# Chromosome 4q25 Variants Are Genetic Modifiers of Rare Ion Channel Mutations Associated With Familial Atrial Fibrillation

Marylyn D. Ritchie, PhD,\* Shane Rowan, MD,† Gayle Kucera, BSC,† Tanya Stubblefield, BSC,† Marcia Blair, MS,† Shannon Carter, BSC,† Dan M. Roden, MD,†‡ Dawood Darbar, MD†

Nashville, Tennessee

**Objectives** 

The aim of this study was to test the hypothesis that 2 common polymorphisms in the chromosome 4q25 region that have been associated with atrial fibrillation (AF) contribute to the variable penetrance of familial AF.

**Background** 

Although mutations in ion channels, gap junction proteins, and signaling molecules have been described for Mendelian forms of AF, penetrance is highly variable. Recent studies have consistently identified 2 common single-nucleotide polymorphisms in the chromosome 4q25 region as independent AF susceptibility alleles.

**Methods** 

Eleven families in which AF was present in  $\geq$ 2 members who also shared a candidate gene mutation were studied. These mutations were identified in all subjects with familial lone AF (n = 33) as well as apparently unaffected family members (age >50 years with no AF; n = 17).

Results

Mutations were identified in SCN5A (n = 6), NPPA (n = 2), KCNQ1 (n = 1), KCNA5 (n = 1), and NKX2.5 (n = 1). In genetic association analyses, unstratified and stratified according to age of onset of AF and unaffected age >50 years, there was a highly statistically significant association between the presence of both common (rs2200733 and rs10033464) and rare variants and AF (unstratified p = 1  $\times$  10<sup>-8</sup>, stratified [age of onset <50 years and unaffected age >50 years] p = 7.6  $\times$  10<sup>-5</sup>) (unstratified p < 0.0001, stratified [age of onset <50 years and unaffected age >50 years] p < 0.0001). Genetic association analyses showed that the presence of common 4q25 risk alleles predicted whether carriers of rare mutations developed AF (p = 2.2  $\times$  10<sup>-4</sup>).

**Conclusions** 

Common AF-associated 4q25 polymorphisms modify the clinical expression of latent cardiac ion channel and signaling molecule gene mutations associated with familial AF. These findings support the idea that the genetic architecture of AF is complex and includes both rare and common genetic variants. (J Am Coll Cardiol 2012; 60:1173–81) © 2012 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is an important and increasing public health problem. The prevalence of AF doubles for each advancing decade of life, and there is widespread agreement that the prevalence is increasing over time (1,2). The risk factors for AF are multifactorial and include male sex, advancing age, coronary artery disease, congestive heart failure, and valvular heart disease. However, a substantial portion of the variability in risk for AF remains unexplained, leading investigators to search for genetic factors.

From the \*Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee; †Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; and the ‡Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennesse. This work was supported by NIH/NHLBI grants HL65962 and HL092217 and by an American Heart Association Established Investigator Award (0940116N). Dr. Rowan has received fellowship support from St. Jude, Medtronic, and Boston Scientific; and speaker fees from Medtronic. Dr. Roden is a consultant for Warner Chilcott, Astellas. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received January 29, 2012; revised manuscript received March 21, 2012, accepted April 23, 2012.

Investigators at the Framingham Heart Study have observed that the odds ratio (OR) of developing AF was 1.8 times higher for subjects with at least 1 parent diagnosed with AF compared with those without such a parental history (3). The OR increased further (to 3.2) if 1 parent was affected before 75 years of age. In a population-based cohort of more

## See page 1184

than 5,000 patients with AF from Iceland, first-degree relatives of patients with AF were 1.77-fold more likely to have AF than the general population, with a relative risk of 4.67 in first-degree relatives of patients <60 years of age (4). Familial aggregation of AF is particularly prominent in patients with idiopathic or so-called lone AF (i.e., early-onset AF without structural heart disease), for which as many as 30% of probands have first-degree relatives with the arrhythmia (5–7).

Although a Mendelian pattern of inheritance has been reported, large AF kindreds such as those used to identify

# Abbreviations and Acronyms

AF = atrial fibrillation

DNA = deoxyribonucleic

OR = odds ratio

acid

SNP = single nucleotide polymorphism

disease genes in other inherited arrhythmia syndromes (e.g., congenital long-QT syndrome) are unusual. A common presentation of the Mendelian form of the arrhythmia is a proband with familial lone AF (6). Mutations in genes encoding cardiac ion channels, gap junction proteins, atrial natriuretic peptide and nucleo-

porins (Nup155) have been reported in isolated cases and small kindreds (8). Although traditional linkage analysis and candidate gene approaches have been successful in identifying monogenic forms of familial lone AF, the mode of transmission for most forms of AF remains unclear, supporting the idea that AF inheritance is complex.

