Gain-of-function mutation S422L in the *KCNJ8*-encoded cardiac K_{ATP} channel Kir6.1 as a pathogenic substrate for J-wave syndromes

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BACKGROUND J-wave syndromes have emerged conceptually to encompass the pleiotropic expression of J-point abnormalities including Brugada syndrome (BrS) and early repolarization syndrome (ERS). *KCNJ8,* which encodes the cardiac K_{ATP} Kir6.1 channel, recently has been implicated in ERS following identification of the functionally uncharacterized missense mutation S422L.

OBJECTIVE The purpose of this study was to further explore *KCNJ8* as a novel susceptibility gene for J-wave syndromes.

METHODS Using polymerase chain reaction, denaturing high-performance liquid chromatography, and direct DNA sequencing, comprehensive open reading frame/splice site mutational analysis of *KCNJ8* was performed in 101 unrelated patients with J-wave syndromes, including 87 with BrS and 14 with ERS. Six hundred healthy individuals were examined to assess the allelic frequency for all variants detected. *KCNJ8* mutation(s) was engineered by site-directed mutagenesis and coexpressed heterologously with SUR2A in COS-1 cells. Ion currents were recorded using whole-cell configuration of the patch-clamp technique.

RESULTS One BrS case and one ERS case hosted the identical missense mutation S422L, which was reported previously. KCNJ8-S422L involves a highly conserved residue and was absent in 1,200 reference alleles. Both cases were negative for mutations in all known BrS and ERS susceptibility genes. K_{ATP} current of the Kir6.1-S422L mutation was increased significantly over the voltage range from 0 to 40 mV compared to Kir6.1-WT channels ($n = 16–21$; $P < .05$).

CONCLUSION These findings further implicate *KCNJ8* as a novel J-wave syndrome susceptibility gene and a marked gain of function in the cardiac K_{ATP} Kir6.1 channel secondary to KCNJ8-S422L as a novel pathogenic mechanism for the phenotypic expression of both BrS and ERS.

KEYWORDS Early ventricular repolarization; Genetic disease; Idiopathic ventricular fibrillation; Ion channel; J-wave syndrome; K_{ATP} channel; Sudden cardiac death

ABBREVIATIONS **BrS** = Brugada syndrome; **ECG** = electrocardiogram; **ERS** = early repolarization syndrome; **ICD** = implantable cardioverter-defibrillator; **VF** = ventricular fibrillation

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Introduction

The early repolarization pattern on 12-lead ECG is characterized by elevation of the QRS–ST junction, better known as the *J point,* and was long considered a benign abnormality common in young healthy men and athletes. However, data from a large multicenter study reported a 30% increase in the prevalence of an early repolarization pattern, especially J-point elevation, in the inferolateral leads of patients with idiopathic ventricular fibrillation (VF) compared with controls.[1] Antzelevitch and Yan[2] recently proposed heritable J-wave syndromes as a new conceptual framework for ECG/arrhythmic phenotypes involving J-point/QRS–ST abnormalities. According to this classification scheme, the heritable J-wave syndromes include Brugada syndrome

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(BrS) and three different subtypes of early repolarization syndrome (ERS) distinguished by spatial localization of the early repolarization pattern.

Over the past decade, molecular sleuthing to elucidate the pathogenic substrates for these J-wave syndromes as well as for idiopathic VF has yielded eight genes associated with either BrS or idiopathic VF: *SCN5A*, *GPD1L*, *CACNA1C*, *CACNB2B*, *SCN1B*, *KCNE3*, *SCN3B*, and *DPP6*.[3–8] Perturbations in *SCN5A* and *SCN3B* have been implicated in both BrS and idiopathic VF without any discernible Brugada ECG pattern evidencing the pleiotropic expression that now is recognized for several channelopathy susceptibility genes.

Recently, the novel missense mutation S422L in the *KCNJ8*-encoded Kir6.1 alpha-subunit of the ATP-sensitive potassium (K_{ATP}) channel was reported in a young female with VF secondary to ERS, but the functional properties of KCNJ8-S422L were not investigated.[9] Notwithstanding, Kir6.1 is clearly expressed in cardiomyocytes,[10–12] although its functional role in ventricular repolarization remains controversial. In this study, we examined *KCNJ8* as a candidate gene involved in the pathogenesis of J-wave syndromes.

Methods

Study participants

We examined a cohort of 101 unrelated J-wave syndrome patients including 87 with BrS and 14 with ERS who were referred to either the Windland Smith Rice Sudden Death Genomics Laboratory at Mayo Clinic, Rochester, Minnesota, or the Molecular Cardiology Laboratory, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, for BrS/idiopathic VF genetic testing. Following receipt of written consent for this protocol, which was approved by the Mayo Foundation Institutional Review Board and Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy, Medical Ethical Committee, genomic DNA was extracted from peripheral blood lymphocytes using the Purgene DNA extraction kit (Gentra, Inc., Minneapolis, MN, USA).

