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Loss of Function ABCC8 Mutations in Pulmonary Arterial Hypertension

Running Title: Bohnen et al.; ABCC8 Mutations in PAH

Michael S. Bohnen, PhD[1], Lijiang Ma, PhD[2], Na Zhu, PhD[2,4], Hongjian Qi, BS[3,4], Conor McClenaghan, PhD[5], Claudia Gonzaga-Jauregui, PhD[6], Frederick E. Dewey, MD[6], John D. Overton, PhD[6], Jeffrey G. Reid, PhD[6], Alan R. Shuldiner, MD[6], Aris Baras, MD[6], Kevin J. Sampson, PhD[1], Marta Bleda, PhD[7], Charaka Hadinnapola, MB, BChir[7], Matthias Haimel, BSc[7], Harm J. Bogaard, MD, PhD[8], Colin Church, PhD[9], Gerry Coghlan, FRCP[10], Paul A. Corris, MB, BS, FRCP[11], Mélanie Eyries, PhD[12], J. Simon R. Gibbs, MD[13], Barbara Girerd, PhD[14], Arjan C. Houweling, MD, PhD[8], Marc Humbert, MD, PhD[14], Christophe Guignabert, PhD[14], David G. Kiely, MD[15], Allan Lawrie, PhD[16], Rob V. MacKenzie Ross, MB, BChir[17], Jennifer M. Martin, BSc(Hons)[7], David Montani, MD, PhD[14], Andrew J. Peacock, MD[9], Joanna Pepke-Zaba, PhD[18], Florent Soubrier, MD, PhD[12], Jay Suntharalingam, MD[17], Mark Toshner, MD[7, 18], Carmen M. Treacy, BSc(Hons)[7], Richard C. Trembath, MD[19], Anton Vonk Noordegraaf, MD, PhD[8], John Wharton, PhD[20], Martin R. Wilkins, MD[20], Stephen J. Wort, PhD[13, 21], Katherine Yates[7], Stefan Gräf, PhD[7,22], Nicholas W. Morrell, MD[7], Usha Krishnan, MD, DM[2], Erika B. Rosenzweig, MD[2], Yufeng Shen, PhD[3,4], Colin G. Nichols, PhD[5], Robert S. Kass, PhD[1], Wendy K. Chung, MD, PhD[2]

[1]Dept of Pharmacology, College of Physicians and Surgeons, [2]Dept of Pediatrics, College of Physicians & Surgeons, [3]Dept of Applied Physics & Applied Mathematics, [4]Dept of Systems Biology, Columbia University, New York, NY; [5]Dept of Cell Biology & Physiology, and the Centre for the Investigation of Membrane Excitability Diseases, Washington University School of Medicine, Washington University in St Louis, St Louis, MO; [6]Regeneron Genetics Center, Regeneron Pharmaceuticals Inc. Tarrytown, NY; [7]Dept of Medicine, [22]Dept of Haematology, University of Cambridge, Addenbrookes Hospital, Hills Road, Cambridge, United Kingdom; [8]VU University Medical Center, Amsterdam, Netherlands; [9]Golden Jubilee National Hospital, Glasgow, Scotland; [10]Royal Free Hospital, London; [11]Newcastle University & The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom; [12]Dépt de génétique, hôpital Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, and UMR_S 1166-ICAN, INSERM, UPMC Sorbonne Universités, Paris, France; [13]National Heart & Lung Institute, Imperial College London, United Kingdom; [14]Université Paris-Sud, Faculté de Médecine, Université Paris-Saclay, AP-HP, Centre de référence de l'hypertension pulmonaire sévère, INSERM UMR_S 999, Hôpital Bicêtre, Le Kremlin-Bicêtre, France; [15]Sheffield Clinical Research Facility, Royal Hallamshire; [16]Dept of Infection, Immunity & Cardiovascular Disease, University of Sheffield; [17]Royal United Bath Hospitals; [18]Papworth Hospital, Cambridge; [19]Division of Genetics & Molecular Medicine, King's College, London, [20]Dept of Medicine, Imperial College London, Hammersmith Campus, London; [21]Royal Brompton Hospital, London, United Kingdom

Correspondence:

Wendy K. Chung, MD PhD.

1150 St. Nicholas Avenue, Room 620

New York, NY 10032

Tel: 212-8515313

Fax: 212-851-5306

E-mail: wkc15@cumc.columbia.edu

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Journal Subject Terms: Pulmonary Hypertension; Ion Channels/Membrane Transport; Genetics; Electrophysiology; Basic Science Research

ABSTRACT:

Background: In pulmonary arterial hypertension (PAH), pathological changes in pulmonary arterioles progressively raise pulmonary artery pressure and increase pulmonary vascular resistance, leading to right heart failure and high mortality rates. Recently, the first potassium channelopathy in PAH, due to mutations in KCNK3, was identified as a genetic cause and pharmacological target.

Methods: Exome sequencing was performed to identify novel genes in a cohort of 99 pediatric and 134 adult onset group I pulmonary arterial hypertension patients. Novel rare variants in the gene identified were independently identified in a cohort of 680 adult onset patients. Variants were expressed in COS cells and function assessed by patch-clamp and rubidium flux analysis.

Results: We identified a de novo novel heterozygous predicted deleterious missense variant c.G2873A (p.R958H) in ABCC8 (ATP-binding cassette, subfamily C, member 8) in a child with idiopathic PAH. We then evaluated all individuals in the original and a second cohort for rare or novel variants in ABCC8 and identified 11 additional heterozygous predicted damaging ABCC8 variants. ABCC8 encodes sulfonylurea receptor 1 (SUR1), a regulatory subunit of the ATP-sensitive potassium channel (K_{ATP}). We observed loss of K_{ATP} function for all ABCC8 variants evaluated, and pharmacological rescue of all channel currents in vitro by the SUR1 activator, diazoxide.

