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Independent Susceptibility Markers for Atrial Fibrillation on Chromosome 4q25

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Background—Genetic variants on chromosome 4q25 are associated with atrial fibrillation (AF). We sought to determine whether there is more than 1 susceptibility signal at this locus.

Methods and Results—Thirty-four haplotype-tagging single-nucleotide polymorphisms (SNPs) at the 4q25 locus were genotyped in 790 case and 1177 control subjects from Massachusetts General Hospital and tested for association with AF. We replicated SNPs associated with AF after adjustment for the most significantly associated SNP in 5066 case and 30 661 referent subjects from the German Competence Network for Atrial Fibrillation, Atherosclerosis Risk In Communities Study, Cleveland Clinic Lone AF Study, Cardiovascular Health Study, and Rotterdam Study. All subjects were of European ancestry. A multimarker risk score composed of SNPs that tagged distinct AF susceptibility signals was constructed and tested for association with AF, and all results were subjected to meta-analysis. The previously reported SNP, rs2200733, was most significantly associated with AF (minor allele odds ratio 1.80, 95% confidence interval 1.50 to 2.15, $P=1.2\times10^{-20}$) in the discovery sample. Adjustment for rs2200733 genotype revealed 2 additional susceptibility signals marked by rs17570669 and rs3853445. A graded risk of AF was observed with an increasing number of AF risk alleles at SNPs that tagged these 3 susceptibility signals.

Conclusions—We identified 2 novel AF susceptibility signals on chromosome 4q25. Consideration of multiple susceptibility signals at chromosome 4q25 identifies individuals with an increased risk of AF and may localize regulatory elements at the locus with biological relevance in the pathogenesis of AF. **(*Circulation*. 2010;122:976-984.)**

Key Words: atrial fibrillation ■ electrophysiology ■ genetics ■ epidemiology ■ risk factors

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Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice and is associated with substantial morbidity[1] and societal healthcare costs.[2] Although many risk factors for AF have been identified, the recognition of a common heritable component underlying AF[3,4] indicates that genetic variation may play a role in its pathogenesis.

Clinical Perspective on p 984

We recently participated in a genome-wide association study that identified a disease-susceptibility locus for AF on chromosome 4q25 in individuals of European and Asian descent.[5] We replicated the association between the most significantly associated single-nucleotide polymorphism (SNP), rs2200733, and AF in a subsequent study of 3508 subjects with AF and 12 173 control subjects from 4 additional cohorts of European ancestry.[6] A meta-analysis of the results from both studies revealed an odds ratio (OR) of 1.9 for the rs2200733 risk allele (95% confidence interval [CI] 1.60 to 2.26, $P=3.3\times10^{-13}$).[6] We and others have again replicated the chromosome 4q25 AF susceptibility locus in subsequent genome-wide association studies for AF.[7–9]

In the present study, we sought to identify whether there are multiple AF susceptibility signals at the 4q25 locus in individuals of European ancestry by performing fine mapping of common SNPs in the region and replicating associations in independent study samples. We further sought to determine whether the consideration of multiple markers associated with AF at this locus could further refine the association signal.

Methods

Study Samples

Detailed descriptions of the study cohorts are provided in the online-only Data Supplement. Individuals in the discovery stage of the analysis were drawn from 2 different samples at the Massachusetts General Hospital (MGH) and pooled for analysis. These samples included patients with lone AF referred to the Cardiac Arrhythmia Service starting in June 2001 in whom AF was documented by ECG before 66 years of age and patients with AF by ECG or history who were admitted to the MGH stroke service between January 1998 and July 2006 with an acute ischemic or hemorrhagic stroke. Referent subjects from MGH were from a large (>18 000 patients) primary care practice serving the hospital catchment area. Absence of AF was documented through interview and from review of medical records, including all available ECGs.

Genetic variants associated with AF in the discovery sample after adjustment for the top SNP (see Statistical Analysis) were genotyped in an independent replication study sample composed of subjects from the German Competence Network for Atrial Fibrillation (AFNET), a national registry of AF patients.[10] AF was confirmed by ECG, and DNA samples were collected from patients with AF in whom onset occurred before 60 years of age. Referent subjects were derived from a community-based epidemiological survey study conducted between 1999 and 2001 of persons living in or near the city of Augsburg, Germany (Cooperative Health Research in the Region of Augsburg [KORA] S4) and were excluded if they reported a history of AF, had signs or symptoms of AF on physical examination, or had an absence of sinus rhythm on a required ECG.[11]

