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Genetics/Genomics

Mutation E169K in Junctophilin-2 Causes Atrial Fibrillation Due to Impaired RyR2 Stabilization

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Objectives This study sought to study the role of junctophilin-2 (JPH2) in atrial fibrillation (AF).

Background JPH2 is believed to have an important role in sarcoplasmic reticulum (SR) Ca^{2+} handling and modulation of ryanodine receptor Ca^{2+} channels (RyR2). Whereas defective RyR2-mediated Ca^{2+} release contributes to the pathogenesis of AF, nothing is known about the potential role of JPH2 in atrial arrhythmias.

Methods Screening 203 unrelated hypertrophic cardiomyopathy patients uncovered a novel JPH2 missense mutation (E169K) in 2 patients with juvenile-onset paroxysmal AF (pAF). Pseudoknock-in (PKI) mouse models were generated to determine the molecular defects underlying the development of AF caused by this JPH2 mutation.

Results PKI mice expressing E169K mutant JPH2 exhibited a higher incidence of inducible AF than wild type (WT)-PKI mice, whereas A399S-PKI mice expressing a hypertrophic cardiomyopathy-linked JPH2 mutation not associated with atrial arrhythmias were not significantly different from WT-PKI. E169K-PKI but not A399A-PKI atrial cardiomyocytes showed an increased incidence of abnormal SR Ca^{2+} release events. These changes were attributed to reduced binding of E169K-JPH2 to RyR2. Atrial JPH2 levels in WT-JPH2 transgenic, nontransgenic, and JPH2 knockdown mice correlated negatively with the incidence of pacing-induced AF. Ca^{2+} spark frequency in atrial myocytes and the open probability of single RyR2 channels from JPH2 knockdown mice was significantly reduced by a small JPH2-mimicking oligopeptide. Moreover, patients with pAF had reduced atrial JPH2 levels per RyR2 channel compared to sinus rhythm patients and an increased frequency of spontaneous Ca^{2+} release events.

Conclusions Our data suggest a novel mechanism by which reduced JPH2-mediated stabilization of RyR2 due to loss-of-function mutation or reduced JPH2/RyR2 ratios can promote SR Ca^{2+} leak and atrial arrhythmias, representing a potential novel therapeutic target for AF. (J Am Coll Cardiol 2013;62:2010–9) © 2013 by the American College of Cardiology Foundation

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and causes significant morbidity and mortality (1). Although the mechanisms behind AF pathogenesis are complex, it is believed that AF is induced and maintained by a combination of re-entry and triggered activity that includes early afteredepolarizations (EADs)

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and delayed afterdepolarizations (DADs) (2,3). Spontaneous diastolic Ca^{2+} release from the sarcoplasmic reticulum (SR) generates a depolarizing Na^{+}/Ca^{2+} exchanger (NCX) current, which may induce DADs and triggered activity (4). The major SR Ca^{2+} release channel responsible for arrhythmogenic Ca^{2+} release is the type 2 ryanodine receptor channel (RyR2) located within junctional membrane complexes (JMCs) (5–7). Defective regulation and activity of RyR2 has been linked to AF in humans and various animal models (4,8,9). In addition, structural changes in the JMC may precipitate destabilization of RyR2 and diastolic Ca^{2+} leakage (10). However, the molecular mechanisms underlying abnormal RyR2 Ca^{2+} leakage from JMCs remain poorly understood, especially in the atrial myocardium.

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Junctophilin-2 (JPH2) plays a critical structural role within JMCs (10,11). In mouse studies, knockdown of JPH2 was associated with loss of JMC numbers, reduced Ca^{2+}-induced Ca^{2+} release, and development of acute heart failure (10). Several mutations in JPH2 were previously identified in patients with hypertrophic cardiomyopathy (HCM) (12,13). Here, we report two novel *JPH2* mutations, one of which, E169K, was identified in a proband with the unusual clinical presentation of juvenile-onset paroxysmal AF (pAF). The proband's father, carrying the same mutation, also exhibited supraventricular tachycardia in addition to HCM. The patient with the other HCM-associated JPH2 mutation, A405S, did not exhibit atrial arrhythmias, similar to all other previously reported HCM patients with JPH2 mutations (12,13).