Conclusions: Novel and rare missense variants in ABCC8 are associated with pulmonary arterial hypertension. Identified ABCC8 mutations decreased K_{ATP} channel function, which was pharmacologically recovered.

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Key words: pulmonary hypertension; genetics, human; electrophysiology; ion channel; pharmacology; ABCC8; SUR1

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare and often fatal disease characterized by distinctive changes in pulmonary arterioles that lead to elevated pulmonary artery pressure and right sided heart failure. Novel treatment options have decreased mortality, but PAH remains a frequently fatal illness. The heterogeneity in disease etiology and nonspecific patient presentation often delays diagnosis, contributing to poor outcomes.

Genetics are recognized to play an important role in the pathogenesis of PAH in patients with and without a family history of disease. Most genetic studies of the disease have been performed in patients with adult-onset disease. Germline mutations in bone morphogenetic protein receptor type 2 (BMPR2), a member of the transforming growth factor β (TGFβ) superfamily of receptors, have been identified as the major genetic cause, including in 70% of inherited and 10–40% of idiopathic cases.[1, 2] Mutations in other TGFβ family members comprise additional rare genetic causes. The prevalence of the disease in children is estimated at 2.2 cases per million, an order of magnitude lower than the estimated prevalence of 15-50 cases per million in adults,[3] and there are few genetic studies of individuals with childhood-onset PAH.

We previously used exome sequencing to identify mutations in the KCNK3 potassium channel as a genetic cause of idiopathic and familial PAH.[4] Furthermore, we proposed KCNK3 as a novel pharmacological target in PAH, as potassium channel currents of select mutant and wildtype KCNK3 channels were pharmacologically remedied by ONO-RS-082, an experimental compound.[4, 5] In the current study, we report a novel association of ABCC8/ SUR1 loss of function mutations in both pediatric and adult onset PAH. ABCC8 encodes sulfonylurea receptor 1 (SUR1), a regulatory subunit of the ATP-sensitive potassium channel (K_{ATP}). We have functionally assessed mutant K_{ATP} channels, and characterized their pharmacological activation.

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METHODS

The sequencing data and methods will be made available to other researchers for purposes of reproducing the results or replicating the procedures. The study was approved by the institutional review board at Columbia University Medical Center. Detailed methods are available as supplemental data.

RESULTS

Inherited and de novo variants in ABCC8

By exome sequencing, we identified a de novo missense variant c.G2873A (p.R958H) in ATP-binding cassette, subfamily C, member 8 (ABCC8) (NM_001287174), which was predicted to be deleterious, in a patient diagnosed with idiopathic PAH at the age of 10 (Table 1). We then examined all CU-PAH patients for rare or novel variants in ABCC8 and identified seven additional rare damaging missense variants, predicted by multiple algorithms to be deleterious in seven unrelated patients with idiopathic, familial, or congenital heart disease associated pulmonary arterial hypertension (Table 1). In one familial case, the p.A240T variant was transmitted from an affected father and also observed in the affected sibling. To replicate the findings in the CU-PAH cohort, we evaluated the UK-PAH cohort and identified three additional rare or novel missense and one splice variant in ABCC8 in three patients with idiopathic and one patient with congenital heart disease associated PAH (Table 1). Five variants – c.A214G (p.N72D), c.G558T (p.E186D), c.G718A (p.A240T), c.G2371C (p.E791Q), and c.T2694+2G -- were novel; four rare variants – c.G331A (p.G111R), c.C403G (p.L135V), c.G2437A (p.D813N), and c.G4414A (p.D1472N) -- have been reported in patients with congenital hyperinsulinism; and two variants – c.C686T (p.T229I) and c.G3941A (p.R1314H) – have been

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reported in patients with transient or permanent neonatal diabetes mellitus.[6-12] Alignment of the ABCC8 sequence revealed that all missense variants occur at amino acid residues conserved across species and in critical domains (Figure 1A and 1B).

All individuals were heterozygous for these rare ABCC8 variants. The L135V, D813N, and R1314H variants were inherited from an unaffected father and represented PAH risk variants in childhood-onset PAH, while E791Q and D1472N were inherited from an unaffected mother and represented PAH risk variants in childhood-onset PAH. The ABCC8 p.R1314H carrier also had a TBX4 c.1106delC: p.S369fs mutation. None of the other ABCC8 carriers had mutations in any known PAH genes. Other predicted deleterious variants throughout the genome carried by the ABCC8 discovery cohort are listed in Supplementary Table S1. Seven of the probands were female and five were male. None of the probands had any evidence of hyperinsulinism or transient/permanent neonatal diabetes mellitus although one adult patient had type 2 diabetes. Six of the patients were children at the time of diagnosis. Three patients responded and four patients did not respond to acute vasodilator testing using inhaled nitric oxide during cardiac catheterization. Two patient had evidence of a cardiac arrhythmia (Table 1).

To estimate the genetic effect size of ABCC8 variants, we selected 33,369 European adult subjects from Exome Aggregation Consortium (ExAC) and 49,630 European ancestry subjects from the Regeneron-Geisinger DiscovEHR study independently as controls,[13] and tested for an excess of rare (minor allele frequency ≤0.1%) predicted deleterious missense variants in cases compared to controls. There were six rare predicted deleterious alleles in 150 PAH cases of European ancestry in the CU-PAH cohort, while 158 unique deleterious variants were observed a total of 295 times in 33,369 controls with exome sequencing in the ExAC dataset and 165 rare unique deleterious variants were observed 712 times in the DiscovEHR study. With binomial

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tests, we observed significant excess of rare predicted deleterious missense variants in ABCC8 in CU-PAH cases when comparing to ExAC controls (P-value = 0.0023, Enrichment rate = 4.5) and to DiscovEHR controls (P-value = 0.022, Enrichment rate = 2.8). We tested the association of predicted benign ABCC8 variants and identified two rare synonymous alleles in cases, 223 unique predicted benign missense variants or synonymous variants observed a total of 512 times in controls, and found no significant difference between the CU-PAH group and ExAC (P-value = 1; relative risk = 0.87).