We performed *in silico* replication of associations between AF and SNPs that represented distinct susceptibility signals in MGH and AFNET in 4 additional study samples with previously genotyped subjects. The Atherosclerosis Risk In Communities (ARIC) study is a prospective population-based study of cardiovascular disease in the United States that consists of participants who were 45 to 64 years old at enrollment. Subjects included in this analysis included those recruited from 3 US communities (suburbs of Minneapolis, Minn; Washington County, Maryland; and Forsyth County, North Carolina) between 1987 and 1989.[12] The Cleveland Clinic Lone AF Study (CCAF) is composed of case subjects with AF in the absence of significant structural heart disease. Referent subjects were population controls from studies 64, 65, 66, and 67 in the Illumina iControl database, a publicly accessible database of genotype and phenotype data from control genome-wide association study populations. The Cardiovascular Health Study (CHS) is a prospective population-based study of cardiovascular disease in individuals 65 years or older recruited from 4 field centers in the United States (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pa).[13] The Rotterdam Study (RS) is a community-based longitudinal study of elderly individuals from a suburb of Rotterdam, Netherlands, founded in 1990 with a focus on identifying determinants of health and cardiovascular, neurogeriatric, bone, and eye diseases.[14]

Prevalent AF was defined as events that occurred in individuals before that individual's DNA collection in cohort studies and on the basis of AF ascertainment in case-control studies. Incident AF was defined as events that occurred after DNA collection among participants free of AF at DNA collection in cohort studies. Subjects were restricted to those of self-reported European descent.

SNP Selection and Genotyping

A 200-kb region extending from the *PITX2* gene to approximately 50 kb beyond the previously reported SNP rs2200733 was considered for fine mapping of the chromosome 4q25 locus. All SNPs on chromosome 4 between positions 111 780 000 and 111 985 000 with a minor allele frequency of 5% or greater were identified from the HapMap CEU data set release 22 (NCBI [National Center for Biotechnology Information] build 36, dbSNP [database of SNPs] build 126). We identified 35 haplotype-tagging SNPs ($r^2\geq0.8$) in this region using the Tagger program within Haploview version 4.0.[15] Additionally, 6 SNPs that were moderately correlated with rs2200733 (r^2 between 0.2 and 0.8) were selected.

We extracted DNA from whole blood of each subject using standard techniques. In the MGH and AFNET samples, genotyping was performed with polymerase chain reaction, iPlex single-base primer extension, and matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry in a 384-well format (Sequenom, San Diego, Calif) according to the manufacturer's instructions. Data were analyzed with SpectroTYPER 3.4 software, and cluster plots were inspected visually and curated manually to confirm genotyping calls. The genotyping platforms for the remaining cohorts were Affymetrix 6.0 (ARIC; Affymetrix, Santa Clara, Calif), Illumina Hap550 version 3 and Illumina Hap610 version 1 (CCAF cases; Illumina Inc, San Diego, Calif), Illumina Hap550 version 1 or 3 (CCAF referents), Illumina 370 CNV (CHS), and Illumina Infinium HumanHap550 version 3 (RS). Only directly genotyped SNPs were included in the analysis, with the exception of rs17570669 during the replication stage in the CCAF sample. Imputation in CCAF was performed with MACH version 1.0.16 (available at http://www.sph.umich.edu/csg/abecasis/MaCH/index.html from the Center for Statistical Genetics of the University of Michigan) with the HapMap CEU reference panel (NCBI build 36; Rsq 0.6144 for cases and 0.7302 for the iControl database controls). In CHS, genotypes for rs17570669 were imputed with BIMBAM version 0.99,[16] but these were not included in the analyses owing to poor imputation quality (ratio of observed to expected genotype variance of 0.11).

The institutional review board or medical ethics committee, as appropriate for each participating institution, approved study procedures. Written informed consent was obtained from all study subjects or their proxies, including consent to use DNA for genetic analyses of cardiovascular disease.

Statistical Analysis

We tested each SNP included in the discovery stage for deviation from Hardy-Weinberg equilibrium using an exact test[17] and excluded

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Table 1. Characteristics of Study Subjects

	Discovery Stage Prevalent Analysis MGH Cohort		Replication Stage													
			Prevalent Analysis								Incident Analysis					
			AFNET Cohort		CCAF Cohort		CHS Cohort		RS Cohort		ARIC Cohort		CHS Cohort		RS Cohort	
	AF	No AF	AF	No AF	AF	No AF	AF	No AF	AF	No AF	AF	No AF	AF	No AF	AF	No AF
No. of subjects	790	1177	2145	4073	496	2971	66	3205	309	5665	743	7184	765	2440	542	5123
Age, y	63±15	67±13	49±14	61±12	58±11	28±22	76±6	72±5	76±8	69±9	57±5	54±6	73±6	72±5	72±8	69±9
Female, %	31	47	27	51	24	62	52	39	53	60	40	54	45	37	54	60
Hypertension, %	49	57	56	18	54	Unknown	52	52	42	33	44	25	59	50	45	32

Data presented as mean±SD or %.

