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Loss-of-Function Mutations in the *KCNJ8*-Encoded Kir6.1 K_{ATP} Channel and Sudden Infant Death Syndrome

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Abstract

Background—Approximately 10% of sudden infant death syndrome (SIDS) may stem from cardiac channelopathies. The *KCNJ8*-encoded Kir6.1 (K_{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 PCR, DHPLC, 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 co-expressed 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 to WT, the pinacidil-activated K_{ATP} current was decreased 45% to 68% for Kir6.1-E322del and 40% to 57% for V346I between -20 mV to 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.

Keywords

sudden death; ion channels; genetics; pediatrics

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Conflict of Interest Disclosures: MJA is a consultant for Biotronik, Boston Scientific, Medtronic, St. Jude Medical, Inc., and Transgenomic. Intellectual property derived from MJA's research program resulted in license agreements in 2004 between Mayo Clinic Health Solutions (formerly Mayo Medical Ventures) and PGxHealth (formerly Genaissance Pharmaceuticals, now recently acquired by Transgenomic). DJT, BHT, AMD, CS, and JCM have no conflicts to disclose. MJA 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.

Introduction

Sudden infant death syndrome (SIDS) is a sudden death of an infant under 1 year of age which remains unexplained following 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 mortality overall in the United States with an estimated incidence of 0.57 per 1,000 live births[2, 3].

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 cardiorespiratory 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 (BrS)[15-17].

The *KCNJ8*-encoded Kir6.1 (K_{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 block[19] and exhibit a maladaptive systemic inflammatory response to infection resulting in sudden premature death following endotoxin-mediated stress[20]. In 2009, a *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 two additional patients with the same missense mutation, S422L, and demonstrated a gain-of-function phenotype for this mutant K_{ATP} channel[22].

Here, 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_{ATP} channel may confer a pathogenic substrate for infant vulnerability in some SIDS cases.

Methods

Sudden Infant Death Syndrome (SIDS) Cohort

Frozen necropsy tissue or autopsy blood from 292 sudden infant death syndrome (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 upon 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 upon the local biases and different degrees and types of death scene and ancillary testing by independent medical examiner offices. 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_{ATP} Channel

Genomic DNA was extracted using the Puregene DNA Isolation Kit (Qiagen, Inc, Valencia, Calif). 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, New Jersey). Primer sequences and PCR/DHPLC conditions are in Table 2.

Cloning of Human *KCNJ8*-Encoded Kir6.1 K_{ATP} Channel and Mutagenesis

Human heart cDNA was created using human heart total RNA[23] 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 mammalian expression vector pIRES2-EGFP (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′-GTGACTGAGGAAGGAGTGTATTCTG-3′

Kir6.1-E332del reverses 5′-CAGAATACACTCCTTCCTCAGTCAC-3′;

Kir6.1-V346I forward 5′-GGCAACACTATTAAAGTAGCTGCTCC-3′

Kir6.1-V346I reverses 5′-GGAGCAGCTACTTTAATAGTGTTGCC-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 co-transfected with the mammalian expression vector pIRES2-EGFP containing human Kir6.1-WT (1 mcg), or 1 mcg of each mutant (Kir6.1-E332del or V346I) with 1 mcg mouse full-length SUR2A cDNA[24] using FuGENE®6 Transfection Reagent (Roche Diagnostics; Indianapolis, IN) according to the manufacturer's instructions. Transfected cells were cultured in 35- mm diameter cell-culture dish with Dulbecco's modified Eagle's medium, as previously described[24].

Electrophysiology and Data Analysis

After 48-72 hours of transfection, cells expressing green fluorescence protein were selected for recording whole cell current at room temperature (22°C ~24°C). Axopath 200A amplifier and pClamp version 10.2 (Axon Instruments, Union City, California, USA) were used. Patch pipettes were drawn from borosilicate glass (World Precision Instruments Incorporated, Sarasota, Florida, USA) with resistance 2 - 3 MΩ when filled with recording solutions. The bath (extracellular) solution contained (in mM) 140 NaCl, 5 KCl, 1 MgCl2, 1 CaCl2, and 10 HEPES, (pH 7.4 set with NaOH). The pipette (intracellular) solution contained (in mM) 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 μM pinacidil (Parke Davis, Ann Arbor, Michigan, USA) was applied to obtain the maximal I_{KATP} occurring within 2 minutes. Then 20 μM 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 (S.E.M.). Determinations of statistical significance were performed using a Student *t* test for comparisons of two groups or using analysis of variance (ANOVA) for comparing multiple groups. A p-value < 0.05 was considered statistically significant.