In 2007, a genome-wide association study in Icelanders identified a locus on chromosome 4q25 associated with AF in subjects of all ages (9). Within this locus, 2 noncoding single-nucleotide polymorphisms (SNPs) were independently associated with AF, and these findings were replicated in 2 populations of European descent and 1 of Asian descent. The SNP most strongly associated with AF, rs2200733, conferred a 1.71-fold increased risk for AF, while the other SNP, rs10033464, conferred a 1.42-fold increased risk. Recently, this association was replicated in a study of 4 large populations with ambulatory AF (10). This association has also been reported for post-cardiac surgery AF, a setting thought to be related to inflammation (11), and has recently been reported to predict the likelihood of successful AF ablation (12). Although mutations in ion channels, gap junction proteins, and signaling molecules have been identified in isolated kindreds with 2 or more members affected with familial lone AF, penetrance in these families is highly variable. One potential explanation for this phenomenon is the coexistence of modifier gene alleles, possibly common SNPs altering AF susceptibility. In this study, we tested the hypothesis that 4q25 genotypes contribute to the variable penetrance of the AF phenotype in familial AF.

### **Methods**

Vanderbilt AF Registry. Between November 2002 and July 2009, subjects with AF were prospectively enrolled in the Vanderbilt AF Registry, which comprises clinical and genetic databases (13). At enrollment, detailed medical and drug histories were obtained from all patients; patients were also asked to complete a symptom questionnaire. Patients were recruited from the Vanderbilt Cardiology and Arrhythmia Clinics, the emergency department, and inpatient services. Echocardiography was performed in all patients at the time of enrollment into the registry. The upper limits of normal for cardiac chamber dimensions are based on age and body surface area. Subjects >18 years of age with diagnoses of AF confirmed by electrocardiography who

presented because of symptoms or who were diagnosed during routine physical examinations were included in the Vanderbilt AF Registry. Subjects were excluded if AF was diagnosed in the setting of recent cardiac surgery or if they were unable to give informed consent or report for follow-up. The study protocol was approved by the Vanderbilt University institutional review board, and participants were enrolled after providing written informed consent.

AF probands and their relatives were clinically classified by a consistently applied set of definitions. AF was defined as the replacement of sinus P waves by rapid oscillations or fibrillatory waves that varied in size, shape, and timing and were associated with an irregular ventricular response when atrioventricular conduction was intact. Documentation of AF on electrocardiography, rhythm strip, event recorder, or Holter monitor recording was necessary. Paroxysmal AF was defined as AF lasting more than 30 s that terminated spontaneously. Persistent AF was defined as AF lasting more than 7 days and requiring either pharmacologic therapy or electrical cardioversion for termination. AF that was refractory to cardioversion or that was allowed to continue was classified as permanent.

**Study cohort.** The relationship between clinical phenotype (familial lone AF) and genotype was determined for probands in whom ion channel and other gene mutations were identified, together with their relatives. A total of 70 subjects in 11 kindreds had complete phenotype and genotype data available for analysis (Table 1), and this group formed the study cohort.

Clinical phenotype. The clinical phenotype evaluated in this study was lone familial AF, defined as AF occurring in patients <66 years of age without hypertension, overt structural heart disease, or thyroid dysfunction (as determined by clinical examination, electrocardiography, echocardiography, and normal thyroid function test results) and AF in 1 or more first-degree relatives (5,6). Left ventricular hypertrophy was defined as a left ventricular mass index ≥150 g/m² (14). All familial lone AF probands had left ventricular mass indexes <150 g/m².

Familial AF was defined as the presence of AF in 1 or more first-degree relatives of the index case. Although the proband in each family had to have lone AF, other family members were classified as affected if they had AF, despite structural heart disease. Family history information was initially obtained from the medical record and was supplemented by a questionnaire detailing medical history, family history, and clinical symptoms. For lone AF probands with positive family histories, family members were contacted, and more detailed personal medical histories were obtained. Family members were then sent a blood kit for deoxyribonucleic acid (DNA) extraction, and written permission was obtained to review their medical records. We routinely evaluate for the presence of asymptomatic AF by providing all unaffected family members who consent to participate in the study with a 7-day full-disclosure continuous monitor. In addition, individuals with symptoms suggestive for AF

