Loss-of-Function Mutations in the KCNJ8-Encoded Kir6.1 K$_{\text{ATP}}$ Channel and Sudden Infant Death Syndrome

David J. Tester, BS*; Bi-Hua Tan, MD, PhD*; Argelia Medeiros-Domingo, MD, PhD; Chunhua Song, MD, PhD; Jonathan C. Makielski, MD; Michael J. Ackerman, MD, PhD

Background—Approximately 10% of sudden infant death syndrome (SIDS) may stem from cardiac channelopathies. The KCNJ8-encoded Kir6.1 (K$_{\text{ATP}}$) channel critically regulates vascular tone and cardiac adaptive response to systemic metabolic stressors, including sepsis. KCNJ8-deficient mice are prone to premature sudden death, particularly with infection. We determined the spectrum, prevalence, and function of KCNJ8 mutations in a large SIDS cohort.

Methods and Results—Using polymerase chain reaction, denaturing high-performance liquid chromatography, and DNA sequencing, comprehensive open reading frame/splice-site mutational analysis of KCNJ8 was performed on genomic DNA isolated from necropsy tissue on 292 unrelated SIDS cases (178 males, 204 white; age, 2.9±1.9 months). KCNJ8 mutations were coexpressed heterologously with SUR2A in COS-1 cells and characterized using whole-cell patch-clamp. Two novel KCNJ8 mutations were identified. A 5-month-old white male had an in-frame deletion (E332del) and a 2-month-old black female had a missense mutation (V346I). Both mutations localized to Kir6.1’s C-terminus, involved conserved residues and were absent in 400 and 200 ethnic-matched reference alleles respectively. Both cases were negative for mutations in established channelopathic genes. Compared with WT, the pinacidil-activated K$_{\text{ATP}}$ current was decreased 45% to 68% for Kir6.1-E332del and 40% to 57% for V346I between ~20 mV and 40 mV.

Conclusions—Molecular and functional evidence implicated loss-of-function KCNJ8 mutations as a novel pathogenic mechanism in SIDS, possibly by predisposition of a maladaptive cardiac response to systemic metabolic stressors akin to the mouse models of KCNJ8 deficiency. (Circ Cardiovasc Genet. 2011;4:510-515.)

Key Words: sudden death ■ ion channels ■ genetics ■ pediatrics

Sudden infant death syndrome (SIDS) is the sudden death of an infant under 1 year of age that remains unexplained after a death scene and medico-legal investigation, including a complete autopsy and clinical history review.1 These perplexing tragedies remain the leading cause of postneonatal infant death and the third leading cause of infant death overall in the United States with an estimated incidence of 0.57 per 1000 live births.2,3

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A triple-risk model for SIDS was proposed suggesting a convergence of a perfect storm involving the triad of the vulnerable infant in the setting of exogenous stressors occurring in a critical development period.4 Since then, several predisposing risk factors for SIDS, including infection and inflammation, have been identified.5–9 Although many pathophysiologic theories, mostly implicating failed defense mechanisms, have been proposed for SIDS, including cardiopulmonary instability, maladaptive sympathetic bias, and coronary artery spasm, decisive pathogenic mechanisms triggering an infant’s sudden death remain unclear.3,10–13 Genetic factors constituting possible underlying vulnerabilities in SIDS victims have been identified in genes involved in neurotransmission, energy metabolism, autonomic response, response to infection, and cardiac action potential duration.3,14 An estimated 10% of SIDS stems from mutations in sudden death predisposing, channelopathy-susceptibility genes that cause potentially lethal, ventricular fibrillation syndromes such as long-QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), and Brugada syndrome.15–17

The KCNJ8-encoded Kir6.1 (K$_{\text{ATP}}$) channel is a critical regulator of vascular tone and cardiac adaptive response to systemic metabolic stressors.18,19 KCNJ8-deficient mice are prone to a high rate of premature sudden death associated with spontaneous ST-segment elevation followed by atrioventricular block19 and exhibit a maladaptive systemic inflammatory response to infection resulting in sudden premature death after endotoxin-mediated stress.20 In 2009, a

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**Table 1. Demographics of SIDS Cohort**

<table>
<thead>
<tr>
<th>SIDS Cases</th>
<th>No. of SIDS</th>
<th>Sex</th>
<th>Age at SIDS death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>292</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Mean, mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>178</td>
<td>2.9±1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td></td>
<td>114</td>
<td>0.1–12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reported ethnicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Black</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hispanic</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Asian</td>
<td>2</td>
</tr>
</tbody>
</table>

SIDS indicates sudden infant death syndrome.