Results

Molecular Characterization of *KCNJ8*-Encoded Kir6.1 K_{ATP} Channel Mutations in SIDS

Following a postmortem molecular analysis two 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 1/204 (0.5%) white infants and 1/76 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 K_{ATP} 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

Non transfected 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 co-expressing the Kir6.1-WT, Kir6.1-E332del or Kir6.1-V346I along with SUR2A were voltage clamped after 48-72 hours of incubation showed robust K currents (Figure 2). The currents in control solutions likely 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 0mV (Figure 3). A partially blocking dose of glibenclmaide (20 μM) blocked both WT and mutant currents (Figure 2 and 3) supporting their identity as I_{KATP}, although glibenclamide at this dose may not be entirely specific.

Discussion

Sudden infant death syndrome (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[3]. Approximately 10% of SIDS infants have mutations in channelopathy-susceptibility genes providing a plausible pathogenic substrate for underlying vulnerability[15, 17, 25]. Here, we add the *KCNJ8*-encoded ATP-sensitive Kir6.1 potassium channel as a novel putative pathogenic substrate for SIDS.

ATP-sensitive potassium channels (K_{ATP}) 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[26]. These channels act as metabolic sensors to help protect against a number of acute metabolic stressors[26], including hyperglycemia, hypoglycemia, ischemia, hypoxia, and inflammation, all of which have been implicated in SIDS. Therefore, the impact of these two loss-of-function mutations may not necessarily be confined to the heart.

In general, K_{ATP} channels are hetero-octamers consisting of four inwardly rectifying pore forming Kir6.x subunits (*KCNJ8*-encoded Kir6.1 or *KCNJ11*-encoded Kir6.2) and four 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 K_{ATP} channels with distinct electrophysiological properties and biological roles[26]. However, given the tissue distribution and architectural heterogeneity in K_{ATP} channels, the two most likely mechanistic explanations for the sudden death in these infants involve either a K_{ATP} channelopathy-mediated lethal ventricular arrhythmia or a K_{ATP} channelopathy-mediated maladaptive cardiac stress response.

It has been accepted widely that Kir6.2/SUR2A forms the cardiac/myocardial K_{ATP} channel. However, several newer studies have suggested that both Kir6.1 and Kir6.2 are highly expressed in the heart[27-29]. Electrophysiological and biochemical studies demonstrate that Kir6.1 and Kir6.2 may co-assemble into functional, heteromeric channel complexes and that dominant-negative Kir6.1 subunits are capable of suppressing sarcolemmal K_{ATP} currents[29, 30]. While over 50 mutations have been identified in *KCNJ11*-encoded 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* [21]. 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[31]. 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 here 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 pro-arrhythmic, loss-of-function cardiac K_{ATP} channel is conceivable. K_{ATP} channels of the cardiomyocyte provide metabolic sensing and cardioprotection[18]. In the heart, K_{ATP} channels are normally closed but open in response to physiological and pathological metabolic stress. Under conditions of increased cardiac demand, the opening of K_{ATP} channels increases outward K^+ 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_{ATP} 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 E. 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_{ATP} 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 following 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 following inoculation with as little as 10 ng of LPS[32]. 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_{ATP} 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 following exposure to septic pathogens, even at sublethal dose (i.e. mild infection). While it is unknown if the two 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

Here, 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_{ATP} channels precipitated a lethal ventricular arrhythmia or a maladaptive cardiac/coronary response to a systemic metabolic stressor remains speculative since *KCNJ8* is expressed in multiple tissues including heart, vascular, and neuronal tissue.

