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Epistatic Effects of Potassium Channel Variation on Cardiac Repolarization and Atrial Fibrillation Risk

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Objectives The aim of this study was to evaluate the role of cardiac K^+ channel gene variants in families with atrial fibrillation (AF).

Background The K^+ channels play a major role in atrial repolarization but single mutations in cardiac K^+ channel genes are infrequently present in AF families. The collective effect of background K^+ channel variants of varying prevalence and effect size on the atrial substrate for AF is largely unexplored.

Methods Genes encoding the major cardiac K^+ channels were resequenced in 80 AF probands. Nonsynonymous coding sequence variants identified in AF probands were evaluated in 240 control subjects. Novel variants were characterized using patch-clamp techniques and in silico modeling was performed using the Courtemanche atrial cell model.

Results Nineteen nonsynonymous variants in 9 genes were found, including 11 rare variants. Rare variants were more frequent in AF probands (18.8% vs. 4.2%, $p < 0.001$), and the mean number of variants was greater (0.21 vs. 0.04, $p < 0.001$). The majority of K^+ channel variants individually had modest functional effects. Modeling simulations to evaluate combinations of K^+ channel variants of varying population frequency indicated that simultaneous small perturbations of multiple current densities had nonlinear interactions and could result in substantial (>30 ms) shortening or lengthening of action potential duration as well as increased dispersion of repolarization.

Conclusions Families with AF show an excess of rare functional K^+ channel gene variants of varying phenotypic effect size that may contribute to an atrial arrhythmogenic substrate. Atrial cell modeling is a useful tool to assess epistatic interactions between multiple variants. (J Am Coll Cardiol 2012;59:1017–25) © 2012 by the American College of Cardiology Foundation

Genetic variation is increasingly recognized to be a significant determinant of human disease. Over the past decade, genome-wide association studies have sought to identify common genetic variants that affect susceptibility to common complex disorders, but the variants identified have generally had only modest individual effect size and collectively explain relatively little of observed heritability (1). With the advent of

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Abbreviations and Acronyms

AF = atrial fibrillation
AP = action potential
APD = action potential duration
CHO = Chinese hamster ovary
DNA = deoxyribonucleic acid
MAF = minor allele frequency
WT = wild-type

new sequencing technologies, it is now feasible and affordable to sequence the entire human exome to look for rare gene-coding sequence variants (1–3). Data analysis has become the major challenge, with thousands of variants detected in every individual person.

Recently, exome sequencing of 237 ion channels in persons with idiopathic epilepsy demonstrated that rare deleterious variants in known Mendelian disease genes were present not only in affected cases but also in a majority of age-and race-matched healthy subjects (4). These important observations clearly demonstrate that single deleterious variants cannot be assumed to be disease-causing and that the total variant burden needs to be considered. Variants in the same gene, or in different genes, can be expected to show epistasis, namely, have additive, neutralizing, or synergistic actions that are nonintuitive and unpredictable based on knowledge of the individual variant effects (5). Consequently, development of in silico methods for modeling the biological effects of multiple variants is critically required to derive meaningful information from genomic sequence data.

Here we have utilized atrial cell modeling in an analysis of variants in multiple cardiac potassium (K^+) channel genes in familial atrial fibrillation (AF). Genetic factors have an important role in the pathogenesis of AF, but the genes involved and mechanistic links with atrial arrhythmogenesis are incompletely understood. Given the fundamental importance of K^+ currents in atrial repolarization, cardiac K^+ channel genes have been considered strong candidates, and mutations in 8 genes have been associated with AF in families or in sporadic cases (6–9). Several common variants that modify susceptibility to AF in the general population have also been identified, including an intronic variant in *KCNN3*, that encodes the calcium-activated small conductance K^+ channel, SK3 (10). We resequenced genes encoding all the major cardiac K^+ currents in a cohort of persons with familial AF and coding sequence variants identified were evaluated in healthy control subjects. Novel variants were characterized using patch-clamp techniques, and an atrial cell model was used to assess the effects of multiple simultaneous variations of K^+ channel activation on atrial action potential (AP) properties. Our data show that multiple K^+ channel variants are frequently present in families with AF and can contribute to an arrhythmogenic atrial substrate.

Methods

Subjects. Study subjects comprised 80 persons (56 males), 26 to 90 years of age (mean 55 years of age) with a family history of AF, defined by AF in 2 or more first-degree relatives. None of the families studied had other inherited cardiac or systemic disorders that would account for AF. Families in which isolated affected members had concurrent risk factors for AF, such as hypertension, were not excluded. All subjects provided informed written consent and were evaluated by history and physical examination, electrocardiography, and transthoracic echocardiography. Two hundred-forty healthy subjects (83 male), 16 to 91 years of age (mean 53 years of age), with no history of cardiovascular disease comprised a control group. All participants were of Caucasian ethnicity. Protocols were approved by St. Vincent's Hospital human research ethics committee.

Mutation screening. Protein-coding sequences of the *KCND3, KCNIP2, KCNA5, KCNQ1*, *KCNH2*, *KCNE1, KCNE2, KCNE3, KCNE4, KCNE5, KCNJ2, KCNJ4,* and *KCNJ14,* genes were polymerase chain reaction amplified from genomic deoxyribonucleic acid (DNA) and sequenced using Big Dye terminator (version 3.1, Applied Biosystems, Foster City, California) and ABI PRISM 3730 DNA Analyzer (Applied Biosystems). Variants identified in AF probands were evaluated in control subjects by sequencing or high-resolution melting, using SensiMix HRM (Quantace, London, United Kingdom) or Lightcycler 480 HRM Master (Roche Diagnostics, Mannheim, Germany) mastermix and a Lightcycler 480 Instrument (Roche Diagnostics).

Cellular electrophysiology. Chinese hamster ovary (CHO) cells were transfected with wild-type (WT) or mutant K^+ channel cDNA clones and currents were recorded using conventional patch-clamp techniques (see Supplementary Methods).

In silico modeling. Code for the Courtemanche atrial cell model (11) was downloaded from the CellML repository and converted into Matlab M-code using cellular open resource (COR) (12). The model was solved using the Matlab ode15s solver with a maximal time-step of 1 ms. Models were equilibrated for 10 s of simulated time at a pacing rate of 1 Hz, and the duration of the last AP (APD) was measured from the time of the peak to the time of 90% repolarization (APD_{90}). The sensitivity of the model to changes in repolarizing K^+ currents was estimated (13). The model was solved for 1,000 consecutive runs. In each run, the 5 K^+ conductances (g_{K1}, g_{to}, g_{Kr}, g_{Ks}, and g_{Kur}) were individually scaled by a random number drawn from a log-normal distribution centered around a mean value of 1 (SD 22%). In each of 1,000 runs, the APD_{90} of the 10th AP was determined and saved with the corresponding set of 5 scaling factors. After 1,000 runs, matrices containing APD_{90} values and scaling factors were log-transformed, centered on their mean values, and normalized to their means. These values were used as inputs for the partial least squares function PLSREGRESS in the Matlab Statistics Toolbox. The output of this function is an array of correlation coefficients that gives the model sensitivity for each K^+ current. To determine the impact of altered repolarization reserve, the sensitivity analysis was repeated while increasing or reducing all K^+ current densities in 10%