ABCC8 expression in human lung

ABCC8 encodes the sulfonylurea receptor 1 (SUR1) protein, a regulatory subunit of the K_{ATP} channel, which associates with the pore-forming Kir6.2 subunit.[14] SUR1 controls cell excitability by regulating trafficking and expression of the K_{ATP} channel, and confers sensitivity of K_{ATP} channels to magnesium-nucleotides and pharmacological modulators. SUR1-dependent K_{ATP} channels are prominent in neuronal and pancreatic tissues, but present in many other tissues, including cardiac atria.[14-16] We demonstrate that ABCC8 is expressed in lungs of patients with PAH and in healthy individuals (Supplementary Figure S1), providing a potential target for influencing and modulating PAH. We replicated our finding of ABCC8 gene expression in a second set of lung samples from heritable PAH patients with known BMPR2 mutations (HPAH) and healthy controls. We observed a significant increase in ABCC8 expression in lungs of BMPR2-associated HPAH patient samples (Supplementary Figure S2).

To determine the cell types expressing SUR1 protein in lungs of idiopathic PAH (IPAH) patients, confocal microscopic analyses and triple labeling with ABCC8, CD68 and SM22 antibodies were used in paraffin-embedded lungs from six IPAH patient lung samples. Strong

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staining for SUR1 was found in a population of alveolar macrophages, and moderate staining for SUR1 was observed in proximal pulmonary arteries (Supplementary Figure S3).

Functional characterization of ABCC8 mutations

We examined the consequence of eight of the twelve identified ABCC8 variants putatively associated with PAH on SUR1 function using two complementary measures of K_{ATP} activity: (1) patch-clamp electrophysiology provided a direct measurement of whole cell K_{ATP} conductance in individual cells across different membrane potentials; and (2) rubidium ($^{86}Rb^{+}$) flux assays provided quantification of channel activity using $^{86}Rb^{+}$ efflux as a measure of macroscopic K_{ATP} conductance from a population of intact cells. By co-expressing Kir6.2 with SUR1 in COS cells, functional K_{ATP} channels were formed in each assay. All SUR1 variants tested demonstrated loss of function in at least one functional assay.

First, we used patch-clamp experiments to directly measure SUR1-dependent K_{ATP} channel activity by applying a voltage ramp in whole-cell conditions (Figure 2A), using an established assay.[17] We maximally activated K_{ATP} channel currents with diazoxide (100μM), a selective SUR1 activator. Once steady-state diazoxide current activation was achieved, glibenclamide (10μM) – which inhibits K_{ATP} channels by binding to the SUR subunit – was co-applied. The glibenclamide-sensitive current was taken as the SUR1-dependent K_{ATP} current.[17] A series of control experiments for assay validation are shown in Supplementary Figures S4 and S5.

Robust SUR1-dependent K_{ATP} currents were measured in cells expressing Kir6.2 and wildtype SUR1 (Figure 2B). By contrast, currents were much smaller in cells expressing A240T SUR1, one of the novel SUR1 variants identified. Similarly small currents were observed in cells expressing D813N or D1472N SUR1, previously reported mutations in congenital

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hyperinsulinism (Figure 2B). Further analysis demonstrated significantly reduced SUR1-dependent currents in six of eight SUR1 mutants functionally evaluated (E186D, A240T, E791Q, D813N, R1314H, and D1472N), and non-significant reductions in L135V and R958H (Figure 2C).

Next, $^{86}Rb^{+}$ efflux rate was recorded as a measure of K_{ATP} channel activity in cells expressing wildtype or mutant SUR1 along with Kir6.2 (Figure 3), in basal metabolic conditions and in metabolic inhibition. Compared to wildtype SUR1, basal conditions yielded marked decreases in efflux rate for the A240T, D813N, and D1472N variants, and smaller decreases for L135V, E791Q, R958H, and R1314H [Figure 3A and 3B; Supplementary Table S1]. In metabolic inhibition (extracellular solution supplemented with 2-deoxy-D-glucose and oligomycin to impair ATP synthesis and relieve K_{ATP} channels from inhibition by intracellular nucleotides), the flux rates for the L135V, A240T, D813N, R958H and D1472N mutants were markedly lower than wildtype (Figure 3C and 3D; Supplementary Table S2). The E791Q and R1314H mutant fluxes were slightly reduced, while no decrease in flux under basal or metabolic inhibition conditions was observed for E186D.

Thus, when combining results from the whole-cell patch clamp and rubidium flux functional assays, there was a significant decrease in basal and/or maximal channel activity for all SUR1 mutants associated with PAH that were functionally tested. SUR1 and K_{ATP} loss of function could result from various mechanisms, but any channel activity present might be augmented by selective potassium channel opening drugs, such as diazoxide. Consistent with this suggestion, all mutants tested were pharmacologically activated by diazoxide (100µM) in rubidium efflux (Figure 4A and 4B; Supplementary Table S3), and whole-cell patch clamp (Figure 4C and 4D; Supplementary Figure S4-F).

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DISCUSSION

Using exome sequencing, we identified de novo and inherited heterozygous mutations in a novel candidate gene, ABCC8, potentially associated with idiopathic, familial, and congenital heart disease associated PAH, in eight independent families, with six of the probands diagnosed as children in the CU-PAH study. We identified an additional four rare or novel predicted damaging missense and splice variants in ABCC8 in a second cohort (UK-PAH) of adult group I PAH patients with idiopathic, familial, congenital heart disease-associated or appetite drug-associated disease. The mutations are incompletely penetrant similar to most other genes for PAH, and penetrance may depend on additional genetic or environmental modifiers. Functional analyses demonstrated reduced ATP-sensitive potassium (K_{ATP}) channel activity in all SUR1 mutants tested and pharmacological rescue of K_{ATP} activity in vitro by diazoxide.