Clinical Characteristics of Familial Lone AF Probands Enrolled in the Table 1 Vanderbilt AF Registry Identified to Have Mutations or Rare Variants in **Ion Channel or Signaling Proteins** Age at Onset LA Size AF Kindred and Age at Diagnosis Rhythm **Echocardiography** Pedigree # (yrs) (yrs) Symptoms (mm) AF027 41 54 PerstAF LVH 46 II-1 Yes 36 PAF 42 III-1 31 Normal Yes AF063 II-1 33 39 PAF Yes Normal 44 48 PAF 41 Yes Normal 42 AF119 1-1 51 54 PerstAF 46 No Normal II-1 28 38 PAF Normal 42 AF240 70 72 50 II-1 PerstAF No LVH III-1 42 42 PΔF Yes Normal 43 III-2 39 42 PAF Normal 42 AF527 54 PerstAF 46 52 LVH 1-1 Yes 56 11-1 56 PAF Yes Normal 44 III-1 25 PAF Normal 42 AF313 56 PermAF MR. LVH 11-1 48 Yes 52 II-1 44 46 PerstAF Yes Normal 42 42 43 PAF Normal 41 Yes 11-4 44 45 PAF Normal 42 Yes AF579 I-1 56 64 PerstAF No LVH 46 48 49 PAF Yes Normal 43 II-3 49 50 PAF No Normal 41 AF673 48 50 PAF Yes Normal 41 AF242 II-1 38 40 PAF Yes 44 Normal 42 44 11-9 PAF Yes Normal 43 III-2 20 26 PAF 39 Normal AF406 78 78 PermAF LVH 48 1-1 No II-4 61 62 Perst A F No LVH 49 52 58 Normal III-1 17 22 PAF 39 Normal Yes III-3 39 42 PAF Normal 42 Yes III-5 42 46 PAF Yes Normal 44 III-7 44 45 PAF Normal 46 AF1111 1-1 58 64 PermAF LVH 50 No II-4 56 60 PAF Yes Normal 44 46 48 Yes Normal

AF = atrial fibrillation; LA = left atrial; LVH = left ventricular hypertrophy; MR = mitral regurgitation; PAF = paroxysmal atrial fibrillation; PerstAF = persistent atrial fibrillation; PermAF = permanent atrial fibrillation.

are also given a 30-day event recorder that can be activated whenever they develop symptoms.

Screening for mutations in ion channel and other genes. Whole blood was collected for genomic DNA extraction and analysis from lone AF probands and as many first-degree relatives as would consent to the study. Mutational analyses of SCN5A (15), KCNQ1 (16), NPPA (16), KCNA5

(17), and *NKX2.5* genes were performed in lone AF probands and all consented family members. The coding and flanking regions were amplified by polymerase chain reaction using primers designed to obtain fragments of appropriate size, as previously described (15–17). Briefly, polymerase chain reaction–amplified DNA fragments were analyzed using the Reveal Discovery System (based on

Table 2	Probands With AF Enrolled in the Vanderbilt AF Registry (Cases) and Controls Who Were Resequenced for Variants in Ion Channel and Non-Ion Channel Genes
---------	--

Gene	Lone AF Cases	Controls
SCN5A	118	460
	257 (typical AF)	
KCNQ1	231	500
NPPA	98	500
	133 (typical AF)	
KCNA5	231	300
	200 (typical AF)	
NKX2.5	160	500

AF = atrial fibrillation

temperature gradient capillary electrophoresis) to identify aberrant conformers, which were then directly sequenced. Table 2 lists the number of lone and typical (non-lone) patients with AF enrolled in the Vanderbilt AF Registry and controls who were screened for genetic variants in the ion channel and non-ion channel genes.

q25 genotypes. Genotyping of the 2 SNPs (rs2200733 and rs10033464) was performed using real-time 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, California) as previously described (18). Seventy subjects (11 familial lone AF probands and 69 family members) underwent genotyping at the 2 common 4q25 SNPs.

Statistical analysis. The goal of these analyses was to determine if there is a statistically significant association between the presence of both common and rare AF variants and the development of AF (i.e., AF disease status). We hypothesized that the common variants on 4q25 influence the development of AF by modulating the rare ion channel and non-ion channel mutations. To test this hypothesis, we first performed a series of chi-square tests (2  $\times$  2 tables) of association (using Yates's correction test for small sample size) with 1 degree of freedom. We initially performed an unstratified analysis, followed by 5 stratified analyses on the basis of the age at onset of AF and the age of the controls. Because most lone AF probands develop AF before the age of 50 years, our first age cutoff for AF age of onset was <50 years, but we also stratified on the basis of an AF age of onset of <40 years. Thus, we stratified by AF age at onset <50 years, AF age at onset <40 years, unaffected subjects age >50 years only, and the 2 possible combinations of these 3 criteria. We collapsed the common variant genotypes into an allelic test of the presence and absence of the variant allele. The nominal p values as well as p values generated with permutation testing were determined.

