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Targeted sequencing in candidate genes for atrial fibrillation: The Cohorts for Heart and Aging Research in Genomic Epidemiology Targeted Sequencing Study

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Abstract

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Appendix Supplementary data: Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.hrthm.2013.11.012

Background—Genome-wide association studies (GWAS) have identified common genetic variants that predispose to atrial fibrillation (AF). It is unclear whether rare and low-frequency variants in genes implicated by such GWAS confer additional risk of AF.

Objective—To study the association of genetic variants with AF at GWAS top loci.

Methods—In the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Targeted Sequencing Study, we selected and sequenced 77 target gene regions from GWAS loci of complex diseases or traits, including 4 genes hypothesized to be related to AF (*PRRX1*, *CAV1*, *CAV2*, and *ZFHX3*). Sequencing was performed in participants with (n = 948) and without (n = 3330) AF from the Atherosclerosis Risk in Communities Study, the Cardiovascular Health Study, the Framingham Heart Study, and the Massachusetts General Hospital.

Results—One common variant (rs11265611; $P = 1.70 \times 10^{-6}$) intronic to *IL6R* (interleukin-6 receptor gene) was significantly associated with AF after Bonferroni correction (odds ratio 0.70; 95% confidence interval 0.58–0.85). The variant was not genotyped or imputed by prior GWAS, but it is in linkage disequilibrium ($r^2 = .69$) with the single-nucleotide polymorphism, with the strongest association with AF so far at this locus (rs4845625). In the rare variant joint analysis, damaging variants within the *PRRX1* region showed significant association with AF after Bonferroni correction ($P = .01$).

Conclusions—We identified 1 common single-nucleotide polymorphism and 1 gene region that were significantly associated with AF. Future sequencing efforts with larger sample sizes and more comprehensive genome coverage are anticipated to identify additional AF-related variants.

Keywords

Arrhythmia; Genetics; Atrial fibrillation; Epidemiology

Introduction

Atrial fibrillation (AF) is the most common arrhythmia and a major public health burden causing high morbidity,[1–3] mortality,[4] and societal cost.[5] Both clinical[6] and genetic[7] factors predispose to AF. By using genome-wide association studies (GWAS), we and others have reported common genetic variants at 9 distinct chromosomal loci that are significantly related to AF.[8–11] Although the heritability of AF has been estimated to be as high as 60%,[12] the known AF-related genetic variants appear to explain only a fraction of the heritability.[13] It has been hypothesized that the "missing heritability" of AF and other common diseases may be explained in part by rare variants with large effects.[7]

The Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE)[14] Targeted Sequencing Study was initiated to identify genetic variants within genes implicated by GWAS. We hypothesized that GWAS loci might harbor functional variants as well as rare and low-frequency variants associated with AF. The analysis evaluated both individual disease-associated variants and joint analysis of low-frequency variants at each locus.

Methods

Study samples

The methods for the CHARGE Targeted Sequencing Study are summarized in the Online Supplement. For AF analyses, cases with AF were derived from both the cohort random sample and all the phenotype groups. In addition, we selected 200 patients from the Massachusetts General Hospital (MGH) with early-onset AF (≤66 years at AF onset and no structural heart disease at diagnosis). Study participants without AF from either the cohort random sample or the other phenotype groups were used as referents (Table 1). Participants

with AF from the MGH were combined with participants with AF from the Framingham Heart Study (FHS) and were then compared with participants without AF from the FHS. This approach has been applied successfully in the past, since both participants from the MHS and participants from the FHS are derived from the same geographic region.[10] Participants with AF from the Atherosclerosis Risk in Communities (ARIC) Study and the Cardiovascular Health Study (CHS) were compared with participants without AF from the ARIC Study and the CHS, respectively.[9] All the participants in this study were of European ancestry. Institutional review boards at all participating centers approved the study, and participants gave informed consent.