ABCC8 encodes the sulfonylurea receptor 1 (SUR1) protein, a K_{ATP} channel subunit. The eleven identified missense ABCC8 variants are all rare, located at residues highly conserved across species, and reside in intracellular and transmembrane domains of SUR1, including nucleotide binding fold regions (Figure 1). ABCC8 is highly expressed in the human brain and endocrine pancreas, and moderately expressed in human lungs[18] (Supplementary Figures S1 and S2). SUR1 expression has been observed in intact rat pulmonary arteries,[19] while K_{ATP} channel activity was shown to be upregulated by elevated shear stress in pulmonary vascular endothelial cells.[20] More recently, SUR1 upregulation by hypoxia was reported in cerebral microvascular endothelial cells.[21, 22] As we have observed upregulation of ABCC8 in BMPR2-associated heritable PAH patient samples (Supplementary Figure S2), as well as SUR1 protein in both alveolar macrophages and proximal pulmonary arteries within the lung (Supplementary Figure S3), further studies may elucidate SUR1's primary physiologic role in the pulmonary

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vasculature, and how exactly its dysfunction and subsequent reduction in K_{ATP} currents contribute to PAH in some patients.

KCNK3, established as the first potassium channelopathy in PAH,[4, 5, 23] is also regulated by hypoxia in pulmonary artery smooth muscle cells, and may contribute to hypoxic pulmonary vasoconstriction.[24] In the lung, SUR1-dependent K_{ATP} channel loss of function alongside KCNK3 channel loss of function represent possible pathogenic mechanisms in PAH, and pharmacologic recovery of channel function a therapeutic avenue.[4, 5, 23] Moreover, heteromeric channel assembly of SUR1 and KCNK3 with related channel subunits is well documented for both K_{ATP} and KCNK3 in various organs.[14, 25, 26] This complementary and redundant potassium channel activity could contribute to the lung-specific phenotype observed clinically in patients with heterozygous ABCC8 or KCNK3 mutation.[5]

Despite loss of ABCC8 function underlying many cases of congenital hyperinsulinism, the patients in our study have no evidence of hyperinsulinemic hypoglycemia or transient/permanent neonatal diabetes mellitus. This raises the question: why do SUR1-dependent PAH patients not have hyperinsulinism, and vice versa, why do hyperinsulinism patients not have evidence of PAH? Ultimately, a combination of genetic, developmental, and environmental factors may determine which patients with ABCC8 mutations develop PAH.

The mechanism of SUR1 loss of function likely varies based on mutation location with the channel subunit. For instance, G111R and D1472N have been previously shown to decrease SUR1 trafficking to the plasma membrane,[7, 12, 27] while nucleotide binding fold mutations, D813N and R958H, may impair magnesium-nucleotide activation. SUR1 mutation severity impacts viability for pharmacological rescue, as previously demonstrated for KCNK3 mutant channels associated with PAH,[4, 5] and for SUR1 mutants associated with congenital

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hyperinsulinism.[28] Alongside pharmacological activation of SUR1-containing K_{ATP} channels, ascertaining the mechanism of loss of function of all SUR1 variants in our study has important implications for disease pathogenesis and the therapeutic potential of K_{ATP} activation in PAH. Understanding mechanism of dysfunction may be accomplished by screening for SUR1 defects in trafficking, gene expression, regulation by nucleotides, and post-translational modifications.

As mainstay treatment in congenital hyperinsulinism, diazoxide administration overcomes disease-causing ABCC8 loss-of-function mutations. We observed variable functional recovery in vitro by diazoxide of each SUR1 mutant tested in our study of PAH patients with ABCC8 mutations. Diazoxide is a SUR1 activator clinically employed as an anti-hypertensive and anti-hyperinsulinism agent. Case reports from many years ago described the successful use of diazoxide to reverse pulmonary hypertension; [29, 30] however, hypoglycemic infants treated with diazoxide have developed pulmonary hypertension.[31] This may be secondary to inadequate diuresis with diazoxide treatment, leading to volume overload following systemic blood volume expansion. Until diazoxide is proven to be safe, we do not recommend diazoxide as a treatment for pulmonary hypertension. While SUR1-dependent K_{ATP} activation is an intriguing potential basis for pulmonary hypertension therapy,[32] ultimately, K_{ATP} channel activators with less pulmonary toxicity may prove useful for pulmonary hypertension treatment.[33]

In conclusion, we have identified mutations in the ABCC8 gene as a potential second potassium channelopathy in PAH, and as a possible therapeutic target.

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Table 1. Pathogenicity Predictions and Clinical Characteristics of Patients with Pulmonary Arterial Hypertension with *ABCC8* Mutations. ExAC frequency lists the allele frequency for all of ExAC followed by the allele frequency in the patient's ethnic **group.**

