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Functional Characterization of *ABCC9* Variants Identified in Sudden Unexpected Natural Death

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Abstract

Background: Genetic variation in ion channel genes ('channelopathies') are often associated with inherited arrhythmias and sudden death. Genetic testing ('molecular autopsies') of channelopathy genes can be used to assist in determining the likely causes of sudden unexpected death. However, different *in silico* approaches can yield conflicting pathogenicity predictions and assessing their impact on ion channel function can assist in this regard.

Methods and Results: We performed genetic testing of cases of sudden expected death in the New York City metropolitan area and found four rare or novel variants in *ABCC9*, which codes for the regulatory SUR2 subunit of K_{ATP} channels. All were missense variants, causing amino acid changes in the protein. Three of the variants (A355S, M941V, and K1379Q) were in cases of infants less than six-months old and one (H1305Y) was in an adult. The predicted pathogenicities of the variants were conflicting. We have introduced these variants into a human SUR2A cDNA, which we coexpressed with the Kir6.2 pore-forming subunit in HEK-293 cells and subjected to patch clamp and biochemical assays. Each of the four variants led to gain-of-function phenotypes. The A355S and M941V variants increased in the overall patch current. The sensitivity of the K_{ATP} channels to inhibitory 'cytosolic' ATP was repressed for the M941V, H1305Y and K1379Q variants. None of the variants had any effect on the unitary K_{ATP} channel current or the surface

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Disclosures
None

expression of K_{ATP} channels, as determined with biotinylation assays, suggesting that all of the variants led to an enhanced open state.

Conclusions: All four variants caused a gain-of-function phenotype. Given the expression of SUR2-containing K_{ATP} channels in the heart and specialized cardiac conduction, vascular smooth muscle and respiratory neurons, it is conceivable that electrical silencing of these cells may contribute to the vulnerability element, which is a component of the triple risk model of sudden explained death in infants. The gain-of-function phenotype of these *ABCC9* variants should be considered when assessing their potential pathogenicity.

Keywords

Sudden death; channelopathy; ion channels

Introduction

Although the incidence of unexplained infant death has decreased significantly since the introduction of safe sleep campaigns, it remains the leading cause of death of apparently healthy infants before their first birthday. The causes of sudden death are largely unknown¹. The “Triple Risk Model” posits that overlapping factors combine to elevate risk, which include i) a critical developmental period during the first year of life, ii) the presence of exogenous stressors (e.g. the sleep position) and iii) underlying vulnerabilities (e.g. an unrecognized pathology)². Sudden unexplained death also occurs in young and older adults where the ‘critical developmental period’ is absent, but the elements involving exogenous stressors and a vulnerable individual remain (the ‘Duel Risk Model’)³. The nature of the vulnerabilities that contribute to sudden death are uncertain, but recent developments have implicated genetic factors as major contributors. With the advent of cheap and reliable sequencing technologies, genetic screening of dozens or hundreds of genes (‘molecular autopsies’) are increasingly being used to supplement classic forensic investigations in order to determine the cause of death. Since genetic variation in ion channel genes (channelopathies) are often associated with inherited arrhythmias⁴, many of these molecular autopsies include ion channel genes (particularly those expressed in the heart). We have followed this approach to perform genetic testing of cases of sudden unexpected death (SUD) in the New York City metropolitan area; initially with a small panel of six channelopathy genes in 274 SUD cases⁵, and subsequently with an expanded panel of 89 genes in 296 cases^{6,7}. The pathogenicity of the variants uncovered is sometimes unclear as different *in silico* analyses can yield conflicting predictions. Functional evaluation of a channelopathy variant is an essential aspect of the clinical validity curation process, as specified in the Standard Operating Procedures of the NIH Clinical Genome Resource Consortium^{8,9}. We have therefore started to systematically evaluate effects of channelopathy variants on channel function, including *SCN5A*¹⁰, *KCNH2*¹¹, *HCN4*¹², and *TRPM4*¹³.

