of AF during the postoperative period, and patients with new-onset AF are more likely to have acute kidney dysfunction after cardiac surgery.[15,16] Because chronic kidney disease is associated with hypertension and high atrial pressure, both of which predispose to AF,[15,17] the development of AF in patients with kidney dysfunction could simply reflect mechanical stress in atrium. However, in our study, even mild kidney dysfunction was associated with the development of AF and the association between kidney dysfunction and AF was significant in multivariate models adjusted for blood pressure and treated hypertension and in those without hypertension. Thromboembolism and a decline in cardiac function with AF may play a role in the subsequent decline in kidney function decline.

Thus, AF may cause kidney dysfunction and vice versa. However, AF and chronic kidney disease share risk factors and putative mechanisms, suggesting that common pathophysiologic processes may drive both outcomes. One possible common link is inflammation. Chronic kidney disease is associated with inflammation, even in the stage of moderate kidney dysfunction,[25] suggesting that persistent inflammation starts early in the process of kidney function decline. Elevated levels of inflammatory markers including C-reactive protein, fibrinogen, and interleukin-6 have been reported in chronic kidney disease.[24,25,39] More severe kidney impairment is associated with a trend towards higher levels of inflammation, and inflammatory markers predict progression of kidney dysfunction.[25,40] Inflammation and oxidant stress also play a significant role in the initiation and perpetuation of AF.[26,27] Elevated inflammatory markers have been associated with new-onset and persistent AF.[26,41] Oxidant stress and changes in gene expression profiles toward a more pro-oxidant state in AF have been reported.[27,42] Therefore, the association between AF and kidney disease may reflect activation of signaling pathways important for inflammation and oxidant stress, although inflammation markers such as C-reactive protein were not measured in this study population. Evidence that administration of anti-inflammatory drugs (eg, glucocorticoids) and drugs with antioxidant properties (such as statins) may prevent AF and also preserve kidney function and prevent proteinuria, further supports the importance of inflammation as a common pathophysiologic link between the pathogenesis of AF and that of chronic kidney disease.[43-46]

Another possible common link between AF and chronic kidney disease is renin-angiotensin-aldosterone system activation.[28-34] The renin-angiotensin-aldosterone system is involved in the pathogenesis of common forms of kidney disease, especially with hypertension,[29] and it is reported that plasma renin activity is inappropriately high in chronic kidney disease.[28] Treatment with renin-angiotensin-aldosterone system modulators, including angiotensin-converting enzyme inhibitors and angiotensin receptor blockers slows progression of chronic kidney disease.[47] Evidence suggesting a role for the renin-angiotensin-aldosterone system in the pathogenesis of AF has been also reported.[30-35] Angiotensin II can increase atrial pressure, promote atrial fibrosis, and modulate ion channels, all of which are involved in atrial structural and electrical remodeling resulting in AF.[31,48,49] Polymorphisms in components of this pathway have been linked to the development of AF.[30] Expression of angiotensin-converting enzyme is increased in atria during AF,[31] and treatment with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers reduces incidence of AF.[32,35] In addition, locally produced angiotensin II induces inflammation.[26,27,29] Between subjects with kidney dysfunction and those without, there were differences in baseline characteristics including age, sex, body mass index, blood pressure, hypertension, and diabetes, all of which are risk factors for AF,[17-19] and thus, the association between kidney dysfunction and subsequent AF may be explained by the differences. However, kidney dysfunction was associated with development of AF in multivariate models adjusted for these factors. Similarly, although baseline characteristics were different between subjects with AF and those without, multivariate models revealed that AF was associated with development of kidney dysfunction.

Our study has some limitations. Although annual health examinations are available for residents in our prefecture, about half of subjects do not receive the examinations and this may result in selection bias. We used an estimated GFR instead of actual measurement,[10,11,50] but this is a common approach in large population studies. Atrial fibrillation was diagnosed based on annual ECG recordings, and the manner and frequency of evaluation supporting AF diagnosis may lead underestimation of AF. However, the incidence of AF in our study was similar to that in other studies in Japan.[51,52] Our study population included more women than men, although an opportunity for this annual health examination is provided equally to men and women. The medical history was self-reported. It has been reported that treatment with angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and antidyslipidemia drugs have beneficial effects on AF and chronic kidney diease,[32-35,53] but we do not have information on individual drug regimens. We did not study the effects of cardiovascular events such as myocardial infarction and heart failure during the follow-up on subsequent AF and kidney disease. This study was conducted in a Japanese population, and further studies in other populations are necessary to generalize our results.

## Conclusions

We found that there is an association between kidney disease and AF, even in the absence of hypertension and

diabetes. We suggest that disrupted multiple signaling pathways may be critical for the pathogenesis of both diseases and, potentially, their interaction. Chronic kidney disease and AF are associated with increased incidence of cardiovascular events and high mortality, and our findings further propose the importance of management of these diseases to prevent subsequent events and to improve mortality.

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# *ACE* I/D polymorphism associated with abnormal atrial and atrioventricular conduction in lone atrial fibrillation and structural heart disease: Implications for electrical remodeling

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**BACKGROUND** The angiotensin-converting enzyme (ACE) gene contains a common polymorphism based on the insertion (I) or deletion (D) of a 287-bp intronic DNA fragment. The D allele is associated with higher ACE activity and thus higher angiotensin II levels. Angiotensin II stimulates cardiac fibrosis and conduction heterogeneity.

**OBJECTIVE** The purpose of this study was to determine whether the *ACE* I/D polymorphism modulates cardiac electrophysiology.

**METHODS** Three different cohorts of patients were studied: 69 patients with paroxysmal lone atrial fibrillation (AF), 151 patients with structural heart disease and no history of AF, and 161 healthy subjects without cardiovascular disease or AF. Patients taking drugs that affect cardiac conduction were excluded from the study. ECG parameters during sinus rhythm were compared among the *ACE* I/D genotypes.

**RESULTS** The *ACE* I/D polymorphism was associated with the PR interval and heart block in the lone AF cohort. In multivariable linear regression models, the D allele was associated with longer PR interval in the lone AF and heart disease cohorts (12.0-ms and 7.1-ms increase per D allele, respectively). P-wave duration showed a similar trend, with increase in PR interval across *ACE* I/D genotypes in the lone AF and heart disease cohorts.

**CONCLUSION** The *ACE* D allele is associated with electrical remodeling in patients with lone AF and in those with heart disease, but not in control subjects. *ACE* activity may play a role in cardiac remodeling after the development of AF and heart disease.

**KEYWORDS** Atrial fibrillation; Angiotensin-converting enzyme; Genetics; Electrocardiogram; Heart block; Arrhythmia

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## Introduction

The renin-angiotensin-aldosterone system (RAAS) plays an important role in regulation of normal cardiovascular function, and disorder of the system is associated with cardiovascular diseases.[1–6] Genetic variants in angiotensin-converting (ACE) enzyme have been associated with cardiovascular disease, including hypertension, myocardial infarction, dilated cardiomyopathy, and left ventricular hypertrophy.[1–3,7] Recent evidence suggests that the *ACE* gene may also play an important role in the pathogenesis of arrhythmias.[4–6]

The *ACE* gene contains a polymorphism based on the presence [insertion (I)] or absence [deletion (D)] of a 287-bp intronic DNA segment, resulting in three genotypes (DD and II homozygotes, and ID heterozygotes).[8] The *ACE* D allele is associated with higher plasma and cardiac *ACE* activity resulting in higher angiotensin II levels, and it has been reported to increase the risk of arrhythmias, including sudden death and atrial fibrillation (AF).[4–6,8,9] RAAS activation plays an important role in structural and electrophysiologic remodeling that is associated with the development of AF.[10] It has been reported recently that angiotensin II also provides an electrophysiologic substrate for arrhythmias by modulation of ion channels and gap junctions.[11–14] Moreover, we recently reported that antiarrhythmic drugs are less effective for AF in subjects carrying the D allele, suggesting the hypothesis that the *ACE* I/D polymorphism modulates cardiac electrophysiology.[15] In the present study, we tested this hypothesis by studying the association of the *ACE* I/D polymorphism with ECG parameters in subjects with AF and in those with heart disease.

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## Methods

### Study population

The study protocol was approved by the Institutional Review Board of Vanderbilt University and Massachusetts General Hospital. All subjects gave written informed con-

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sent. This study included three cohorts: (1) patients with lone AF enrolled in the Vanderbilt or Massachusetts AF Registry, which is composed of clinical and genetic registries[16,17]; (2) patients with structural heart disease but no personal or family history of AF[18]; and (3) 161 healthy volunteers with no significant medical history, normal physical examinations, and no personal or family history of AF.[19] At enrollment into the AF Registries, all subjects completed a symptom questionnaire to assess for symptomatic AF burden, which is measured by using an algorithm for scoring the frequency, duration, and severity of symptoms.[15] Although AF symptoms are important when evaluating response to drug therapies, for the purposes of this study, the AF burden score was limited to the frequency and duration of AF.

*Lone AF* was defined as AF occurring in patients younger than 65 years without hypertension or overt structural heart disease by clinical examination, ECG, and echocardiography. Patients with lone AF who had 12-lead ECGs showing sinus rhythm were included in the lone AF cohort. Patients taking drugs that affect cardiac conduction, including antiarrhythmic drugs, beta-blockers, calcium channel blockers, and digoxin, were excluded from the study.

### ECG measurements

All 12-lead ECGs were recorded with the patient resting supine, using an analog system at 25 mm/s paper speed, 10 mm/mV gain, and 40-Hz low-pass filter setting. P-wave duration and PR intervals were measured on all possible leads by an experienced observer blinded to all clinical details using a semi-automated digitizing program with electronic calipers.[20]

To determine interobserver variability, a second observer made independent blinded P-wave duration determinations of 20 randomly selected ECGs. Intraobserver variability was evaluated by P-wave duration analysis of 30 randomly assigned ECGs, again in a blinded fashion by a single observer. Interobserver measurement error was avoided by using the measurements of the same experienced observer for statistical comparisons.

### Determination of ACE genotype

*ACE* I/D genotypes were determined by polymerase chain reaction as described previously.[15] Briefly, a set of primers was designed to encompass the polymorphic region in intron 16 of the *ACE* gene (sense primer 5′ CTGGAGACCACTCCCATCCTTTCT 3′, antisense primer 5′ GATGTGGCCATCACATTCGTCAGAT 3′). The products were separated by electrophoresis on 2% agarose gel and identified by ethidium bromide staining. Each sample found to be DD was verified using insertion specific primers (sense primer 5′ TGGGACCACAGCGCCCGCCACTAC3′, antisense primer 5′ TCGCCAGCCCTCCCATGCCCATAA 3′).

### Statistical analysis

Statistical analysis was performed using the SPSS statistical package, release 16.0.1 (SPSS, Inc., Chicago, IL, USA). All values are expressed as mean ± SD for continuous variables and proportions for categorical variables. Hardy-Weinberg equilibriums were performed using Chi-square test. Statistical tests of parameters by genotypes were analyzed using Fisher exact test for categorical variables and analysis of variance for continuous variables.

Associations of ECG measurements with *ACE* genotypes were adjusted for variables affecting cardiac conduction using a linear regression model. All factors adjusted for were determined *a priori.* Modulating factors for the lone AF cohort included age, length of time with paroxysmal AF (AF burden), heart rate, and left atrial size. For healthy controls, factors included age and heart rate. For patients with cardiovascular disease, factors adjusted for included age, heart rate, history of hypertension, and congestive heart failure. PR interval and P-wave durations were checked for normal gaussian distributions; no transformations were required. A two-sided significance of 0.05 was used for all analyses.