Nucleotide and AA variant	c.A214G: p.N72D	c.G331A: p.G111R	c.C403G: p.L135V	c.G558T: p.E186D	c.C686T: p.T229I	c.G718A: p.A240T	c.G2371C: p.E791Q	c.G2437A: p.D813N	c.G2873A: p.R958H	c.G3941A: p.R1314H	c.G4414A:p .D1472N	c.T2694+2G: p.NA
ExAC_Freq	0	0.00002/ 0.00001	0.00003/ 0.00006	0	0.000008/ 0.00001	0	0	0.0001/ 0.0012	0.00002/ 0.00001	0.00002/ 0.00001	0	0
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Hispanic	Asian	Caucasian	Caucasian	Caucasian	Caucasian
MetaSVM_pred	D	D	D	D	D	D	D	D	D	D	D	N/A
CADD_phred	22.8	28.4	20.5	19.46	29.4	35	32	35	27.9	23	22.4	25.1
Cohort	UK	UK	CU	CU	UK	CU	CU	CU	CU	CU	CU	UK
PAH type	CHD associated	Idiopathic	Idiopathic	Idiopathic	Idiopathic	Familial; Sister and father with PAH	Familial; two deceased affected siblings not tested	Idiopathic	Idiopathic	CHD-associated	Idiopathic	Idiopathic
Inheritance/ segregation	Unknown	Unknown	Paternal	Unknown	Unknown	Paternal; father and affected sister carry p.A240T	Maternal; affected siblings unavailable for analysis	Paternal	De novo	Paternal	Maternal	Unknown
Gender	F	M	M	M	F	F	F	M	F	M	F	F
Age of diagnosis	35	64	5	79	34	Unknown	14	12	10	<1	9	60
RAP M (mmHg)	N/A	11	9	N/A	9	N/A	N/A	25	6	8	7	7
PAP M (mmHg)	N/A	44	55	N/A	45	N/A	N/A	54	56	54	37	36
AOP M (mmHg)	N/A	Unknown	78	N/A	Unknown	N/A	N/A	48	88	57	67	Unknown
PVRi (U*m2)	N/A	19	25	N/A	13	N/A	N/A	29	16	13.6	17	19
Art Sat %	N/A	97	91	N/A	98	N/A	N/A	93	91	97	96	97
PCWPm (mmHg)	N/A	15	8	N/A	10	N/A	N/A	N/A	8	8	N/A	6
CI (L/min/m2)	N/A	1.5	2.8	N/A	2.7	N/A	N/A	N/A	2.8	3.5	2	1.6
Response to acute vasodilator test	N/A	Unknown	Yes	Unknown	No	Unknown	Unknown	No	Yes	No	Yes	No
Other genetic variants in PAH genes	None	None	None	None	None	None	None	None	None	TBX4 c.1106delC: p.S369fs	None	None
Cardiac arrhythmias; other conditions	Large atrial septal defect	Type 2 Diabetes Mellitus	Bigeminy, 1st degree heart block	None	Hearing loss Hypothyroid Lipodermato-sclerosis ESRD Raynaud syndrome	None	None	Atrial flutter Nonspecific intra-ventricular block Autism	None	Ventricular septal defect	None	None

Abbreviations: AA= amino acid, M=male, F=female, N/A=not available, RAP M=mean right atrial pressure, PAP M=mean pulmonary arterial pressure, AOP M=mean aortic pressure, PVRi=pulmonary vascular resistance index, Art Sat=arterial oxygen saturation, PCWPm=mean pulmonary capillary wedge pressure, CI=cardiac index. UK=United Kingdom cohort, CU=Columbia University cohort

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FIGURE LEGENDS

Figure 1. Topologic analysis of the SUR1 protein encoded by ABCC8, and sequence alignment of ABCC8 across species. Panel A shows the topology of the SUR1 protein. The 17 transmembrane segments are grouped into transmembrane domains (TMD): TMD0, TMD1, and TMD2. The two nucleotide-binding fold domains (NBF1 & 2) are indicated. Variants N72D, G111R, L135V, and E186D are located in TMD0; T229I and A240T are located in the cytoplasmic loop, L0; E791Q, D813N, and R958H are located in NBF1; R1314H and D1472N are located in NBF2. The position of each mutation is indicated by a red circle. Panel B shows the alignment of human ABCC8-encoding SUR1 protein with 14 different species, demonstrating conservation across species of each amino acid found mutated in this study.

Figure 2. Electrophysiological consequence of SUR1 mutations on K_{ATP} (SUR1+Kir6.2) channel function. Whole-cell voltage clamp was used to measure expressed wildtype (WT) versus mutant K_{ATP} channel currents containing SUR1+Kir6.2 in COS7 cells. Panel A shows a wildtype SUR1-dependent K_{ATP} current trace. A voltage ramp from -120mV to +60mV over 1 second was applied every 3 seconds, from a -80mV holding potential. For all sample current traces, the vertical scale is 100 pA/pF, and the horizontal scale is 20mV. Panel B shows SUR1-dependent current traces of mutant K_{ATP} channels containing A240T, D813N, or D1472N SUR1 as indicated. Panel C summarizes SUR1-dependent K_{ATP} current densities (pA/pF) for the eight SUR1 mutants evaluated and wildtype, measured at -40mV (indicated by the black arrow in Panel A); 8 to 30 cells were studied per condition. Data are shown as means; T bars indicate

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standard errors. Asterisks indicate P<0.05 for the comparison between wildtype SUR1 and each mutant, as calculated by a one-way ANOVA and post-hoc Tukey test.

Figure 3. Functional impact of SUR1 mutations on macroscopic K_{ATP} (SUR1+Kir6.2) channel activity. $^{86}Rb^{+}$ efflux was measured over time from COSm6 cells expressing K_{ATP} channels containing SUR1+Kir6.2. Panel A shows basal efflux for wildtype (WT, black curve) versus mutant (colored curves) SUR1-containing K_{ATP} channels, and GFP-alone (gray curve). Panel B displays the mean rate constants for K_{ATP}-dependent $^{86}Rb^{+}$ efflux under basal conditions. Panel C shows efflux from cells exposed to solution containing oligomycin and 2-deoxy-D-glucose to induce metabolic inhibition of cells, thereby relieving K_{ATP} channels from intracellular inhibition by ATP. Wildtype versus mutant SUR1-containing K_{ATP} channels, and GFP-alone, are compared. The inset shows exponential fits to early time points which were used to derive the efflux rate constants (see Supplementary Table S3). Panel D shows the mean rate constants for K_{ATP}-dependent $^{86}Rb^{+}$ efflux under metabolic inhibition conditions. For each condition, 7 to 10 cell populations were studied. Data are shown as means; T bars indicate standard errors. Asterisks indicate P<0.05 for the comparison between wildtype SUR1 and each mutant, as calculated by a one-way ANOVA and post-hoc Tukey test.

