0.92	1.14 (1.04-1.23)	5.1×10^{-3}	1.06 (1.02-1.12)	5.2×10^{-3}
rs3853445§	T / C	111761487	0.74	1.23 (1.18-1.28)	1.8×10^{-19}	1.12 (1.08-1.18)	**1.0×10^{-7}**

Approximate Conditional Analysis							
SNP		**Chr 4q25 HG19 Position**	**AF risk allele frequency**	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
rs1448818†	C / A	111570223	0.25	1.14 (1.09-1.19)	7.3×10^{-9}	1.12 (1.08-1.17)	**1.6×10^{-8}**
rs6817105//	C / T	111705768	0.14	1.64 (1.55-1.73)	1.8×10^{-74}	1.58 (1.50-1.65)	**5.1×10^{-95}**
rs4032974‡	C / T	111732536	0.09	1.20 (1.12-1.28)	2.6×10^{-8}	1.07 (1.03-1.11)	1.5×10^{-3}
rs6838973§	C / T	111765495	0.44	1.20 (1.16-1.27)	8.8×10^{-20}	1.11 (1.08-1.15)	**6.0×10^{-9}**

All models adjusted for age, sex and significant principal components of ancestry.

rs6817105, rs17570669, and rs3853445 have previously been reported as distinct AF susceptibility signals and were forced into the traditional conditional analysis model.

*Discovery data from Ellinor et al (12)

†In linkage disequilibrium with one another ($r^2 > 0.3$)

‡In linkage disequilibrium with one another ($r^2 > 0.3$)

§In linkage disequilibrium with one another ($r^2 > 0.3$)

//Same SNP

Table 3

Marginal and SNP-adjusted associations between distinct AF susceptibility SNPs and AF in the AFGen consortium and BioBank Japan sample.