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References

1. Willinger M, James LS, Catz C. Defining the sudden infant death syndrome (SIDS): deliberations of an expert panel convened by the National Institute of Child Health and Human Development. Pediatric Pathology. 1991; 11:677–684. [PubMed: 1745639]

2. Dwyer T, Ponsonby AL, Blizzard L, Newman NM, Cochrane JA. The contribution of changes in the prevalence of prone sleeping position to the decline in sudden infant death syndrome in Tasmania. Jama. 1995; 273:783–789. [PubMed: 7861572]

3. Kinney HC, Thach BT. The sudden infant death syndrome. New England Journal of Medicine. 2009; 361:795–805. [PubMed: 19692691]

4. Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. Biology of the Neonate. 1994; 65:194–197. [PubMed: 8038282]

5. Hoffman HJ, Damus K, Hillman L, Krongrad E. Risk factors for SIDS. Results of the National Institute of Child Health and Human Development SIDS Cooperative Epidemiological Study. Annals of the New York Academy of Sciences. 1988; 533:13–30. [PubMed: 3048169]

6. Hoffman HJ, Hillman LS. Epidemiology of the sudden infant death syndrome: maternal, neonatal, and postneonatal risk factors. Clinics in Perinatology. 1992; 19:717–737. [PubMed: 1464187]

7. Ponsonby AL, Dwyer T, Gibbons LE, Cochrane JA, Wang YG. Factors potentiating the risk of sudden infant death syndrome associated with the prone position. New England Journal of Medicine. 1993; 329:377–382. see comment. [PubMed: 8326970]

8. Fleming PJ, Blair PS, Bacon C, Bensley D, Smith I, Taylor E, Berry J, Golding J, Tripp J. Environment of infants during sleep and risk of the sudden infant death syndrome: results of 1993-5 case-control study for confidential inquiry into stillbirths and deaths in infancy. Confidential Enquiry into Stillbirths and Deaths Regional Coordinators and Researchers. Bmj. 1996; 313:191–195. see comment. [PubMed: 8696193]

9. Getahun D, Amre D, Rhoads GG, Demissie K. Maternal and obstetric risk factors for sudden infant death syndrome in the United States. Obstetrics & Gynecology. 2004; 103:646–652. [PubMed: 15051553]

10. Valdes-Dapena MA. Editorial: Sudden, unexpected and unexplained death in infancy--a status report--1973. New England Journal of Medicine. 1973; 289:1195–1197. [PubMed: 4754967]

11. Guntheroth WG. Theories of cardiovascular causes in sudden infant death syndrome. Journal of American College of Cardiology. 1989; 14:443–447.

12. Kanda I, Endo M. Coronary artery spasm: a hypothesis on prevention by progesterone. Medical Hypotheses. 1997; 49:183–185. [PubMed: 9278934]

13. Yun AJ, Lee PY. Sudden death among infants and adults: companion disorders of maladaptive sympathetic bias. Medical Hypotheses. 2004; 62:857–860. [PubMed: 15142636]

14. Weese-Mayer DE, Ackerman MJ, Marazita ML, Berry-Kravis EM. Sudden Infant Death Syndrome: review of implicated genetic factors. American Journal of Medical Genetics. 2007; Part A. 143A:771–788. [PubMed: 17340630]

15. Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, Towbin JA. Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. Jama. 2001; 286:2264–2269. [PubMed: 11710892]

16. Tester DJ, Ackerman MJ. Sudden infant death syndrome: How significant are the cardiac channelopathies? Cardiovascular Research. 2005; 67:388–396. [PubMed: 15913580]

17. Arnestad M, Crotti L, Rognum TO, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Wang DW, Rhodes TE, George AL Jr, Schwartz PJ. Prevalence of long-QT syndrome gene variants in sudden infant death syndrome. Circulation. 2007; 115:361–367. see comment. [PubMed: 17210839]

18. Zingman LV, Alekseev AE, Hodgson-Zingman DM, Terzic A. ATP-sensitive potassium channels: metabolic sensing and cardioprotection. Journal of Applied Physiology. 2007; 103:1888–1893. [PubMed: 17641217]

19. Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K, Koseki H, Iwanaga T, Nakaya H, Seino S. Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. Nature Medicine. 2002; 8:466–472.