The *ABCC9* gene encodes the SUR2 protein, which is an accessory subunit that combines with Kir6 pore-forming subunits to form a functional ATP-sensitive K⁺ (K_{ATP}) channel¹⁴. The most commonly studied SUR2 splice variants are SUR2A and SUR2B, which differ in their distal C-terminal 42 amino acids as a result of alternative exon usage¹⁴. The K_{ATP}

channels in the cardiac ventricle and specialized cardiac conduction system are respectively composed of Kir6.2/SUR2A and Kir6.1/Kir6.2/SUR2B subunit combinations¹⁴⁻¹⁶ and they modulate excitability during elevated heart rates and participate in protective responses against stress^{17, 18}. SUR2 subunits are also components of K_{ATP} channels in vascular smooth muscle and the vascular endothelium, where they modulate vascular membrane potential and contractions¹⁹⁻²². Additionally, SUR2 is expressed in several types of neurons, including central respiratory neurons²³⁻²⁶. In anaesthetized cats, K_{ATP} channels have been shown to contribute to the spontaneous activity and excitability of expiratory neurons - even during normoxic conditions²⁷. In mice, deficiency of *Abcc9* cause the onset of spontaneous coronary vasospasms that resemble Prinzmetal angina²⁸, whereas in humans, genetic variants in *ABCC9* have been linked to dilated cardiomyopathy, myocardial infarction, familial atrial fibrillation and Cantú syndrome²⁹⁻³². Our genetic testing studies are continually expanding and we have uncovered rare and novel variants in the *ABCC9* gene. The purpose of this study was to functionally evaluate these variants.

Materials and Methods

This study is exempt from human subject research approval requirements because cadaver specimens were used for genetic analysis. New York City Office of the Chief Medical Examiner (NYC-OCME) approved this study for diagnosis of the underlying causes of sudden expected death (SUD). Methods are available in the supplemental data document.

Results

ABCC9 variants associated with SUD

Four *ABCC9* variants were identified while performing variant classification of data from our genetic screening using a cardiac arrhythmogenic testing panel in ~300 cases of SUD⁶. The demographics and circumstances of death of the four cases are given in Table 1. Three of the cases were infants less than six months old, and one was an adult male. There was no dominance in terms of race or gender. Variant information is provided in Table 2, which lists the genome location, affected amino acids, and minor allele frequency as reported in gnomAD. These were all novel or rare variants. We used a variety *in silico* approaches to predict pathogenicity, but these predictions were often contradictory, leading us initially to classify these variants as “variants of uncertain significance” (VUS). Functional evaluation of a channelopathy variant is a necessary element in the clinical validity curation process^{3, 9} and the purpose of these studies was therefore to examine the effects of these variants on K_{ATP} channel function.

Location of amino acids in the SUR2A channel subunit

SUR2, the gene product of *ABCC9* and a subunit of vascular, muscle and neuronal K_{ATP} channels, consists of an extracellular N-terminus, a group of five transmembrane (TM) helices (TMD0), an intracellular linker to a second group of TM regions (TMD1), an intracellular region that contains a nucleotide binding fold (NBF1), a third group of TM regions (TMD2), and a C-terminal intracellular region that contains a second nucleotide binding fold (NBF2). The four identified variants each led to a missense nucleotide change,

causing amino acid substitutions in the protein region that is common between SUR2A and SUR2B. The membrane topology of SUR2 and the positions of the affected amino acids are shown in Figure 1. The positions of the residues are also depicted in sequence alignments of SUR1, SUR2A and SUR2B from several species (Figure S1). The human SUR2B is 99% identical in amino acid sequence to SUR2A, while SUR1 has an overall 67% sequence identity with SUR2A. The SUR2 A355, M941 and K1379 residues are completely preserved in SUR1 and across several species, whereas SUR2's H1305 corresponds to an asparagine (N) in SUR1. Although *ABCC8* (SUR1) variants are frequently associated with blood glucose and insulin release disorders such as familial hyperinsulinemic hypoglycemia and permanent or transient neonatal diabetes mellitus³³, we did not find evidence for disease association of corresponding residues in SUR1 (A359, M959, N1337 and K1411).

