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A Genome-Wide Association Study to Identify Genomic Modulators of Rate Control Therapy in Patients with Atrial Fibrillation

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Abstract

For many patients with atrial fibrillation (AF), ventricular rate control with atrioventricular (AV) nodal blockers is considered first-line therapy, though response to treatment is highly variable. Using an extreme phenotype of failure of rate control necessitating AV nodal ablation and pacemaker implantation, we conducted a genome wide association study (GWAS) to identify genomic modulators of rate control therapy. Cases included 95 patients who failed rate control therapy. Controls (N=190) achieved adequate rate control therapy with 2 AV nodal blockers using a conventional clinical definition. Genotyping was performed on the Illumina 610-Quad platform, and results were imputed to the 1000 Genomes reference haplotypes. 554,041 single

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nucleotide polymorphisms (SNPs) met criteria for minor allele frequency (>0.01), call rate ($>95\%$), and quality control, and 6,055,224 SNPs were available after imputation. No SNP reached the canonical threshold for significance for GWAS of $P < 5 \times 10^{-8}$. Sixty-three SNPs with $P < 10^{-5}$ at 6 genomic loci were genotyped in a validation cohort of 130 cases and 157 controls. These included 6q24.3 (near *SAMD5/SASH1*, $P = 9.36 \times 10^{-8}$), 4q12 (*IGFBP7*, $P = 1.75 \times 10^{-7}$), 6q22.33 (*C6orf174*, $P = 4.86 \times 10^{-7}$), 3p21.31 (*CDCP1*, $P = 1.18 \times 10^{-6}$), 12p12.1 (*SOX5*, $P = 1.62 \times 10^{-6}$), and 7p11 (*LANCL2*, $P = 6.51 \times 10^{-6}$). However, none of these were significant in the replication cohort or in a meta-analysis of both cohorts. In conclusion, we identified several potentially important genomic modulators of rate control therapy in AF, particularly *SOX5*, which was previously associated with resting heart rate and PR interval. However these failed to reach genome-wide significance.

Keywords

atrial fibrillation; genomics; rate control

Introduction

Atrial fibrillation (AF) is the most common sustained clinical arrhythmia, affecting an estimated 6 million patients in the United States. Given the increasing incidence of AF observed over the past 2 decades, its prevalence in the United States is projected to increase to 12 to 16 million by the year 2050.¹ For many patients with AF, pharmacological therapy with atrioventricular (AV) nodal blockers is a first-line therapeutic strategy to control ventricular rate and prevent symptoms and systolic heart failure (HF) associated with rapid ventricular rates. This is generally accomplished with the administration of calcium channel blockers, betaadrenergic blockers, and/or digitalis, which slow conduction in the AV node. Rate control therapy has been shown to be equivalent at preventing clinical outcomes to a rhythm control strategy, in which antiarrhythmic drugs (AADs) and electrical cardioversions are used to maintain sinus rhythm.² However, a substantial minority (~30%) of patients fail rate control therapy, either due to persistent symptoms or inability to control the ventricular rate despite therapeutic doses of multiple AV nodal blockers.³ Genetic differences might explain some of the inter-individual variability in the response to rate control therapy. We recently demonstrated that a single nucleotide polymorphism (SNP) in the $\beta 1$ -adrenergic receptor gene (*ADRB1*) is associated with response to rate control therapy.⁴ Recent genome wide association studies (GWASs) have identified genomic predictors of resting heart rate in sinus rhythm^{5–8} and PR interval.⁹ These findings suggest that response to rate control therapy may also be genotype-dependent.^{10,11} The goal of this study was to conduct a GWAS comparing AF patients who responded effectively and ineffectively to ventricular rate control therapy. Given that the efficacy of rate control therapy is highly variable and is a complex clinical phenotype with multiple potential confounders, we chose an extreme phenotype for our analysis: failure of multiple AV nodal blockers necessitating AV node ablation and pacemaker implantation.

Methods

The discovery cohort consisted of patients in the Vanderbilt AF Registry (VAFR). As previously described, patients with AF have been recruited from Vanderbilt arrhythmia and general cardiology clinics, the emergency department, and inpatient services since April, 2000.¹² The replication cohort consisted of patients selected from the Vanderbilt University Medical Center DNA Biobank (“BioVU”).¹³ This resource consists of de-identified medical records of Vanderbilt inpatients and outpatients and DNA extracted from blood that is left over after routine laboratory testing and scheduled to be discarded. The Vanderbilt University Institutional Review Board has approved VAFR and BioVU. All patients in VAFR gave written informed consent. Given their de-identified nature, patient records and biological samples in BioVU fall under the designation of “non-human subjects” under Title 45, Code of Federal Regulations, Part 46, and individual informed consent is not obtained. BioVU includes a mechanism to exclude subjects known to be part of prospective registries like VAFR; thus, samples from patients in VAFR were not included in the BioVU replication set.