To determine how the E169K mutation in *JPH2* might cause atrial arrhythmias, we generated genetically modified mouse lines carrying the E169K or A399S (A405S in humans) mutation in JPH2. Subsequently, pseudoknock-in (PKI) mice with total cardiac JPH2 levels similar to those of nontransgenic (NTg) mice were generated by crossing JPH2 transgenic (Tg) mice with inducible, cardiac-specific JPH2 knockdown mice. The E169K-PKI but not A399S-PKI mice exhibited an enhanced susceptibility to pacing-induced AF. Atrial myocytes from E169K-PKI but not A399S-PKI mice showed an increased frequency of spontaneous SR Ca^{2+} release events. Interestingly, the JPH2 E169K mutation but not the A399S mutation decreased the binding affinity of JPH2 to RyR2. To further examine the importance of JPH2 loss-of-function in AF, atrial JPH2 levels in mice were shown to have an inverse correlation with AF inducibility.

Addition of a small peptide containing the E169 region of JPH2 was able to ameliorate the Ca^{2+} spark frequency in atrial cardiomyocytes isolated from JPH2 knockdown mice, and to normalize the open probability (Po) of RyR2 channels from JPH2 knockdown mice. Finally, to assess the importance of JPH2 in clinical AF, JPH2 protein levels were assessed in atrial samples from non-HCM patients with pAF. JPH2:RyR2 ratios were reduced significantly in pAF patients compared with those in sinus rhythm, and isolated atrial cardiomyocytes showed an increased frequency of potential proarrhythmic SR Ca^{2+} release events, consistent with enhanced RyR2 activity.

Together, these data suggest that reduced JPH2-mediated stabilization of RyR2 (either because of the unique inherited loss-of-function E169K mutation in JPH2 or because of the reduced JPH2 expression levels per RyR2 channel) can promote abnormal RyR2-mediated SR Ca^{2+} release events associated with AF. These findings may open new avenues for the development of anti-AF drugs that target either JPH2 or RyR2 abnormalities, to reduce spontaneous proarrhythmic diastolic SR Ca^{2+} release events.

Abbreviations and Acronyms

- **AF** = atrial fibrillation
- **Ca^{2+}** = calcium
- **DAD** = delayed afterdepolarizations
- **EAD** = early afterdepolarizations
- **HCM** = hypertrophic cardiomyopathy
- **JMC** = junctional membrane complex
- **JPH2** = junctophilin-2
- **NCX** = Na^{+}/Ca^{2+} exchanger
- **NTg** = nontransgenic
- **PKI** = pseudoknock-in
- **RyR2** = ryanodine receptor type 2
- **SR** = sarcoplasmic reticulum
- **Tg** = transgenic
- **WT** = wild type

Methods

Expanded methods can be found in the Online Appendix.

Genetic analysis. Comprehensive encoding region genetic analysis of *JPH2* was accomplished by polymerase chain reaction, denaturing high performance liquid chromatography, and direct DNA sequencing as described previously (12).

Generation of animals. All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine, conforming to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 85-23, revised 1996). Generation of Tg mice, including the WT-Tg and E169K and A399S JPH2 mutant Tg and short hairpin RNA (shRNA) knockdown mice, is described in detail in the Online Appendix. Pseudoknock-in mice were generated by crossing the JPH2 Tg mice (WT and E169K and A399S mutants) with an inducible cardiac-specific JPH2 knockdown mouse line. The offspring were given doses of tamoxifen to induce shRNA-mediated knockdown of total JPH2 levels to protein levels similar to those in NTg mouse hearts. Experiments were performed between 2 and 3 weeks post-knockdown when JPH2 levels were stable.

Programmed electrical stimulation. Atrial and ventricular intracardiac electrocardiography (ECG) traces were recorded using a 1.1F octapolar electrode catheter (EPR-800, Millar Instruments, Houston, Texas) inserted into the right ventricle via the right jugular vein, as described previously (14). Atrial