Mutational analysis

Comprehensive open reading frame/splice site mutational analysis of *KCNJ8* was performed using polymerase chain reaction (PCR), denaturing high-performance liquid chromatography, and direct DNA sequencing as described previously.[13] Six hundred healthy individuals (1,200 reference alleles), including 100 African-Americans and 200 Caucasians from the Human Genetic Cell Repository and 300 additional European Caucasian controls, were examined to assess allelic frequency for all nonsynonymous variants detected.

Cloning of human KCNJ8 and mutagenesis

Human heart cDNA was created using human heart total RNA[14] and SuperScript First-Strand cDNA Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, USA). The reaction was performed according to the manufacturer's protocol. The *KCNJ8* (Kir6.1) gene was amplified from human heart cDNA by PCR using forward primer 5′-ATGTTGGCCAGAAAGAGTATCATC-3′ and reverse primer 5′-TCATGATTCCGATGTGTTTTGATT-3′. The human Kir6.1 PCR product was first TOPO cloned into pCR2.1 vector (Invitrogen) and then subcloned into mammalian expression vector pIRES2-EGFP (Clontech, Palo Alto, CA, USA) by a single EcoRI site. Kir6.1-S422L was generated using a Quick Change Site-Directed Mutagenesis kit (Stratagene) with the following primers: Kir6.1-S422L forward 5′-CCAGAAGGAAATCAAAACACATTGGAATCA-3′ and Kir6.1-S422L reverse 5′-TGATTCCAATGTGTTTTGATTTCCTTCTGG-3′. The cDNA sequence of Kir6.1-WT and Kir6.1-S422L in the constructs was verified by sequencing analysis.

Transfection and cell culture

COS-1 cells were cotransfected with the mammalian expression vector pIRES2-EGFP containing human Kir6.1-WT (1 μg) or human Kir6.1-S422L (1 μg) with 1 μg mouse full-length SUR2A cDNA[15] using FuGENE6 Transfection Reagent (Roche Diagnostics, Indianapolis, IN,USA) according to the manufacturer's instructions. Transfected cells were cultured in 35-mm-diameter cell culture dishes with Dulbecco's modified Eagle medium, as previously described.[15]

Electrophysiology and data analysis

After 48 to 72 hours of transfection, the cells expressing green fluorescence protein were selected for recording the whole cell current at room temperature (22°–24°C). An Axopath 200A amplifier and pClamp version 10.2 (Axon Instruments, Union City, CA, USA) were used. Patch pipettes were drawn from borosilicate glass (World Precision Instruments, Inc., Sarasota, FL, USA) with a resistance of 2 to 3 MΩ when filled with recording solutions. The bath

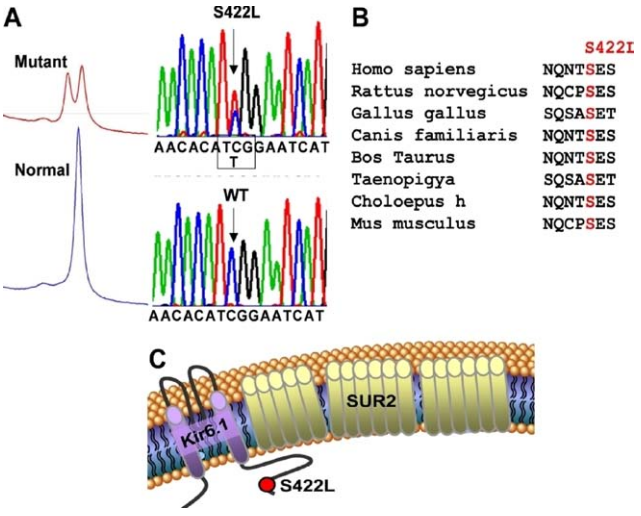

Figure 1 **A: Top:** The proband's denaturing high-performance liquid chromatography profile and DNA sequence chromatogram showing a C>T substitution at position 1265 of *KCNJ8* resulting in a Serine (S) to Leucine (L) substitution at position 422 (S422L). **Bottom:** Normal denaturing high-performance liquid chromatography profile and DNA sequence chromatogram. **B:** Conservation across species for S422 in Kir6.1. **C:** Linear topology of the Kir6.1/SUR2 complex showing the predicted localization of the S422L missense mutation to the C-terminus. SUR2 = sulfonylurea receptor type 2.