Cases included patients in whom rate control therapy failed necessitating AV node ablation and permanent pacemaker implantation. Controls were AF patients who achieved criteria for successful rate control (ventricular rate ≤ 80 beats per minute at rest and no higher than 110 beats per minute during a 6 minute walk test), as outlined in the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) study,¹⁴ with ≥ 2 AV nodal blockers. The discovery cohort included 285 patients with persistent AF. Of these, 95 failed rate control therapy necessitating AV node ablation and pacemaker implantation (cases) and 190 were successfully rate-controlled with ≥ 2 AV nodal blockers (controls). The validation cohort consisted of 130 cases and 157 controls defined with the same criteria. Baseline clinical characteristics are presented in Table 1.

Genomic DNA was extracted from whole blood using standard techniques. Genotyping in the discovery cohort was performed for 620,902 variants with the Illumina 610-Quad platform (San Diego, CA). We performed quality control of these data by excluding samples with $>5\%$ missing genotypes, we estimated probability of identity by descent to check for family relationships, and agreement with database sex using X chromosome heterozygosity rates in PLINK.¹⁵ We dropped 2 samples for missing data, no samples for relationships above the 1st cousin level, and no samples for sex discordance. For SNPs within the set of high-quality samples, we dropped 43,807 SNPs for missing $>5\%$ genotypes, 54,558 monomorphic and rare SNPs with minor allele frequencies (MAF) $<1\%$, and 1,601 SNPs for Hardy-Weinberg equilibrium test P-values smaller than 1×10^{-6} . We also removed 2,718 palindromic SNPs to facilitate genotype imputation, and removed 15,553 non-autosomal SNPs. In the discovery cohort, 502,665 SNPs met the pre-specified criteria for inclusion in the analysis.

We imputed genotypes from the 1000 Genomes cosmopolitan reference panel of haplotypes using IMPUTE.¹⁶ A total of 6,055,224 genotyped and imputed SNPs with MAF $\geq 5\%$ and information scores >0.4 were included in primary discovery analyses. Logistic regression models were fit for each SNP using SNPTEST,¹⁶ regressing the response to rate control

outcome onto genotype, age, and sex. To evaluate quality control we calculated the genomic control parameter¹⁷ for tests at genotyped and imputed autosomal SNPs and $\lambda = 1.025$, suggesting that there were no strong unaccounted for biases or confounders (Supplementary Figure 1).

We ranked SNPs by P-value, and conducted secondary analyses adjusting for the index, or most significant SNP in each genomic region within 1 megabase. We then selected a set of haplotype tag SNPs for the CEU reference population to tag a 100kb region centered on each index SNP with $r^2 \geq 0.8$, and MAF ≥ 0.05 , and genotyped the index and tag SNPs in the replication samples (130 BioVU cases and 157 BioVU controls) using the Sequenom MassArray genotyping platform (Sequenom, Inc., San Diego, CA). A total of 63 SNPs at 6 genomic regions were selected for replication experiments (Table 2, Figure 1a–e).

Quality control procedures in the replication cohort were conducted as in the discovery cohort. No samples failed quality control. Two SNPs failed missing data filters. We then imputed the candidate regions to the 1000 Genomes cosmopolitan reference haplotypes with IMPUTE, and conducted logistic regression analysis in the replication samples using SNPTEST. We then conducted fixed-effects inverse variance weighted meta-analysis with METAL¹⁸ to obtain combined evidence of effects and significance from discovery and replication samples.

Results

In the discovery analysis, SNPs in 6 genomic regions were associated with rate control therapy failure (Table 2). No SNP reached the canonical threshold for significance for GWAS of $P < 5 \times 10^{-8}$ in the discovery analysis (Figure 2). Multiple SNPs in the 6q24.3, 6q22.33, 4q12, 12p12.1, 3p21.31, and 7p11 regions were nominally significantly associated (Figures 1a–e).

None of the 63 SNPs genotyped in the replication cohort were significantly associated with failure of rate control therapy (Table 3). Using the genotypes for these 63 SNPs, we imputed 2792 SNPs. The region near the *IGFBP7* gene on chromosome 4 was the only region with p-values smaller than 0.05 in the replication cohort.

A meta-analysis was performed for the association of the imputed SNPs with failure of rate control therapy in the discovery and replication cohort (225 cases and 347 controls). None of the SNPs were significantly associated with failure of rate control therapy, and the most significantly associated SNPs with p-values smaller than 10^{-4} were all in the 6q24 region between the *SAMD5* and *SASH1* genes (Table 4).