*KCNJ8* missense mutation was identified in 14-year-old female with idiopathic ventricular fibrillation with prominent early repolarization, implicating a pathogenic mutation in *KCNJ8* for the first time in human disease.21 Subsequently, we identified 2 additional patients with the same missense mutation, S422L, and demonstrated a gain-of-function phenotype for this mutant K$_{\text{ATP}}$ channel.22 We demonstrate that akin to the mouse models of *KCNJ8* deficiency and sudden death, loss-of-function mutations in the *KCNJ8*-encoded Kir6.1 K$_{\text{ATP}}$ channel may confer a pathogenic substrate for infant vulnerability in some SIDS cases.

**Methods**

**SIDS Cohort**

Frozen necropsy tissue or autopsy blood from 292 SIDS cases (Table 1) were submitted to the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory for postmortem genetic testing. Since the diagnosis of SIDS across the United States is not based on mandatory and standardized procedures in different medical examiner systems, this SIDS cohort may be heterogeneous and not uniformly classified as these cases may be classified variously based on the local biases and different degrees and types of death scene and ancillary testing by independent medical examiners. Nonetheless, the enrollment criterion was a comprehensive medico-legal autopsy-negative sudden unexplained death of an infant <1 year of age, including a negative toxicology screen and death scene investigation. Infants with asphyxia or specific disease causing death were excluded. This Mayo Foundation Institutional Review Board–approved anonymous necropsy study only had limited medical information such as the sex, ethnicity and age at the time of death available. Time of day, medication use, infection, and position at death were unavailable.

**Postmortem Mutational Analysis of the *KCNJ8*-Encoded Kir6.1 K$_{\text{ATP}}$ Channel**

Genomic DNA was extracted using the Puregene DNA Isolation Kit (Qiagen, Inc., Valencia, CA). Comprehensive coding region point-mutation analysis of *KCNJ8* was performed using polymerase chain reaction (PCR), denaturing high-performance liquid chromatography (DHPLC), and direct DNA sequencing as previously described.16 Control genomic DNA from 200 ostensibly healthy white and 100 healthy black subjects was acquired from the Human Genetic Cell Repository sponsored by the National Institute of General Medical Sciences and the Coriell Institute for Medical Research (Camden, NJ). Primer sequences and PCR/DHPLC conditions are in Table 2.

**Cloning of Human *KCNJ8*-Encoded Kir6.1 K$_{\text{ATP}}$ Channel and Mutagenesis**

Human heart cDNA was created using human heart total RNA23 and SuperScript First-Strand cDNA Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol. The *KCNJ8* (Kir6.1) gene was amplified and subcloned into the mammalian expression vector p RES2-EFGP (Clontech, Pal Alto, CA) as previously described.22 Mutations were introduced into the human Kir6.1 by using a Quick Change Site-Directed Mutagenesis kit (Stratagene). The following primer pairs were used to mutate the targeted sites in the cDNA:

- Kir6.1-E332del forward 5’-GTGACTGAGGAAGAGTGATTTCG-3’
- Kir6.1-E332del reverse 5’-CAGAAATACACTCTCCGTTGAC-3’
- Kir6.1-V346I forward 5’-GGAACACTATTAAAGTGAACGTC-3’
- Kir6.1-V346I reverse 5’-GGACAGCTTATTTATAGTGTTG-3’

The cDNA sequences of Kir6.1 WT, Kir6.1-E332del and Kir6.1-V346I in the constructs were verified by sequencing analysis.