## Results

### Clinical characteristics

Among 399 patients with lone AF and 385 heart disease patients without AF in the original cohorts, 69 patients with lone AF and 151 heart disease patients were eligible for this study. The reasons for exclusion from this analysis included concomitant medications that modulate cardiac conduction and failure to record an ECG during sinus rhythm. Table 1 lists the clinical characteristics of the study subjects. The mean symptomatic AF burden score in the lone AF cohort at enrollment was high, but, more importantly, it was similar across the three ACE I/D genotypes.

### *ACE* I/D genotype and ECG parameters

The frequencies of the II, ID, and DD genotypes for the lone AF cohort (22%, 53%, and 25%, respectively), the heart disease cohort (16%, 49%, and 35%, respectively), or the control cohort (18%, 48%, and 34%, respectively) did not deviate significantly from those predicted by Hardy-Weinberg criteria (27%, 50%, and 23% respectively; P = NS for each). The intraclass correlation coefficient for the ECG measurement between the two observers was 0.98, indicating excellent agreement and reproducibility of data.

In the lone AF cohort, patients with the DD genotype had longer PR intervals and a higher incidence of heart block (bundle branch block or prolonged PR interval ≥200 ms; Table 2). To determine whether conduction in the atria is modulated by the *ACE* I/D polymorphism, P-wave duration was compared among the genotypes. P-wave duration was not different among the *ACE* I/D genotypes. The *ACE* I/D genotypes were not associated with heart rate, QRS interval, QRS axis, or QT interval in any of the cohorts.

In multivariable linear regression models, the *ACE* I/D polymorphism was associated with the PR interval in the lone AF and heart disease cohorts (Figure 1). With each D allele, the PR interval prolonged by an average of 12.0 ms and 7.1 ms in the lone AF and heart disease cohorts, respectively. The *ACE* I/D polymorphism was not signifi-

**Table 1** Clinical characteristics of study subjects

| Variable | All patients (n = 69) | *ACE* I/D genotype DD (n = 17) | ID (n = 38) | II (n = 14) | *P* value |
|---|---|---|---|---|---|
| Lone AF Patients | | | | | |
| Age at AF onset (years) | 38 ± 14 | 43 ± 13 | 37 ± 14 | 35 ± 13 | .12 |
| Duration after AF diagnosis (years) | 4 ± 8 | 4 ± 7 | 3 ± 6 | 8 ± 11 | .06 |
| AF burden score[15] | 12 ± 6 | 11 ± 5 | 12 ± 6 | 11 ± 6 | .77 |
| Male sex [n (%)] | 33 (72%) | 6 (55%) | 21 (78%) | 6 (75%) | .34 |
| Left atrial size (mm) | 36 ± 6 | 38 ± 7 | 35 ± 5 | 37 ± 6 | .37 |
| Ejection fraction (%) | 59 ± 6 | 62 ± 5 | 58 ± 6 | 58 ± 6 | .11 |
| | All patients (n = 151) | *ACE* I/D genotype DD (n = 54) | ID (n = 77) | II (n = 20) | *P* value |
| Heart Disease Patients without AF | | | | | |
| Age (years) | 55 ± 16 | 55 ± 16 | 54 ± 16 | 57 ± 14 | .69 |
| Male sex [n (%)] | 63 (42%) | 25 (46%) | 32 (42%) | 6 (30%) | .45 |
| Hypertension [n (%)] | 92 (61%) | 37 (70%) | 42 (55%) | 13 (65%) | .2 |
| Diabetes [n (%)] | 31 (21%) | 14 (26%) | 13 (17%) | 4 (20%) | .42 |
| Coronary artery disease [n (%)] | 52 (34%) | 16 (30%) | 30 (39%) | 6 (30%) | .49 |
| Congestive heart failure [n (%)] | 39 (26%) | 14 (26%) | 17 (22%) | 8 (40%) | .26 |
| Ejection fraction (%) | 44 ± 17 | 42 ± 19 | 44 ± 17 | 44 ± 15 | .82 |
| | All subjects (n = 118) | *ACE* I/D genotype DD (n = 41) | ID (n = 53) | II (n = 24) | *P* value |
| Controls without AF or Heart Disease | | | | | |
| Age (years) | 27 ± 6 | 27 ± 6 | 27 ± 6 | 28 ± 5 | .82 |
| Male sex [n (%)] | 46 (39) | 16 (39) | 21 (40) | 9 (38) | .98 |

AF = atrial fibrillation; I/D = insertion/deletion polymorphism.

cantly related to PR interval in the healthy cohort. P-wave duration showed a similar trend, with an increase in PR interval across the *ACE* I/D genotypes in the lone AF and heart disease cohorts (Figure 2). P-wave duration was significantly longer in subjects with the D allele than in those without the D allele (DD/ID vs II) in the lone AF cohort ($P$ = .04), but not in the heart disease or healthy control cohorts ($P$ = NS for each). With each D allele, P-wave duration prolonged by an average of 4.6 ms and 2.1 ms in the lone AF and heart disease cohorts, respectively. The *ACE* I/D polymorphism was not related to P-wave duration in the healthy cohort.

## Discussion

Recent studies strongly support the concept that genetic variation may be important in the pathogenesis of cardiac disorders, including arrhythmias, and the importance of genetic medicine in clinical practice is increasingly being recognized.[21–23] Mutations in genes encoding cardiac ion channels and associated proteins have been identified in inherited arrhythmia syndromes, including AF.[21,22] In population association studies, certain polymorphisms (ion channels, *ACE*, $\beta_2$-adrenergic receptors) have been associated with increased risk of arrhythmias and sudden cardiac death.[5,6,21,24] Furthermore, contribution of genetic variants to ECG variation in the general population has been reported. Polymorphisms in the cardiac potassium channel (KCNH2) and nitric oxide synthase 1 adaptor protein (NOS1AP) genes have been associated with QT-interval variation.[25,26] In some cases, the genetic variants are unmasked only when the "reserve" is reduced, for example, exposure to QT-prolonging drugs in patients with formes frustes of the congenital long QT syndrome.[27] In this study, we show for the first time that the *ACE* I/D polymorphism is associated with atrial and atrioventricular conduction in patients with lone AF and in those with heart disease, but not in healthy subjects. Our findings suggest that the RAAS plays an important role in electrical remodeling after development of AF and heart disease. The *ACE* D allele is associated with higher plasma concentration of ACE,[14] higher cardiac ACE concentration,[21] and increased renal ACE mRNA expression.[22] Thus, subjects with the D allele may be exposed to higher angiotensin II levels than are those with the I allele. Myocardial fibrosis is strongly correlated with RAAS activation, especially angiotensin II and aldosterone, and chronic exposure to high levels of circulating and/or tissue angiotensin may predispose to both left ventricular hypertrophy and myocardial fibrosis.[23]

AF is a highly heterogeneous disorder, and there is increasing evidence that activation of the RAAS plays a role in the pathophysiology of AF. The *ACE* I/D polymorphism is associated with serum and cardiac ACE activity, and subjects with the ID or DD genotype have approximately

**Table 2** Electrocardiographic parameters across ACE I/D genotypes

| Variable | All patients | ACE I/D genotype | | | *P* value |
|---|---|---|---|---|---|
| | | DD | ID | II | |
| Patients with Lone AF | | | | | |
| Heart rate (bpm) | 65 ± 12 | 64 ± 15 | 66 ± 11 | 65 ± 12 | .83 |
| P-wave duration (ms) | 109 ± 17 | 110 ± 16 | 111 ± 18 | 101 ± 16 | .17 |
| PR interval (ms) | 166 ± 26 | 177 ± 27 | 166 ± 26 | 150 ± 22 | .02 |
| Heart block [n (%)] | 8 (11) | 6 (33) | 2 (5) | 0 (0) | .002 |
| QRS interval (ms) | 89 ± 9 | 92 ± 9 | 89 ± 9 | 89 ± 9 | .51 |
| QRS axis (°) | 37 ± 35 | 38 ± 38 | 37 ± 35 | 41 ± 34 | .88 |
| QT interval (ms) | 402 + 36 | 400 + 37 | 400 + 36 | 410 + 34 | .67 |
| QTc | 408 ± 24 | 408 ± 28 | 411 ± 22 | 398 ± 25 | .20 |
| Heart Disease Patients without AF | | | | | |
| Heart rate (bpm) | 78 ± 20 | 79 ± 19 | 77 ± 20 | 78 ± 25 | .79 |
| P-wave duration (ms) | 104 ± 19 | 105 ± 16 | 105 ± 21 | 100 ± 17 | .54 |
| PR interval (ms) | 157 ± 29 | 162 ± 29 | 155 ± 30 | 149 ± 23 | .15 |
| Heart block [n (%)] | 12 (8) | 7 (13) | 5 (7) | 0 (0) | .15 |
| QRS interval (ms) | 97 ± 23 | 98 ± 27 | 95 ± 22 | 98 ± 20 | .69 |
| QRS axis (°) | 27 ± 51 | 32 ± 61 | 26 ± 45 | 20 ± 42 | .72 |
| QT interval (ms) | 387 + 55 | 386 + 59 | 385 + 53 | 394 + 54 | .57 |
| QTc | 430 ± 35 | 435 ± 42 | 425 ± 30 | 436 ± 34 | .31 |
| Controls without AF or Heart Disease | | | | | |
| Heart rate (bpm) | 66 ± 11 | 64 ± 11 | 67 ± 10 | 67 ± 11 | .42 |
| P-wave duration (ms) | 103 ± 12 | 102 ± 13 | 103 ± 13 | 104 ± 10 | .99 |
| PR interval (ms) | 152 ± 23 | 152 ± 23 | 154 ± 24 | 148 ± 19 | .56 |
| Heart block [n (%)] | 4 (3) | 2 (5) | 2 (4) | 0 (0) | .57 |
| QRS interval (ms) | 87 ± 10 | 87 ± 8 | 87 ± 9 | 89 ± 13 | .91 |
| QRS axis (°) | 72 ± 20 | 75 ± 15 | 70 ± 22 | 71 ± 19 | .32 |
| QT interval (ms) | 393 + 34 | 389 + 33 | 396 + 34 | 392 + 34 | .68 |
| QTc | 407 ± 19 | 407 ± 18 | 405 ± 18 | 417 ± 19 | .63 |

AF = atrial fibrillation; I/D = insertion/deletion polymorphism; QTc = corrected QT interval.

25% and 50% higher ACE levels, respectively, than do those with the II genotype.[8] It has been reported that genetic variants in *ACE* are associated with increased AF susceptibility,[5,6] and in this study we found that the polymorphism was also associated with cardiac conduction defects in patients with AF and those with heart disease. Our results are consistent with prior studies, which demonstrated that increased expression of ACE in cardiac tissues of genetically engineered mice results in the development of conduction abnormalities and AF.[28,29] Activation of the RAAS initiates a cascade of processes resulting in hypertrophy, fibroblast proliferation, accumulation of collagen, and apoptosis, all of which predispose to a reduction in conduction velocity.[30] Therefore, slow conduction associated with the D allele may result from electrophysiologic remodeling due to higher ACE levels and increased activation of the RAAS. Because blockade of the RAAS attenuates atrial fibrosis,[31] ACE inhibitors and angiotensin II receptor blockers may also prevent or reduce conduction defects in AF. Moreover, evidence that angiotensin II affects cardiac electrophysiology by modulation of ion channels and by gap junction remodeling, which in turn impairs cell-to-cell impulse conduction, further supports our findings.[11–14]

In this study, the *ACE* I/D polymorphism correlated with cardiac conduction in the atrium and conduction system, but not in the ventricle. The density of angiotensin II receptors is higher in atrial than in ventricular tissue, suggesting that the atrium is more vulnerable to angiotensin effects.[32] An alternative explanation is that rapid electrical activity during AF leads to greater electrical remodeling resulting in conduction defects in the atrium and conduction system than in the ventricle, as the ventricle is partially protected by atrioventricular block. The *ACE* I/D polymorphism may have effects on cardiac electrophysiology in the absence of AF or heart disease, but the effects may be subtle and thus may be difficult to detect on the conventional surface ECG. To address this issue, further studies using more advanced techniques such as signal-averaged ECG and intracardiac electrograms, which were not performed in this study, in a large number of subjects may be important.