Discussion

We conducted a GWAS for failure of rate control therapy in patients with AF, with cases defined as patients who required AV nodal ablation and pacemaker implantation, and controls as patients who met AFFIRM criteria for successful rate control with ≥ 2 AV nodal blockers. Although we found SNPs at 6 genomic loci near statistical significance in the

discovery cohort, these failed to replicate in the replication cohort or in the combined meta-analysis of both cohorts.

The clinical response to AV nodal blockers in patients with AF managed with a rate control strategy is highly heterogeneous and multifactorial. Several recent studies have led us to hypothesize that genetic factors contribute to this variability. Eijgelsheim and colleagues conducted a meta-analysis of GWASs of resting heart rate in 38,991 subjects of European descent.⁵ They discovered 6 novel loci associated with resting heart rate and confirmed 3 previously described loci. Three large studies in 2010 used GWAS to analyze variability in PR interval. In one of these, Pfeufer et al⁹ performed a meta-analysis of GWASs of PR interval that included 28,517 European subjects. They found 9 loci associated with PR interval (including *SOX5*), of which 5 were also associated with AF. We previously conducted a separate GWAS of PR interval in 2334 subjects drawn from BioVU, and replicated the finding that *SCN10A* variants are associated with variability in the PR interval.¹⁹ While the vast majority of subjects in these studies had no prior history of AF, the finding that genetic variants play a role in resting heart rate and AV nodal conduction led us to believe that they might also modify the response to rate control therapy in AF. For example, genetic variants in adrenergic receptor genes are associated with response to β -blockers in patients with hypertension and heart failure²⁰ and also modify heart rate response to β -blockade in healthy subjects.²¹ We previously studied whether common polymorphisms in the *ADRB1* gene (encoding the cardiac β 1 receptor) are associated with response to rate control therapy in the Vanderbilt AF registry. Carriers of the Arg389Gly variant were more likely than wild-type individuals to achieve AFFIRM criteria for successful rate control.⁴ These studies led us to hypothesize that additional genetic variants are associated with successful rate control therapy in patients with AF.

In the current study, SNPs at 6 genomic regions were close to our pre-specified criteria for genome-wide statistical significance in the discovery cohort, although we failed to replicate these findings in the replication cohort. The first region was Chr6q24.3 near the sterile alpha motif containing 5 (*SAMD5*) and sterile alpha motifs and SH3 domain-containing protein 1 (*SASH1*) genes. *SASH1* is ubiquitously expressed with highest tissue level in lung, placenta, spleen, and thymus. It is down-regulated in the majority of breast tumors (74%) in comparison with corresponding normal breast epithelial tissue.²² Reduced expression is also reported in a majority of lung tumors, thyroid tumors and in a few colon cancers. *SAMD5* is ubiquitously expressed, particularly in the esophagus, colon, ileum, oral mucosa, and kidney. While little has been published about the protein's function, *SAMD5* expression was significantly decreased in human eye trabecular meshwork cells treated with *PITX2* small interfering RNAs.²³ *PITX2* encodes the paired-like homeodomain 2 transcription factor which is not only important for proper eye development but is also crucial for left-right patterning and development of the pulmonary myocardium during cardiogenesis, and has been robustly associated with AF risk and response to therapy for AF.^{10,11,24,25}

Two SNPs at Chr4q12 (near *IGFBP7*) were associated with failed rate control in the discovery cohort. *IGFBP7* is expressed in many tissues including heart, and a splice site mutation was associated with retinal arterial macroaneurysm and supraaortic pulmonic stenosis in 8 Saudi families.²⁶ Although the function of *IGFBP7* is incompletely understood,

it has been implicated as a tumor suppressor gene that binds vascular endothelial growth factor (VEGF)-A and is involved in angiogenesis in breast and colorectal cancer, meningioma, and melanoma. *IGFBP7* was also found to play a role in physiological angiogenesis during embryogenesis in mice.²⁷ In addition, a morpholino knock-down model of the *IGFBP7* ortholog in zebrafish demonstrated a complex phenotype that included global defects in blood vessel formation as well as pericardial edema.²⁷ Therefore, *IGFBP7* plays a vital role in angiogenesis in zebrafish and mice, and in humans has been linked to structural cardiac disease.

An intronic SNP in the CUB domain-containing protein (CDCP)-1 gene was associated with failure of rate control therapy in the discovery cohort. This gene, which has not been extensively studied, encodes a protein involved with cell adhesion and has been implicated as an oncogene.²⁸ Its potential role in cardiac pathophysiology has not been determined.