**Transfection and Cell Culture**

COS-1 cells were cotransfected with the mammalian expression vector p RES2-EFGP containing human Kir6.1 WT (1 μg), or 1 μg of each mutant (Kir6.1-E332del or V346I) with 1 μg mouse full-length SUR2A cDNA24 using FuGENE® 6 Transfection Reagent (Roche Diagnostics;}

Using polymerase chain reaction (PCR) reactions were performed in 20-μL volumes using 50 ng of DNA, 16 pmol of each primer, 200 μmol/L of each dNTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), and 1.0 U of AmpliTaq Gold (ABI, Foster City, CA). PCR amplification was performed using a DNA Engine Tetrad thermal cycler (MJ Research, Waltham, MA).

Thermal cycling method 100: 94°C for 5 minutes, followed by 5 cycles of 94°C for 20 seconds, 58°C for 20 seconds, and 72°C for 30 seconds; additional 35 cycles of 94°C for 20 seconds, 58°C for 20 seconds, and 72°C for 30 seconds, and a final extension of 72°C for 10 minutes.

Thermal cycling method a58: 94°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and a final extension of 72°C for 10 minutes.

Denaturing high-performance liquid chromatography (DHPLC) was performed using a 5% buffer B/minute gradient. The temperature followed by start and stop % buffer B at which the gradient was performed is indicated in the table.

**Table 2. Oligonucleotide Primers, PCR, and DHPLC Conditions for Mutational Analysis of *KCNJ8***

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward Primer (5’-3’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
<th>Size, bp</th>
<th>MgCl$_2$, mmol</th>
<th>Thermal Cycling Method</th>
<th>Gradient 1, Temp °C, %B</th>
<th>Gradient 2, Temp °C, %B</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNCJ8-1a</td>
<td>GACGGAGAGGGACAGG7TGGAG</td>
<td>ATGATAAGCGAAGGACGAGGA</td>
<td>322</td>
<td>2 mmol/L</td>
<td>100</td>
<td>62, 58–68</td>
<td>66, 51–61</td>
</tr>
<tr>
<td>KNCJ8-1b</td>
<td>CCACCGCTGCTGATCTTATAC</td>
<td>CTACGTGTGATAGTGTGGTTC</td>
<td>331</td>
<td>2 mmol/L</td>
<td>A58</td>
<td>54, 58–68</td>
<td>60, 53–63</td>
</tr>
<tr>
<td>KNCJ8-2a</td>
<td>GCAAGCTTATAGTCTTGGTGGT</td>
<td>TCTGCTCTTCTTCTGAGCGCT</td>
<td>301</td>
<td>2 mmol/L</td>
<td>A58</td>
<td>58, 57–67</td>
<td></td>
</tr>
<tr>
<td>KNCJ8-2b</td>
<td>CCATACGCTTTGTAGGATCTC</td>
<td>CAGAATACCTTGTTGCTTGCTG</td>
<td>326</td>
<td>2 mmol/L</td>
<td>A58</td>
<td>60, 56.4–66.4</td>
<td>62, 53.9–63.9</td>
</tr>
<tr>
<td>KNCJ8-2c</td>
<td>GGTTCTACTACCAACAGCTC</td>
<td>GCACCGTGGAGAGGACTAC</td>
<td>340</td>
<td>2 mmol/L</td>
<td>A58</td>
<td>60, 58–68</td>
<td></td>
</tr>
<tr>
<td>KNCJ8-2d</td>
<td>AAGAGGAGGTATCTCTGTTG</td>
<td>TCACATTCTGATATAAAACGTGC</td>
<td>324</td>
<td>2 mmol/L</td>
<td>A58</td>
<td>58.5, 58–68</td>
<td></td>
</tr>
</tbody>
</table>

Using polymerase chain reaction (PCR) reactions were performed in 20-μL volumes using 50 ng of DNA, 16 pmol of each primer, 200 μmol/L of each dNTP, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), and 1.0 U of AmpliTaq Gold (ABI, Foster City, CA). PCR amplification was performed using a DNA Engine Tetrad thermal cycler (MJ Research, Waltham, MA).

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Thermal cycling method a58: 94°C for 15 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension of 72°C for 10 minutes.