Other factors are important for electrical and structural remodeling. In this study, normal volunteers without a history of AF or cardiac disease were younger than those with lone AF or those with cardiac disease, and aging may also be associated with *ACE* I/D polymorphism–mediated conduction slowing. However, in multivariate models adjusted for age and other variables, the association remained significant. Although the *ACE* D allele has been linked with hypertension,[7] we found that the *ACE* I/D polymorphism was associated with cardiac conduction in patients with lone AF who do not have a history of hypertension. Although lone AF patients have no overt structural heart disease, a prior study has shown subclinical atrial structural abnormalities in patients with lone AF refractory to antiarrhythmic

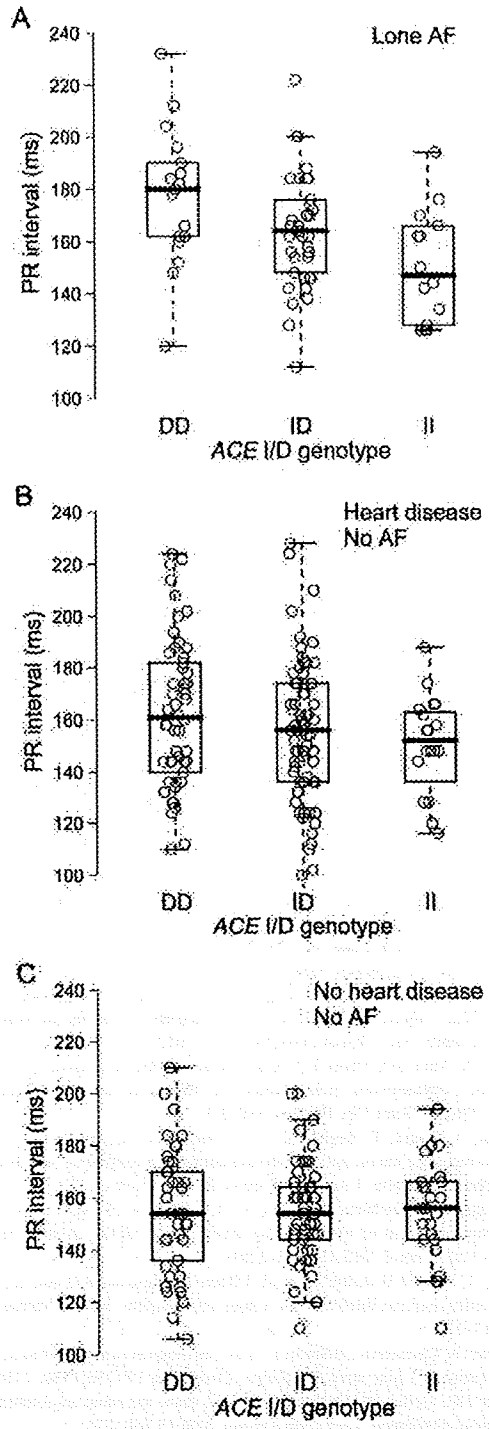


**Figure 1** *ACE* I/D genotype and PR interval in **(A)** patients with lone AF ($P$ = .02), **(B)** patients with heart disease and no history of AF ($P$ = .04), and **(C)** healthy subjects without AF or heart disease ($P$ = .84). ACE = angiotensin-converting enzyme; AF = atrial fibrillation; I/D = insertion/deletion polymorphism.

drugs.[33] Thereby, it is possible that the *ACE* D allele is associated with structural remodeling rather than electrical remodeling. Atrial stretch may cause P-wave prolongation and further increases local synthesis of angiotensin II,[34] but left atrial size was similar among the *ACE* I/D genotypes. Our study has several limitations. This study excluded patients taking atrioventricular node–modifying drugs so that the exclusive effects of *ACE* I/D polymorphism on cardiac electrophysiology could be evaluated. Although this could have introduced a selection bias in the study cohorts, it is unlikely because the frequencies of the *ACE* I/D genotypes were unchanged after the exclusion compared to those in the

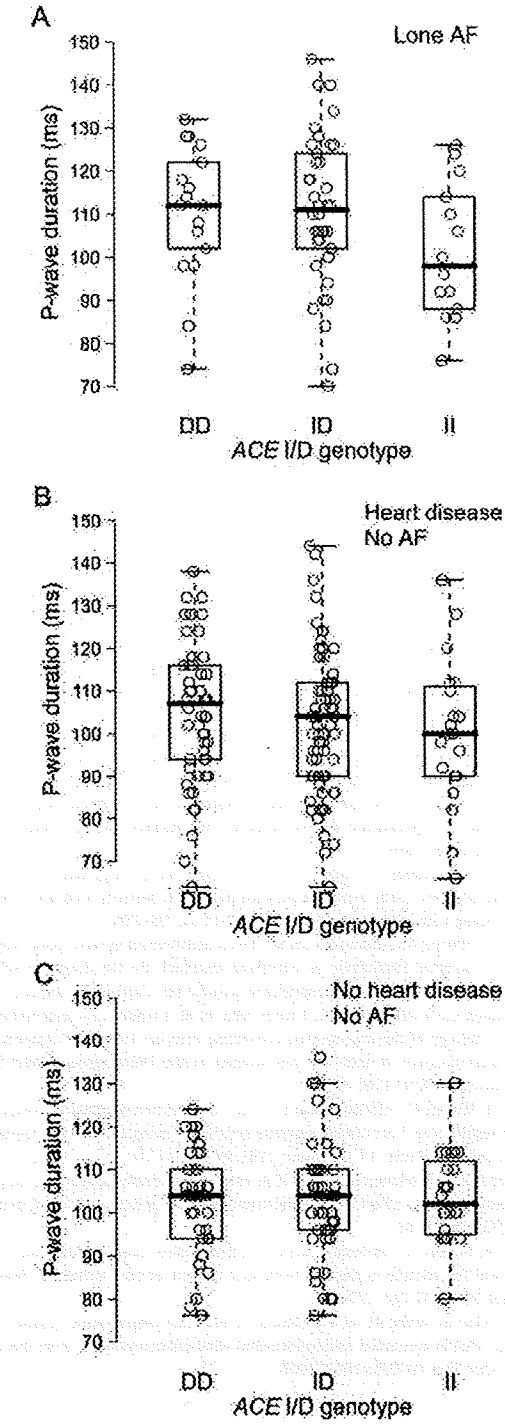


**Figure 2** *ACE* I/D genotype and P-wave duration in **(A)** patients with lone AF ($P$ = .18), **(B)** patients with heart disease and no history of AF ($P$ = .17), and **(C)** healthy subjects without AF or heart disease ($P$ = .25). ACE = angiotensin-converting enzyme; AF = atrial fibrillation; I/D = insertion/deletion polymorphism.

original populations. Furthermore, the study cohorts were in Hardy-Weinberg equilibrium, providing further evidence for the absence of this selection bias. Although the DD genotype was associated with increased risk of AF in a prior study,[5] the frequency of *ACE* I/D genotypes was similar in AF patients and controls in our study, similar to a study by Tsai et al.[6] They also reported two-way gene–gene interactions between *ACE* I/D polymorphism and angiotensinogen gene haplotypes, which may explain this discrepancy.[6] Our study only included patients with lone AF, and further study will be required to evaluate if the *ACE* I/D polymorphism also modulates ECG parameters in patients with AF associated with cardiovascular disease.

## Conclusion

Our data support the hypothesis that the *ACE* I/D polymorphism modulates cardiac electrophysiology in patients with lone AF and in those with cardiac disease, consistent with the known effect of the D allele on ACE activity. This study provides further evidence for the role of activation of the RAAS in the pathophysiology of cardiac disorders. Therapies modulating the RAAS may be useful in preventing the development of conduction abnormalities in patients with AF in addition to preventing AF and other cardiac diseases.[35,36]

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# Mutations in Sodium Channel β1- and β2-Subunits Associated With Atrial Fibrillation

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***Background***—We and others have reported mutations in the cardiac predominant sodium channel gene *SCN5A* in patients with atrial fibrillation (AF). We also have reported that *SCN1B* is associated with Brugada syndrome and isolated cardiac conduction disease. We tested the hypothesis that mutations in the 4 sodium channel β-subunit genes *SCN1B–SCN4B* contribute to AF susceptibility.

***Methods and Results***—Screening for mutations in the 4 β-subunit genes was performed in 480 patients with AF (118 patients with lone AF and 362 patients with AF and cardiovascular disease) and 548 control subjects (188 ethnically defined anonymized subjects and 360 subjects without AF). The effects of mutant β-subunits on *SCN5A* mediated currents were studied using electrophysiological studies. We identified 2 nonsynonymous variants in *SCN1B* (resulting in R85H, D153N) and 2 in *SCN2B* (R28Q, R28W) in patients with AF. These occur at residues highly conserved across mammals and were absent in control subjects. In 3 of 4 mutation carriers, the ECGs showed saddleback-type ST-segment elevation in the right precordial leads. Transcripts encoding both *SCN1B* and *SCN2B* were detected in human atrium and ventricle. In heterologous expression studies using Chinese hamster ovary cells, the mutant β1- or β2-subunits reduced *SCN5A*-mediated current and altered channel gating compared with coexpression of wild-type subunits.

***Conclusions***—Loss of function mutations in sodium channel β-subunits were identified in patients with AF and were associated with a distinctive ECG phenotype. These findings further support the hypothesis that decreased sodium current enhances AF susceptibility. ***(Circ Arrhythmia Electrophysiol.* 2009;2:268-275.)**

**Key Words:** arrhythmia ■ sodium channel ■ electrophysiology ■ genetics ■ mutations ■ atrial fibrillation

Risk factors for the development of atrial fibrillation (AF) include male sex, increasing age, hypertension, type II diabetes, metabolic syndrome, and obesity as well as underlying heart disease.[1–3] In addition to these risk factors, multiple studies now support a genetic contribution to AF susceptibility. In isolated patients and families, mutations in multiple ion channel genes including *KCNQ1*, *KCNE2*, *KCNJ2*, *KCNA5*, *SUR2A*, and *SCN5A* as well as the gap junction gene *GJA5* and the nuclear protein NUP155 have been associated with AF.[4–6] In addition, linkage of AF to 4 further loci has been reported, although the disease genes in the loci have not yet been identified.[4] In population studies, increased AF susceptibility has been associated with common polymorphisms in ion channels (*KCNE1*, *KCNE5*, *SCN5A*), a G-protein subunit (*GNB3*), angiotensinogen (*AGT*), and a locus near the atrial transcription factor *PITX2*.[4,5,7]

**Editorial see p 215**
**Clinical Perspective on p 275**

Sodium channels are multisubunit protein complexes composed of pore-forming α-subunits, auxiliary function-modifying β-subunits,[8] and multiple other proteins.[9] In humans, 4 sodium channel β-subunits (β1 to β4, encoded by *SCN1B* to *SCN4B*) have been identified. They share a common predicted protein topology with an extracellular immunoglobulin-like domain, a single transmembrane spanning segment, and an intracellular C-terminal domain.[8] Functions attributed to β-subunits include an increase in sodium channel expression at the cell surface, modulation of channel gating and voltage dependence, and a role in cell adhesion and recruitment of cytosolic proteins such as ankyrin G.[8]

Mutations in *SCN5A*, encoding the predominant cardiac sodium channel α-subunit, cause a range of inherited arrhyth-

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mia diseases including the long-QT syndrome, the Brugada syndrome, progressive cardiac conduction disease, and sick sinus syndrome.[10] Moreover, mutations in *SCN1B* and *SCN4B* have also been implicated in the Brugada syndrome and/or conduction disease, and long-QT syndrome, respectively.[11,12] In addition, *SCN5A* mutations and polymorphisms have been associated with AF, and we recently reported *SCN5A* mutations in 5.9% of patients with AF.[4,5,13] Taken together, these data suggest β-subunits as candidates for AF pathogenesis. Therefore, we have tested this hypothesis by screening sodium channel β-subunit genes for variants in patients with AF and control subjects.