the SNP if $P \leq 1\times10^{-4}$ in referent subjects. This corresponds to an experiment-wide Hardy-Weinberg significance threshold of $0.005/41 \approx 1\times10^{-4}$. We tested the remaining SNPs for association with AF in the MGH sample using logistic regression assuming an additive genetic model and subsequently adjusted for the genotype of the top SNP to identify independent associations with AF. Significance thresholds were adjusted for multiple testing by use of the Bonferroni method, with an experiment-wide error rate of 0.05. Thirty-four SNPs passed quality control measures, and therefore, the adjusted significance threshold was $P<0.001$ (0.05/34). We then tested SNPs significantly associated with AF, after adjusting for the top SNP in the MGH discovery sample, for association with AF in the AFNET sample.[18]

To identify SNPs that represented distinct AF susceptibility signals, we calculated pairwise linkage disequilibrium measures r^2 and D′ in the MGH and AFNET samples and constructed linkage disequilibrium blocks using Haploview[15] with previously described definitions.[19] We inferred haplotypes composed of SNPs located on the same block from unphased data using an expectation-maximization algorithm and tested haplotypes for association with AF using a weighted logistic regression model with adjustment for genotypes of the remaining SNPs that marked separate AF susceptibility signals.[20] Haplotypes were weighted according to the posterior probability of possible haplotype pairs for each individual subject.[20]

The SNPs associated with AF in both the MGH and AFNET samples that were markers for independent signals were then assessed for association with AF in the additional replication cohorts by use of logistic regression in samples with prevalent AF (CCAF, CHS, and RS) and Cox proportional hazards regression in samples with incident AF (ARIC, CHS, and RS). Individuals were censored at death, loss to follow-up, or date of last contact. Person-time for the incident analyses began at the time of DNA collection. Associations were adjusted for significant principal components of race for those studies in which population structure was associated with AF. Both prevalent and incident associations were subjected to meta-analysis by an inverse-variance weighted fixed-effects method.[21]

Because indirectly measured haplotype phasing is accompanied by uncertainty, we used the combination of genotypes at SNPs that marked each distinct AF susceptibility signal to define a multimarker variable for each individual. We assessed the association between each multimarker combination of genotypes and AF relative to the most common combination of genotypes for these SNPs, allowing for separate effects for each genotype combination. The effects were meta-analyzed as described above. In samples with incident AF, the time-dependent area under the receiver operating characteristic curve was estimated for models with and without the multimarker variables included.[22]

For the discovery stage of the analysis in the MGH sample of 790 cases and 1177 referent subjects, we estimated that we would have 41% power to detect ORs of 1.5 for risk alleles with frequencies of 5% and 80% power to detect ORs of 1.5 for risk alleles with frequencies of at least 10%, assuming a 2-sided α-level of 0.001 and population disease prevalence of 1%.[23]

Statistical analyses were performed with PLINK version 1.06,[24] SAS version 9.1.3 (SAS Institute, Cary, NC), and R version 2.11 (The R Project for Statistical Computing; available at http://www.R-project.org). Regional association plots were prepared with SNAP.[18]

Results

A total of 790 subjects with AF and 1177 referent subjects from MGH were included in the discovery stage of the analysis. Among the 790 case subjects, 488 were from the MGH lone-AF cohort, and 302 were from the MGH stroke cohort (Table 1). The overall call rate for the 34 SNPs tested for association with AF was 98.9%.

There was a strong association between AF and SNPs on chromosome 4q25 (Table 2; Figure 1A; online-only Data Supplement Table I). Among the 34 SNPs examined, 15 exceeded the significance threshold of $P<0.001$ after adjustment for age, sex, and hypertension. The most significant association with AF observed at this locus was with the previously reported SNP, rs2200733, with an OR for the minor T allele of 1.80 (95% CI 1.50 to 2.15, $P=1.2\times10^{-10}$). A second SNP previously reported to confer an independent risk of AF, rs10033464,[5] was not significantly associated with AF in the present sample (OR for minor T allele 1.07, 95% CI 0.84 to 1.35, $P=0.59$).

We then performed analyses with adjustment for rs2200733 genotype. The 4 SNPs most significantly associated with AF were all located within 30 kb of one another and telomeric to rs2200733 (Figure 1; online-only Data Supplement Table II). Associations between these 4 SNPs and AF after adjustment for age, sex, hypertension, and rs2200733 genotype (rs17570669: OR for minor T allele 0.60, 95% CI 0.46 to 0.78, $P=2.0\times10^{-4}$; rs4124163: OR for minor G allele 0.56, 95% CI 0.39 to 0.81, $P=2.3\times10^{-3}$; rs6838973: OR for minor T allele 0.77, 95% CI 0.67 to 0.89, $P=3.4\times10^{-4}$; and rs3853445: OR for minor C allele 0.75, 95% CI 0.64 to 0.89, $P=6.9\times10^{-4}$) were similar to unadjusted associations (online-only Data Supplement Table II). The minor alleles for each of these 4 SNPs were associated with a lower risk of AF. Although rs3853445 and rs6838973 were both associated with AF before adjustment for rs2200733, the association between rs17570669 and AF was not evident until after adjustment for rs2200733. There was a suggestion of a separate signal associated with AF centromeric to rs2200733; however, no SNPs in this region were significantly associated with AF after adjustment for multiple comparisons (Figure 1; online-only Data Supplement Table II).