Denaturing high-performance liquid chromatography (DHPLC) was performed using a 5% buffer B/minute gradient. The temperature followed by start and stop % buffer B at which the gradient was performed is indicated in the table.
Indianapolis, IN) according to the manufacturer’s instructions. Transfected cells were cultured in 35-mm diameter cell-culture dish with Dulbecco modified Eagle’s medium, as previously described.24

Electrophysiology and Data Analysis
After 48 to 72 hours of transfection, cells expressing green fluorescence protein were selected for recording whole cell current at room temperature (22°C). Axopatch 200A amplifier and pClamp version 10.2 (Axon Instruments, Union City, CA) were used. Patch pipettes were drawn from borosilicate glass (World Precision Instruments Incorporated, Sarasota, FL) with resistance 2 to 3 mol/L when filled with recording solutions. The bath (extracellular) solution contained (in mmol) 140 NaCl, 5 KCl, 1 MgCl2, 1 CaCl2, and 10 HEPES (pH 7.4 set with NaOH). The pipette (intracellular) solution contained (in mmol) 120 K-aspartate, 25 KCl, 1 MgCl2 10 EGTA, and 10 HEPES (pH 7.2 set with KOH). The whole-cell current was generated by clamp pulses from a holding potential of 40 mV to voltages ranging from 80 to 40 mV in 20-mV steps for 260 ms, filtered at 1 KHz and sampled at 5 kHz. Data were digitally stored for off-line analysis using pClamp10.2 software (Axon Instruments Inc). Baseline current was recorded after cell membrane rupture, and extracellular 100 μmol/L pinacidil (Parke Davis, Ann Arbor, MI) was applied to obtain the maximal I KATP occurring within 2 minutes. Then, 20 μmol/L glibenclamide (Sigma-Aldrich) was used to show that the current has this characteristic of I KATP. The I KATP was normalized by cell capacitance to obtain current density.

Statistical Analysis
Data are shown as the mean value with bars representing the standard error of the mean (SEM). Determinations of statistical significance were performed using a Student t test for comparisons of 2 groups or using ANOVA for comparing multiple groups. A probability value of <0.05 was considered statistically significant.

Results

Molecular Characterization of KCNJ8-Encoded Kir6.1 KATP Channel Mutations in SIDS
After postmortem molecular analysis, 2 distinct and novel putative SIDS-causing KCNJ8 mutations (E332del in a 5-month-old white male and V346I in a 2-month-old black female) were identified in 1 of 204 (0.5%) white infants and 1 of 76 black infants (1.3%, Table 3). Figure 1 details the molecular characterization and the location of the two KCNJ8 mutations that alter highly conserved residues, were absent in 600 reference alleles (200 healthy white, 100 healthy black subjects), and localized to the C-terminus of the Kir6.1 KATP channel. These KCNJ8 mutation positive SIDS cases were mutation negative for all known LQTS- and CPVT-susceptibility genes (data not shown). Due to the anonymous nature of this necropsy study, we were unable to determine if these KCNJ8 mutations were spontaneous germline or familial inherited mutations.

SIDS-Associated Mutations in KCNJ8 Show Loss of Current Density
Nontransfected COS-1 cells showed only passive linear current (data not shown). Neither SUR2 nor Kir6.1 expressed alone produce currents in COS-1 cells (data not shown). COS-1 cells transiently coexpressing the Kir6.1-WT, Kir6.1-E332del or Kir6.1-V346I along with SUR2A were voltage-clamped after 48 to 72 hours of incubation showed robust K currents (Figure 2). The currents in control solutions probably represent partially activated I KATP, and although there was a trend for the mutants to produce smaller currents, it did not reach statistical significance. Pinacadil, an I KATP opener, was added to fully activate the current, and showed that Kir6.1-WT produced significantly larger currents than the mutants, with Kir6.1-E332del producing only 47% of WT and Kir6.1-V346I 55% of WT at 0 mV (Figure 3). A partially blocking dose of glibenclamide (20 μmol/L) blocked both WT and mutant currents (Figures 2 and 3), supporting their identity as I KATP, although glibenclamide at this dose may not be entirely specific.