Effects of the SUR2A variants on K_{ATP} current amplitude

To examine the variant phenotypes, we co-expressed Kir6.2 with SUR2A cDNAs in HEK293 cells. This subunit combination gives rise to K_{ATP} channel currents resembling those present in the cardiac ventricle¹⁶. Patch clamp recordings were made in excised patches with the inside-out configuration³⁴, in which the cytosolic face of the excised membrane patch faces the experimental bath solution. Typical for when measuring K_{ATP} channels with this method, channel activity was present immediately after patch excision into a bath solution devoid of ATP, and channel activity was rapidly blocked when applying 1 mM 'cytosolic' ATP by using a rapid solution exchange system (Figure 2). For these studies, we have generated a human SUR2A construct, which resulted in smaller patch currents compared to the rat SUR2A construct that we have used in past studies^{35, 36}. For wild-type Kir6.2/SUR2A, 26 of 38 excised patches exhibited none, or only a few, active K_{ATP} channels under ATP-free conditions, whereas 12/38 patches exhibited robust currents with six or more active channels. This result suggests that clustering occurs and we have consequently limited subsequent analysis to patches that contain six or more channels. When examining the *ABCC9* variants, we found that the proportion of patches that contain six or more active channels did not differ from wild type (Table S1). In the presence of 1 mM ATP, K_{ATP} channel activity is not completely blocked and residual channel activity remains. We analyzed the unitary current amplitudes of these residual channels with all-point histograms (Figure 2). In aggregate, this analysis showed that the variants had no significant effects on K_{ATP} channel unitary currents (Figure 3A), suggesting that the conductance properties of the channel was unchanged. When examining the amount of current upon patch excision (the mean patch current) it was apparent that the currents of the A355S and M941V variants were larger when compared to WT (Figure 2). Indeed, summary data showed that currents of the A355S and M941V variants were statistically increased compared to WT (Figure 3B).

Effects of the SUR2A variants on ATP-sensitivity

Prototypically, the open probability of K_{ATP} channels is regulated by the intracellular ATP:ADP:AMP ratio¹⁴. We examined the nucleotide sensitivity of K_{ATP} channels by step-wise changing the 'cytosolic' ATP concentration, which led to a progressive corresponding changes in K_{ATP} channel mean patch current (Figure 4A). K_{ATP} channels comprised of wild-type SUR2A were half-maximally blocked by $\mu 30 \mu M$ ATP, whereas the K1379Q SUR2A variant was obviously less sensitive to ATP, requiring $\sim 100 \mu M$ ATP for the same degree of

block (arrows in Figure 4A). When plotting the mean patch current as a function of the ATP concentrations and subjecting data points to curve fitting to a logistic function, the ATP needed for half-maximal block (IC_{50}) was 41.4 μ M for WT and 139.8 μ M for the K1379Q variant (with slope factors of 0.99 and 0.83, respectively) (Figure 4B). When performing this analysis for all recorded data, we found that three variants, M941V, H1305Y and K1379Q, were significantly less sensitive to inhibitory ATP when compared to WT (Figure 4C).

Surface expression of K_{ATP} channels

Since the number of channels at the cell surface can determine the current amplitude, we used biochemical methods to determine this variable. Surface proteins of live transfected cells were labeled with biotin, whereupon cells were lysed, surface proteins were extracted by precipitation with NeutrAvidin-coated agarose beads, and followed by immunoblotting with anti-Kir6.2 antibodies to determine the amount of surface K_{ATP} channels (Figure 5A). Relative to the total amount of Kir6.2 in the cell lysates, none of the variants had obvious effects on the surface K_{ATP} channel expression (Figure 5B), suggesting that trafficking mechanisms were unaffected.

Discussion

Our studies have uncovered four *ABCC9* variants in four out of ~300 cases of SUD. All of the variants led to a gain-of-function phenotype of K_{ATP} channels.