Sequencing, functional annotation, and data analysis

Sequencing methods, single-nucleotide polymorphism (SNP) functional annotation, and data analysis methods are described in detail in the Online Supplement. Briefly, approximately 2 Mb of target regions were captured by using a customized NimbleGen Capture array and sequenced by using the ABI SOLiD V4.0 platform. The raw short reads were aligned to the reference human genome by BFAST.[15] SAMtools[16] was used to pile up aligned reads and call variants with quality filters. We then performed quality control procedures on the resulting data. Variants were categorized as known or novel by comparison with the dbSNP database and the 1000 Genomes Project.[17] The functional effect of identified variants on the encoded proteins was predicted by using the ANNOVAR software package.[18] We selected and sequenced 77 targeted regions in this study. Thirty-three of these regions were previously implicated by GWAS to be associated with 1 of 14 investigated phenotypes. The remaining 44 targeted regions were pleiotropic loci that were associated with multiple phenotypes. In particular, 4 regions were specifically selected for AF[7]: *ZFHX3*, *PRRX1*, *CAV1*, and *CAV2*. By balancing gene size and space restrictions due to assay design, for AF we limited the analysis to the exonic regions of the 4 candidate genes. The location of capture probes is shown in Online Supplemental Figure 1.

For common variants (minor allele frequency [MAF] ≥ 1%), we performed both unweighted analyses and analyses weighted by the sampling probabilities to account for the study design. We report *P* values from unweighted analysis (which provides more power) and β estimates from the weighted analysis (which reflects population estimates of effect sizes).[19] For rare variants (MAF <1%), we used a modified version of the sequence kernel association test to aggregate all the variants within each region and tested its association with AF.[20] Our analyses were restricted to the exonic variants only. In our secondary analyses, we restricted the analyses to damaging variants only, which were defined as those that were nonsense variants or located in the splicing sites or missense variants but predicted to cause damaging effects to the encoded proteins by PolyPhen.[21]

Results

We identified a total of 52,736 variants across all sequenced individuals, including 4800 common variants and 47,936 rare variants. In particular, we identified 62 common variants and 818 rare variants within the 4 AF targeted genes.

Common variant results

No SNP within any of the 4 AF targeted regions was significantly associated with AF after Bonferroni correction for multiple testing (cutoff *P* value = $0.05/62 = 8.06 \times 10^{-4}$). Only 4 SNPs reached nominal significance, including 2 SNPs located 46 kb upstream of *PRRX1* (rs577676 and rs576736), 1 located in the 5′ untranslated region of *CAV1* (rs45498702), and 1 missense SNP in *ZFHX3* (rs2213978). The regional association plots are shown in Figure 1, which provides a detailed overview of the variation at each locus.

We then expanded the analysis to variants in other targeted regions. Despite the complex design of our study, no obvious relevant population substructure was recognized (genomic control factor $\lambda = 1.047$; Online Supplemental Figure 2). Figure 2 shows the distribution of common variant associations across the 77 targeted regions in chromosomal order. Only 1 SNP, rs11265611 ($P = 1.7 \times 10^{-6}$), was significantly associated with AF after Bonferroni correction for multiple testing (cutoff P value = $0.05/4800 \approx 1.0 \times 10^{-5}$). The SNP was widely observed in patients from the FHS and MGH (MAF = 49%) and participants of the 1000 Genomes Project (MAF = 43%). However, it was filtered out in the ARIC Study and the CHS owing to the stringent quality control filters. The SNP is located in the first intron of *IL6R* (interleukin-6 receptor gene), which encodes the interleukin 6 receptor. It was not genotyped or imputed by prior GWAS, but it is in moderate linkage disequilibrium with rs4845625 ($r^2 = .69$), an SNP that was previously reported to be associated with AF.[22] We then performed conditional analysis by conditioning on rs4845625. The SNP, rs1 1265611, was still significantly associated with AF ($P = 6.2 \times 10^{-6}$) after adjusting the effect of rs4845625, suggesting that it might be an independent signal. The 10 SNPs with the smallest P values overall and the variants in AF-related target regions reaching nominal significance are presented in Table 2.

Rare variant results

Table 3 displays the association results of rare variants with AF for the 4 targeted regions. None of them showed association with AF when all the exonic variants were included. However, 1 gene, *PRRX1*, was found to be associated with AF when only damaging variants were included ($P = .01$). The gene encodes a homeodomain transcription factor, and a common variant 46 kb upstream of this gene was found to be associated with AF in our prior GWAS.[9] A total of 16 exonic rare variants were found within the *PRRX1* region (Online Supplemental Table 1). These included 9 missense variants, 6 synonymous variants, and 1 nonsense variant. Three of these variants have already been reported in the dbSNP database. Six of 9 missense variants were predicted to cause damaging effects to the encoded protein.[23] The nonsense variant (chr1: 170695522, G → A) resulted in a truncation of the last 25 amino acids of *PRRX1*.