Table 3. SIDS-Associated KCNJ8-Encoded Kir6.1 KATP Channel Mutations

<table>
<thead>
<tr>
<th>Sex</th>
<th>Ethnicity</th>
<th>Age</th>
<th>Status</th>
<th>Exon</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>White</td>
<td>5 mo</td>
<td>SIDS</td>
<td>2</td>
<td>c. del995_997 GAA</td>
<td>p. delE332</td>
<td>C-terminus</td>
</tr>
<tr>
<td>Female</td>
<td>Black</td>
<td>2 mo</td>
<td>SIDS</td>
<td>2</td>
<td>c. 1036 G&gt;A</td>
<td>p. V346I</td>
<td>C-terminus</td>
</tr>
</tbody>
</table>

Figure 1. Molecular characterization and localization of the novel sudden infant death syndrome (SIDS)-associated KCNJ8-encoded Kir6.1 KATP channel mutations. Illustrated are the direct DNA sequence chromatograms for delE332 and V346I along with the predicted linear KCNJ8 (Kir6.1) channel topology (not drawn to scale) showing the localization of the mutations as indicated by the yellow circles.
Discussion

SIDS may occur when an infant with an underlying vulnerability is exposed to exogenous stressors during a critical developmental period. Accordingly, SIDS does not occur in normal infants but rather only in vulnerable infants with an underlying abnormality. Approximately 10% of SIDS infants have mutations in channelopathy-susceptibility genes providing a plausible pathogenic substrate for underlying vulnerability. We add the KCNJ8-encoded ATP-sensitive Kir6.1 potassium channel as a novel putative pathogenic substrate for SIDS.

ATP-sensitive potassium channels (KATP) are present in many tissues including pancreatic islets, brain, heart, skeletal muscle, and vascular smooth muscle and are thought to couple the cellular metabolic state to the cell's membrane excitability and contractility. These channels act as metabolic sensors to help protect against a number of acute metabolic stressors, including hyperglycemia, hypoglycemia, ischemia, hypoxia, and inflammation, all of which have been implicated in SIDS. Therefore, the impact of these 2 loss-of-function mutations may not necessarily be confined to the heart.

In general, KATP channels are hetero-octamers consisting of 4 inwardly rectifying pore forming Kir6.x subunits (KCNJ8-encoded Kir6.1 or KCNJ11-encoded Kir6.2) and 4 regulatory sulfonylurea receptors (SURs), members of the ATP-binding cassette (ABC) transporter family of proteins (ABCC8-encoded SUR1, SUR2A, or SUR2B, alternative splice forms encoded by ABCC9), in which different combinations of Kir6.x and SURx subunits have been proposed to comprise tissue specific KATP channels with distinct electrophysiological properties and biological roles. However, given the tissue distribution and architectural heterogeneity in KATP channels, the 2 most likely mechanistic explanations for the sudden death in these infants involve either a KATP channelopathy-mediated lethal ventricular arrhythmia or a KATP channelopathy-mediated maladaptive cardiac stress response.

It has been accepted widely that Kir6.2/SUR2A forms the cardiac/myocardial KATP channel. However, several newer studies have suggested that both Kir6.1 and Kir6.2 are highly expressed in the heart. Electrophysiological and biochemical studies demonstrate that Kir6.1 and Kir6.2 may coassemble into functional, heteromeric channel complexes and that dominant-negative Kir6.1 subunits are capable of suppressing sarcolemmal KATP currents. Although more than 50 mutations have been identified in KCNJ11-encoded

Figure 2. Representative whole-cell current traces of Kir6.1-WT, Kir6.1-E332del, and Kir6.1-V346I in control, Pinacidil (Pin) and Glibenclamide (Glib). A, Examples of whole-cell current traces of Kir6.1-WT recorded in control (left), after 1 to 2 minutes of perfusion with 100 μmol/L Pin (middle), and in the presence of 20 μmol/L Glib (right). B, Example of whole-cell current traces of Kir6.1-E332del recorded in control (left), after 1 to 2 minutes of perfusion with 100 μmol/L Pin (middle), and in the presence of 20 μmol/L Glib (right). C, Examples of whole-cell current traces of Kir6.1-V346I recorded in control (left), after 1 to 2 minutes of perfusion with 100 μmol/L Pin (middle), and in the presence of 20 μmol/L Glib (right).