Several SNPs at Chr12p12.1 (near *SOX5*) fell just short of genome wide significance in the discovery cohort. This gene encodes a transcription factor that is expressed in many tissues, including the heart. *Sox5* deficient mice displayed congenital skeletal and spinal cord deformities, and *Sox5*^{-/-} knock-out mice died in utero of apparent heart failure.²⁹ In humans, polymorphisms near *SOX5* were associated with resting heart rate and PR interval in separate GWASs,^{5,9} although the mechanism by which this gene influences cardiac electrophysiology remains unknown.

As with all retrospective, nonrandomized studies, ours is susceptible to selection bias and the effects of unknown confounders. We found several loci associated with failure of rate control therapy in our discovery cohort but we were unable to replicate these findings in the replication cohort. There are no standardized and validated criteria to define successful ventricular rate control during AF. While the AFFIRM criteria have been widely used, their correlation with outcomes is unknown. In addition, more recent studies have suggested that there is no difference in outcomes between a strict versus lenient target heart rate response during AF.³⁰ Success of rate control therapy is a difficult phenotype to study, as it is inherently subjective and influenced by many factors. Even though we attempted to uniformly apply the AFFIRM criteria to define the success of rate control therapy, it was up to individual practitioners to evaluate the success or failure. Other latent sources of confounding include patient compliance with medications and the ability to tolerate effective doses of AV nodal blockers. We attempted to address some of these confounders by choosing an extreme phenotype, the need for AV nodal ablation and pacemaker implantation, as the definition of failure of rate control in this study. However, even this is subject to bias since individual providers might have different thresholds for AV nodal ablation. Finally, our study was relatively small and susceptible to type 2 error.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Manhattan plot of results for genome-wide association with failure of rate control therapy in patients with atrial fibrillation. The canonical threshold for genome-wide significance, $P < 5 \times 10^{-8}$, is indicated by the red line. The threshold for inclusion of variants in the validation experiment, $P < 5 \times 10^{-5}$, is indicated by the blue line.

Figure 2.

Plots of the top five most significant genomic regions associated with failure of rate control therapy in patients with atrial fibrillation: a) Chr6q24.3 (near *SAMD5/SASH1*); b) 4q12 (*IGFBP7*); c) 6q22.33 (*C6orf174*); d) 3p21.31 (*CDCPI*); and e) 12p12.1 (*SOX5*).

Table 1

Baseline clinical characteristics for cases in whom rate control therapy was ineffective and in controls with adequate rate response.

	Discovery		Validation		P value*	Controls (N=157)	P value*
	Cases (N=95)	Controls (N=190)	Cases (N=130)	Controls (N=157)			
Age (years)	69.2±9.8	65.8±10.3	73.4±11.6	56.8±11.3	<0.001		
Male	60%	78%	53.1%	67.5%	0.001		
Hypertension	64%	69%	68%	66%	0.45		
Diabetes	31%	19%	32%	18%	0.01		
Coronary Artery Disease	32%	30%	72%	14%	<0.001		
Myocardial Infarction	30%	14%	13%	5.5%	0.03		
Heart Failure	30%	22%	81%	9.2%	<0.001		

* Chi-square test for discrete variables, Mann-Whitney U test for continuous variables.

Table 2

Association results for index SNPs in six genomic regions from in the discovery cohort.

Region	SNP	Nearest gene	Effect/referent	Effect Allele	Frequency	Information	OR	95% CI	p-value
6q24	rs1534773	<i>SAMD5/SASH1</i>	T/C	0.82	0.99	0.28	(0.18–0.45)	9.36×10^{-8}	
4q12	rs4242007	<i>IGFBP7</i>	C/G	0.05	0.90	7.11	(3.41–14.84)	1.75×10^{-7}	
6q22	rs1512450	<i>C6orf174</i>	G/C	0.68	0.99	0.36	(0.24–0.55)	1.11×10^{-6}	
3p21	rs55891698	<i>CDCP1</i>	T/C	0.05	0.80	6.2	(2.98–13.21)	1.18×10^{-6}	
12p12	rs7967643	<i>SOX5</i>	T/C	0.82	0.99	0.31	(0.19–0.50)	1.62×10^{-6}	
7p11	rs815929	<i>LANCL2</i>	A/C	0.89	0.89	0.27	(0.15–0.47)	6.51×10^{-6}	

CI: confidence interval. OR: odds ratio. SNP: single nucleotide polymorphism.

Table 3

The 20 most significant SNPs from the replication cohort. All SNPs are in the chromosome 4 *IGFBP7* region.