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Table 2. Fine Mapping of the Locus for AF on Chromosome 4q25 in the Discovery Sample From MGH

SNP	Position	Minor/Major Allele	Minor Allele Frequency, %		Adjusted OR (95% CI)*	*P*
			AF	No AF		
rs17554590	111 782 351	G/C	1.7	2.0	0.97 (0.59–1.61)	0.91
rs2595098	111 782 931	A/T	4.8	7.1	0.63 (0.46–0.84)	2.9×10^{-3}
rs1448818	111 789 672	C/A	30.1	25.7	1.24 (1.07–1.44)	4.7×10^{-3}
rs12498374	111 803 868	T/C	25.0	19.9	1.32 (1.12–1.55)	7.0×10^{-4}
rs1448822	111 820 547	A/G	36.0	30.6	1.26 (1.09–1.45)	1.5×10^{-3}
rs13120244	111 823 793	A/G	10.0	12.4	0.83 (0.67–1.03)	0.09
rs1900827	111 843 188	T/C	40.0	31.2	1.41 (1.23–1.62)	7.8×10^{-7}
rs4371683	111 846 216	A/C	40.1	31.6	1.40 (1.22–1.61)	1.5×10^{-6}
rs17042026	111 851 823	A/G	26.1	16.8	1.64 (1.39–1.92)	2.8×10^{-9}
rs12646859	111 854 082	G/T	14.1	14.7	0.99 (0.82–1.19)	0.89
rs10222783	111 854 275	T/C	3.6	2.3	1.56 (1.02–2.37)	0.04
rs2595085	111 856 222	G/C	40.2	31.8	1.40 (1.22–1.60)	2.0×10^{-6}
rs1448817	111 860 502	G/A	38.0	27.6	1.51 (1.31–1.74)	8.4×10^{-9}
rs11098090	111 875 857	C/T	14.8	14.2	1.04 (0.86–1.26)	0.68
rs4307025	111 876 952	A/T	37.8	27.4	1.50 (1.31–1.73)	1.2×10^{-8}
rs2634071	111 888 669	T/C	28.9	19.2	1.60 (1.37–1.87)	2.0×10^{-9}
rs2723333	111 918 540	A/G	8.9	12.0	0.74 (0.59–0.92)	6.7×10^{-3}
rs1906615	111 921 247	T/G	30.1	20.6	1.54 (1.32–1.80)	2.6×10^{-8}
rs2200733	111 929 618	T/C	21.5	11.7	1.80 (1.50–2.15)	1.2×10^{-10}
rs13143308	111 933 868	T/G	31.7	21.1	1.60 (1.38–1.86)	9.5×10^{-10}
rs13105878	111 937 596	A/C	7.8	10.8	0.73 (0.58–0.93)	9.7×10^{-3}
rs11931959	111 939 134	G/A	38.2	28.5	1.47 (1.28–1.69)	7.8×10^{-8}
rs10033464	111 940 210	T/G	9.2	8.5	1.07 (0.84–1.35)	0.59
rs3855819	111 946 612	G/C	14.3	13.2	1.09 (0.90–1.32)	0.38
rs6533531	111 951 414	G/T	47.3	37.0	1.43 (1.24–1.64)	5.9×10^{-7}
rs3853444	111 953 585	C/T	29.7	30.8	0.95 (0.82–1.10)	0.49
rs17570669	111 956 331	T/A	7.2	8.5	0.87 (0.67–1.12)	0.28
rs13130446	111 958 605	T/C	49.8	49.8	1.02 (0.89–1.16)	0.80
rs10516564	111 958 741	G/A	29.1	30.0	0.93 (0.80–1.08)	0.33
rs3866834	111 963 462	A/G	33.9	34.0	1.02 (0.89–1.18)	0.79
rs4124163	111 965 048	G/A	3.0	5.0	0.61 (0.42–0.87)	6.1×10^{-3}
rs3853445	111 980 936	C/T	20.3	26.2	0.71 (0.61–0.84)	4.1×10^{-5}
rs6838901	111 984 764	C/G	13.6	14.6	0.93 (0.77–1.13)	0.47
rs6838973	111 984 944	T/C	34.6	41.5	0.75 (0.66–0.86)	4.8×10^{-5}

Genomic position from NCBI build 36. OR is the OR that corresponds to the minor allele.
*Adjusted for age, sex, and hypertension.