Figure 3. KCNJ8 mutant channels decreased $I_{\text{KATP}}$. A, Summary data of the whole-cell current voltage plot for $I_{\text{KATP}}$ after maximal activation by 100 μmol/L Pin at the voltages tested (insert). $n=14–21$ cells; *statistically significant differences of $I_{\text{KATP}}$ for mutants versus WT channels. B, Bar graphs show the mean $I_{\text{KATP}}$ densities before and after perfusion of 100 μmol/L of Pin, as well as after partial block by 20 μmol/L Glib for Kir6.1-WT, Kir6.1-E332del, and Kir6.1-V346I channels. Currents were elicited by a test pulse from holding potential of −40 mV to 0 mV for 260 ms ($n=14–21$ cells).
Kir6.2 as the pathogenic basis for neonatal diabetes (gain-of-function) or congenital hyperinsulinism (loss-of-function), neither disorder has been associated with cardiac arrhythmias.

Instead, KCNJ8-encoded Kir6.1 has been implicated recently as the pathogenic basis for idiopathic ventricular fibrillation (IVF) associated with prominent early repolarization in a case report involving a 14-year-old female presenting with cardiac arrest while playing a video game, who was identified as having a heterozygote missense mutation (S422L) in KCNJ8. Defined as spontaneous ventricular fibrillation in the absence of a readily identifiable abnormality responsible for the arrhythmia, IVF has a mean age of 35 to 40 years. However, infantile IVF has been reported in a 9-month-old female and a 12-month-old male. Without successful resuscitation, these infants would have satisfied the definition of SIDS.

In contrast to this previously published gain-of-function mutation, Kir6.1-S422L,21,22 both of the SIDS-associated KCNJ8 mutations discovered in the present study exhibited a marked loss-of-function phenotype. Just as both gain- and loss-of-function mutations have been identified in other potassium channels, a cogent argument for a proarrhythmic, loss-of-function phenotype. Just as both gain- and loss-of-function mutations have been identified in other potassium channels, a cogent argument for a proarrhythmic, loss-of-function phenotype. Just as both gain- and loss-of-function mutations have been identified in other potassium channels, a cogent argument for a proarrhythmic, loss-of-function phenotype. Under conditions of increased cardiac demand, the opening of K<sub>ATP</sub> channels increases outward K<sup>+</sup> currents and accelerates cardiac repolarization, thus limiting intracellular calcium transients and calcium overload and thereby successfully suppressing arrhythmias triggered by calcium dependent afterdepolarizations.18,27 Loss of cardiac K<sub>ATP</sub> channel function could prevent this response to such metabolic stress thereby precipitating a potentially lethal ventricular arrhythmia.

Besides sudden death secondary to a lethal ventricular arrhythmia, the electrophysiological phenotype of marked loss-of-function could be consistent with sudden death secondary to a lethal maladaptive cardiac/coronary response to metabolic stress as seen in KCNJ8 knock-out mouse models. Mice lacking Kir6.1 have a high rate of premature sudden death associated with spontaneous ST-segment elevation and atrioventricular block, with most mice dying between 5 and 6 weeks after birth.19 Induced vasospasm using methylergometrine, a known vasoconstrictor of vascular smooth muscle, precipitated their cardiac death with concomitant changes in ST-segment, suggesting dysregulation of vascular tone of the coronary arteries as a potential mechanism of death in some of the KCNJ8 (Kir6.1) knockout mice.19