SNP	Effect/referent	Effect Allele	Frequency	Information	OR	95% CI	p-value
rs4865181	A/G	0.22	0.96	0.57	(0.38–0.85)	0.006	
rs13119745	T/C	0.27	0.61	0.51	(0.31–0.83)	0.007	
chr4:57950946	T A/T	0.43	0.45	1.89	(1.17–3.03)	0.009	
rs6554428	T/C	0.55	0.90	1.55	(1.11–2.15)	0.010	
rs13108835	C/A	0.55	0.90	1.55	(1.11–2.15)	0.010	
rs11133483	A/G	0.57	0.97	1.54	(1.11–2.13)	0.010	
rs11133484	G/A	0.57	0.97	1.54	(1.11–2.13)	0.010	
rs7661524	C/G	0.55	0.91	1.54	(1.11–2.14)	0.010	
rs7681360	A/G	0.57	0.97	1.53	(1.11–2.12)	0.010	
rs11133482	T/C	0.57	0.97	1.53	(1.11–2.12)	0.010	
rs13129156	G/A	0.55	0.93	1.53	(1.1–2.12)	0.011	
rs9993153	A/G	0.55	0.92	1.53	(1.1–2.13)	0.011	
rs13147020	G/A	0.57	0.98	1.52	(1.1–2.11)	0.011	
rs7670481	T/C	0.54	0.83	1.55	(1.1–2.19)	0.013	
rs6825609	A/G	0.56	0.95	1.50	(1.09–2.08)	0.014	
rs4865182	A/G	0.24	1.00	0.62	(0.43–0.91)	0.015	
rs4075349	T/C	0.41	0.95	0.68	(0.49–0.94)	0.020	
rs1714007	G/A	0.65	1.00	1.49	(1.06–2.08)	0.020	
rs1714006	G/A	0.65	1.00	1.49	(1.06–2.08)	0.020	
rs1718829	C/A	0.65	0.99	1.49	(1.06–2.08)	0.020	

CI: confidence interval. OR: odds ratio. SNP: single nucleotide polymorphism.

Table 4

Results from the meta-analysis of discovery and replication association summary statistics. All of the most significant results come from the region between the *SAM5D/SASH1* genes on chromosome 6.

SNP	effect/reference	OR	95% CI	P-value	Direction
rs1534774	C/G	0.42	(0.28–0.62)	1.67×10^{-5}	--
rs1534773	T/C	0.53	(0.39–0.71)	1.69×10^{-5}	--
rs9386204	A/G	0.43	(0.29–0.63)	2.10×10^{-5}	--
rs1358689	T/C	0.43	(0.29–0.63)	2.19×10^{-5}	--
rs2328840	A/C	0.43	(0.29–0.64)	2.58×10^{-5}	--
rs9390526	T/C	0.43	(0.29–0.64)	2.83×10^{-5}	--
rs9322117	A/C	0.54	(0.4–0.72)	3.01×10^{-5}	--
rs9322118	T/C	0.54	(0.4–0.72)	3.02×10^{-5}	--
rs9377097	A/G	0.44	(0.29–0.64)	3.03×10^{-5}	--
rs1881827	A/G	0.44	(0.3–0.64)	3.11×10^{-5}	--
rs1997380	T/C	1.88	(1.39–2.53)	3.30×10^{-5}	++
rs1404488	A/G	2.30	(1.55–3.41)	3.53×10^{-5}	++
rs4896956	T/G	1.85	(1.38–2.47)	3.57×10^{-5}	++
rs7765323	C/G	1.84	(1.38–2.46)	3.67×10^{-5}	++
rs4896958	T/C	0.54	(0.41–0.73)	3.90×10^{-5}	--
rs1406709	A/T	0.54	(0.4–0.72)	3.94×10^{-5}	--
rs9403916	T/C	0.54	(0.41–0.73)	3.96×10^{-5}	--
rs4896957	T/C	0.54	(0.41–0.73)	3.96×10^{-5}	--
rs1406710	A/T	1.85	(1.38–2.49)	4.05×10^{-5}	++
rs9403915	T/C	0.55	(0.41–0.73)	4.21×10^{-5}	--
rs9403913	T/G	0.55	(0.41–0.73)	4.28×10^{-5}	--
rs9322115	A/T	1.91	(1.4–2.61)	4.66×10^{-5}	++
rs4896955	T/C	0.55	(0.41–0.74)	6.04×10^{-5}	--
rs2876678	A/G	1.81	(1.35–2.42)	7.15×10^{-5}	++

CI: confidence interval. OR: odds ratio. SNP: single nucleotide polymorphism.