Permutation testing was performed to correct for multiple testing, while controlling for the familial relationships in the kindreds. The process involved removing the AF affection status within each family and randomly reassigning in the same proportion of affected and unaffected individuals. This was carried out in each family, 1,000 times, to create 1,000 null datasets. In each random dataset, we performed association analysis and recorded the chi-square statistic. After 1,000 analyses, we had an empirical distribution from which to assign a permutation p value, which is adjusted for the relatedness in the dataset. In addition, we performed the analyses by way of the presence or absence of rare variants (ignoring the common variant).

As a follow-up, we performed a second series of chisquare analyses (using Yates's correction as appropriate). Here, we restricted the dataset to include only those subjects who possessed a rare variant in the ion channel or non-ion channel genes sequenced; these analyses included 48 subjects. In this set, we performed the identical series of chi-square analyses as described previously, although here, we were looking at the association of the common variant with AF status; all subjects possessed a rare variant in this set. We performed the unstratified analysis first, followed by 5 stratified analyses. We again stratified by AF age at onset <50 years, AF age at onset <40 years, unaffected individuals age >50 years only, and the two possible combinations of those 3 criteria. All chi-square tests of association were performed in JMP version 8.0 (SAS Institute Inc., Cary, North Carolina).

# Results

**Study cohort.** Our candidate gene approach to identifying novel mutations or rare variants associated with AF identified 11 familial lone AF probands who had 1 or more first-degree relatives with the arrhythmia. The total study cohort consisted of 70 subjects in 11 kindreds who had complete phenotype and genotype data available for analysis (Table 2).

Novel SCN5A variants. During the initial 3-year enrollment period, 396 patients with AF were approached, and 375 (95%) agreed to participate in the Vanderbilt AF Registry (15). Within this cohort, 118 patients (31%) had familial lone AF. As previously reported, resequencing identified 8 novel variants in 10 probands (2.7%) that were not found in the population-based controls (0%) (p = .001) (15). Genotyping was performed in 6 of these kindreds with 2 or more affected subjects with AF (Fig. 1). The novel variants identified affect highly conserved residues in the SCN5A protein. All probands identified were heterozygous for the mutation.

KCNQ1 variant. In this study cohort of 231 subjects with familial lone AF, screening for KCNQ1 mutations in genomic DNA identified a unique sequence variant in 1 case of familial AF (AF313) (16). In the proband, a 9-bp duplication resulting in insertion of the amino acids isoleucine, alanine, and proline at locations 54 to 56 (IAP54-56) in the N-terminus of the KCNQ1 protein was identified. The variant was also confirmed in 3 affected family mem-

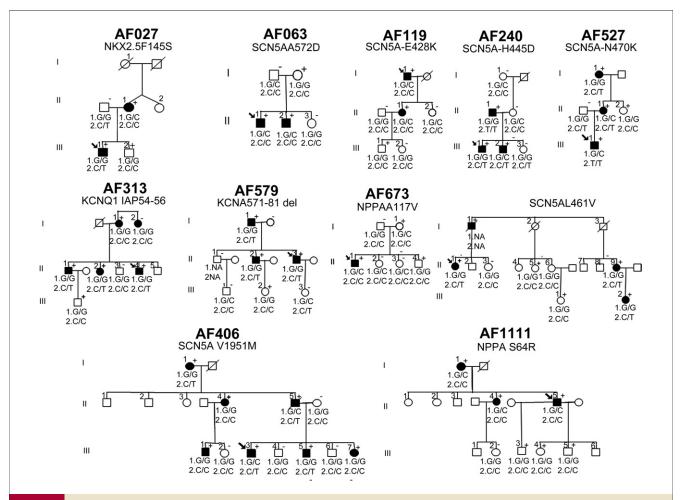


Figure 1 Pedigrees of 12 Familial AF Kindreds With Rare Ion Channel and Signaling Molecule Variants

Squares indicate male family members, circles indicate female family members, and symbols with slashes indicate deceased family members. Arrows indicate the probands. Solid symbols indicate the presence of atrial fibrillation (AF), and open symbols indicate unaffected family members. The presence (+) or absence (-) of a rare variant is indicated for persons with deoxyribonucleic acid samples available for testing. Numerals 1 (rs10033464) and 2 (rs2200733) refer to the genotype for the single-nucleotide polymorphisms on chromosome 4q25 that been associated with AF. NA = genotype not available.

bers. The sequence change was not found in Caucasian, Han Chinese, or Asian population controls but was identified in 2.1% (2 of 94) of African American subjects. Because the controls were obtained from the anonymous Coriell repository, no clinical information about these subjects is available.

NPPA variants. Genomic DNA sequencing of NPPA identified 2 novel missense mutations in 2 Caucasian AF kindreds; S64R in AF1111 (16) and A117V in AF673 (Fig. 1). The S64R mutation was also confirmed in 2 affected family members but was absent in unaffected family members. Furthermore, both mutations were not identified in Caucasian, Han Chinese, Asian, or African American population controls. The A117V mutation was identified in a family with lone AF (Fig. 1). The kindred included 6 family members, 3 of whom were heterozygous for the mutation, with the proband presenting with early-onset paroxysmal lone AF.

