Circulation: Genomic and Precision Medicine

ORIGINAL ARTICLE

Loss-of-Function *ABCC8* Mutations in Pulmonary Arterial Hypertension

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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, because of 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 PAH 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* 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 SUR1 (sulfonylurea receptor 1)—a regulatory subunit of the ATP-sensitive potassium channel. We observed loss of ATP-sensitive potassium channel 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 PAH. Identified *ABCC8* mutations decreased ATP-sensitive potassium channel function, which was pharmacologically recovered.

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Key Words: electrophysiology ■ genetics ■ humans ■ hypertension, pulmonary ■ ion channels

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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 *BMPR2* (bone morphogenetic protein receptor type 2)—a member of the TGF-β (transforming growth factor-β) superfamily of receptors—have been identified as the major genetic cause, including in 70% of inherited and 10% to 40% of idiopathic cases.[1,2] Mutations in other TGF-β 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 to 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 subfamily K member 3) potassium channel as a genetic cause of idiopathic and familial PAH.[4] Furthermore, we proposed KCNK3 as a novel pharmacological target in PAH because potassium channel currents of select mutant and wild-type 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* (ATP-binding cassette, subfamily C, member 8)/SUR1 (sulfonylurea receptor 1) loss-of-function mutations in both pediatric and adult-onset PAH. *ABCC8* encodes 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.

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 the Columbia University Medical Center. Detailed methods are available in the Data Supplement.

RESULTS

Inherited and De Novo Variants in *ABCC8*

By exome sequencing, we identified a de novo missense variant c.G2873A (p.R958H) in *ABCC8* (NM_001287174), which was predicted to be deleterious, in a patient diagnosed with idiopathic PAH at the age of 10 years (Table). We then examined all CU-PAH (Columbia University PAH patient cohort) patients for rare or novel variants in *ABCC8* and identified 7 additional rare damaging missense variants, predicted by multiple algorithms to be deleterious in 7 unrelated patients with idiopathic, familial, or congenital heart disease-associated PAH (Table). In 1 familial case, the p.A240T variant was transmitted from an affected father and was also observed in the affected sibling. To replicate the findings in the CU-PAH cohort, we evaluated the UK-PAH cohort (United Kingdom PAH) and identified 3 additional rare or novel missense and 1 splice variant in *ABCC8* in 3 patients with idiopathic and 1 patient with congenital heart disease-associated PAH (Table). Five variants—c.A214G (p.N72D), c.G558T (p.E186D), c.G718A (p.A240T), c.G2371C (p.E791Q), and c.T2694+2G—were novel; 4 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 2 variants—c.C686T (p.T229I) and c.G3941A (p.R1314H)—have been 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, whereas 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 Table I in the Data Supplement. Seven of the probands were women, and 5 were men. None of the probands had any evidence of hyperinsulinism or transient/permanent neonatal diabetes mellitus although 1 adult patient had type 2 diabetes mellitus. Six of the patients were children at the time of diagnosis. Three patients responded, and 4 patients did not respond to acute vasodilator testing using inhaled NO during cardiac catheterization. Two patients had evidence of a cardiac arrhythmia (Table).

To estimate the genetic effect size of *ABCC8* variants, we selected 33 369 European adult subjects from Exome Aggregation Consortium 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 with controls. There were 6 rare predicted deleterious alleles in 150 PAH cases of European ancestry

Table. **Pathogenicity Predictions and Clinical Characteristics of Patients With Pulmonary Arterial Hypertension With *ABCC8* Mutations**