Figure 4. Pharmacological recovery of mutant K_{ATP} (SUR1+Kir6.2) channels. Diazoxide restores function of K_{ATP} channels (SUR1/Kir6.2) containing mutant SUR1. Panel A shows rubidium efflux in the presence of diazoxide 100µM for wildtype (WT) SUR1 (black curve), mutant SUR1-containing K_{ATP} channels (colored curves), and GFP-alone (gray curve). Panel B shows average efflux rates for wildtype and mutant K_{ATP} channels in basal (gray) versus

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diazoxide 100µM (black) conditions. For each condition, 7 to 9 cell populations were studied. Panel C depicts whole-cell drug time courses of wildtype and selected mutant K_{ATP} channel currents with varying degrees of pharmacological activation. Time course depicts before drug application (gray, control), during diazoxide 100µM application (black), and during co-application of glibenclamide 10µM with diazoxide 100µM (red). The vertical scale is 25 pA/pF, and the horizontal scale is 1 minute. Arrows indicate the maximal steady-state current-density (pA/pF) achieved during diazoxide 100µM application. Panel D summarizes current density (pA/pF at -40mV) for wildtype and each mutant SUR1-containing K_{ATP} channel, in control (gray) and diazoxide 100µM (black) conditions; 6 to 30 cells were studied per condition. Data are shown as means; T bars indicate standard errors. Asterisks denote $P<0.05$ for the comparison of basal and diazoxide (Panel B), or control and diazoxide (Panel D), calculated by the paired Student's t-test.