The *ABCC9* variants have gain-of-function phenotypes

The total amount of current through a given class of ion channels is determined by:

$$I = N \cdot i \cdot p_o \dots \dots \dots (1),$$

where I is the total current, N is the number of functional channels, i is the unitary current through every channel, and p_o is the open probability. None of the variants affected the unitary current amplitudes (Figure 3A) or the number of surface channels (Figure 5B). Thus, each variant led to a significant increase in the channel open probability, but apparently through different mechanisms. The M941V variant caused both an increase in mean patch current and rendered the channel less sensitive to inhibitory intracellular ATP. Several cryo-EM structures have been resolved SUR1, but not for SUR2 to date. The residue corresponding to M941 is not present in the SUR1 structures (Figure 6A), suggesting that this residue exists in a disordered (and most likely a highly flexible) region of the subunit. We performed homology modeling of SUR2A based on a cryo-EM structure of SUR1 (PDB: 6C3O; see Figure S2 for the model quality metrics), which indeed predicts that M941 is present within a flexible intracellular loop that is distant from any of the nucleotide binding sites (Figure 6B). Our previous studies have demonstrated the existence of two coil-coiled domains in the SUR2 protein region that spans M941, and we have shown that the glycolytic enzyme aldolase interacts with the K_{ATP} channel via these coil-coiled domains³⁷. It is possible, therefore, that variants in this region may alter ATP-sensitivity and/or preferential regulation of the K_{ATP} channel by glycolytic enzymes³⁸. The residues in SUR1 (N1337 and K1411) that correspond to SUR2's H1305 and K1379, are located in the

vicinity of the nucleotide binding site of NBF2, but not sufficiently close to directly interfere with ATP/ADP binding to this site (Figure S3). Thus, the apparent change in ATP-sensitivity of these variant is likely due to long-distance interactions or it may be indirect. The A355S variant also led to a large increase in mean patch current, but without significantly affecting the ATP sensitivity. The A355 residue is located within a transmembrane α -helix near the extracellular surface of SUR2A and it is likely that mutagenesis of this residue causes a conformational change that promotes and increased open probability.

Functional classification of the ABCC9 variants

Determining the cause of sudden death in an infant, young person or adult, when all available tests are inconclusive or negative, remains a major challenge in forensic pathology. Genetic testing, or a ‘molecular autopsy’, has taken on an increasingly important role, but is faced with unique challenges since computational algorithms often provide conflicting predictions of pathogenicity. Functional characterization of ion channelopathy variants is a necessary element (along with variant rarity, and clinical, familial and segregation data) to assist forensic pathologists with a clinical diagnosis and to determine variant pathogenicity³. Here, we show that four rare or novel *ABCC9* variants found in SUD cause a significant gain-of-function of K_{ATP} channels. Diagnosticians need to include this information when deciding, based on the standard operating procedures of the NIH Clinical Genome Resource Consortium⁹, whether to reclassify these variants as ‘variants of uncertain significance’ or ‘likely pathogenic’.

Is it possible for the ABCC9 variants to contribute to SUD?