Discussion

By analyzing common genetic variants in the coding regions or in proximity to genes selected for targeted sequencing in almost 1000 individuals with AF and more than 3300 referents without AF, we could identify a single association that withstood correction for multiple comparisons, the SNP rs11265611. The association was still significant, although attenuated after adjusting the effect of a previously reported AF-associated SNP.[22] The SNP rs11265611 was intronic to the gene *IL6R*, which encodes the IL6R. Interleukin-6 is an inflammatory cytokine that, through its receptor (IL6R), triggers the production of acute phase reactants.[24,25] Inflammation is thought to play a central role in the initiation and maintenance of AF in many individuals.[26–29] However, none of the common variants within the 4 AF targeted regions showed significant association with AF.

As in many other complex genetic disorders, the genetic variability of AF is not explained fully by the common variants identified to date.[7] Several groups have advanced the hypothesis that rare variants might contribute to explaining genetic variability, both of AF and other complex traits.[30,31] Sequencing genes implicated in GWAS revealed additional independent rare variants in the genes *NOD*, *IL23R*, *CARD9*, and at least 5 more genes.[32] One hypothesis is that an individual rare genetic variant confers a stronger risk of disease than a common variant, for example, those identified by GWAS.[32] In our study, we found that damaging rare variants within the *PRRX1* region were associated with AF. The

association, however, disappeared when all the exonic variants were included, suggesting that the rare variant test is susceptible to the inclusion of unrelated variants.

Nine genetic loci have been found to be significantly related to AF.[8–11] However, 6 of these loci were located in the intronic gene regions whereas the remaining 3 loci were located in the intergenic regions. Given that these loci did not directly affect the encoded proteins, they are likely associated with AF through other mechanisms, which present a great challenge to identifying causal variants for AF. Additional efforts, including fine mapping of the discovered loci and deep resequencing of putative candidate genes, are thus necessary to uncover the genetic architecture underlying AF.

In our current approach, we balanced dense coverage of targeted regions vs limited array capacities. As with GWAS for more common alleles, it can be expected that hypothesis-free, large-scale approaches will lead to the detection of novel variants, some of them rare. Such approaches will include exome-wide sequencing in sufficient numbers of AF cases and controls, genotyping by newly designed arrays that primarily contain rare alleles, such as the Illumina Exome Chip,[33] or ultimately the sequencing of the entire genome in large samples.

Conclusions

We identified 1 common SNP and 1 genomic region that were significantly associated with AF. On the basis of our experience with increasing sample sizes in GWAS, we anticipate that robust novel rare and low-frequency variants for AF will be detected by broader efforts including exome-wide sequencing and exome chip genotyping in studies involving greater numbers of individuals with AF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AF	atrial fibrillation
ARIC	Atherosclerosis Risk in Communities
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CHS	Cardiovascular Health Study
FHS	Framingham Heart Study
GWAS	genome-wide association studies
IL6R	interleukin-6 receptor
MAF	minor allele frequency
MGH	Massachusetts General Hospital
SNP	single-nucleotide polymorphism