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	White	White	White	White	White	White	Hispanic	Asian	White	White	White	White
MetaSVM_pred	D	D	D	D	D	D	D	D	D	D	D	NA
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; 2 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
Sex	F	M	M	M	F	F	F	M	F	M	F	F
Age of diagnosis, y	35	64	5	79	34	Unknown	14	12	10	<1	9	60
RAP M, mm Hg	NA	11	9	NA	9	NA	NA	25	6	8	7	7
PAP M, mm Hg	NA	44	55	NA	45	NA	NA	54	56	54	37	36
AOP M, mm Hg	NA	Unknown	78	NA	Unknown	NA	NA	48	88	57	67	Unknown
PVRi, U×m^2	NA	19	25	NA	13	NA	NA	29	16	13.6	17	19
Art Sat, %	NA	97	91	NA	98	NA	NA	93	91	97	96	97
PCWPm, mm Hg	NA	15	8	NA	10	NA	NA	NA	8	8	NA	6
CI, L/min per m^2	NA	1.5	2.8	NA	2.7	NA	NA	NA	2.8	3.5	2	1.6
Response to acute vasodilator test	NA	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, first-degree heart block	None	Hearing loss, hypothyroid lipodermatosclerosis ESRD, Raynaud syndrome	None	None	Atrial flutter, nonspecific intra ventricular block, autism	None	Ventricular septal defect	None	None

ExAC frequency lists the allele frequency for all of the ExAC followed by the allele frequency in the patient's ethnic group. AA indicates Amino Acid; AOP M, mean aortic pressure; Art Sat, Arterial Oxygen Saturation; CADD, combined annotation dependent deletion; CHD, congenital heart disease; CI, cardiac index; CU, Columbia University; D, deleterious; ESRD, end stage renal disease; ExAC, Exome Aggregation Consortium; f, female; freq, frequency; m, male; MetaSVM, meta support vector machine; NA, not available; PAH, pulmonary arterial hypertension; PAP M, mean pulmonary arterial pressure; PCWPm, mean pulmonary capillary wedge pressure; phred, phred quality score for sequence quality; pred, prediction; PVRi, pulmonary vascular resistance index; RAP M, mean right atrial pressure; TBX4, T-Box Protein 4; and UK, United Kingdom.