ABCC9 codes for SUR2A and SUR2B, which respectively are components of K_{ATP} channels in the heart/skeletal muscle and in vascular smooth muscle¹⁴. Genetic studies with mice, as well as human genetic variation, has demonstrated roles for SUR2 in spontaneous vasospasms, dilated cardiomyopathy, myocardial infarction, familial atrial fibrillation and Cantú syndrome²⁸⁻³². In addition, SUR2 mRNA and protein is also widely expressed in neurons and glial cells in the brain²³⁻²⁶. Importantly, K_{ATP} channels in central respiratory neurons continuously modulate and contribute to periodic adjustment of neuronal excitability. Moreover, recent studies show that K_{ATP} channels are also present in expiratory neurons of adult cats and contribute to the control of excitability - even during normoxia²⁷. Overactive K_{ATP} channels in smooth muscle may lead to loss of vascular pressor responses, whereas gain-of-function of K_{ATP} channels in central respiratory neurons may lead to electrical silencing and loss of respiratory regulation. A central feature of sudden infant death syndrome is unexpected death during sleep, which may have a respiratory component. While our studies cannot directly determine if (or how) the gain-of-function *ABCC9* variants found in our studies (together with other unknown factors²) contributed to unexpected death, loss of respiratory control is a real possibility; especially when considering that three of the four victims were under the age of 6-months old at the time of death.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Duncan JR, Byard RW. Sudden Infant Death Syndrome: An Overview In: Duncan JR, Byard RW, eds. SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future. Adelaide (AU); 2018.
2. Filiano JJ, Kinney HC. A perspective on neuropathologic findings in victims of the sudden infant death syndrome: the triple-risk model. *Biol Neonate*. 1994;65(3-4):194–197. [PubMed: 8038282]
3. Gando I, Yang HQ, Coetzee WA. Functional significance of channelopathy gene variants in unexplained death. *Forensic Sci Med Pathol*. 12 13 2018;293:37–46.
4. Skinner JR, Winbo A, Abrams D, Vohra J, Wilde AA. Channelopathies That Lead to Sudden Cardiac Death: Clinical and Genetic Aspects. *Heart Lung Circ*. 1 2019;28(1):22–30. [PubMed: 30389366]
5. Wang D, Shah KR, Um SY, et al. Cardiac channelopathy testing in 274 ethnically diverse sudden unexplained deaths. *Forensic Sci Int*. 4 2014;237:90–99. [PubMed: 24631775]
6. Lin Y, Williams N, Wang D, et al. Applying High-Resolution Variant Classification to Cardiac Arrhythmogenic Gene Testing in a Demographically Diverse Cohort of Sudden Unexplained Deaths. *Circ Cardiovasc Genet*. 12 2017;10(6).
7. Tang Y, Williams N, Sampson BA. Genetic testing in sudden unexpected natural death in the young: New York City Office of Chief Medical Examiner's experience and perspective. *Forensic Sci Med Pathol*. 12 7 2018.
8. Strande NT, Riggs ER, Buchanan AH, et al. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. *Am J Hum Genet*. 6 1 2017;100(6):895–906. [PubMed: 28552198]
9. Educational and Training Materials: Standard Operating Procedures, Version 6. Clinical Genome Resource. Available at: <https://www.clinicalgenome.org/curation-activities/gene-disease-validity/educational-and-training-materials/standard-operating-procedures/>.
10. Gando I, Morganstein J, Jana K, McDonald TV, Tang Y, Coetzee WA. Infant sudden death: Mutations responsible for impaired Nav1.5 channel trafficking and function. *Pacing Clin Electrophysiol*. 6 2017;40(6):703–712. [PubMed: 28370132]
11. Dong J, Williams N, Cerrone M, et al. Molecular autopsy: using the discovery of a novel de novo pathogenic variant in the KCNH2 gene to inform healthcare of surviving family. *Heliyon*. 12 2018;4(12):e01015. [PubMed: 30582040]
12. Dong J, Subbotina E, Williams N, Sampson BA, Tang Y, Coetzee WA. Functional reclassification of variants of uncertain significance in the HCN4 gene identified in sudden unexpected death. *Pacing Clin Electrophysiol*. 12 22 2018.
13. Subbotina E, Williams N, Sampson BA, Tang Y, Coetzee WA. Functional characterization of TRPM4 variants identified in sudden unexpected natural death. *Forensic Sci Int*. 10 24 2018;293:37–46. [PubMed: 30391667]
14. Foster MN, Coetzee WA. KATP Channels in the Cardiovascular System. *Physiol Rev*. 1 2016;96(1):177–252. [PubMed: 26660852]
15. Bao L, Kefaloyianni E, Lader J, et al. Unique properties of the ATP-sensitive K(+) channel in the mouse ventricular cardiac conduction system. *Circ Arrhythm Electrophysiol*. 12 2011;4(6):926–935. [PubMed: 21984445]
16. Babenko AP, Gonzalez G, Aguilar-Bryan L, Bryan J. Reconstituted human cardiac KATP channels: functional identity with the native channels from the sarcolemma of human ventricular cells. *Circ Res*. 1998;83(11):1132–1143. [PubMed: 9831708]