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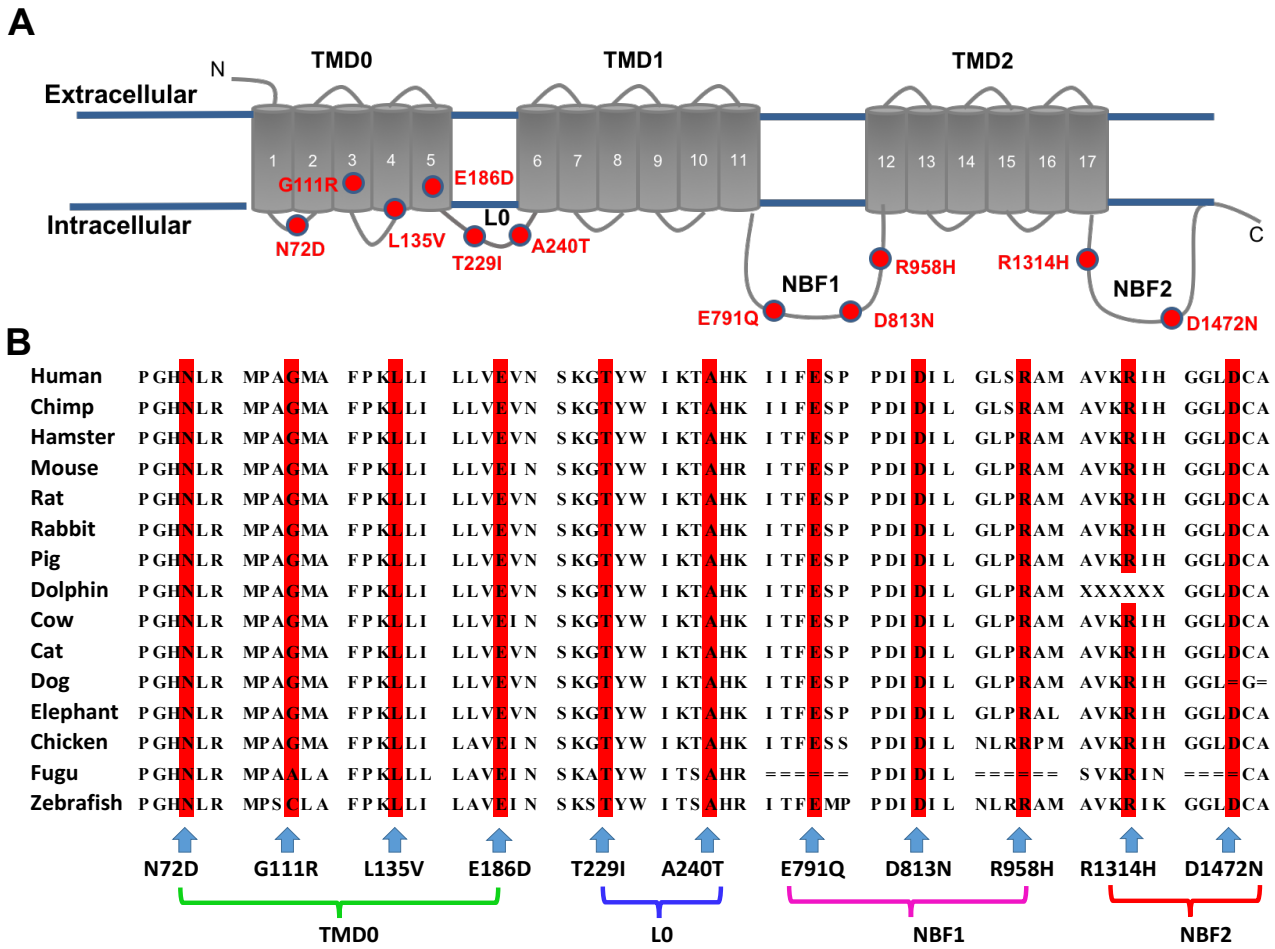
A
N
TMD0
TMD1
TMD2
Extracellular
Intracellular
1 2 3 4 5
6 7 8 9 10 11
12 13 14 15 16 17
G111R
E186D
L0
N72D
L135V
T229I
A240T
C
NBF1
R958H
R1314H
NBF2
E791Q
D813N
D1472N
B
Human PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK IIFESP PDIDIL GLSRAM AVKRIH GGLDCA
Chimp PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK IIFESP PDIDIL GLSRAM AVKRIH GGLDCA
Hamster PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAM AVKRIH GGLDCA
Mouse PGHNLR MPAGMA FPKLLI LLVEIN SKGTYW IKTAHR ITFESP PDIDIL GLPRAM AVKRIH GGLDCA
Rat PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAM AVKRIH GGLDCA
Rabbit PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAM AVKRIH GGLDCA
Pig PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAM AVKRIH GGLDCA
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Cat PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAM AVKRIH GGLDCA
Dog PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAM AVKRIH GGL=G=
Elephant PGHNLR MPAGMA FPKLLI LLVEVN SKGTYW IKTAHK ITFESP PDIDIL GLPRAL AVKRIH GGLDCA
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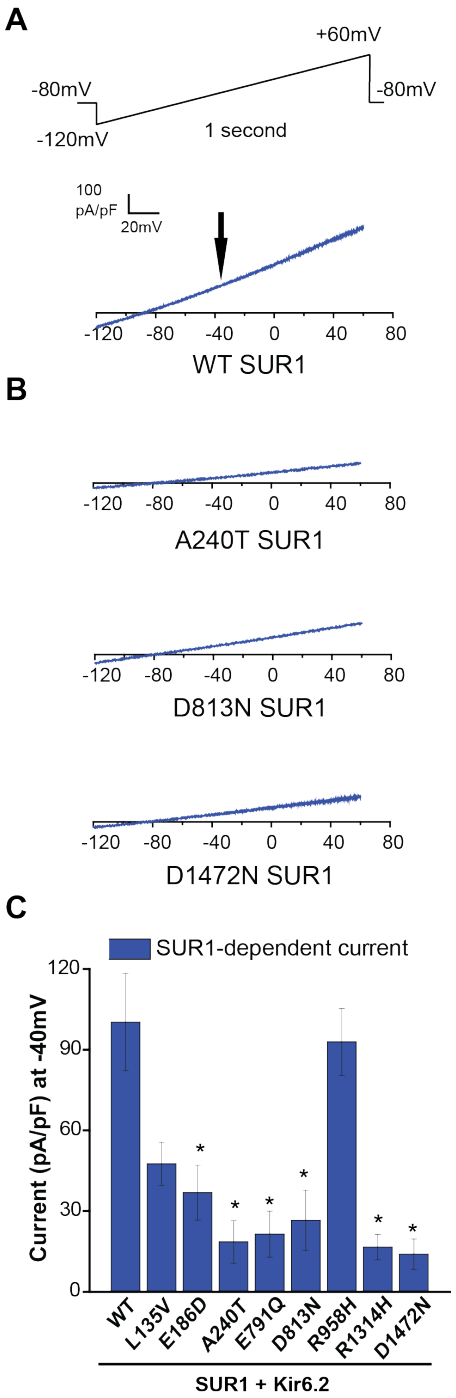
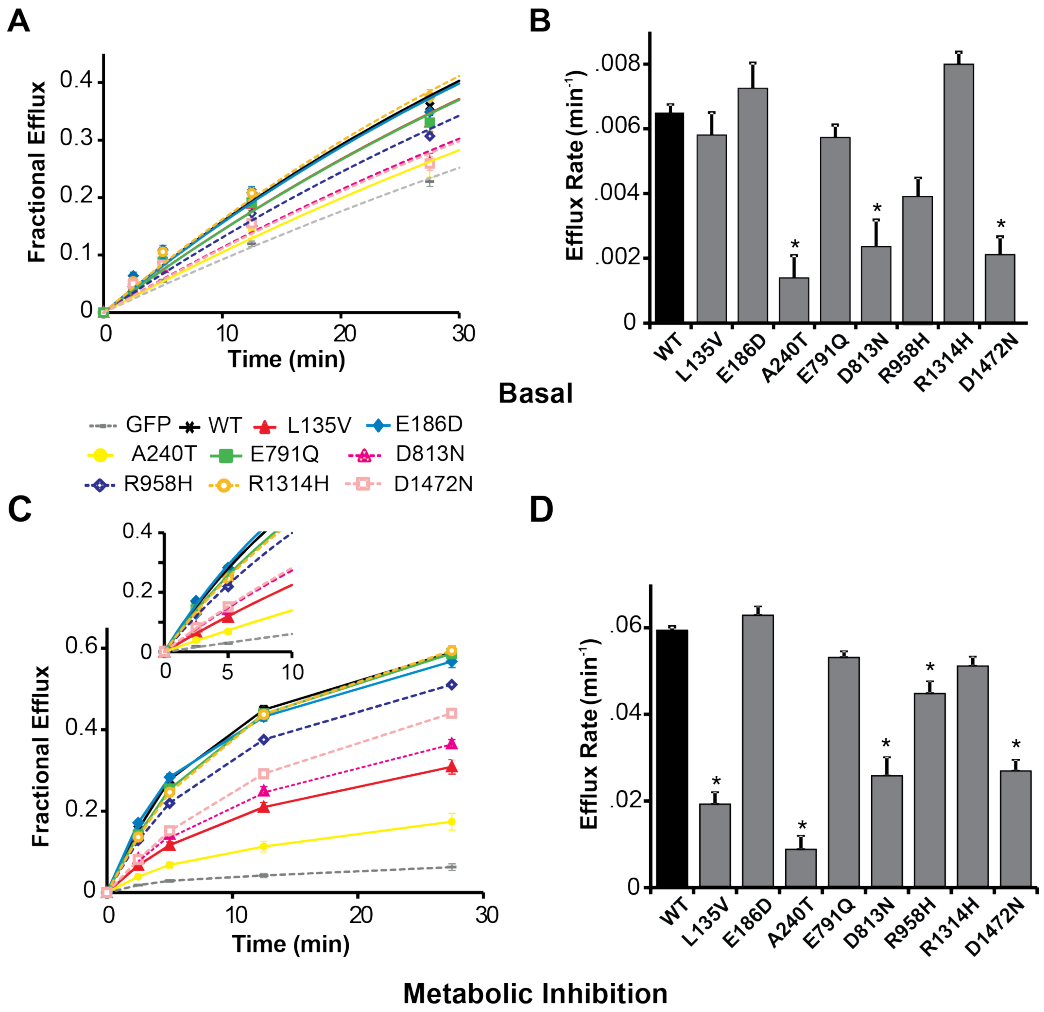
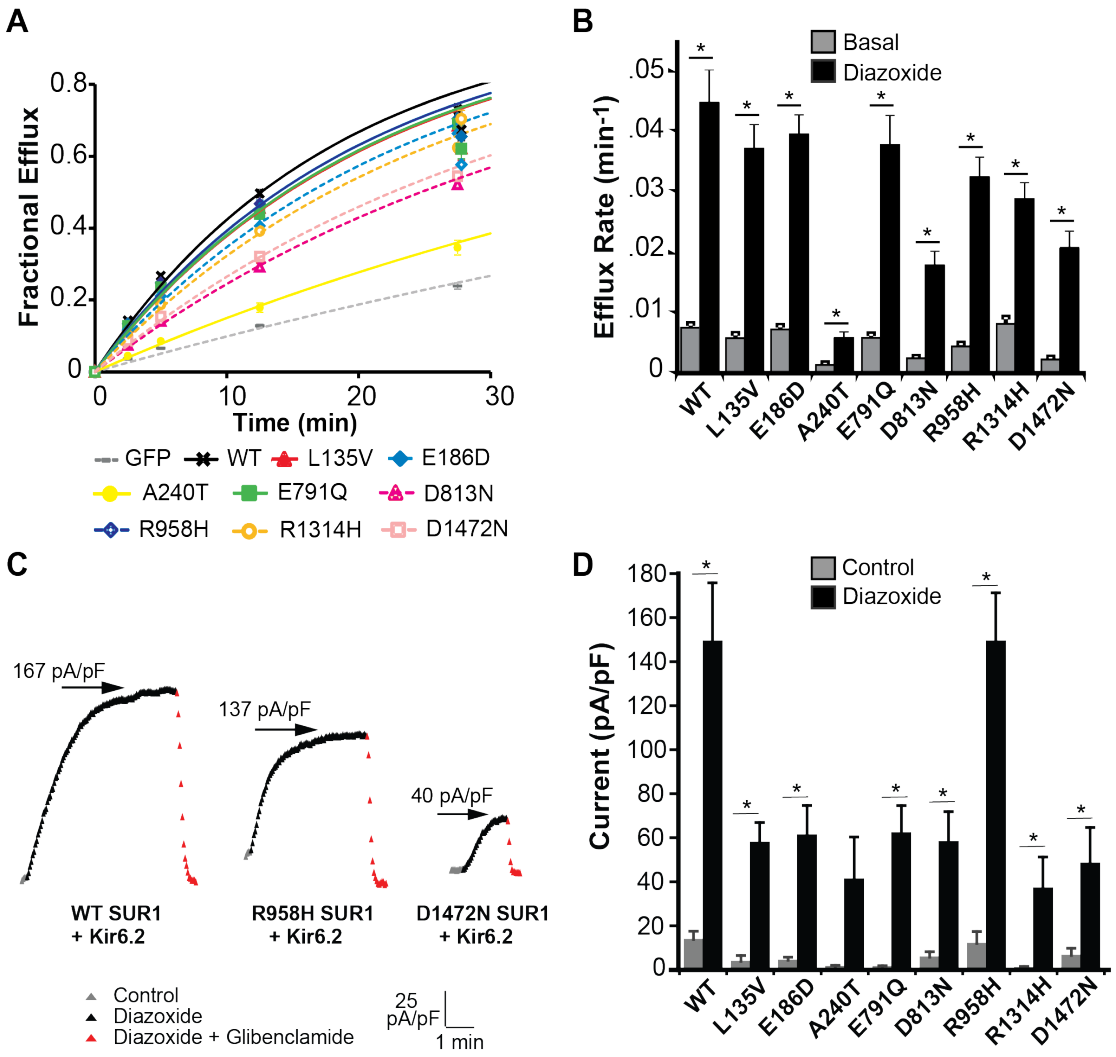
Fugu PGHNLR MPAALA FPKLLL LAVEIN SKATYW ITSAHR ======= PDIDIL ======= SVKRIN ====CA
Zebrafish PGHNLR MPSCLA FPKLLI LAVEIN SKSTYW ITSAHR ITFEMP PDIDIL NLRRAM AVKRIK GGLDCA
N72D G111R L135V E186D T229I A240T E791Q D813N R958H R1314H D1472N
TMD0 L0 NBF1 NBF2