		AFGen								BioBank Japan				
				Marginal analysis*		SNP adjusted – Chromosome 4q25 only†		SNP adjusted – All loci			SNP adjusted – Chromosome 4q25 only†		SNP adjusted – All loci	
SNP	**Chromosomal locus**	**AF risk / referent allele**	**AF risk allele frequency***	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***	**Allele frequency**	**RR (95% CI)**	***P***	**RR (95% CI)**	***P***
rs6666258	1q21 / *KCNN3-PMVK*	C / G	0.30	1.18 (1.13-1.23)	2.0×10^{-14}	–	–	1.12 (1.08-1.16)	1.6×10^{-8}	0.02	–	–	1.23 (0.91-1.67)	0.18
rs3903239	1q24 / *PRRX1*	G / A	0.45	1.14 (1.10-1.18)	9.1×10^{-11}	–	–	1.09 (1.06-1.13)	5.9×10^{-7}	0.54	–	–	1.12 (1.04-1.20)	2.0×10^{-3}
rs1448818	4q25 / *PITX2*	C / A	0.25	1.14 (1.09-1.19)	7.3×10^{-9}	1.12 (1.08-1.17)	2.0×10^{-8}	1.12 (1.08-1.17)	3.1×10^{-8}	0.25	0.98 (0.90-1.06)	0.56	0.97 (0.89-1.06)	0.49
rs6817105	4q25 / *PITX2*	C / T	0.13	1.64 (1.55-1.73)	1.8×10^{-74}	1.56 (1.49-1.64)	3.3×10^{-74}	1.60 (1.52-1.68)	1.2×10^{-80}	0.47	1.92 (1.76-2.10)	5.1×10^{-48}	1.90 (1.74-2.08)	5.4×10^{-46}
rs4400058	4q25 / *PITX2*	A / G	0.10	1.18 (1.11-1.26)	1.1×10^{-07}	1.33 (1.26-1.41)	6.9×10^{-23}	1.31 (1.24-1.39)	4.1×10^{-20}	0.22	1.43 (1.30-1.58)	1.8×10^{-12}	1.42 (1.29-1.57)	6.2×10^{-12}
rs6838973	4q25 / *PITX2*	C / T	0.57	1.21 (1.16-1.26)	8.8×10^{-20}	1.13 (1.09-1.17)	8.1×10^{-12}	1.13 (1.09-1.18)	7.1×10^{-12}	0.48	1.12 (1.04-1.21)	2.2×10^{-3}	1.11 (1.04-1.20)	3.9×10^{-3}
rs3807989	7q31 / *CAV1*	G / A	0.60	1.14 (1.10-1.19)	9.6×10^{-11}	–	–	1.05 (1.01-1.09)	6.3×10^{-3}	0.66	–	–	1.25 (1.16-1.35)	3.0×10^{-9}
rs10821415	9q22 / *C9orf3*	A / C	0.42	1.13 (1.08-1.18)	$7.9\times10^{-}09$	–	–	1.10 (1.06-1.15)	2.1×10^{-7}	0.26	–	–	1.08 (0.99-1.17)	0.07
rs10824026	10q22 / *SYNPO2L*	A / G	0.84	1.17 (1.11-1.24)	1.7×10^{-08}	–	–	1.16 (1.10-1.22)	4.9×10^{-9}	0.60	–	–	0.94 (0.88-1.01)	0.10
rs1152591	14q23 / *SYNE2*	A / G	0.48	1.13 (1.09-1.18)	6.2×10^{-10}	–	–	1.10 (1.06-1.14)	1.1×10^{-7}	0.38	–	–	1.02 (0.95-1.09)	0.63
rs7164883	15q24 / *HCN4*	G / A	0.16	1.16 (1.10-1.22)	1.3×10^{-08}	–	–	1.13 (1.08-1.18)	3.1×10^{-7}	0.11	–	–	0.97 (0.86-1.08)	0.57
rs2106261	16q22 / *ZFHX3*	T / C	0.18	1.24 (1.17-1.30)	3.2×10^{-16}	–	–	1.17 (1.12-1.23)	6.4×10^{-12}	0.33	–	–	1.28 (1.19-1.38)	7.0×10^{-11}

Analyses adjusted for age and sex (AFGen and BioBank Japan), and significant principal components of ancestry (AFGen).

*Discovery data from Ellinor et al (12)

†SNPs representing distinct susceptibility signals derived from the two conditional analysis approaches.

Table 4

Association between multimarker risk scores and AF in the AFGen consortium and BioBank Japan sample.

	AFGen		BioBank Japan	
Allele score	**Beta (SE)**	***P***	**Beta (SE)**	***P***
Chromosome 4q25				
Unweighted	0.21 (0.01)	2.6×10^{-73}	0.26 (0.02)	2.7×10^{-38}
Weighted	0.98 (0.04)	1.4×10^{-107}	1.19 (0.07)	2.6×10^{-58}
All AF loci				
Unweighted	0.13 (<0.01)	9.6×10^{-107}	0.15 (0.01)	2.0×10^{-37}
Weighted	0.86 (0.03)	9.0×10^{-148}	0.99 (0.06)	7.6×10^{-63}

Beta coefficients correspond to the estimated log-relative risk of AF associated with each one-unit increase in the score. Selected SNPs are listed in Table 3. All models adjusted for age and sex (AFGen and BioBank Japan), and principal components of ancestry (AFGen). Weighting based on regression estimate from conditional analysis for SNPs on chromosome 4q25 or from Ellinor et al (12) for other SNPs and applied as follows: 0.13*rs3903239_G, 0.17*rs6666258_C, 0.12*rs1448818_C, 0.48*rs6817105_C, 0.25*rs4400058_A, 0.11*rs6838973_C, 0.13*rs3807989_G, 0.12*rs10821415_A, 0.16*rs10824026_A, 0.13*rs1152591_A, 0.15*rs7164883_G, 0.21*rs2106261_T.