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Characteristics of the 2145 case and 4073 referent subjects in the AFNET sample are displayed in Table 1. In addition to rs2200733, 3 of the 4 SNPs associated with AF in the MGH discovery cohort analysis passed quality control measures with an overall call rate of 94.4% and were tested for association with AF. As in the MGH sample, with adjustment for age, sex, and hypertension, rs2200733 ($P=3.8\times10^{-52}$), rs3853445 ($P=2.14\times10^{-7}$), and rs6838973 ($P=1.45\times10^{-8}$) were associated with AF, but rs17570669 ($P=0.28$) was not. After additional adjustment for rs2200733 genotype, significant associations with AF were observed for the remaining 3 SNPs (rs17570669: minor T allele OR 0.64, 95% CI 0.54 to 0.77, $P=5.3\times10^{-7}$; rs3853445: minor C allele OR 0.82, 95% CI 0.74 to 0.91, $P=1.1\times10^{-4}$; and rs6838973: minor T allele OR 0.81, 95% CI 0.74 to 0.89, $P=5.4\times10^{-6}$).

Pairwise linkage disequilibrium measures revealed that SNPs rs3853445 and rs6838973 were moderately correlated (r^2 0.43 and 0.41 in MGH and AFNET, respectively) and located on the same haplotype block in both samples, which suggests that associations between each of these 2 SNPs and AF represented the same signal (online-only Data Supplement Table III). Because rs6838973 was not genotyped directly in the remaining replication samples, rs3853445 was used for subsequent analyses as a marker for this susceptibility signal. In contrast, there was a very low level of correlation between the remaining SNPs ($r^2<0.11$ for all

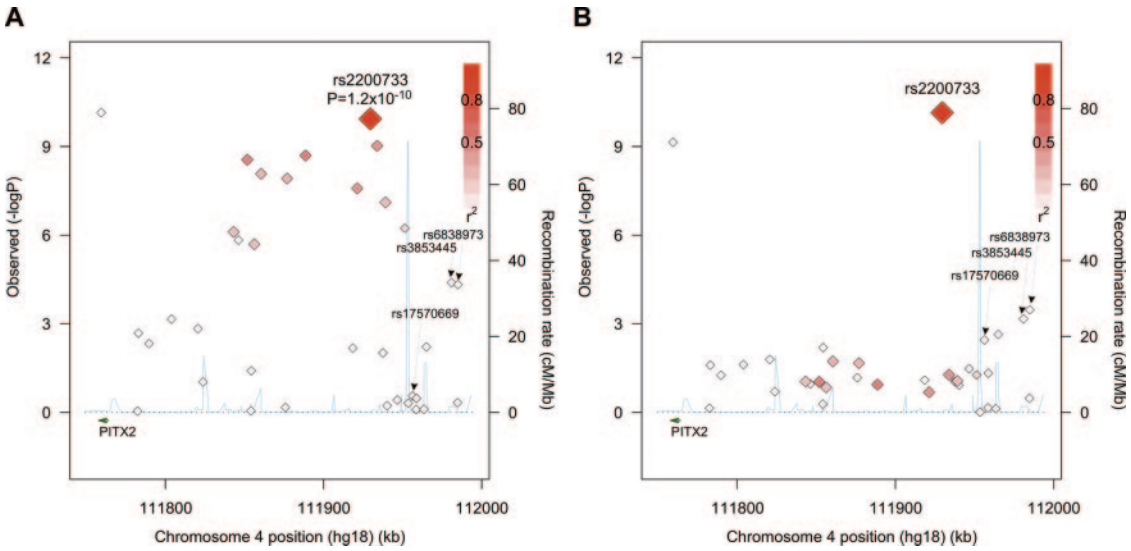

Figure 1. Regional plot of SNPs associated with prevalent AF in the MGH discovery sample. A, Associations between SNPs included in the analysis and AF in the MGH sample, adjusted for age, sex, and hypertension. B, Associations after additional adjustment for rs2200733 genotype, with the rs2200733 position corresponding to the unadjusted association significance level. SNPs are plotted according to their genomic position (NCBI build 36) and $-\log_{10}$ probability value for the association. The intensity of shading for each SNP corresponds to the strength of linkage disequilibrium (r^2) relative to rs2200733. Estimated recombination rates are shown by the blue line. *PITX2* is indicated by the dark green arrow. Linkage disequilibrium and recombination rates are based on CEU HapMap release 22. SNPs that were associated with AF after adjustment for rs2200733 genotype and meta-analysis of results from both the MGH and AFNET samples are labeled. Figures were prepared with SNAP.[18]

pairwise comparisons), as expected on the basis of the haplotype-tagging SNP selection method.