KCNA5 variant. We resequenced KCNA5 in 231 subjects with familial lone AF and identified a novel 33-bp coding region deletion in 2 Caucasian probands with early-onset lone AF. The variant results in the deletion of 11 amino acids at positions 71 to 81 of the N-terminus (71-81del) (17). The sequence change was not found in 200 patients with typical AF and 300 ethnically matched population controls. The proband in this kindred (AF579) presented with symptomatic paroxysmal familial lone AF, and his AF is currently managed with sotalol. A family history also showed that most of the affected family members developed familial lone AF at relatively young ages, and most presented with symptomatic paroxysmal AF.

**NKX2.5** variant. NKX2.5 was screened in 160 familial lone AF patients. We identified 1 novel nonsynonymous variant (F145S) that was not identified in more than 300 control patients with no history of AF. The proband in this kindred (AF027) presented with early-onset symptomatic familial

Table 3

An Interaction Between Rare (Ion Channel and Non-Ion Channel) and Common Genetic Variants to Determine Susceptibility to the Development of Familial AF

Chromosome 4q25 SNPs	AF by Age 50 Years	No AF by Age 50 Years
Absent	0	13
Present	21	8*

 $^*p=1.21\times 10^{-5}$  comparing the presence of both common and rare variants in 11 kindreds with familial AF.

AF = atrial fibrillation; SNP = single-nucleotide polymorphism.

lone paroxysmal AF. The proband's father also presented with familial lone AF in his 40s, and his brother, who carries the variant, has not been diagnosed with AF at age 52 years.

4q25 genotypes. Table 3 shows a significant interaction between common and rare genetic variants on the basis of an AF age of onset <50 years. However, when we stratified on the basis of AF age of onset <40 years and the ages of unaffected subjects in the families, there remained a significant association between the presence of common and rare genetic variants (Table 4). Furthermore, when we examined the genetic association of rare variants alone (ignoring common variants) unstratified and stratified by age of onset and the ages of unaffected subjects in the families, there remained a highly statistically significant association between the presence of both common and rare variants and AF disease status (Table 5). ORs were calculated for the test of co-occurrence of rare and common variants, in the unstratified and stratified analyses. The ORs ranged from 4 to 452 for the different strata; these values are far in excess of the 1.4 to 1.7 reported for studies in the general population, thus supporting the idea that these common variants modulate penetrance of the AF phenotype in these kindreds. ORs could not be calculated for the presence of common variants in those subjects with rare variants only because the contingency table cells contained zero counts.

We performed a logistic regression analysis with 2 main effect terms (presence or absence of rare variants and presence or absence of common variants) in addition to a multiplicative interaction term (presence or absence of rare variants  $\times$  presence or absence of common variants). On the basis of the likelihood ratio test, there was a highly significant interaction (p = 0.002).

# **Discussion**

The present findings provide strong evidence that 2 common AF-associated polymorphisms act as genetic modifiers of the clinical expression of latent cardiac ion channel and signaling gene mutations associated with familial AF. Furthermore, our findings strongly support the idea that AF inheritance is complex and non-Mendelian.

Although the potential mechanism of action of variants at the chromosome 4q25 locus tagged by the 2 noncoding SNPs remains unknown, it is quite likely mediated through effects on the paired-like homeodomain transcription factor 2 (PITX2) gene. PITX2 plays a critical role in left-right asymmetry, and loss of the pitx2c isoform in mice can lead to right atrial isomerization and failure to suppress a default pathway for sinus node development of the pulmonary myocardium (19-21). Importantly, this area is now well recognized as a common source for ectopic atrial activity necessary for the initiation and propagation of AF (22). Microarray analysis of pitx2 null-mutant and control mice hearts has revealed up-regulation of Kcnq1, a potassium channel gene that has been associated with gain-of-function mutations in familial AF (19,20). More recently, Kirchhof et al. (23) performed expression arrays in pitx2+/- mice demonstrating alteration in a number of cellular and molecular pathways in addition to potassium channels, which might provide an explanation for why mutations in ion channels are associated with familial AF. These recent data strongly support our hypothesis that the 4q25 AF susceptibility alleles likely mediate their effects by the modulation of cardiac ion channel expression and signaling proteins in the heart.