## Methods

### Study Subjects

The study protocol was approved by the Institutional Review Board of Vanderbilt University, and all subjects gave informed consent. This study included 2 sets of patients with AF: (1) 375 patients including 118 patients with lone AF and 257 patients with AF and cardiovascular disease from the Vanderbilt AF Registry (356 whites [94%], 19 blacks [5%], 3 Hispanics [0.8%], 1 Asian [0.3%]), and (2) 105 patients from the Vanderbilt Cardiac Surgery Registry (101 whites [96%], 3 blacks [3%], 1 Hispanic [1%]) who had not had AF before or during surgery and in whom AF was documented in the postoperative period.[13,14]

### Control Populations

There were 3 sets of controls in this study:

1. There were 188 ethnically identified but otherwise anonymized subjects (white, black, Hispanic, Asian; n=47 for each group) from the Coriell Cell Repositories (Camden, NJ).
2. For the lone AF control subjects, we used 94 subjects (51 whites [54%], 43 blacks [46%]) who on screening had no significant medical history, normal physical examinations, and no personal or family history of AF.[15] The subjects were matched on the basis of age, sex, and ethnicity to the lone AF cohort.
3. For the group with AF in association with heart disease or other risk factors, we included 266 patients (211 whites [81%], 51 blacks [19%]) from the Cardiac Surgery Registry who had no personal or family history of AF and no post cardiac operative AF.[13,14] The patients were matched for age, sex, ethnicity, and ejection fraction (±5%) to the cohort with AF and heart disease.

### Resequencing and Follow-Up Genotyping

The coding regions and flanking intronic sequences of all 4 β-subunit genes, including exon 3A of β1B,[16] were resequenced in all of the AF (n=488 patients; 118 patients with lone AF, 257 patients with AF and cardiovascular disease, 105 patients with AF in the postoperative period) and the Coriell control (n=188 subjects) cohorts in the Vanderbilt DNA Sequencing Facility or the National Heart, Lung, and Blood Institute–supported Resequencing and Genotyping Service at the J. Craig Venter Institute. The patient controls (94 healthy subjects and 266 control subjects from the Cardiac Surgery Registry) were genotyped at the variant sites identified in the AF cohort using the MassArray SNP genotyping system (Sequenom Inc, San Diego, Calif) at the Vanderbilt DNA Resource Core. Proband samples were included in triplicate as positive controls.

### Frequency of ST-Segment Elevation

A high prevalence of right precordial ST-segment elevation has recently been reported in a group of patients with lone AF.[17,18] We therefore studied the frequency of the ST-segment elevation in lone AF patients and lone AF control subjects (healthy subjects).

### Quantitative Real-Time Polymerase Chain Reaction

Poly $A^+$ RNA pooled separately from atria and ventricles of healthy hearts from ≥15 whites (Clontech, Mountain View, Calif) was analyzed. cDNA was synthesized from 2 μg of the RNA and used as template. Genes of interest subcloned into the pEGFP-IRES vector (*SCN1B*, *SCN2B*, *SCN5A*; Clontech) or the pRC-CMV vector (β-actin; Invitrogen, Carlsbad, Calif) were used for absolute quantification. Real-time polymerase chain reaction (PCR) was performed with predesigned TaqMan assays (*SCN1B*, Hs00168897_m1; *SCN2B*, Hs00394952_m1; *SCN5A*, Hs00965681_m1; β-actin, Hs99999903_m1) using the 7900HT Real-Time Instrument (Applied Biosystems, Foster City, Calif).

### Functional Analysis

Full-length human *SCN1B* cDNA (Gen Bank accession No. NM_001037) and *SCN2B* cDNA (NM_004588) subcloned into a bicistronic vector (pEGFP-IRES, Clontech) also carrying GFP were supplied by Dr Alfred George, Jr. Mutations were prepared using the QuickChange II XL site-directed mutagenesis kit (Stratagene, La Jolla, Calif) and were verified by resequencing. The *SCN5A* cDNA (NM_198056) was subcloned into the pBK-CMV vector (Stratagene). *SCN1B* or *SCN2B* constructs (1 μg) were cotransfected with the plasmid encoding *SCN5A* (1 μg) in Chinese hamster ovary (CHO) cells. When *SCN5A* DNA was transfected without β-subunits, the pEGFP-IRES vector was cotransfected to identify fluorescent cells for voltage-clamp.

Whole-cell voltage-clamp was performed at room temperature using an Axopatch 200B amplifier and pClamp9.2 software (Molecular Devices, Union City, Calif) as described previously.[12] Transfected cells were clamped with ≈1.0-mol/LΩ glass microelectrodes and were held at a resting potential of −120 mV. Data for voltage dependence were fitted with the Boltzmann equation: $y=(1+\exp([V-V_{1/2})/k])^{-1}$, where $V_{1/2}$ is the voltage required to achieve half-maximal conductance or channel availability and k is the slope factor. Pulse protocols are shown as insets in the Figures.

### Statistical Analysis

Data are presented as mean±SEM. Student unpaired *t* test, 1-way ANOVA, or Fisher exact test was used to test for significant differences. A value of $P<0.05$ was considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

## Results

Resequencing the AF population identified 2 nonsynonymous variants in the reference *SCN1B* sequence and 2 in *SCN2B* in 3 white and 1 black subject. These variants were absent in the Coriell controls and in the AF population control subjects, including a total of 309 whites and 141 blacks. Resequencing *SCN5A* in these 4 patients carrying β-subunit mutations did not identify any coding region or splice junction variant. No AF-unique nonsynonymous variant was identified in *SCN3B* or *SCN4B*.

### Clinical Features

Clinical features are described in Table 1 and as follows:

- Patient 1: A heterozygous missense mutation in exon 3 of *SCN1B* (c.254G→A) resulting in p.R85H was identified in a 68-year-old white woman with paroxysmal AF and moderate aortic stenosis (pressure gradient, 31 mm Hg; aortic valve area, 0.82 $cm^2$) (Figure 1). There was no history of hypertension. AF was diagnosed when she was 58 years old. The 12-lead ECG showed saddleback-type ST-segment elevation in leads $V_1$ to $V_3$ (Figure 1). The

**Table 1. Clinical Characteristics of Patients Carrying β-Subunit Mutations**

| Patient No. | Sex | Age at Onset, y | Type of AF | PR Interval, ms | QRS Interval, ms | QTc, ms | ST-Segment Elevation* | LVDD, mm | LVEF, % | LA, mm | Nucleotide Substitution | Amino Acid Substitution |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 58 | Paroxysmal | 140 | 83 | 385 | Yes | 50 | 55 | 47 | SCN1B 254G→A | β1 R85→H |
| 2 | F | 35 | Paroxysmal | 132 | 84 | 400 | No | 46 | 65 | 48 | SCN1B 457G→A | β1 D153→N |
| 3 | M | 55 | Paroxysmal | 200 | 80 | 354 | Yes | 50 | 55 | 40 | SCN2B 82C→T | β2 R28→W |
| 4 | M | 57 | Paroxysmal | 180 | 88 | 398 | Yes | 49 | 60 | 42 | SCN2B 83G→A | β2 R28→Q |

LA indicates left atrium; LVDD, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; QTc, corrected QT interval by Bazett formula.
*ST-segment elevation in the right precordial leads.

ST-segment elevation was evident both during AF and sinus rhythm with beat-to-beat and day-to-day variability. She did not have ischemic heart disease, congestive heart failure, electrolyte abnormality, or antiarrhythmic drug therapy to explain the ST-segment elevation. Amiodarone failed to maintain sinus rhythm and did not exacerbate ST-segment elevation. Echocardiography revealed left atrial enlargement. No family member had documented AF, although her grandmother and daughter had a history of stroke. Her father had a history of myocardial infarction. Mutations in *SCN1B* have been previously reported in the generalized epilepsy with febrile seizures plus (GEFS+) syndrome, and R85H was initially found in a patient with GEFS+.[19,20] However, there was no personal or family history of seizures in this or any of the other 3 patients having β-subunit mutations described here.

- Patient 2: A heterozygous missense mutation in exon 4 of *SCN1B* (c.457G→A) resulting in p.D153N was identified in a 57-year-old black woman with paroxysmal lone AF (Figure 1). AF was initially diagnosed when she was 35 years old. Her ECG was normal and did not show ST-segment elevation in the right precordial leads or any conduction abnormality. Echocardiography revealed left atrial enlargement. When she was 54 years old, she had episodes of paroxysmal AF with rapid ventricular responses, unresponsive to sotalol, propafenone, and amiodarone; there was no ST-segment elevation during therapy with antiarrhythmic drugs. She underwent atrioventricular nodal ablation followed by implantation of dual-chamber pacemaker. There was no family history of AF, although her mother had hypertension and a pacemaker.
- Patient 3: A heterozygous missense mutation in exon 2 of *SCN2B* (c.82C→T) resulting in p.R28W was identified in a 61-year-old white man with paroxysmal AF and hypertension (Figure 2). AF was initially diagnosed when he was 55 years old. The ECG showed saddleback-type ST-segment elevation in the right precordial leads during sinus rhythm with a prolonged PR interval of 220 ms. The magnitude of ST-segment elevation showed day-to-day variability. Echocardiography was normal. Holter recording during sinus rhythm did not reveal atrial tachycardia. Sotalol failed to maintain sinus rhythm and did not exacerbate ST-segment elevation. There was no family history of AF.
- Patient 4: A heterozygous missense mutation in exon 2 of *SCN2B* (c.83G→A) resulting in p.R28Q was identified in a 57-year-old white man with paroxysmal lone AF (Figure 2). AF was initially diagnosed when he was 57 years old. There was saddleback-type ST-segment elevation in the right precordial leads. Echocardiography revealed slight