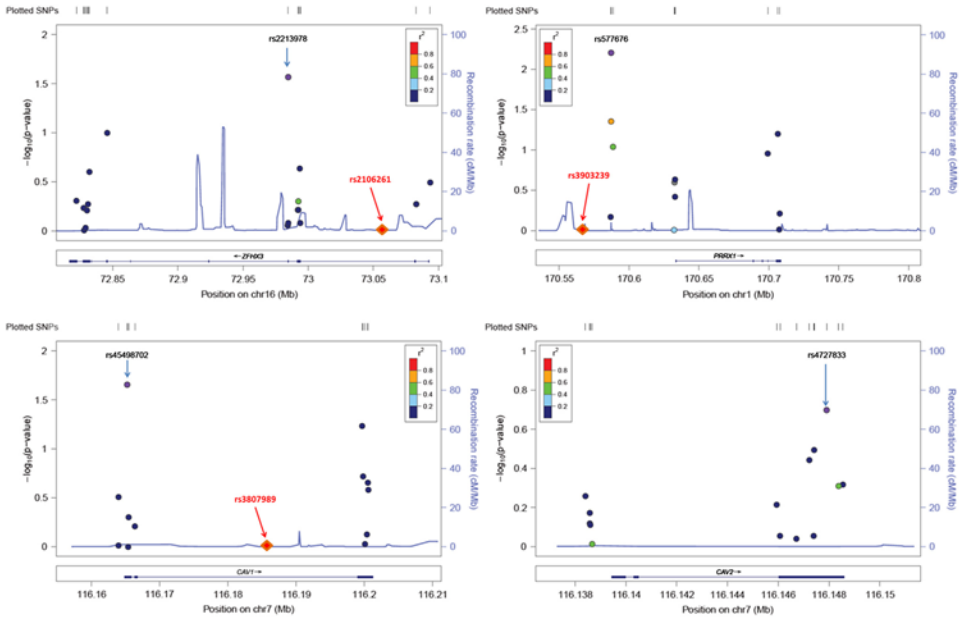

Figure 1.

Regional association plots of atrial fibrillation (AF)–related genes. Common variants identified by sequencing in each of the 4 AF-related genes are plotted according to their chromosomal position on the x-axis (NCBI Build 37, 2009). The y-axis provided the $-\log_{10}$ *P* value of each variant's association with AF. Variants are clustered within the exons of these genes and colored on the basis of the linkage disequilibrium (r^2) to the most significant single-nucleotide polymorphism (SNP; purple point) in each gene region. The diamond symbols indicate the location of the original genome-wide association study (GWAS) signal (the most significant SNP) relative to the sequence regions. Of note, the GWAS SNPs were located in introns or intergenic regions and thus not directly sequenced here. Also, *CAV1* and *CAV2* genes are adjacent, which is why a GWAS SNP is present only in the *CAV1* plot.

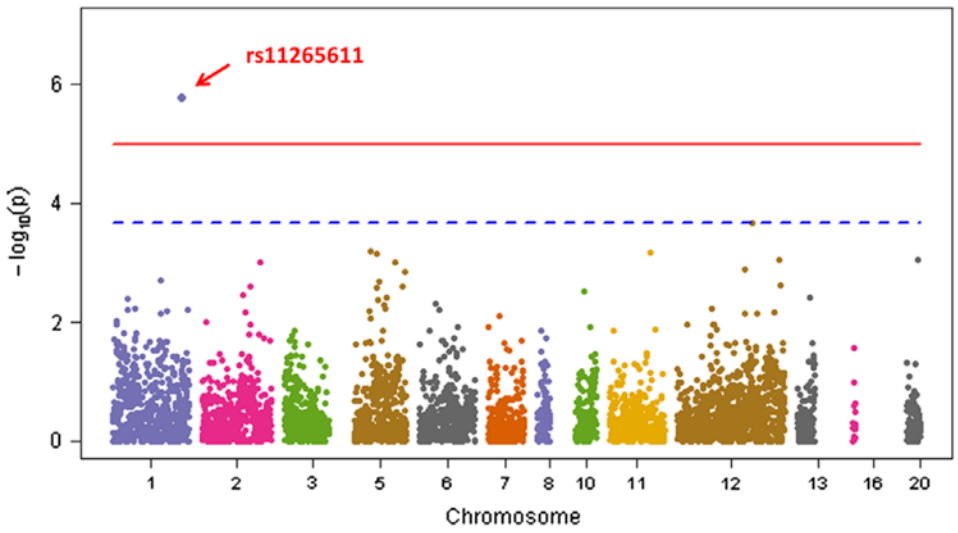

Figure 2.

Manhattan plot of common variants. All common single-nucleotide polymorphisms (SNPs; n = 4800) were analyzed by using unweighted models. The $-\log_{10} P$ value of each SNP is plotted according to its chromosomal position. The red horizontal line indicates the Bonferroni corrected threshold of significance at $P = 1.0 \times 10^{-5}$, whereas the blue horizontal line indicates the 1 false-positive threshold at $P = 1/4800 = 2.1 \times 10^{-4}$.

Table 1

Clinical characteristics of the study samples by cohort

	No. of individuals			Clinical characteristic		
Cohort	**Total**	**With AF**	**Without AF**	**Age (y)***	**Women**	**Hypertension**
ARIC Study	1952	245	–	57.4 ± 5.6	87 (35.5%)	116 (47.3%)
		–	1707	54.4 ± 5.6	868 (50.8%)	488 (28.5%)
CHS	1132	351	–	73.0 ± 5.8	185 (52.7%)	221 (63.0%)
		–	781	72.3 ± 5.3	422 (54.0%)	412 (52.8%)
FHS	994	152	–	69.1 ± 9.4	63 (41.4%)	63 (41.4%)
		–	842	60.8 ± 10.2	449 (53.3%)	228 (27.1%)
MGH	200	200	–	52.9 ± 11.1	37 (18.5%)	50 (25.0%)

Values are presented as mean ± SD and as n (%).