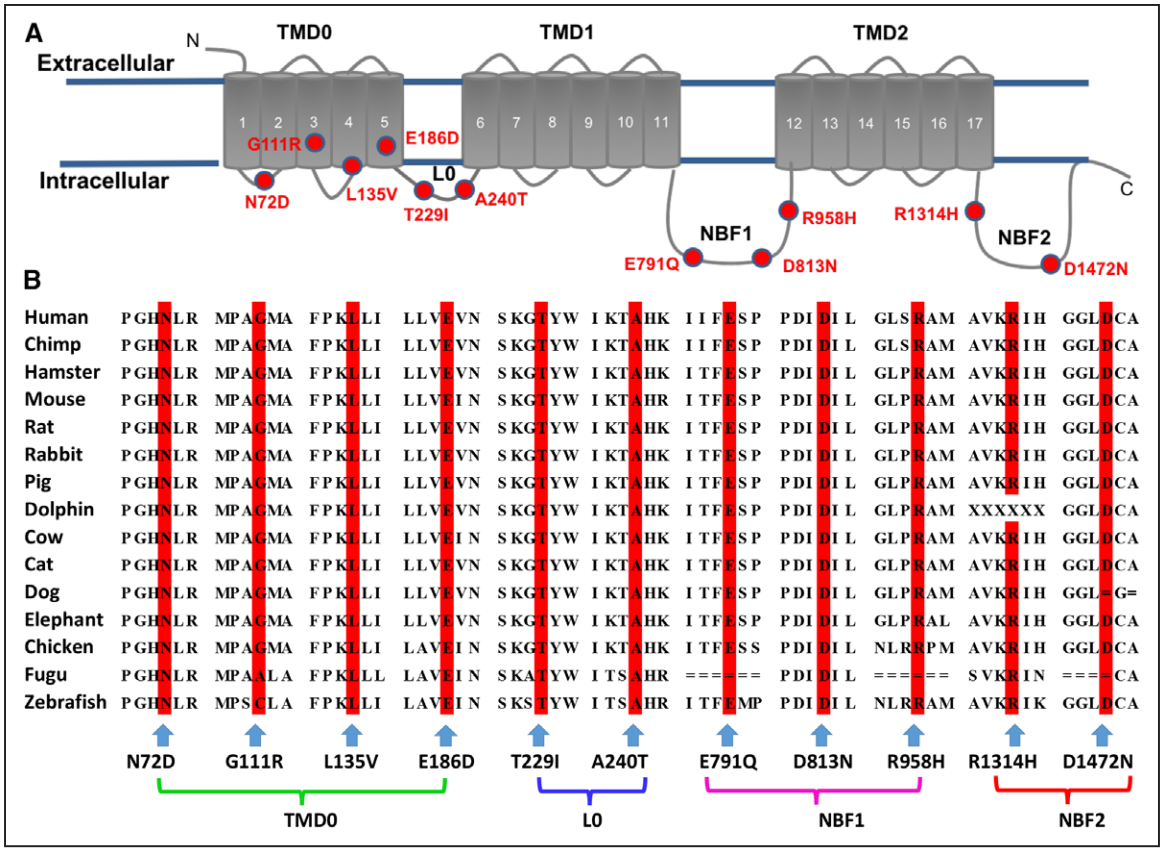

Figure 1. Topologic analysis of the SUR1 (sulfonylurea receptor 1) protein encoded by *ABCC8* and sequence alignment of *ABCC8* across species. **A**, Topology of the SUR1 protein. The 17 transmembrane segments are grouped into transmembrane domains (TMDs): TMD0, TMD1, and TMD2. The 2 nucleotide-binding fold domains (NBFs: NBF1 and NBF2) 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. **B**, Alignment of human *ABCC8*-encoding SUR1 protein with 14 different species, demonstrating conservation across species of each amino acid found mutated in this study.

in the CU-PAH cohort, whereas 158 unique deleterious variants were observed a total of 295× in 33 369 controls with exome sequencing in the Exome Aggregation Consortium dataset, and 165 rare unique deleterious variants were observed 712× in the DiscovEHR study. With binomial tests, we observed significant excess of rare predicted deleterious missense variants in *ABCC8* in CU-PAH cases when comparing with Exome Aggregation Consortium controls (P=0.0023; enrichment rate, 4.5) and to DiscovEHR controls (P=0.022; enrichment rate, 2.8). We tested the association of predicted benign *ABCC8* variants and identified 2 rare synonymous alleles in cases, 223 unique predicted benign missense variants or synonymous variants observed a total of 512× in controls, and found no significant difference between the CU-PAH group and Exome Aggregation Consortium (P=1; relative risk, 0.87).

ABCC8 Expression in Human Lung

ABCC8 encodes the 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 (Figure I in the Data Supplement), 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 and healthy controls. We observed a significant increase in *ABCC8* expression in lungs of BMPR2-associated heritable PAH patient samples (Figure II in the Data Supplement).

To determine the cell types expressing SUR1 protein in lungs of idiopathic PAH patients, confocal microscopic analyses and triple labeling with ABCC8, CD68 (Cluster of Differentiation 68), and SM22 (Smooth Muscle-22) antibodies was used in paraffin-embedded lungs from 6 idiopathic PAH patient lung samples. Strong staining for SUR1 was found in a population of alveolar macrophages, and moderate staining for SUR1 was observed in proximal pulmonary arteries (Figure III in the Data Supplement).

Functional Characterization of *ABCC8* Mutations

We examined the consequence of 8 of the 12 identified *ABCC8* variants putatively associated with PAH on SUR1 function using 2 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 coexpressing 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 1 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 (100 μM)—a selective SUR1 activator. Once steady-state diazoxide current activation was achieved, glibenclamide (10 μM), which inhibits K_{ATP} channels by binding to the SUR subunit, was coapplied. 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 Figures IV and V in the Data Supplement.