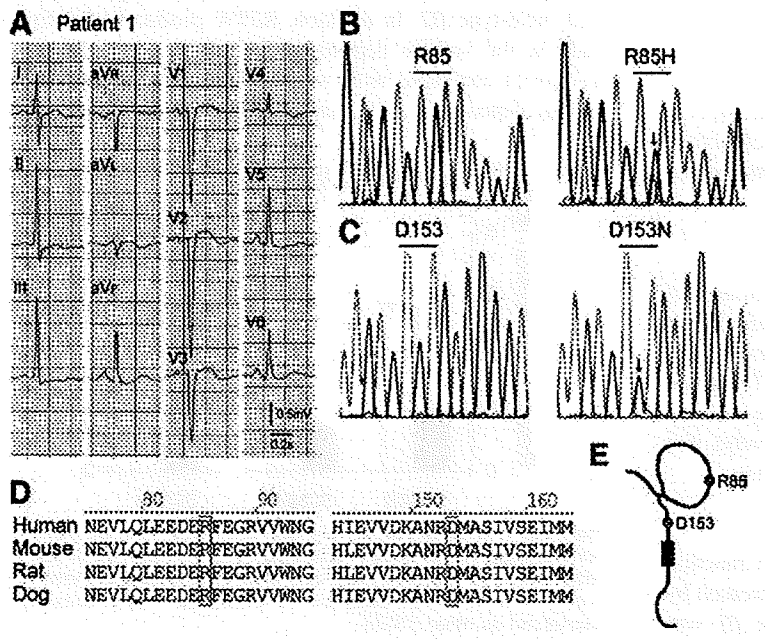


**Figure 1.** *SCN1B* mutations in patients with AF. A, 12-lead ECG in patient 1 shows ST-segment elevation in leads $V_1$ to $V_3$. B, Heterozygous single-nucleotide change in *SCN1B* (c.254G→A) results in p.R85H in patient 1. Left and right panels indicate sequences in a control subject and the patient, respectively. C, Heterozygous single-nucleotide change in *SCN1B* (c.457G→A) results in p.D153N in patient 2. Arrows in B and C indicate heterozygous mutations. D, Alignment of β1 amino acid sequences in human, mouse, rat, and dog. Sites of the mutations are indicated by boxes. E, Locations of mutations in the predicted topology of the β1-subunit (circles).

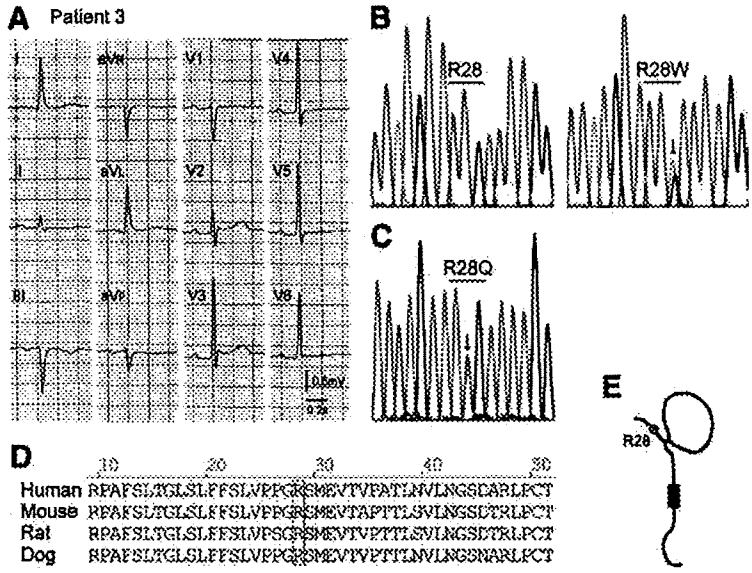


**Figure 2.** *SCN2B* mutations in patients with AF. A, 12-lead ECG in patient 3 shows ST-segment elevation in leads $V_1$ to $V_3$. B. Heterozygous single-nucleotide change in *SCN2B* (c.82C→T) results in p.R28W in patient 3. C, c.83G→A results in p.R28Q in patient 4. Arrows in B and C indicate heterozygous mutations. D, Alignment of β2 amino acid sequences in human, mouse, rat, and dog. R28 is indicated by box. E, Location of mutations in the predicted topology of the β2-subunit (circle).

left atrial enlargement. He did not receive any antiarrhythmic drugs to restore AF. His father and mother had AF and coronary heart disease.

There was no history of ventricular tachyarrhythmias or syncope in any of the 4 patients. Electrophysiological study has not been performed in any of the patients. DNA was not available in any family members of the 4 probands.

### ST-Segment Elevation in Lone AF

Right precordial ST-segment elevation during sinus rhythm was identified more frequently in patients with lone AF (8/118, 6.8%) than in control subjects (1/94, 1.1%, $P<0.05$). The 8 patients in the lone AF group included 1 with the *SCN2B* mutation described above and 1 with a H445D *SCN5A* mutation.[13] None of the 8 patients except for the *SCN2B* mutation carrier with a long PR interval (described above) showed a conduction abnormality.

### Conservation of Mutated Amino Acids

The sites of the mutations identified here, R85 and D153 in β1, and R28 in β2 (Figure 1 and 2) are completely conserved across human, dog, rat, and mouse sequences, suggesting that these amino acids are functionally important.

### Real-Time PCR in Human Heart

As a first step to establishing the functional significance of *SCN1B* and *SCN2B* in the genesis of AF, we studied their expression in atrial tissue. Figure 3 shows that the transcripts were readily detected in both atrium and ventricle. The abundance of *SCN1B* and *SCN5A* transcripts was greater in ventricle than atrium (68% and 35% of ventricle, respectively), but *SCN2B* transcript levels were similar in the 2 chambers.

### Electrophysiology

Each of the 4 mutant subunits generated a loss of function phenotype. Peak sodium current amplitude was increased by 75% at a test pulse of −30 mV when wild-type β1 was coexpressed with *SCN5A* (Table 2, Figure 4, $P<0.001$). This effect was markedly blunted with the D153N mutation (24% increase versus *SCN5A* alone, $P<0.05$) and absent with the R85H mutation, resulting in smaller sodium current amplitude for the mutants than wild-type β1 ($P<0.001$ for each). D153N did not affect the voltage dependence of sodium channel activation or inactivation compared to wild-type β1. However, R85H resulted in a positive shift of both voltage dependence of activation (+10.6 mV, $P<0.001$) and of inactivation (+6.2 mV, $P<0.001$) compared with wild-type β1. There was no difference in persistent sodium current among wild-type (1.0±0.1%), D153N (0.9±0.1%), and R85H β1 (1.1±0.2%).

In contrast to β1, wild-type β2 did not increase peak sodium current amplitude compared with *SCN5A* alone (Table 3, Figure 5, $P$=NS). However, coexpression of R28W or of R28Q reduced peak current amplitude by 30% ($P<0.05$) and by 36% ($P<0.01$) at −30 mV, respectively, compared with wild-type β2. In addition, R28W produced a positive shift in the voltage dependence of activation (+5.1 mV, $P<0.001$) compared with wild-type but did not affect the voltage dependence of inactivation ($P$=NS). R28Q produced

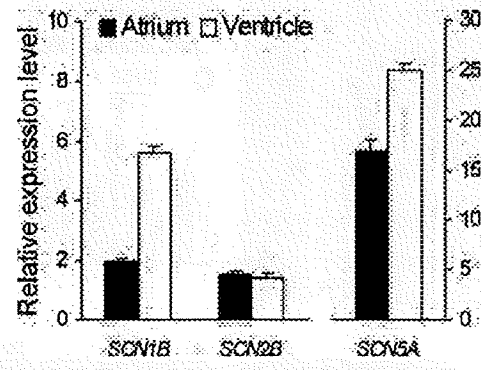


**Figure 3.** Expression profile of *SCN1B*, *SCN2B*, and *SCN5A* in nondiseased human heart tissues using real-time PCR. Graph represents the relative expression levels normalized to those of β actin in atrium (filled bars) and ventricle (open bars). Data are expressed as mean±SEM.

**Table 2. Biophysical Parameters for β1 Variants Associated With AF**

| | Peak Current Density at −30 mV | | Voltage Dependence of Activation | | | Voltage Dependence of Inactivation | | |
|---|---|---|---|---|---|---|---|---|
| | pA/pF | n | $V_{1/2}$ (mV) | *k* (mV) | n | $V_{1/2}$ (mV) | *k* (mV) | n |
| *SCN5A* | −161.0±20.4 | 18 | −38.7±0.5 | 7.7±0.3 | 18 | −86.9±1.5 | 7.1±0.7 | 18 |
| *SCN5A*/WT β1 | −281.7±21.7* | 37 | −48.9±0.8* | 8.5±0.5 | 37 | −93.6±0.8* | 6.8±0.4 | 36 |
| *SCN5A*/R85H β1 | −158.5±16.8† | 35 | −38.3±0.7† | 8.1±0.5 | 35 | −87.4±0.6† | 8.5±0.3 | 34 |
| *SCN5A*/D153N β1 | −200.4±18.2*† | 27 | −50.8±0.9* | 8.7±0.6 | 27 | −95.0±0.7* | 7.5±0.5 | 26 |

*$P<0.05$ versus *SCN5A*.
†$P<0.05$ versus *SCN5A*/WT β1.

a positive shift in both of the voltage dependence of activation (+7.4 mV, $P<0.001$) and of inactivation (+2.8 mV, $P<0.01$) compared with wild-type β2. There was no difference in persistent sodium current among wild-type (1.2±0.3%), R28W (1.1±0.3%), and R28Q β2 (1.2±0.2%).

## Discussion

In this report, we describe rare nonsynonymous variants in *SCN1B* and *SCN2B* in patients with AF. These variants affect highly conserved residues and were not present in large control populations. Thus, *SCN1B* and *SCN2B* are candidate genes for increasing AF susceptibility. The findings that *SCN1B* and *SCN2B* are expressed in atrium, and that mutant β1 and β2 produced loss-of-function effects on *SCN5A*-mediated currents further supports the association of the variants with AF.

The reported effects of coexpressing β1 on *SCN5A* channels are controversial.[8] Some groups have reported that β1 increases *SCN5A* currents without affecting the voltage dependence of gating or kinetics, whereas others have reported β1-mediated changes in channel gating and/or kinetics.[8] In some reports, β1 has no effect on *SCN5A*-mediated current.[8] In β1 null mice, an increase in sodium current amplitude without a change in channel gating or kinetics has been reported.[21] In our experiments, wild-type β1 increased *SCN5A* currents and modulated channel gating, and the p.R85H and p.D153N mutants showed loss of β1 function with significantly decreased current amplitudes.

The effects of β2 on *SCN5A* currents have been less extensively studied. Whereas one group reported that β2 has no effect on *SCN5A* currents using heterologous expression,[22] another group reported a negative shift of the voltage dependence of activation.[23] Sinus node dysfunction has been reported in β2 null mice.[24] In the present study, whereas β2 had no effects on *SCN5A* currents except for a minor positive shift of the voltage dependence of inactivation, both the p.R28W and p.R28Q mutants strikingly decreased peak sodium current amplitude. The patients had no evidence of sinus node dysfunction.