AF = atrial fibrillation; ARIC = Atherosclerosis Risk in Communities; CHS = Cardiovascular Health Study; FHS = Framingham Heart Study; MGH = Massachusetts General Hospital.

*Age in the FHS was assessed at the time when DNA was drawn.

Table 2

Common variant results

			ARIC Study		CHS		FHS/MGH		Meta-analysis				
SNP	**Chr**	**Targeted region**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**β**	**SE**	**OR**	**95% CI**	***P***
Ten most significant associations across all 77 target gene regions													
rs11265611	1	*IL6R*	–	–	–	–	−0.35	0.10	−0.35	0.10	0.70	0.58–0.85	1.7×10^{-6}
rs855222	12	*IGF1*	–	–	0.38	0.13	–	–	0.38	0.13	1.45	1.15–1.88	2.1×10^{-4}
rs114779117	5	*MEF2C*	0.77	0.65	0.70	0.39	0.90	0.39	0.80	0.25	2.22	1.35–3.64	6.3×10^{-4}
rs10769256	11	*SPI1*	–	–	–	–	−0.29	0.10	−0.29	0.10	0.75	0.61–0.91	6.5×10^{-4}
rs79435800	5	*NIPAL4*	0.18	0.33	0.47	0.28	0.36	0.26	0.35	0.17	1.42	1.03–1.97	7.0×10^{-4}
rs72525420	20	*JAG1*	–	–	–	–	0.40	0.17	0.40	0.17	1.49	1.06–2.09	8.9×10^{-4}
rs9805001	12	*C12orf51*	−1.59	0.87	−0.81	0.61	−0.98	0.76	−1.04	0.42	0.35	0.16–0.80	9.0×10^{-4}
rs76416019	5	*ADAM19*	−0.00	0.31	0.40	0.27	0.30	0.26	0.25	0.16	1.29	0.94–1.77	9.7×10^{-4}
rs72965937	2	*IRS1*	0.52	0.34	0.39	0.25	0.59	0.25	0.50	0.16	1.64	1.21–2.33	9.7×10^{-4}
rs1520220	12	*IGF1*	0.36	0.16	0.19	0.14	0.22	0.13	0.25	0.08	1.28	1.09–1.51	1.3×10^{-3}
Nominally significant SNPs within AF-related target regions													
rs577676	1	*PRRX1*	−0.12	0.13	0.01	0.11	−0.38	0.11	−0.16	0.06	0.85	0.75–0.96	6.2×10^{-3}
rs45498702	7	*CAV1*	–	–	−0.13	0.26	−0.72	0.26	−0.43	0.19	0.65	0.45–0.94	2.2×10^{-2}
rs2213978	16	*ZFHX3*	−0.31	0.37	−0.35	0.32	−0.23	0.37	−0.30	0.20	0.74	0.50–1.10	2.7×10^{-2}
rs576736	1	*PRRX1*	0.12	0.13	−0.01	0.11	0.29	0.12	0.13	0.07	1.14	1.00–1.31	4.4×10^{-2}

AF = atrial fibrillation; ARIC = Atherosclerosis Risk in Communities; Chr = chromosome; CHS = Cardiovascular Health Study; CI = confidence interval; FHS = Framingham Heart Study; MGH = Massachusetts General Hospital; OR = odds ratio; SE = standard error; SNP = single-nucleotide polymorphism.

Table 3

Rare variant results of AF targeted genes

Targeted region	Chr	Start position*	Stop position*	Exonic variants		Damaging variants	
				P	CMAF	*P*	CMAF
PRRX1	1	170,586,793	170,708,641	.64	0.02	.01	0.0009
ZFHX3	16	72,820,963	73,093,634	1.00	0.15	.99	0.0102
CAV2	7	116,138,344	116,148,695	.67	0.01	.58	0.0007
CAV1	7	116,163,739	116,201,338	.98	0.04	.52	0.0002

AF = atrial fibrillation; Chr = chromosome; CMAF = cumulative minor allele frequency.

*The chromosomal positions were based on the reference human genome (NCBI Build 37, 2009).