17. Zingman LV, Hodgson DM, Bast PH, et al. Kir6.2 is required for adaptation to stress. *Proc.Natl.Acad.Sci.U.S.A.* 2002;99(20):13278–13283. [PubMed: 12271142]
18. Zingman LV, Zhu Z, Sierra A, et al. Exercise-induced expression of cardiac ATP-sensitive potassium channels promotes action potential shortening and energy conservation. *J Mol Cell Cardiol.* 7 2011;51(1):72–81. [PubMed: 21439969]
19. Davies NW, Standen NB, Stanfield PR. Atp-Dependent Potassium Channels of Muscle-Cells - Their Properties, Regulation, and Possible Functions. *J Bioenerg Biomembr.* 8 1991;23(4):509–535. [PubMed: 1917907]
20. Yoshida H, Feig JE, Morrissey A, Ghiu IA, Artman M, Coetzee WA. KATP channels of primary human coronary artery endothelial cells consist of a heteromultimeric complex of Kir6.1, Kir6.2, and SUR2B subunits. *J Mol Cell Cardiol.* 10 2004;37(4):857–869. [PubMed: 15380676]
21. Morrissey A, Rosner E, Lanning J, et al. Immunolocalization of K(ATP) channel subunits in mouse and rat cardiac myocytes and the coronary vasculature. *BMC Physiol.* 2005;5(1):1. [PubMed: 15647111]
22. Tinker A, Aziz Q, Li Y, Specterman M. ATP-Sensitive Potassium Channels and Their Physiological and Pathophysiological Roles. *Compr Physiol.* 9 14 2018;8(4):1463–1511. [PubMed: 30215858]
23. Pelletier MR, Pahapill PA, Pennefather PS, Carlen PL. Analysis of single K(ATP) channels in mammalian dentate gyrus granule cells. *J Neurophysiol.* 2000;84(5):2291–2301. [PubMed: 11067973]
24. Zawar C, Plant TD, Schirra C, Konnerth A, Neumcke B. Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. *Journal Of Physiology-London.* 1999;514(2):327–341.
25. Zhou M, He HJ, Tanaka O, Sekiguchi M, Kawahara K, Abe H. Localization of the ATP-sensitive K(+) channel regulatory subunits SUR2A and SUR2B in the rat brain. *Neurosci Res.* 8 31 2012.
26. Nelson PT, Jicha GA, Wang WX, et al. ABCC9/SUR2 in the brain: Implications for hippocampal sclerosis of aging and a potential therapeutic target. *Ageing Res Rev.* 7 27 2015.
27. Pierrefiche O, Bischoff AM, Richter DW. ATP-sensitive K⁺ channels are functional in expiratory neurones of normoxic cats. *J Physiol.* 7 15 1996;494 (Pt 2):399–409. [PubMed: 8842000]
28. Chutkow WA, Pu J, Wheeler MT, et al. Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K(ATP) channels. *J Clin Invest.* 7 2002;110(2):203–208. [PubMed: 12122112]
29. Huang Y, McClenaghan C, Harter TM, et al. Cardiovascular consequences of K_{ATP} overactivity in Cantu syndrome. *JCI Insight.* 8 9 2018;3(15).
30. Bienengraeber M, Olson TM, Selivanov VA, et al. ABCC9 mutations identified in human dilated cardiomyopathy disrupt catalytic K_{ATP} channel gating. *Nat Genet.* 4 2004;36(4):382–387. [PubMed: 15034580]
31. Minorette P, Falcone C, Aldeghi A, et al. A novel Val734Ile variant in the ABCC9 gene associated with myocardial infarction. *Clin Chim Acta.* 8 2006;370(1-2):124–128. [PubMed: 16563363]
32. Olson TM, Alekseev AE, Moreau C, et al. KATP channel mutation confers risk for vein of Marshall adrenergic atrial fibrillation. *Nat Clin Pract Cardiovasc Med.* 2 2007;4(2):110–116. [PubMed: 17245405]
33. Aittoniemi J, Fotinou C, Craig TJ, de WH, Proks P, Ashcroft FM. Review. SUR1: a unique ATP-binding cassette protein that functions as an ion channel regulator. *Philos.Trans.R.Soc.Lond B Biol.Sci* 2009;364(1514):257–267. [PubMed: 18990670]
34. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Archives-European Journal of Physiology.* 1981;391:85–100. [PubMed: 6270629]
35. Yang HQ, Jana K, Rindler MJ, Coetzee WA. The trafficking protein, EHD2, positively regulates cardiac sarcolemmal K_{ATP} channel surface expression: role in cardioprotection. *FASEB J* 3 2018;32(3):1613–1625. [PubMed: 29133341]
36. Yoshida H, Bao L, Kefaloyianni E, et al. AMP-activated protein kinase connects cellular energy metabolism to K_{ATP} channel function. *J Mol Cell Cardiol.* 2 2012;52(2):410–418. [PubMed: 21888913]