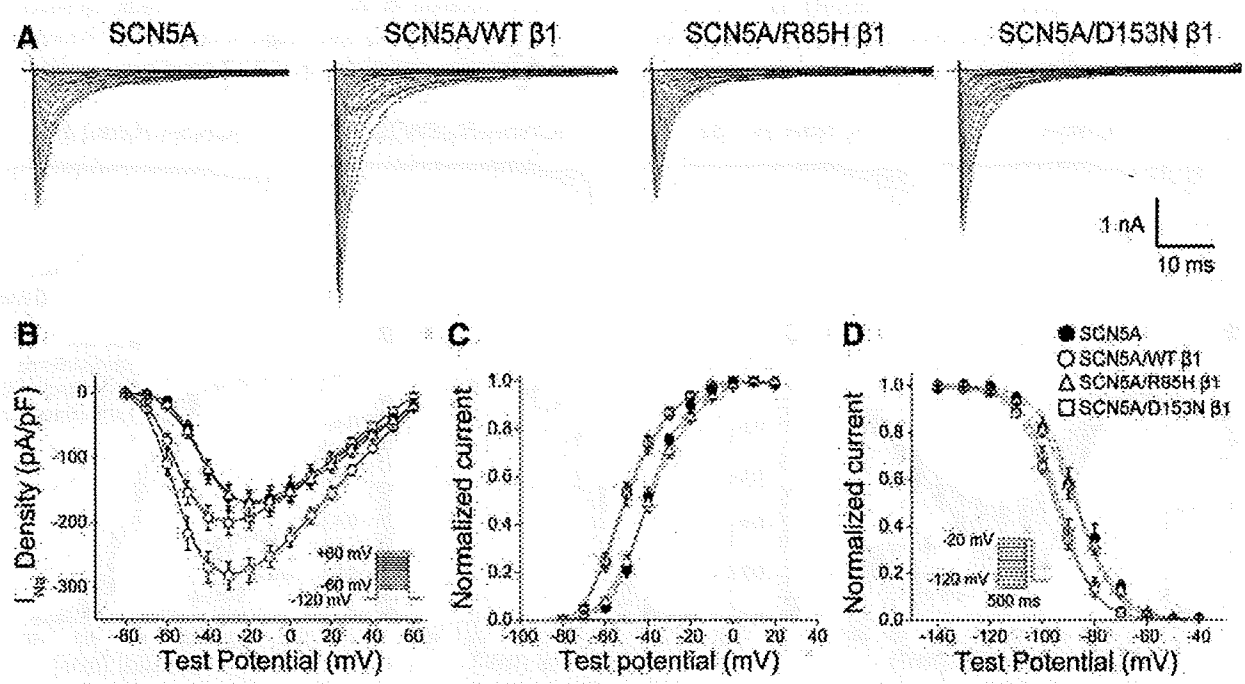


**Figure 4.** Electrophysiological characteristics of β1-subunit variants in CHO cells expressing *SCN5A* and β1-subunits. A, Representative current traces. B, Current-voltage relationships of *SCN5A* alone (filled circles), *SCN5A* coexpressed with wild-type (WT) β1 (open circles), *SCN5A* coexpressed with R85H β1 (open triangles), and *SCN5A* coexpressed with D153N β1 (open squares). Voltage dependence of activation (C) and inactivation (D) are shown.

**Table 3. Biophysical Parameters for β2 Variants Associated With AF**

| | Peak Current Density at −30 mV | | Voltage Dependence of Activation | | | Voltage Dependence of Inactivation | | |
|---|---|---|---|---|---|---|---|---|
| | pA/pF | n | $V_{1/2}$ (mV) | *k* (mV) | n | $V_{1/2}$ (mV) | *k* (mV) | n |
| *SCN5A* | −157.0±13.6 | 25 | −39.6±0.5 | 7.6±0.2 | 25 | −84.4±0.7 | 6.9±0.3 | 24 |
| *SCN5A*/β2 WT | −161.6±13.3 | 27 | −38.0±0.6 | 6.2±0.2 | 27 | −80.2±0.6* | 6.8±0.3 | 26 |
| *SCN5A*/R28W β2 | −112.6±12.2*† | 31 | −32.9±0.8*† | 8.3±0.4 | 31 | −79.4±0.7 | 7.5±0.4 | 29 |
| *SCN5A*/R28Q β2 | −103.4±11.8*† | 29 | −30.6±0.6*† | 6.3±0.2 | 29 | −77.4±0.7*† | 7.2±0.3 | 26 |

*$P<0.05$ versus *SCN5A*.
†$P<0.05$ vs *SCN5A*/WT β2.

All of 4 mutations identified in *SCN1B* and *SCN2B* were located in the extracellular domain, which has a critical role in modulation of cell surface expression and gating of sodium channel.[25] In previous studies of skeletal muscle and neuronal sodium channel α-subunits, deletion of the intracellular domain of the β1-subunit had no effect on its modulation of α-subunit function, whereas deletions within the extracellular domain block modulation.[26–28] Our recent study, which describes loss-of-function mutations in *SCN1B* in the extracellular domain, supports functional importance of the extracellular domain.[12] However, it is also possible that specific residues may not be as important as preservation of overall structural motifs because β-subunit modulates sodium channel via the membrane anchor plus additional intracellular or extracellular regions.[29]

Variation in *SCN5A* is associated with AF.[4,5] Loss-of-function mutations in *SCN5A* have been associated with AF as well as with dilated cardiomyopathy, sinus node dysfunction, and/or conduction disease.[30] Screening for *SCN5A* variants in a large AF cohort, which was also used for this study, found *SCN5A* mutations in 5.9% of those with AF.[13] A common polymorphism in *SCN5A* (H558R) has also been associated with AF susceptibility,[4] although this was not reproduced in another study.[31]

Sodium channels play a critical role not only in the initiation of the action potential but also in the maintenance of the action potential dome,[10] and loss of sodium channel function can cause shortening of refractoriness and slowing of conduction.[32] Shortening of refractory period by a reduction in inward current and/or an increase in outward current has been proposed as creating a substrate for reentry,[32] and this concept has been supported by evidence that loss-of-function mutations in *SCN5A* or gain-of-function mutations in potassium channel genes that shorten action potential duration contribute to AF susceptibility.[31,33–35] Slow conduction, which is also promoted by decreased sodium current, is another important substrate for reentry.[32] Thus, mutations in *SCN1B* and *SCN2B* that reduce sodium current can generate an AF-prone substrate through multiple mechanisms even in the presence of other susceptibility modifiers.

The clinical features of the AF cases we identified here appear to share molecular and pathophysiologic characteristics with the Brugada syndrome, characterized by ST-segment elevation in the right precordial leads, episodes of ventricular fibrillation, and occasionally AF.[10,36] Moreover, loss-of-function mutations in *SCN5A* have been reported in

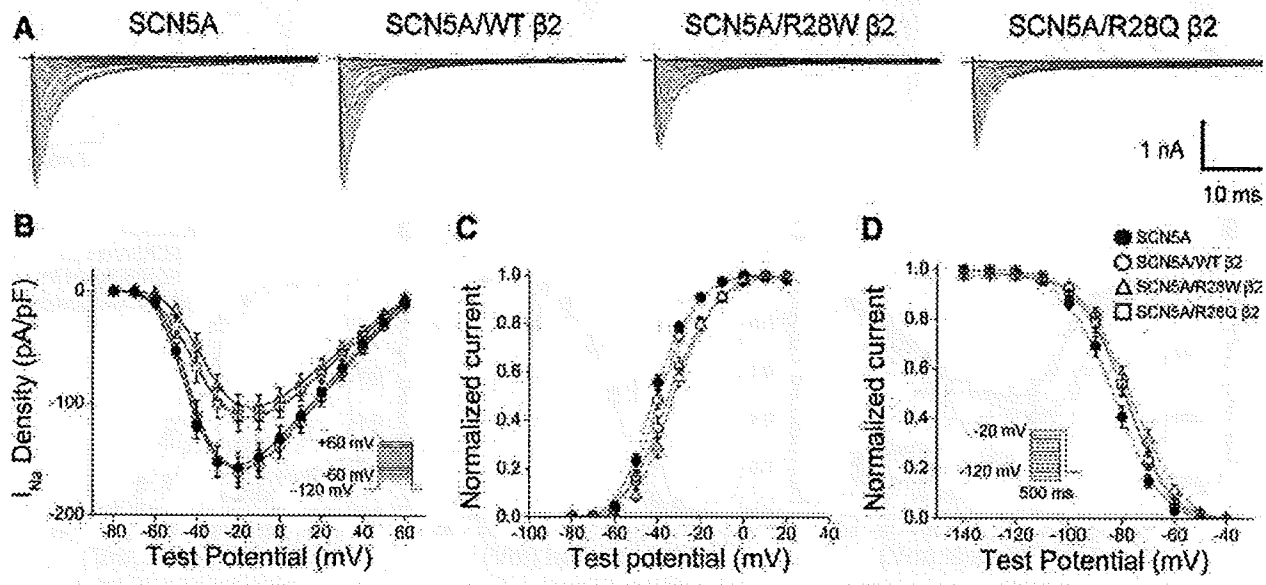


**Figure 5.** Electrophysiological characteristics of β2-subunit variants in CHO cells expressing *SCN5A* and β2-subunits. A, Representative current traces. B, Current-voltage relationships of *SCN5A* alone (filled circles), *SCN5A* coexpressed with wild-type (WT) β2 (open circles), *SCN5A* coexpressed with R28W β2 (open triangles), and *SCN5A* coexpressed with R28Q β2 (open squares). Voltage dependence of activation (C) and inactivation (D) are shown.

the Brugada syndrome as well as in AF,[4,5,10] and we have recently reported a loss-of-function mutation in *SCN1B* in the Brugada syndrome.[12] Brugada-type ST-segment elevation, similar to this study, has been reported in patients with lone AF, and a genetic etiology is suggested by a high frequency of a family history of AF, although no molecular mechanism was identified in previous studies.[17,18] Taken together, these data implicate loss of sodium channel function due to β-subunit mutations as a further mechanism underlying the Brugada-type ECG and AF susceptibility. We also identified ST-segment elevation in other subjects with AF (more commonly than in control subjects), but mutations in *SCN5A*, *SCN1B*, or *SCN2B* were only identified in a minority; thus other genetic mechanisms probably play a role.[10]

Sodium channel–blocking drugs are widely used to restore and maintain sinus rhythm in paroxysmal AF. They are also used to exaggerate or unmask ST-segment elevation in Brugada syndrome, where they can increase ventricular arrhythmia susceptibility. Therefore, these drugs may be proarrhythmic (or at least ineffectual) in cases of AF—such as those we describe here—in which decreased sodium current plays a role in pathogenesis of the arrhythmia. ST-segment elevation in the right precordial leads may be useful to identify such patients.

Mutations in *SCN1B* were originally identified in familial epilepsy, GEFS+.[19] However, there was no history of epilepsy in our patients carrying mutations including R85H, previously reported as an epilepsy mutation.[20] In addition, there was no history of seizure disorder in patients with *SCN1B* mutations in conduction disease and Brugada syndrome that we have recently described.[12] Conversely, to our knowledge, defects in cardiac function have not been investigated in *SCN1B* mutation carriers presenting with epilepsy, and AF has not been described in the family with R85H and seizures.[19,20] The mechanism underlying this difference between the brain and heart phenotypes is not known, but sex, age, and genetic modifiers (eg, common polymorphisms) are commonly invoked as modulators of such clinical phenotypes. One possibility is that the Sudden Unexpected Death in Epilepsy (SUDEP) syndrome is a cardiac arrhythmia manifestation of β-subunit or other mutations contributing to epilepsy.[37]

### Limitations

Screening for β-subunit genes was performed in large cohorts including ethnically defined and population-matched controls, and mutations were identified only in patients with AF. However, it is difficult to have controls definitely free from AF. We believe a cohort of patients with heart disease undergoing cardiac surgery but without AF is a very robust control set. Linkage or segregation analysis was not conducted because DNA was not available in family members of affected patients. The variants are rare and thus genetic variants in β-subunit genes may not be responsible in a large number of patients with AF. Evidence supporting a critical role of β-subunits in AF includes expression of *SCN1B* and *SCN2B* in atrium and loss of sodium channel function in the heterologous expression studies. The functional analyses used a conventional heterologous expression system, where the environment is different from that in the native cardiomyocyte, and other proteins associated with the sodium channel complex (including other β-subunits) are absent. Nevertheless, the in vitro characteristics of the mutations were consistent with the phenotype in the patients, further supporting the disease causality of the mutations. The observed alterations in gating indicate that the mutant subunits are expressed and probably coassemble with *SCN5A* to form dysfunctional channels.