20. Kane GC, Lam CF, O'Cochlain F, Hodgson DM, Reyes S, Liu XK, Miki T, Seino S, Katusic ZS, Terzic A. Gene knockout of the KCNJ8-encoded Kir6.1 K(ATP) channel imparts fatal susceptibility to endotoxemia. FASEB Journal. 2006; 20:2271–2280. [PubMed: 17077304]

21. Haissaguerre M, Chatel S, Sacher F, Weerasooriya R, Probst V, Loussouarn G, Horlitz M, Liersch R, Schulze-Bahr E, Wilde A, Kaab S, Koster J, Rudy Y, Le Marec H, Schott JJ. Ventricular

fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. Journal of Cardiovascular Electrophysiology. 2009; 20:93–98. [PubMed: 19120683]

22. Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, Kroboth SL, Song C, Zhou Q, Kopp D, Schwartz PJ, Makielski JC, Ackerman MJ. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac KATP channel Kir6.1 as a pathogenic substrate for J-wave syndromes. Heart Rhythm. 2010; 7:1466–1471. [PubMed: 20558321]
23. Foell JD, Balijepalli RC, Delisle BP, Yunker AMR, Robia SL, Walker JW, McEnery MW, January CT, Kamp TJ. Molecular heterogeneity of calcium channel beta-subunits in canine and human heart: evidence for differential subcellular localization. Physiological Genomics. 2004; 17:183–200. [PubMed: 14762176]
24. Chutkow WA, Makielski JC, Nelson DJ, Burant CF, Fan Z. Alternative splicing of sur2 Exon 17 regulates nucleotide sensitivity of the ATP-sensitive potassium channel. Journal of Biological Chemistry. 1999; 274:13656–13665. [PubMed: 10224138]
25. Tester DJ, Dura M, Carturan E, Reiken S, Wronska A, Marks AR, Ackerman MJ. A mechanism for sudden infant death syndrome (SIDS): Stress-induced leak via ryanodine receptors. Heart Rhythm. 2007; 4:733–739. [PubMed: 17556193]
26. Miki T, Seino S. Roles of KATP channels as metabolic sensors in acute metabolic changes. Journal of Molecular & Cellular Cardiology. 2005; 38:917–925. [PubMed: 15910876]
27. Zhang H, Flagg TP, Nichols CG. Cardiac sarcolemmal K(ATP) channels: Latest twists in a questing tale! Journal of Molecular & Cellular Cardiology. 2010; 48:71–75. [PubMed: 19607836]
28. Singh H, Hudman D, Lawrence CL, Rainbow RD, Lodwick D, Norman RI. Distribution of Kir6.0 and SUR2 ATP-sensitive potassium channel subunits in isolated ventricular myocytes. Journal of Molecular & Cellular Cardiology. 2003; 35:445–459. [PubMed: 12738227]
29. van Bever L, Poitry S, Faure C, Norman RI, Roatti A, Baertschi AJ. Pore loop-mutated rat KIR6.1 and KIR6.2 suppress KATP current in rat cardiomyocytes. American Journal of Physiology - Heart & Circulatory Physiology. 2004; 287:H850–859. [PubMed: 15044189]
30. Cui Y, Giblin JP, Clapp LH, Tinker A. A mechanism for ATP-sensitive potassium channel diversity: Functional coassembly of two pore-forming subunits. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:729–734. [PubMed: 11136227]
31. Chockalingam P, Clur SAB, Reimer AG, Blom NA, Wilde AA. Idiopathic ventricular fibrillation in two infants, not always idiopathic on follow-up. Heart Rhythm. 2009; 6:1501–1503. [PubMed: 19695965]
32. Croker B, Crozat K, Berger M, Xia Y, Sovath S, Schaffer L, Eleftherianos I, Imler JL, Beutler B. ATP-sensitive potassium channels mediate survival during infection in mammals and insects. Nature Genetics. 2007; 39:1453–1460. [PubMed: 18026101]
33. Shi W, Cui N, Wu Z, Yang Y, Zhang S, Gai H, Zhu D, Jiang C. Lipopolysaccharides up-regulate Kir6.1/SUR2B channel expression and enhance vascular KATP channel activity via NF-kappaB-dependent signaling. Journal of Biological Chemistry. 2010; 285:3021–3029. [PubMed: 19959479]

Sudden infant death syndrome (SIDS) is a sudden death of an infant under 1 year of age which remains unexplained following 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 KCNJ8-encoded Kir6.1 (K_{ATP}) channel is a critical regulator of vascular tone and cardiac adaptive response to systemic metabolic stressors. 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 following endotoxin-mediated stress. A gain-of-function missense mutation in 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 KCNJ8 deficiency and sudden death, loss-of-function mutations in the KCNJ8-encoded Kir6.1 K_{ATP} channel may confer a pathogenic substrate for infant vulnerability in some SIDS cases. The identification and functional characterization of 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.