Investigating the genetic basis for AF presents a number of important challenges (8). The paroxysmal nature and variable symptoms in AF, a high prevalence in the general population, and a late age of onset in many patients all make assignment of the correct clinical phenotype challenging. This complexity has compelled a search for new, more effective methods for investigating the genetics of AF. One approach is to use the families of affected patients as an enriched target population for the definition and evaluation of intermediate or endophenotypes, such as prolonged signal-averaged P-wave duration (24), which are causally related to the poorly penetrant classic clinical syndromes

Table 4 Genetic Association Between Common and Rare Variants Unstratified and Stratified by Age of Onset and Age of Unaffected Members of the Families

		Presence of Both Common and		Permutation
Stratification	Sample Size	Rare Variants (p Value)	OR (95% CI)	p Value
Unstratified	70	$9.21 imes10^{-10}$	53 (10-273)	< 0.001
Age of onset <50 yrs	64	$9.41 imes 10^{-8}$	39 (8-205)	< 0.001
Age of onset <40 yrs	57	$ extbf{4.13} imes extbf{10}^{-6}$	51 (6-452)	< 0.001
Unaffected age >50 yrs	48	$8.63 imes 10^{-7}$	41 (7-239)	< 0.001
Age of onset $<$ 50 yrs, unaffected age $>$ 50 yrs	42	$\textbf{1.21}\times\textbf{10}^{-5}$	31 (5-179)	< 0.001
Age of onset $<$ 40 yrs, unaffected age $>$ 50 yrs	35	$ extbf{1.62} imes extbf{10}^{- extbf{4}}$	40 (4-385)	< 0.001

Table 5	Genetic Association of Rare Variants Alone (Ignoring Common Variants) Unstratified and Stratified by Age of Onset and Age of Unaffected Members of the Families			
	Stratification	Sample Size	Presence/Absence of Rare Variants Only (p Value)	OR (95% CI)
Unstratified		70	$2.2  imes \mathbf{10^{-4}}$	19 (2.4-155)
Age of onset <50yrs		64	0.003	14 (1.7-117)
Age of onset <40 yrs		57	0.04	8.8 (1.1-74)
Unaffected age >50 yrs		48	$\textbf{2.1}\times\textbf{10}^{-\textbf{4}}$	26 (3.0-227)
Age of onset <50 yrs, unaffected age >50 yrs		42	0.002	20 (2.2-173)
Age of onset <40 yrs, unaffected age >50 yrs		35	0.013	12 (1.3-109)

Abbreviations as in Table 4.

(25). Because of these limitations, researchers have begun to apply other approaches to identify genes that may be associated with AF. A logical consequence of the availability of comprehensive genomic maps is the advent of high-density genome-wide searches for modest gene effects using large-scale testing of SNPs. This approach has identified common variants on the chromosome 4q25 locus and more recently common SNPs on chromosomes 16q22 (26) and 1q21 (27) that confer an increased risk (OR: 1.2 to 1.4) for AF across multiple different populations.

One of the most challenging problems with non-Mendelian genetic approaches is the differentiation of signal from noise. Association studies may be confounded by etiologic heterogeneity, population stratification, or falsepositives, while the bias toward small effects may miss major genetic contributions relevant only in a subset of study subjects. It may also prove difficult to define the fundamental mechanisms of any true associations, because the responsible gene may be remotely linked to the locus identified. The development of more efficient models for the rapid validation of genomewide association study results and improved understanding of the underlying biology will facilitate the interpretation of such studies. In our study, however, variants identified in our screening are likely to be causative, because we demonstrated cosegregation of the variants with AF in multiple kindreds (where available), the variants were absent in a large control population, and all novel variants are located in highly conserved regions of the ion channel and signaling protein. This supports a link between disturbed ion channel and protein function and AF.

Studies of kindreds with AF suggest a genetic basis for the condition, and mutations in several cardiac potassium channel genes have been linked to familial AF (28,29). Although specific mutations in the *KCNQ1* gene have been observed in families with AF, the role of such mutations in AF remains unclear; often such isolated or "private" mutations are in residues of unknown function, effects on channel conductance are variable, and in most cases it may be difficult to discriminate rare polymorphisms of no functional significance from true mutations. However, the low prevalence of *KCNQ1* mutations in large AF cohorts suggests that mutations in this gene are not a major cause of AF

(30,31). In AF313, multiple family members presented with early-onset lone AF, and our segregation analysis supports the novel *KCNQ1* mutation as being causative for AF. Furthermore, the absence of the *KCNQ1* variant (IAP54-56) in non–African-American control groups excludes the possibility that this mutation is a common polymorphism in this population; this finding is consistent with a disease-associated mutation. The *KCNQ1*–IAP54-56 variant, however, was identified in 2.1% of healthy African American subjects, suggesting that it may be a common risk allele in this population.