## Conclusions

In summary, we have identified mutations in sodium channel β1- and β2-subunit genes in patients with AF and have shown that sodium currents were reduced and channel gating was altered when the mutant β1 or β2 was coexpressed with *SCN5A*, compared with coexpression with wild-type β-subunits. Three of 4 mutation carriers showed ST-segment elevation in the right precordial leads, further implicating loss of sodium current as a disease mechanism for AF. We speculate that sodium channel blockers may have proarrhythmic effects in cases of AF in which decreased sodium current plays a role in pathogenesis of the arrhythmia.

## Acknowledgments

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## Disclosures

None.

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## CLINICAL PERSPECTIVE

There is a positive family history in many patients with atrial fibrillation (AF), especially lone AF. Recent genetic studies have identified both rare and common genetic variants that appear to predispose to the arrhythmia, and this includes variants in *SCN5A*, encoding the cardiac sodium channel pore-forming α-subunit. Sodium channels are multiprotein complexes, and so in this study, 4 function-modifying sodium channel β-subunit genes (*SCN1B* to *SCN4B*) were screened for mutations in a large number of patients with lone AF and AF associated with cardiovascular disease. This screening effort identified 4 subjects with mutations resulting in changes in amino acids highly conserved across species in *SCN1B* and *SCN2B*. All 4 mutations showed decreased sodium current, a change similar to that seen with loss-of-function mutations in *SCN5A* and *SCN1B* in Brugada syndrome. AF is relatively common in the Brugada syndrome, and 3 of the 4 AF patients carrying a mutation in a β-subunit gene showed Brugada syndrome–like ST-segment elevation, further reinforcing the idea that loss of sodium channel function increases AF susceptibility. Indeed, in some series, saddleback or other ST-segment deformities are reported in up to 10% of patients with lone AF, suggesting that these patients represent a distinct subgroup of AF due to reduced sodium current through mutations in *SCN5A*, *SCN1B*, *SCN2B*, or other sodium channel–associated protein genes. Exposure to sodium channel blockers could be used to identify this subgroup, although long-term therapy with these drugs would be undesirable because they can increase ventricular arrhythmia susceptibility in Brugada syndrome.

nature medicine

# Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans

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**Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a potentially lethal inherited arrhythmia syndrome in which drug therapy is often ineffective. We discovered that flecainide prevents arrhythmias in a mouse model of CPVT by inhibiting cardiac ryanodine receptor–mediated $Ca^{2+}$ release and thereby directly targeting the underlying molecular defect. Flecainide completely prevented CPVT in two human subjects who had remained highly symptomatic on conventional drug therapy, indicating that this currently available drug is a promising mechanism-based therapy for CPVT.**

CPVT is an inherited arrhythmia syndrome characterized by a normal baseline electrocardiogram (ECG), polymorphic ventricular tachycardia induced by adrenergic stress in the absence of structural heart disease, and a high mortality rate in young individuals[1]. Treatment with β-adrenergic blockers reduces arrhythmia burden and mortality but is not completely effective[1], and implantable cardioverter defibrillators (ICDs) are used for the prevention of sudden death. However, painful appropriate or inappropriate defibrillation shocks can trigger further adrenergic stress and arrhythmias, and deaths have occurred despite appropriate ICD shocks[2,3]. In such instances, stellate ganglionectomy[4] or even cardiac transplantation[5] have been considered. Two CPVT disease-related genes have been identified: *RYR2*, encoding the cardiac ryanodine receptor $Ca^{2+}$ release channel (RyR2), and *CASQ2*, encoding cardiac calsequestrin[6,7]. Mutations in these genes destabilize the RyR2 $Ca^{2+}$ release complex[8,9]. In mice with CPVT-linked mutations, catecholamines cause spontaneous sarcoplasmic reticulum $Ca^{2+}$ release resulting in delayed after depolarizations (DADs), and they produce triggered activity in myocytes and polymorphic ventricular tachycardia in *vivo*[10,11]. Here we identify a therapy that directly targets the underlying arrhythmia mechanisms: we found that flecainide, an approved antiarrhythmic drug known to block sodium channels, showed remarkable efficacy in suppressing spontaneous sarcoplasmic reticulum $Ca^{2+}$ release by inhibiting RyR2. Flecainide treatment completely prevented adrenergic stress–induced arrhythmias in a mouse model of CPVT and in humans with *CASQ2* or *RYR2* mutations that are refractory to standard drug treatment.

The local anesthetic tetracaine has been used to inhibit RyR2 and suppress spontaneous sarcoplasmic reticulum $Ca^{2+}$ release in isolated myocytes[12]. However, tetracaine causes a rebound increase in sarcoplasmic reticulum $Ca^{2+}$ release events during prolonged exposure[13], effective inhibitory concentrations[14] are too high for clinical use, and systemic administration is contraindicated in humans. We searched among clinically available antiarrhythmic drugs for a more useful RyR2 inhibitor and found that flecainide inhibited RyR2 more potently than tetracaine and by a different mechanism. Whereas tetracaine caused long-lived channel closings, flecainide reduced the duration of channel openings and did not affect closed channel duration (**Supplementary Fig. 1** online). Flecainide's inhibitory potency was higher when RyR2 was activated by high luminal $Ca^{2+}$ concentration mimicking spontaneous sarcoplasmic reticulum $Ca^{2+}$ releases that trigger premature heart beats (**Fig. 1a,b**; half-maximal inhibitory concentration = 15 ± 3 μM), compared to when RyR2 was activated by high cytosolic $Ca^{2+}$ concentration, such as would occur during a normal heart beat (**Supplementary Fig. 1a**; half-maximal inhibitory concentration = 55 ± 8 μM).

We next tested whether RyR2 block by flecainide translates into an inhibition of spontaneous sarcoplasmic reticulum $Ca^{2+}$ release in ventricular myocytes isolated from mice with gene-targeted deletion of *Casq2* (*Casq2*$^{-/-}$ mice), a model of *CASQ2*-linked CPVT[10]. Upon catecholaminergic challenge with isoproterenol, *Casq2*$^{-/-}$ myocytes exhibit frequent spontaneous sarcoplasmic reticulum $Ca^{2+}$ releases that can trigger premature beats (**Fig. 1c** and ref. 10). Flecainide significantly suppressed the rate of spontaneous $Ca^{2+}$ releases from the sarcoplasmic reticulum by 39% (**Fig. 1d**), even though $Ca^{2+}$ content in sarcoplasmic reticulum was not significantly changed (**Supplementary Fig. 2a,b** online). The reduction of spontaneous $Ca^{2+}$ releases by flecainide remained significant even after $Na^+$ and $Ca^{2+}$ were removed from the extracellular bath solution (47% reduction, $P = 0.009$, **Supplementary Fig. 2c,d**), indicating that inhibition of trans-sarcolemmal $Na^+$ or $Ca^{2+}$ fluxes did not contribute to the reduced spontaneous $Ca^{2+}$ releases; that is, indicating that flecainide does not act by blocking $Na^+$ channels. Compared to vehicle, flecainide significantly decreased diastolic sarcoplasmic reticulum $Ca^{2+}$ leak in isoproterenol-stimulated *Casq2*$^{-/-}$ myocytes (**Supplementary Fig. 2e,f**; $P = 0.02$). Indeed, in contrast to tetracaine treatment[13], flecainide treatment did not result in a compensatory increase in sarcoplasmic

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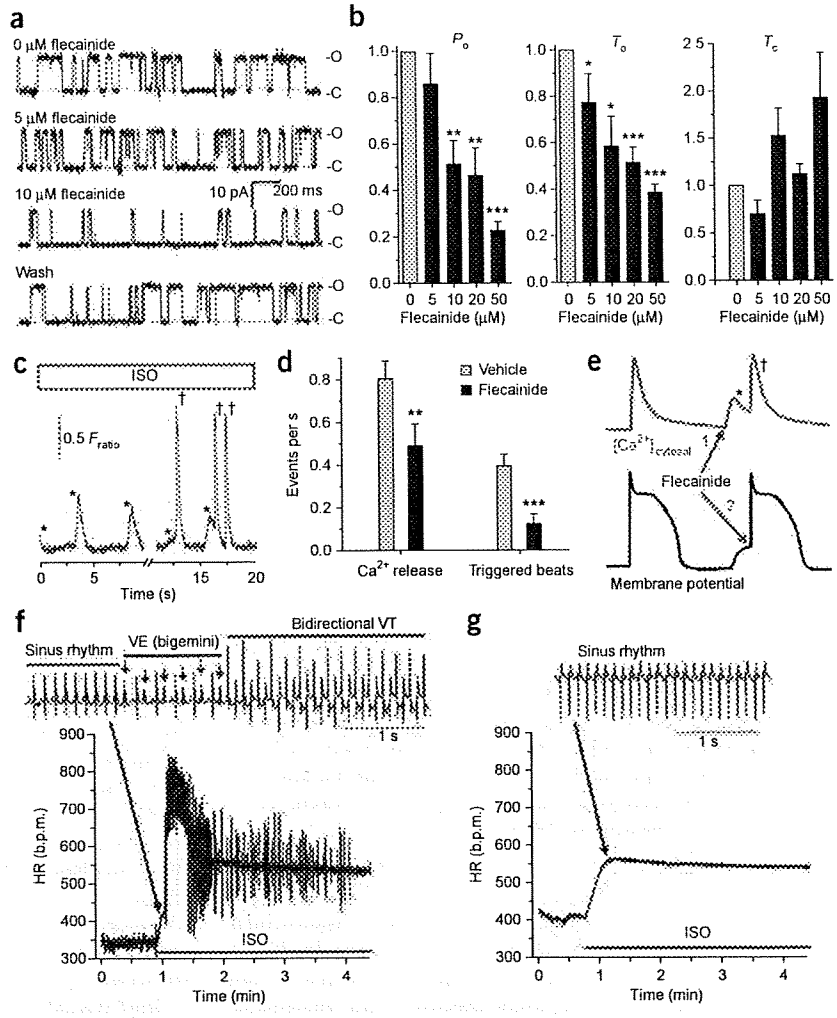