We therefore tested the 3 independent susceptibility signals for association with AF by modeling the 2 independent SNPs along with inferred rs3853445 and rs6838973 haplotypes (Table 3). The haplotype consisting of the minor alleles for both rs3853445 and rs6838973 (CT) occurred with a frequency of 23% in the MGH sample and 26% in the AFNET sample and conferred reduced odds of AF relative to the major allele haplotype (TC) after adjustment for rs2200733 and rs17570669 genotype (combined OR 0.78, 95% CI 0.71 to 0.87, $P=1.75\times10^{-6}$). The remaining 2 susceptibility signals marked by rs2200733 and rs17570669 remained significantly associated with AF in the combined analysis, although the association for rs17570669 was attenuated in the MGH sample.

Associations between SNPs that represented distinct AF susceptibility signals in the MGH and AFNET samples were then tested for replication in the ARIC, CCAF, CHS, and RS study samples. Characteristics of these samples are displayed in Table 1. In general, the associations were replicated in the prevalent AF samples but not in the incident AF samples, although effect estimates tended to be in the same direction as those observed in the discovery sample (Figure 2).

We then calculated the relative risk of AF for each multimarker combination of genotypes for SNPs that tagged each of the 3 AF susceptibility signals relative to the most common combination of genotypes in each of the study samples (Figure 3). The multimarker analysis indicated a graded risk of AF that generally corresponded to the number of AF risk alleles, although the risks varied within strata defined by numbers of risk alleles, and CIs overlapped for

Table 3. Associations Between AF and rs2200733, rs17570669, and Common rs3853445 | rs6838973 Haplotypes in the MGH and AFNET Samples

	MGH			AFNET			Combined†	
Variant	Haplotype Frequency	OR (95% CI)	*P*	Haplotype Frequency	OR (95% CI)	*P*	OR (95% CI)	*P*
rs2200733	. . .	1.87 (1.54–2.27)	2.4×10^{-10}	. . .	2.64 (2.32–3.00)	3.4×10^{-49}	2.37 (2.13–2.64)	3.1×10^{-56}
rs17570669	. . .	0.74 (0.55–1.01)	0.06	. . .	0.70 (0.58–0.85)	2.8×10^{-4}	0.71 (0.61–0.84)	4.3×10^{-5}
rs3853445 | rs6838973 Haplotype								
TC	0.60	Reference	. . .	0.56	Reference	. . .	Reference	. . .
TT	0.16	0.92 (0.75–1.13)	0.45	0.17	0.99 (0.86–1.13)	0.89	0.97 (0.87–1.09)	0.59
CC	0.01	1.24 (0.58–2.66)	0.57	0.01	1.87 (1.21–2.91)	5.0×10^{-3}	1.69 (1.16–2.47)	7.0×10^{-3}
CT*	0.23	0.75 (0.63–0.90)	1.6×10^{-3}	0.26	0.80 (0.71–0.90)	2.7×10^{-4}	0.78 (0.71–0.87)	1.75×10^{-6}

Adjusted for age, sex, and hypertension. OR is the OR that corresponds to the minor allele or specified haplotype.

*Composed of the minor alleles for each respective SNP.

†Meta-analysis performed using a fixed-effects method.

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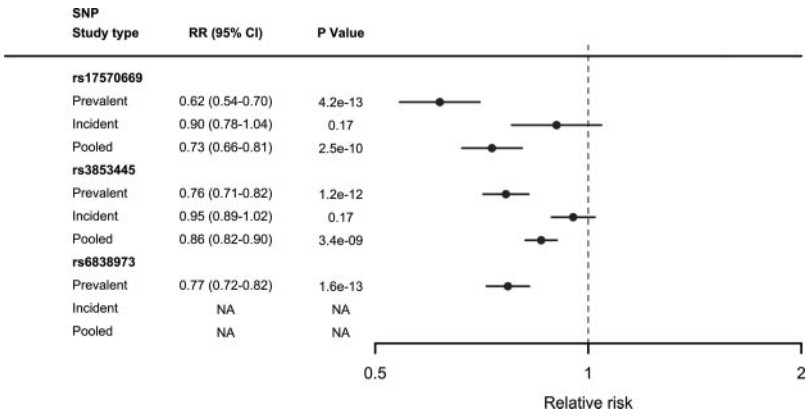

Figure 2. SNPs associated with AF after adjustment for rs2200733 genotype. SNPs associated with AF after adjustment for rs2200733 genotype in the MGH discovery sample were tested for association in the replication samples. The meta-analyzed effects are plotted according to prevalent (OR), incident (hazard ratio), or pooled (relative risk [RR]) analysis status. Associations were adjusted for age, sex, and hypertension (MGH, AFNET, ARIC, CHS, and RS) or sex only (CCAF). Samples with prevalent AF included MGH, AFNET, CHS, CCAF, and RS. Samples with incident AF included ARIC, CHS, and RS. NA indicates not available.