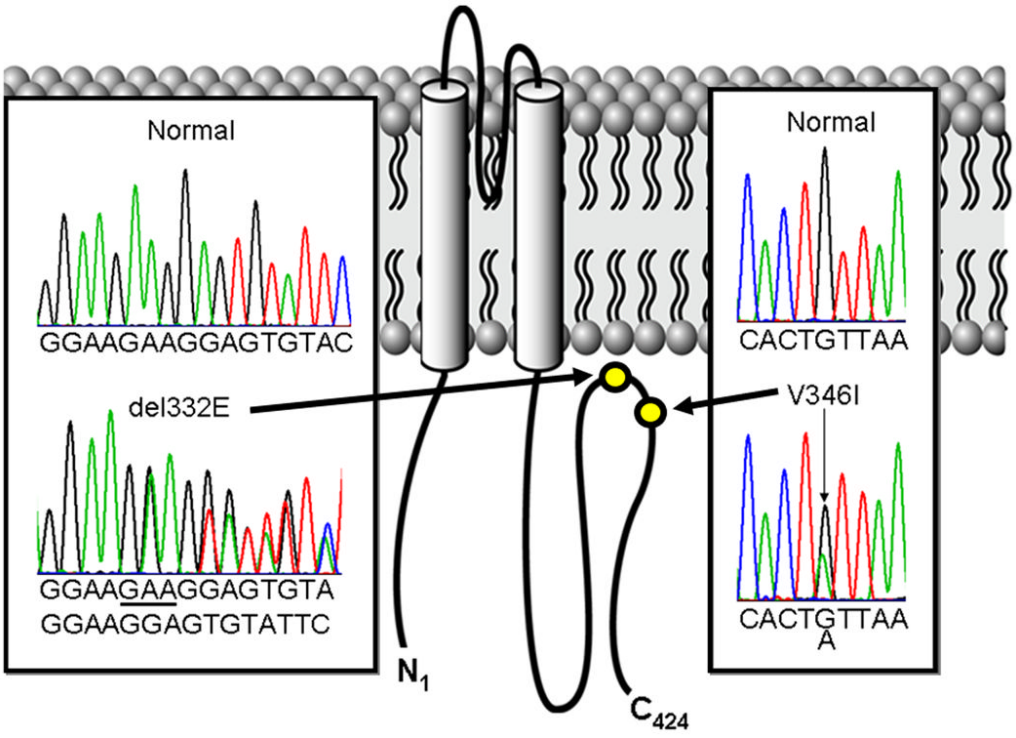

Figure 1.

Molecular Characterization and Localization of the Novel SIDS-Associated *KCNJ8*-Encoded Kir6.1 K_{ATP} 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.

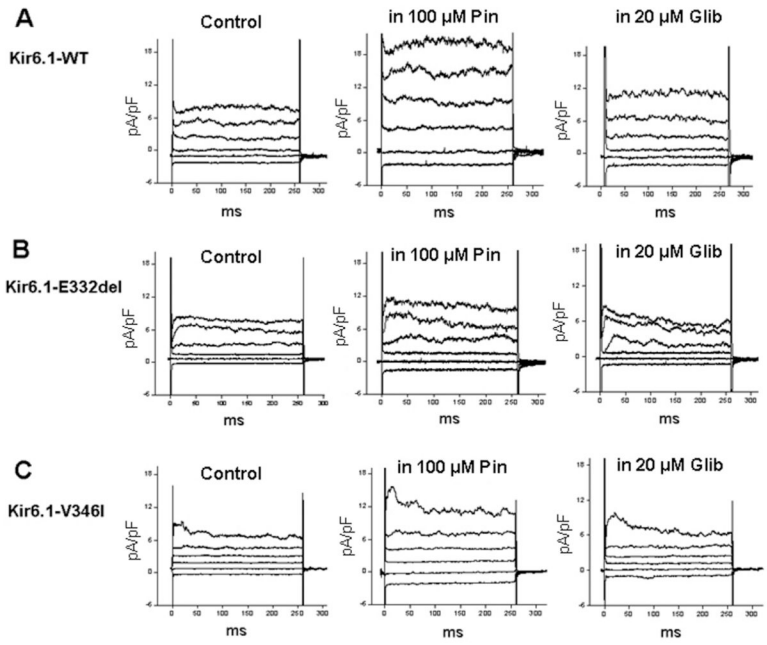

Figure 2.