Alternatively, in these KCNJ8 knockout mice, acute septic shock induced by endotoxin challenge with Escherichia coli lipopolysaccharide (LPS) elicited decreased coronary flow, ischemic cardiac dysfunction, marked ST-segment elevation, and premature death, suggesting that loss of the KCNJ8-encoded Kir6.1 K<sub>ATP</sub> channel confers fatal susceptibility to endotoxemia.20 A randomly generated mouse mutant (mayday) having a homozygous deletion of KCNJ8’s exon 1 and much of exon 2 was vulnerable to sudden death after cytomegalovirus (CMV) infection. Death occurred before high CMV viral titers were achieved, occurred abruptly without evidence of precursory sickness, and these sudden deaths were attributed to infections that would normally resolve uneventfully. Further, the mayday mice exhibited a 20,000-fold increased sensitivity to LPS with 10% of the mayday mice dying suddenly after inoculation with as little as 10 ng of LPS.22 Recently, the septic pathogen LPS has been shown to upregulate Kir6.1/SUR2B channels in a concentration-dependent manner in vascular tissue.33

Through this pathogen-induced upregulation, KCNJ8-encoded Kir6.1 K<sub>ATP</sub> channels exert a critical role in preventing coronary vasospasm thereby maintaining the patency of coronary arteries and myocardial perfusion. This protective, adaptive response is vital to surviving the systemic metabolic stress associated with the innate immune response.33 Extrapolating from these mouse models, one could surmise that KCNJ8 loss-of-function mutations in humans might create a vulnerable host with susceptibility for sudden death after exposure to septic pathogens, even at sublethal dose (ie, mild infection). Although it is unknown if the 2 SIDS victims with KCNJ8 mutations identified here had a mild infection at their time of death, reportedly approximately half of SIDS cases have a seemingly trivial infection at the time of death.3

### Conclusions

We report the first direct molecular and functional evidence implicating loss-of-function KCNJ8 mutations as a potential pathogenic substrate for SIDS. Whether these mutant K<sub>ATP</sub> channels precipitated a lethal ventricular arrhythmia or a maladaptive cardiac/coronary response to a systemic metabolic stressor remains speculative because KCNJ8 is expressed in multiple tissues including heart, vascular, and neuronal tissue.

### Sources of Funding

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### Disclosures

Dr Ackerman is a consultant for Biotronik, Boston Scientific, Medtronic, St Jude Medical, Inc, and Transgenomic. Intellectual property derived from Dr Ackerman’s research program resulted in license agreements in 2004 between Mayo Clinic Health Solutions (formerly Mayo Medical Ventures) and PGxHealth (formerly Geonnaissance Pharmaceuticals, now recently acquired by Transgenomic). Drs Tester, Tan, Meideiros-Domingo, Song, and Makielski have no conflicts to disclose. Dr Ackerman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

### References

Sudden infant death syndrome (SIDS) is a sudden death of an infant under 1 year of age which remains unexplained after a death scene and medico-legal investigation including a complete autopsy and clinical history review. These perplexing tragedies remain the leading cause of postneonatal infant death and the third leading cause of infant mortality overall in the United States. Approximately 10% to 15% of sudden infant death syndrome (SIDS) stems from inheritable sudden cardiac death related disorders, including long QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia. The \textit{KCNJ8}-encoded Kir6.1 (\(K_{\text{ATP}}\)) channel is a critical regulator of vascular tone and cardiac adaptive response to systemic metabolic stressors. \textit{KCNJ8}-deficient mice are prone to a high rate of premature sudden death associated with spontaneous ST-segment elevation followed by atrioventricular block and exhibit a maladaptive systemic inflammatory response to infection resulting in sudden premature death after endotoxin-mediated stress. A gain-of-function missense mutation in \textit{KCNJ8} has been implicated recently in the pathogenesis of idiopathic ventricular fibrillation and early repolarization syndrome. Here, we demonstrate for the first time that, akin to the mouse models of \textit{KCNJ8} deficiency and sudden death, loss-of-function mutations in the \textit{KCNJ8}-encoded Kir6.1 \(K_{\text{ATP}}\) channel may confer a pathogenic substrate for infant vulnerability in some SIDS cases. The identification and functional characterization of \textit{KCNJ8} mutations in SIDS adds to the compendium of channelopathies that may underlie some cases of sudden unexplained death during infancy and may have further implications for genetic testing for such cases.
Loss-of-Function Mutations in the KCNJ8-Encoded Kir6.1 $K_{ATP}$ Channel and Sudden Infant Death Syndrome
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