many of the multimarker groups owing to small sample sizes. The relative risks of AF for the 3 most frequent rs2200733/rs17570669/rs3853445 genotype combinations (relative to the most common genotype combination CC/AA/TT, composed of 4 AF risk alleles; 38% of subjects) were 0.90 (95% CI 0.82 to 0.99, CC/AA/CT [3 risk alleles, 26% of subjects]), 1.74 (95% CI 1.48 to 2.03, CT/AA/CT [4 risk alleles, 5% of subjects]), and 2.26 (95% CI 2.02 to 2.53, CT/AA/TT [5 risk alleles, 10% of subjects]). The greatest relative risk was observed with the combination comprised of both AF risk alleles at each of the 3 SNPs, TT/AA/TT, which occurred in approximately 1% of subjects (relative risk 6.02, 95% CI 4.56 to 7.96). The associations, stratified by prevalent or incident status, are displayed in online-only Data Supplement Figure II.

In samples with incident AF, the time-dependent area under the curve for a model with age, sex, and hypertension was 0.70 (95% CI 0.69 to 0.72) and 0.68 (95% CI 0.66 to 0.70) over the follow-up periods in ARIC and RS, respectively. The area under the curve increased to 0.72 (95% CI 0.70 to 0.73) and 0.70 (95% CI 0.68 to 0.72), respectively, after the addition of the multimarker allele combinations.

Discussion

In the present study sample of subjects from MGH, the previously reported SNP, rs2200733, remained the variant most significantly associated with AF even after consideration of other SNPs at the chromosome 4q25 locus. In addition to this signal, we identified 2 novel AF susceptibility signals after adjustment for rs2200733 genotype in a meta-analysis of 5856 subjects with AF and 31 838 without AF, all of whom were of European ancestry. A multimarker risk score composed of SNPs that tagged each of these 3 AF susceptibility signals on chromosome 4q25 identified indi-

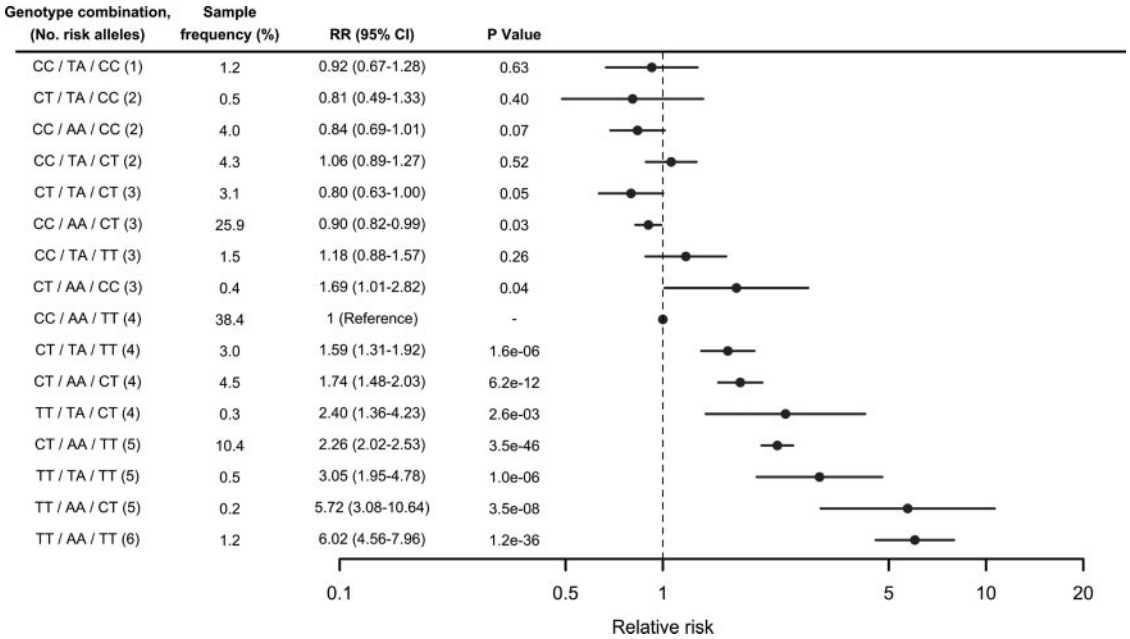

Figure 3. Multimarker risk score for AF based on combined rs2200733, rs17570669, and rs3853445 genotypes. The meta-analyzed relative risk (RR) of AF for each multimarker combination of rs2200733, rs17570669, and rs3853445 genotypes relative to the most common multimarker combination is shown. Only multimarker combinations with an average sample frequency of ≥0.2% are displayed, although the effects were adjusted for all potential combinations, as well as age, sex, and hypertension, or sex only (CCAF). Individuals with incomplete genotypes were not included. Risk alleles for AF were the minor T allele for rs2200733, the major A allele for rs17570669, and the major T allele for rs3853445.

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viduals at varying risk of developing AF, which approximately corresponded to the number of AF risk alleles present.