**Figure 1** Flecainide inhibits RyR2 $Ca^{2+}$ release channels, reduces spontaneous $Ca^{2+}$ release events and triggered beats and prevents ventricular tachycardia in a CPVT mouse model. **(a)** Representative examples of the activity of single sheep RyR2 channels incorporated in lipid bilayers in response to 0, 5 and 10 μM flecainide followed by washout. C, closed; O, open. **(b)** Average concentration dependence of flecainide's effect on RyR2 open probability ($P_o$), mean open time ($T_o$) and mean closed time ($T_c$). Data are expressed relative to values at 0 μM flecainide, with absolute $P_o = 0.12 \pm 0.07$, $T_o = 13.5 \pm 4.4$ ms and $T_c = 146 \pm 56$ ms. RyR2 channels were activated by 1 mM $Ca^{2+}$ on the *trans* (luminal) side with *cis* (cytosolic) at 0.0001 $Ca^{2+}$ mM (to model resting $Ca^{2+}$ concentration). $n = 3$–8 experiments per concentration; $^*P < 0.02$, $^{**}P < 0.01$ and $^{***}P < 0.001$. **(c,d)** Effects of flecainide on isoproterenol (ISO)-stimulated *Casq2*$^{-/-}$ myocytes. **(c)** Representative examples of spontaneous sarcoplasmic reticulum $Ca^{2+}$ release events (*) and triggered beats (†). $F_{ratio}$, Fura-2 fluorescence ratio, which is proportional to free intracellular $Ca^{2+}$ concentration (see **Supplementary Methods** online). **(d)** Flecainide (6 μM, >10 min incubation) significantly decreased spontaneous sarcoplasmic reticulum $Ca^{2+}$ release events and triggered beats ($n = 44$ cells) compared to vehicle ($n = 45$ cells). $^{**}P = 0.0078$ and $^{***}P < 0.001$. **(e)** Cartoon illustrating the dual mechanism of flecainide action: (1) inhibition of the sarcoplasmic reticulum $Ca^{2+}$ release (*) that causes delayed after depolarizations and (2) inhibition of the premature beats (†) that are triggered by delayed after depolarizations. **(f)** Heart rate response to ISO (1.5 mg $kg^{-1}$) challenge in a *Casq2*$^{-/-}$ mouse. As illustrated in the ECG trace, the rapid and irregular heart rate (HR) was the result of numerous repetitive ventricular extrasystoles (VE, arrows) and the induction of ventricular tachycardia (VT). b.p.m., beats per minute. **(g)** Heart rate of the same mouse after it received flecainide (20 mg $kg^{-1}$) 15 min before ISO challenge. All data are means ± s.e.m.; for experimental protocols and statistical analysis see **Supplementary Methods**. Animal experiments were approved by the Vanderbilt University Medical Center Institutional Animal Care and Use Committee.



reticulum $Ca^{2+}$ content and prevented spontaneous $Ca^{2+}$ releases even after prolonged application (**Supplementary Fig. 3** online). These findings are consistent with the idea that flecainide inhibits RyR2 by a different mechanism than tetracaine (**Supplementary Fig. 1**).

A major consequence of spontaneous premature sarcoplasmic reticulum $Ca^{2+}$ release in intact myocytes is the activation of electrogenic $Na^+$-$Ca^{2+}$ exchange, which in turn causes membrane depolarizations termed DADs[15]. DADs of sufficient amplitude activate voltage-gated $Na^+$ channels and trigger full action potentials. Flecainide reduced triggered beats by 69% (**Fig. 1d**), a higher percent inhibition than its reduction of spontaneous $Ca^{2+}$ release events (39%; **Fig. 1d**). These data are consistent with flecainide's known inhibition of $Na^+$ channels to prevent triggered beats[16]. Taken together, these results indicate a dual mode of flecainide action in CPVT: suppression of spontaneous sarcoplasmic reticulum $Ca^{2+}$ release events via RyR2 inhibition and suppression of triggered beats via $Na^+$ channel block (**Fig. 1e**).

We next examined whether this dual mechanism of flecainide action translates into therapeutic efficacy. In a *Casq2*$^{-/-}$ mouse, catecholamine challenge induced an irregular heart rhythm (**Fig. 1f**) resulting from frequent ventricular extrasystoles occurring after each normal 'sinus' beat (bigemini), which quickly degenerated into polymorphic ventricular tachycardia with alternating upward and downward deflections of the QRS complex (bidirectional ventricular tachycardia), the hallmark of CPVT[1]. After flecainide treatment, the heart rate remained regular (with a sinus rhythm) and catecholamine challenge did not induce ventricular arrhythmias (**Fig. 1g**). Flecainide completely suppressed ventricular tachycardia in all 12 mice tested and ventricular extrasystole in 11 out of 12 mice ($P < 0.001$). In the remaining mouse, flecainide reduced the number of isoproterenol-induced ventricular extrasystoles by 99.5%. Flecainide was equally effective in preventing exercise-induced polymorphic or bidirectional ventricular tachycardia in conscious *Casq2*$^{-/-}$ mice; a single administration of flecainide (20 mg per kg body weight intraperitoneally) resulted in serum flecainide concentrations of 2.5 ± 0.2 μM (1.2 ± 0.08 mg $l^{-1}$) 1 h later, when exercise-induced ventricular tachycardia was completely prevented. Protection from ventricular tachycardia persisted for up to 6 h, with no rebound increase in ventricular tachycardia observed during a 20-h follow-up period (**Supplementary Fig. 4** online). Lidocaine, a $Na^+$ channel blocker that does not inhibit RyR2 channels[14] and lacks

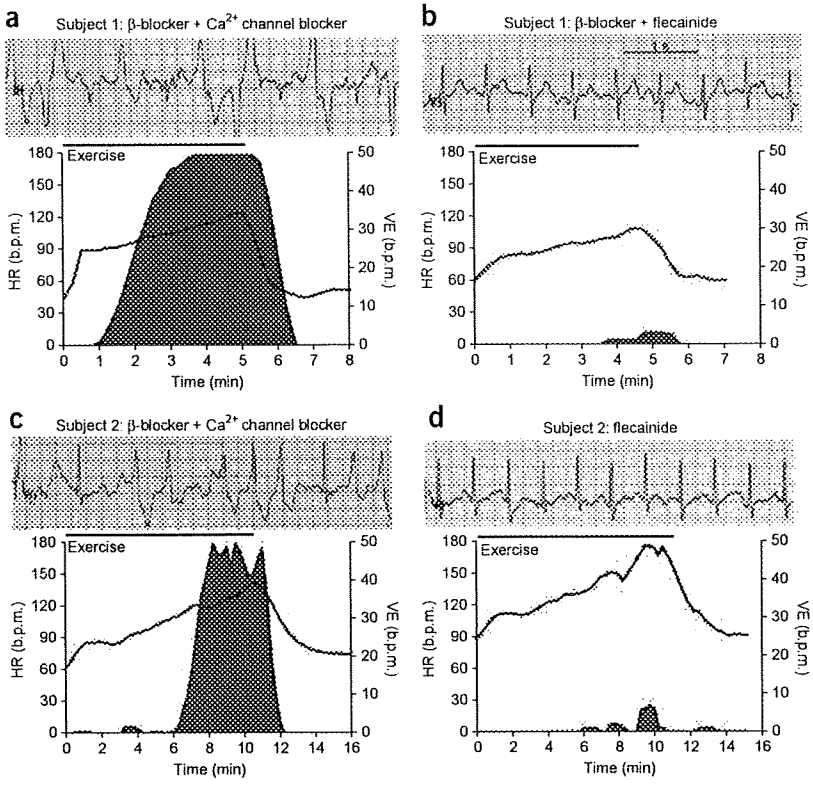


**Figure 2** Flecainide treatment prevents exercise-induced ventricular arrhythmia in two subjects with CPVT refractory to conventional drug therapy. (**a–d**) Effect of flecainide treatment in a 12-year-old boy (subject 1) with a *CASQ2* mutation (**a,b**) and in a 36-year-old female (subject 2) with an *RYR2* mutation (**c,d**). Top of each panel, representative ECGs recorded during maximum stress; bottom of each panel, heart rate and rate of ventricular extrasystoles during an exercise test. Blue area indicates arrhythmia burden. Exercise protocol and ventricular extrasystole analysis are as previously described[4]. Drug therapy during each exercise test: metroprolol 100 mg $d^{-1}$ plus verapamil 120 mg $d^{-1}$ (**a**), metroprolol 100 mg $d^{-1}$ plus flecainide 150 mg $d^{-1}$ for 7 weeks (**b**), bisoprolol 5 mg $d^{-1}$ plus verapamil 240 mg $d^{-1}$ (**c**) or flecainide 150 mg $d^{-1}$ for 8 weeks (**d**). Experiments involving human subjects were reviewed and approved by the University of Amsterdam Academic Medical Center Institutional Medical Ethical Review Board. Both human subjects (or their parents) provided informed consent.

clinical efficacy in CPVT[17], reduced the number of ventricular extrasystoles, but did not prevent exercise-induced ventricular tachycardia (**Supplementary Fig. 5** online), further indicating that RyR2 inhibition is crucial for flecainide's antiarrhythmic efficacy in CPVT.

Given flecainide's efficacy in the mouse model, we tested the drug in two subjects with treatment-refractory CPVT. The first subject was a 12-year-old boy, homozygous for a *CASQ2* missense mutation[18], who suffered from repeated appropriate ICD shocks despite maximal conventional therapy with the β-adrenergic receptor blocker metoprolol and the $Ca^{2+}$ channel blocker verapamil. ICD shocks were prevented by bed rest, although nonsustained ventricular tachycardia persisted, as documented by examination of ECG recordings stored in the ICD (data not shown). Treadmill exercise reproducibly induced arrhythmias in this subject (**Fig. 2a**). A therapeutic trial of flecainide (initially 2 mg per kg body weight per day for 6 weeks, then 3 mg per kg body weight per day) was begun, and verapamil was discontinued. Examination of ICD records at 3 weeks and 12 weeks after starting flecainide revealed a complete absence of ventricular tachycardia episodes (data not shown). Repeat exercise tests at 7 weeks and 12 weeks after the start of flecainide treatment showed complete suppression of ventricular tachycardia and marked reduction in the number of ventricular extrasystoles (**Fig. 2b**).

The second subject was a 36-year-old woman heterozygous for the CPVT-linked *RYR2* S4124G mutation. The subject experienced exercise-induced ventricular tachycardia while on maximum tolerated therapy with a β-blocker and a $Ca^{2+}$-channel blocker (**Fig. 2c**). As in the first subject, flecainide substantially reduced arrhythmia burden at rest and with exercise, even after β-blocker and $Ca^{2+}$-channel blocker treatment was stopped (**Fig. 2d**). Notably, flecainide prevented ventricular tachycardia even though the subject's maximal heart rate was much higher during the second exercise test (**Fig. 2d**) than during the previous exercise test (**Fig. 2c**) owing to the discontinuation of β-blocker therapy.

In summary, we report a previously unrecognized inhibitory action of flecainide on RyR2 channels, which, together with flecainide's inhibition of $Na^+$ channels, allowed us to directly target the underlying mechanism responsible for CPVT[11]. This targeted therapy with flecainide successfully prevented CPVT in two individuals that had remained highly symptomatic on conventional drug therapy. Flecainide can be proarrhythmogenic in some settings (for example, after myocardial infarction), and thus routine flecainide use cannot be recommended until further clinical studies more precisely define its risks and benefits in humans with CPVT. Our data provide proof of principle for the antiarrhythmic efficacy of inhibiting defective RyR2 $Ca^{2+}$ release channels in humans and identify a currently available drug as a promising mechanism-based therapy in CPVT.

*Note: Supplementary information is available on the Nature Medicine website.*

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AUTHOR CONTRIBUTIONS

H.W. and H.S.H. conducted and analyzed the *in vivo* mouse ECG experiments, N.C. and H.S.H. conducted and analyzed the isolated myocyte experiments, D.L. conducted and analyzed the RyR2 single-channel experiments, A.A.M.W. conducted and analyzed the human studies, S.S.D. analyzed the flecainide concentrations, D.E.R. and H.J.D. provided the ECG telemetry analysis software, H.W. and N.C. wrote the initial draft of the manuscript, D.M.R. edited the manuscript, and B.C.K. designed and supervised the overall project, and prepared the final manuscript and figures.