37. Hong M, Kefaloyianni E, Bao L, et al. Cardiac ATP-sensitive K⁺ channel associates with the glycolytic enzyme complex. *FASEB J.* 7 2011;25(7):2456–2467. [PubMed: 21482559]
38. Weiss JN, Lamp ST. Cardiac ATP-sensitive K⁺ channels. Evidence for preferential regulation by glycolysis. *J Gen Physiol.* 11 1989;94(5):911–935. [PubMed: 2512370]

Highlights

- Channelopathies are often associated with inherited arrhythmias and sudden death.
- Genetic testing can assist in determining the likely causes of sudden unexpected death.
- We identified four *ABCC9* variants in sudden unexpected death.
- All four variants caused a gain-of-function phenotype of K_{ATP} channels.

Figure 1:

Topological representation of the human SUR2 subunit, indicating the positions of amino acids affected by the variants. SUR2A is shown as a representative example, which combines with Kir6.x subunits to form a K_{ATP} channel. SUR consists of three groups of transmembrane domains (TMD0, TMD1 and TMD2). Two large intracellular nucleotide binding folds (NB1 and NBF2) each contains Walker A and Walker B domains that coordinate nucleotide binding.

Figure 2:

Representative K_{ATP} channel current traces. HEK293 cells were transfected with Kir6.2 plus human SUR2A cDNAs and K_{ATP} channel activity was recorded in the excised inside-out patch clamp mode at a membrane potential of -80 mV. Shown are representative currents of channels composed of wild type SUR2A, SUR2-A355S and SUR2-M941V immediately after patch excision into ATP-free batch solutions and when applying 1 mM ATP to the 'cytosolic' face of the membrane. All-point histograms (right panels) were constructed from residual channels still open in the presence of 1 mM ATP.

Figure 3: Effects of *ABCC9* variants on K_{ATP} channel properties. Shown are summary data of A) the unitary currents at -80 mV and B) the mean patch current under ATP-free conditions. The genotypes shown are wild type (WT; $n=12$), A355S ($n=5$), M941V ($n=6$), H1305Y ($n=7$), and K1355Q ($n=12$). Data are shown as means and SEMs. * $p < 0.05$ with 1W-ANOVA, followed by a Dunnett's test vs. WT

Figure 4:

Effects of SUR2 variants on the sensitivity of K_{ATP} channels to 'cytosolic' ATP. HEK-293 cells were transfected with Kir6.2 plus SUR variants and K_{ATP} channels were recorded in the excised inside-out patch clamp configuration. A) Representative examples of currents generated by wild type SUR2A (WT) and SUR2A-K1379Q. The horizontal bars indicate the rapid application of ATP (concentrations in μM). The dotted line indicates the zero current level and the white arrows depict a rough estimate of half-maximal current. B) The normalized currents in panel A (I/I_{max}) were plotted as a function of the ATP concentration and data points were subjected to curve fitting to a logistic function $1/(1 + (\text{ATP}/\text{IC}_{50})^h)$, where ATP is the bath ATP concentration, IC_{50} the ATP value needed for half-maximal block and h a slope factor. C) Summary data (means and SEM) of aggregate IC_{50} values for SUR genotypes, wild type (WT; $n=12$), A355S ($n=5$), M941V ($n=6$), H1305Y ($n=7$), and K1355Q ($n=12$). * $p < 0.05$ with 1W-ANOVA, followed by a Dunnett's test vs. WT

Figure 5.