A
+60mV
-80mV
-80mV
-120mV
1 second
100
pA/pF
20mV
-120 -80 -40 0 40 80
WT SUR1
B
-120 -80 -40 0 40 80
A240T SUR1
-120 -80 -40 0 40 80
D813N SUR1
-120 -80 -40 0 40 80
D1472N SUR1
C
SUR1-dependent current
Current (pA/pF) at -40mV
120
90
60
30
0
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N
SUR1 + Kir6.2


A
Fractional Efflux
0.4
0.3
0.2
0.1
0
0
10
20
30
Time (min)
B
Efflux Rate (min-1)
.008
.006
.004
.002
0
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N
Basal
GFP
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N
C
Fractional Efflux
0.6
0.4
0.2
0
0
10
20
30
Time (min)
0.4
0.2
0
0
5
10
D
Efflux Rate (min-1)
.06
.04
.02
0
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N
Metabolic Inhibition


A
Fractional Efflux
0.8
0.6
0.4
0.2
0
0
10
20
30
Time (min)
GFP
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N
B
Efflux Rate (min-1)
.05
.04
.03
.02
.01
0
Basal
Diazoxide
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N
C
167 pA/pF
137 pA/pF
40 pA/pF
WT SUR1
+ Kir6.2
R958H SUR1
+ Kir6.2
D1472N SUR1
+ Kir6.2
Control
Diazoxide
Diazoxide + Glibenclamide
25
pA/pF
1 min
D
Current (pA/pF)
180
160
140
120
100
80
60
40
20
0
Control
Diazoxide
WT
L135V
E186D
A240T
E791Q
D813N
R958H
R1314H
D1472N