Several limitations of the present study warrant consideration. First, most of the AF kindreds were small, and therefore, formal segregation analysis was limited. Data for some unaffected family members were not available. Second, no novel mutations were identified in the ethnically defined controls. However, comprehensive resequencing of the individual gene in the population-based controls was not performed, and this may condition interpretation of the results of this study. Although it is possible that additional novel ion channel and other gene mutations may have been identified, and our focused strategy of control genotyping may underestimate the true prevalence of rare polymorphisms, the association of the mutations with AF is supported primarily by segregation analysis and the fact that the mutations occurred in a highly conserved region of the protein. Third, population stratification may confound our results, especially across families. However, because our

Table 6

Genetic Association of Common Variants in the Subset of Data With All Subjects Harboring Rare Variants: Unstratified and Stratified by Age of Onset and Age of Unaffected Members of the Families

Stratification	Sample Size	Common Variants in Subjects With Rare Variants Only (p Value)
Unstratified	48	$ extbf{2.79} imes extbf{10}^{-7}$
Age of onset $<$ 50 yrs	42	$ extbf{4.41} imes extbf{10}^{-6}$
Age of onset <40 yrs	36	$ extbf{1.46} imes extbf{10}^{- extbf{4}}$
Unaffected age >50 yrs	34	3.4 $\times$ 10 <sup>-4</sup>
Age of onset <50 yrs, unaffected age >50 yrs	28	0.001
Age of onset $<$ 40 yrs, unaffected age $>$ 50 yrs	22	0.01

Table 7 Allele Frequencies of Chromosome 4q25 Single-Nucleotide Polymorphi	
--	--

Variant	Genotype	Number of Alleles	Allele Frequency
rs10033464	С	22	0.16
	G	114	0.83
	Т	2	0.01
rs2200733	С	114	0.83
	Т	24	0.17

cohort came from a fairly homogenous population of individuals of European descent, we believe that this is unlikely to bias our results. Furthermore, data on the common 4q25 allele frequencies (as shown in Table 7) would suggest otherwise.

Clinical implications. The idea that a combination of common and rare variants contributes to the very common phenotype of AF may have significant impact on the assessment and management of patients not only with familial AF but also the more common forms of the arrhythmia. Our study has demonstrated that the penetrance of genes encoding rare ion channel and signaling proteins is modulated by common variants on chromosome 4q25, supporting the idea that AF inheritance is complex (i.e., a single genetic variant does not predict the phenotype). Currently there is no clinical role for genetic testing in most patients with AF (32). However, a case can be made to screen candidate AF genes in those patients who present with early-onset lone AF, especially if there is a strong family history of the arrhythmia. Identification of a rare genetic variant in lone AF probands may be followed by consideration of screening family members to identify other carriers and also genotyping at the 4q25 AF locus. Identification of family members who are at risk for developing AF is important, because therapies known to reduce the incidence of AF, such as angiotensin-converting enzyme inhibitors, could be considered.

# **Conclusions**

We have shown that 2 common AF-associated polymorphisms can act as genetic modifiers of the clinical severity of familial AF. One interpretation of our findings is that in kindreds with familial AF, the arrhythmia develops in subjects who have the rare AF-associated variants as well as susceptibility alleles determined by the 4q25 locus. Our findings support the general concept that common variants may act as significant modifiers of the effects of rare variants and that the genetic architecture of common human phenotypes includes both rare and common variants (33).

Reprint requests and correspondence: Dr. Dawood Darbar, Vanderbilt University School of Medicine, Division of Cardiovascular Medicine, 2215B Garland Avenue, Room 1285A MRB IV, Nashville, Tennessee 37323-6602. E-mail: dawood.darbar@vanderbilt.edu.