Surface expression of K_{ATP} channels. HEK-293 cells were transfected with Kir6.2 plus SUR variants and surface expression was determined using biotinylation methods. A) Live cells were surface biotinylated and surface proteins were isolated with agarose-NeutrAvidin beads. Shown are representative Western blots of total Kir6.2 and β -actin expression in lysates of cells co-expressing Kir6.2 and with SUR2A variants, as well as immunoblots of the biotinylated protein fraction (surface proteins). B) Quantification of total and surface Kir6.2 levels. Total protein was normalized to β -actin relative to wild type, whereas surface Kir6.2 levels are expressed as a function of total Kir6.2 protein. Shown are averaged data (means and SEMs) Kir6.2 and SUR2A variants: wild type (WT) (n=3), A355S (n=3), M941V (n=3), H1305Y (n=3), and K1355Q (n=3). $P > 0.05$ with 1W-ANOVA.

Figure 6:

Cryo-EM structures of Kir6.2/SUR1 channels and molecular model of human SUR2A. A) Coordinates of the Kir6.2/SUR1 cryo-EM structure (PDB: 6c3o) in 'top' view (from the extracellular side) displayed using UCSF Chimera. Highlighted are residues (corresponding SUR2 residues in brackets) A359 (A355), N1337 (H1305) and K1411 (K1379). Residue M959 (M941) was not present in the structure. The four SUR1 chains are displayed in different colors. B) The same structure as in panel A, but turned by 90° on the X-axis to display the channel from the 'side'. C) Overlay of SUR1 chain G (from PDB: 6c3o; yellow) and a molecular model of a SUR2A subunit (cyan), calculated using SWISS-MODEL when using PDB 6c3o chain G as a template. D) Molecular model of the SUR2A subunit, displaying the positions and localization of the variants described in this study.

Table 1:

Patient demographics

Case	Age	Sex	Ethnicity	Notes
A	34y	M	Asian	34 y/o heavy smoker Asian male found dead in bed w/ unknown medical history. Negative autopsy
B	2m	M	Hispanic	A full term 11 week-old boy with no prior medical history found dead in his bassinette. Baby was put to sleep on his right side and was found with no vital signs the next morning.
C	2m	F	White	Full term with unremarkable medical history. Died in mother's arm before feeding. Fussy and crying with difficulty feeding for two days prior to death. Objection to autopsy
D	4m	M	Black	Unknown medical history. Recently seen for cough. The patient became unresponsive at day care, with conflicting stories as to whether it happened while he was asleep or while feeding. Histology is essentially negative with some minor upper respiratory inflammation.

Table 2:

Variant information and classification

Case	Nucleotide	gnomAD	Amino Acid	ClinVar	Other variants	Prediction	Original Classification
A	g.12:21968807G>A	East Asian: 6/19,944 African: 1/24,944 Overall: 7/282,502 (0.002%)	p.His1305Tyr	Not present	None	Neutral [†] , Damaging [‡] , Benign* Polymorphism**	VUS
B	g.12:22001129T>C	European: 1/21,640 Overall: 1/251,384 (0.0004%)	p.Met941Val	Not present	PRKAG2 (p.Glu185Val)	Neutral [†] , Tolerated [‡] , Benign* Disease Causing**	VUS
C	g.12:21965059T>G	Not present	p.Lys1379Gln	Not present	AKAP9 (p.Ile3647Thr) RYR2 (p.Ser756Asn) MYH6 (p.Thr627Asn)	Neutral [†] , Tolerated [‡] , Benign* Disease Causing**	VUS
D	g.12:22063861C>A	Latino: 2/34,492 European: 5/113,360 Overall: 7/250,866 (0.003%)	p.Ala355Ser	45382	None	Neutral [†] , Damaging [‡] , Damaging* Disease Causing**	VUS

The GenBank IDs of the nucleotide and protein are respectively are NM_005691.3 and NP_005682.2. Nucleotide position is referenced to the human genome build GRCh37. The population allele frequency is estimated from The Genome Aggregation Database (gnomAD) database (<http://gnomad.broadinstitute.org/>), searched on 12/26/2018. The ClinVar IDs refer to the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>). Predictions were made using PolyPhen-2*, Provean[†] or SIFT[‡] (<http://provean.jcvi.org/index.php>) or Mutation taster** (<http://www.mutationtaster.org/>). VUS = variant of uncertain significance.