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 μM Pin (middle) and in the presence of 20 μM 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 μM Pin (middle) and in the presence of 20 μM 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 μM Pin (middle) and in the presence of 20 μM Glib (right).

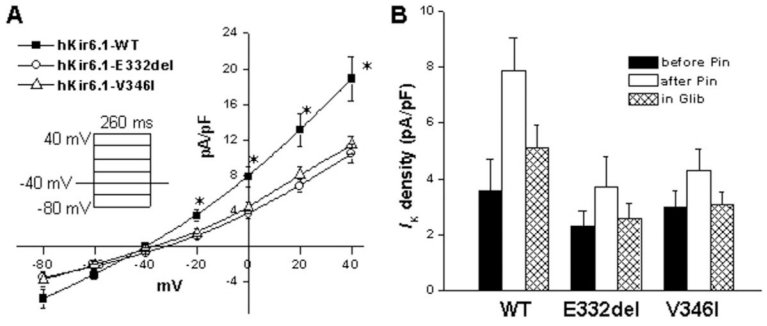

Figure 3.

KCNJ8 mutant channels decreased I_{KATP}. (A) Summary data of the whole cell current voltage plot for I_{KATP} after maximal activation by 100 μM Pin at the voltages tested (*insert*, n=14-21 cells, *Statistically significant differences of I_{KATP} for mutants vs. WT channels). (B) Bar graphs show the mean I_{KATP} densities before and after perfusion of 100 μM of Pin, as well as after partial block by 20 μM 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).

Table 1

Demographics of SIDS cohort

	SIDS Cases
Number of SIDS	292
Gender	
Male	178
Female	114
Age at SIDS Death	
Mean (months)	2.9 ± 1.9
(Range)	(0.1-12)
Reported Ethnicity	
White	204
Black	76
Hispanic	10
Asian	2

Table 2

Oligonucleotide primers, PCR and DHPLC conditions for mutational analysis of *KCNJ8*

Exon	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Size (bp)	MgCl (mM)	Thermal Cycling Method	Gradient 1 (Temp°C; %B)	Gradient 2 (Temp°C;%B)
KCNJ8-1a	GACGGAGAGGCAGGTGAG	ATGATAGCGAAGAGCAGCCA	322	2mM	100	62, 58-68	66, 51-61
KCNJ8-1b	CACACGCTGGTCATCTTTAC	CTTACTGTGTTAGGTGTTGTTC	331	2mM	A58	54, 58-68	60, 53-63
KCNJ8-2a	GCAAGCATTTAATAGTCTTTGG	TCTGCCCTTCTGTGAGCCT	301	2mM	A58	58, 57-67	
KCNJ8-2b	CCATCACGGTTTTGATTCTC	CAGAAAAATGTTATTGCTCTCG	326	2mM	A58	60, 56.4-66.4	62, 53.9-63.9
KCNJ8-2c	GGTTCCTATTCACCAACTGG	GCACCGTGGAGCAGCTAC	340	2mM	A58	60, 58-68	
KCNJ8-2d	AAGAAGGAGTGTATTCTGTGG	TCAAAACTTGATAAAAGACTGTC	324	2mM	A58	58.5,58-68	

PCR reactions were performed in 20μL volumes using 50ng of DNA, 16pmol of each primer, 200μM of each dNTP, 50mMol/L KCl, 10mMol/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 5 min, followed by 5 cycles of 94°C for 20s, 64°C for 20s and 72°C for 30s; additional 35 cycles of 94°C for 20s 62°C for 20s, 72°C for 30s, and a final extension of 72°C for 10 min.

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

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 3

SIDS-Associated KCNJ8-Encoded Kir6.1 K_{ATP} Channel Mutations

Sex	Ethnicity	Age	Status	Exon	Nucleotide Change	Amino Acid Change	Location
Male	White	5 months	SIDS	2	c. del995_997 GAA	p. delE332	C-terminus
Female	Black	2 months	SIDS	2	c. 1036 G>A	p. V346I	C-terminus