#### **REFERENCES**

- Lloyd-Jones DM, Wang TJ, Leip EP, et al. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. Circulation 2004;110:1042-6.
- Miyasaka Y, Barnes ME, Gersh BJ, et al. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. Circulation 2006; 114:119–25.
- Fox CS, Parise H, D'Agostino RB Sr, et al. Parental atrial fibrillation as a risk factor for atrial fibrillation in offspring. JAMA 2004;291: 2851-5.
- 4. Arnar DO, Thorvaldsson S, Manolio TA, et al. Familial aggregation of atrial fibrillation in Iceland. Eur Heart J 2006;27:708–12.
- Darbar D, Herron KJ, Ballew JD, et al. Familial atrial fibrillation is a genetically heterogeneous disorder. J Am Coll Cardiol 2003;41: 2185–92.
- Ellinor PT, Yoerger DM, Ruskin JN, Macrae CA. Familial aggregation in lone atrial fibrillation. Hum Genet 2005;118:179–84.
- Marcus GM, Smith LM, Vittinghoff E, et al. A first-degree family history in lone atrial fibrillation patients. Heart Rhythm 2008;5: 826-30.
- 8. Darbar D. Genetics of atrial fibrillation: rare mutations, common polymorphisms, and clinical relevance. Heart Rhythm 2008;5:483-6.
- Gudbjartsson DF, Arnar DO, Helgadottir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. Nature 2007;488:353–7.
- Kaab S, Darbar D, van Noord C, et al. Large scale replication and meta-analysis of variants on chromosome 4q25 associated with atrial fibrillation. Eur Heart J 2009;30:813–9.
- Body SC, Collard CD, Shernan SK, et al. Variation in the 4q25 chromosomal locus predicts atrial fibrillation after coronary artery bypass graft surgery. Circ Cardiovasc Genet 2009;2:499-506.
- Husser D, Adams V, Piorkowski C, Hindricks G, Bollmann A. Chromosome 4q25 variants and atrial fibrillation recurrence after catheter ablation. J Am Coll Cardiol 2010;55:747–53.
- Darbar D, Motsinger AA, Ritchie MD, Gainer JV, Roden DM. Polymorphism modulates symptomatic response to antiarrhythmic drug therapy in patients with lone atrial fibrillation. Heart Rhythm. 2007;4:743–9.
- Levy D, Savage DD, Garrison RJ, Anderson KM, Kannel WB, Castelli WP. Echocardiographic criteria for left ventricular hypertrophy: the Framingham Heart Study. Am J Cardiol 1987;59:956–60.
- Darbar D, Kannankeril PJ, Donahue BS, et al. Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation. Circulation. 2008;117:1927–35.
- Abraham RL, Yang T, Blair M, Roden DM, Darbar D. Augmented potassium current is a shared phenotype for two genetic defects associated with familial atrial fibrillation. J Mol Cell Cardiol 2010;48:181–90.
- Yang T, Yang P, Roden DM, Darbar D. Novel KCNA5 mutation implicates tyrosine kinase signaling in human atrial fibrillation. Heart Rhythm 2010;7:1246-52.
- Pfeufer A, Jalilzadeh S, Perz S, et al. Common variants in myocardial ion channel genes modify the QT interval in the general population: results from the KORA study. Circ Res 2005;96:693–701.
- Tessari A, Pietrobon M, Notte A, et al. Myocardial Pitx2 differentially regulates the left atrial identity and ventricular asymmetric remodeling programs. Circ Res 2008;102:813–22.
- Wang J, Klysik E, Sood S, Johnson RL, Wehrens XH, Martin JF. Pitx2 prevents susceptibility to atrial arrhythmias by inhibiting left-sided pacemaker specification. Proc Natl Acad Sci U S A 2010;107: 9753–8.
- Mommersteeg MT, Brown NA, Prall OW, et al. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium. Circ Res 2007;101:902-9.
- 22. Haissaguerre M, Jais P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998;339:659–66.
- Kirchhof P, Kahr PC, Kaese S, et al. PITX2c is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression. Circ Cardiovasc Genet 2011;4:123–33.
- Darbar D, Hardy A, Haines JL, Roden DM. Prolonged signalaveraged P-wave duration as an intermediate phenotype for familial atrial fibrillation. J Am Coll Cardiol 2008;51:1083–9.

- 25. Benjamin EJ, Chen PS, Bild DE, et al. Prevention of atrial fibrillation: report from a national heart, lung, and blood institute workshop. Circulation 2009;119:606–18.
- 26. Gudbjartsson DF, Holm H, Gretarsdottir S, et al. A sequence variant in ZFHX3 on 16q22 associates with atrial fibrillation and ischemic stroke. Nat Genet 2009;8:876–8.
- Ellinor PT, Lunetta KL, Glazer NL, et al. Common variants in KCNN3 are associated with lone atrial fibrillation. Nat Genet 2010; 42:24044.
- 28. Chen YH, Xu SJ, Bendahhou S, et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. Science 2003;299:251-4.
- Olson TM, Alekseev AE, Liu XK, et al. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. Hum Mol Genet 2006;15:2185–91.
- 30. Ellinor PT, Moore RK, Patton KK, Ruskin JN, Pollak MR, Macrae CA. Mutations in the long QT gene, KCNQ1, are an uncommon cause of atrial fibrillation. Heart 2004;90:1487–8.
- Ellinor PT, Petrov-Kondratov VI, Zakharova E, Nam EG, Macrae CA. Potassium channel gene mutations rarely cause atrial fibrillation. BMC Med Genet 2006;7:70.
- 32. Ackerman MJ, Priori SG, Willems S, et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies. Heart Rhythm 2011;8:1308–39.
- Johansen CT, Wang J, Lanktree MB, et al. Excess of rare variants in genes identified by genome-wide association study of hypertriglyceridemia. Nat Genet 2010;42:684-7.

**Key Words:** atrial fibrillation ■ genetics ■ mutations ■ polymorphisms.