The present results reinforce the association between chromosome 4q25 and AF and extend the knowledge base by defining the genetic architecture of this locus and its relation to AF.[5–8] Specifically, SNPs rs17570669, rs3853445, and rs6838973 were confined to a 30-kb region from the 200-kb region assayed and located within 50 kb telomeric of rs2200733. The locus studied in the present analysis is marked by regions that appear to be phylogenetically conserved (online-only Data Supplement Figure III).[25] Indeed, there is emerging evidence that highly conserved noncoding regions may act as regulatory elements and underlie phenotypic diversity.[26,27] However, the mechanism by which genetic variation at the chromosome 4q25 locus leads to AF remains unknown.

The lack of significant association in the incident AF samples between AF and SNPs rs17570669 and rs3853445, after adjustment for rs2200733 genotype, may reflect reduced power in the incident stratum, an absence of true association when modeled in this fashion, interactions between variants and unmeasured clinical factors that differ between prevalent and incident AF, or other phenotypic heterogeneity between prevalent and incident AF. A multimarker risk score composed of AF risk alleles at SNPs that tagged these 3 susceptibility signals identified individuals predisposed to the development of AF, which raises the possibility that consideration of multiple genetic variants at the chromosome 4q25 locus may help improve risk stratification of individuals at risk for AF.[28] Whether the consideration of the 3 signals identified in the present analysis will contribute to AF prediction in the context of additional variants associated with AF[7–9,29,30] is presently unclear but merits examination in larger prospective data sets.

Study Limitations

The present analysis was restricted to individuals of European descent, and therefore, the findings may not be generalizable to individuals of other races and ethnicities. Although we could not assess for population stratification in the MGH and AFNET samples, the relative homogeneity of the present study cohorts limits the likelihood of such confounding. Furthermore, we adjusted analyses for population structure in replication cohorts when there was evidence of association with AF. We did not restrict the age of subjects included in the analysis. Although the chromosome 4q25 region has been associated with AF in subjects with a diverse spectrum of presumed causes,[7,9,31,32] including those with lone AF[9] and typical forms of AF,[7] sources of heterogeneity in the discovery cohort that were not accounted for may have affected the SNPs selected for replication. The haplotype and multimarker risk score analyses represent multiple testing, and several subgroups were of small sample size, as reflected by the wide CIs that accompanied some effect estimates; positive associations may represent "winner's curse."[33] Although we did not adjust for multiple testing at this stage, the observed associations are supported by the fact that SNPs tested in these analyses were selected after conservative Bonferroni-adjusted association thresholds in the discovery analysis. Moreover, patterns of association for these subgroups were consistent across independent study samples. We are unable to rule out longer-distance associations at the locus beyond the boundaries of the present SNP selection region. Lastly, although we cannot exclude the possibility that all of the identified SNPs merely tag a single, common element associated with AF, the low correlation between the SNPs identified in the present analysis and the haplotype association analysis suggest that these SNPs represent 3 independent signals at the chromosome 4q25 locus that affect AF risk.

Conclusions

We confirmed the strong association between AF and rs2200733 at the chromosome 4q25 locus and identified 2 novel disease susceptibility signals that are associated with AF. Simultaneous consideration of these signals identifies individuals with an increased risk for AF.

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Disclosures

Dr Greenberg serves as a consultant/advisory board member for Hoffman-LaRoche. Dr Boerwinkle serves as a consultant/advisory board member for Celera Diagnostics. Dr Smith serves as a consultant/advisory board member for Esperion Therapeutics. The remaining authors report no conflicts.

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CLINICAL PERSPECTIVE

Atrial fibrillation (AF) is a heritable disorder with evidence of genetic susceptibility. Common single-nucleotide polymorphisms (SNPs) in a noncoding region on chromosome 4q25 have been associated with AF. We sought to determine whether more than 1 AF susceptibility signal exists at this locus. We genotyped SNPs at the chromosome 4q25 locus in 790 case subjects and 1177 control subjects. After adjustment for the genotype of the most significantly associated SNP, the SNPs that remained significantly associated with AF were replicated in an additional 5066 subjects with AF and 30 661 without AF. We identified 3 distinct AF susceptibility signals, 2 of which have not been described previously. A multimarker risk score composed of SNPs tagging each of these 3 AF susceptibility signals identified individuals at varying risk of developing AF. Among the 1% of subjects homozygous for AF risk alleles at SNPs tagging each susceptibility signal, the risk of AF was markedly increased relative to those with the most common genotypes at these SNPs (relative risk 6.02, 95% confidence interval 4.56 to 7.96, $P=1.2\times10^{-36}$). Consideration of multiple susceptibility signals at the chromosome 4q25 locus identifies individuals with a markedly increased risk of AF and may facilitate the localization of regulatory elements at this locus with particular biological relevance in the pathogenesis of AF.

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