Robust SUR1-dependent K_{ATP} currents were measured in cells expressing Kir6.2 and wild-type 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 hyperinsulinism (Figure 2B). Further analysis demonstrated significantly reduced SUR1-dependent currents in 6 of 8 SUR1 mutants functionally evaluated (E186D, A240T, E791Q, D813N, R1314H, and D1472N) and nonsignificant 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 wild-type or mutant SUR1 along with Kir6.2 (Figure 3), in basal metabolic conditions and in metabolic inhibition. Compared with wild-type 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; Table I in the Data Supplement). 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 wild type (Figure 3C and

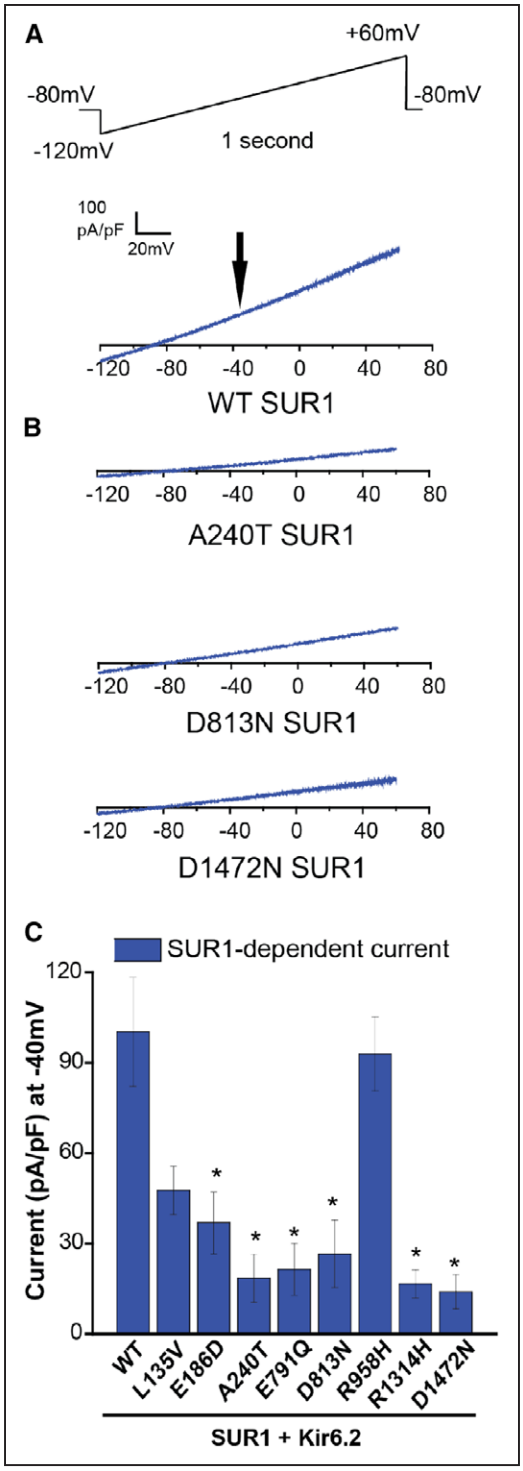

Figure 2. Electrophysiological consequence of SUR1 (sulfonylurea receptor 1) mutations on K_{ATP} (SUR1+Kir6.2) channel function. Whole-cell voltage clamp was used to measure expressed wild-type (WT) vs mutant K_{ATP} channel currents containing SUR1+Kir6.2 in COS7 cells. **A**, WT SUR1-dependent K_{ATP} current trace. A voltage ramp from –120 to +60 mV during 1 s was applied every 3 s, from a –80-mV holding potential. For all sample current traces, the vertical scale is 100 pA/pF, and the horizontal scale is 20 mV. **B**, SUR1-dependent current traces of mutant K_{ATP} channels containing A240T, D813N, or D1472N SUR1 as indicated. **C**, SUR1-dependent K_{ATP} current densities (pA/pF) for the 8 SUR1 mutants evaluated and WT, measured at –40 mV (indicated by the black arrow in **A**); 8 to 30 cells were studied per condition. Data are shown as means; T bars indicate SEs. **P*<0.05 for the comparison between WT SUR1 and each mutant, as calculated by a 1-way ANOVA and post hoc Tukey test.

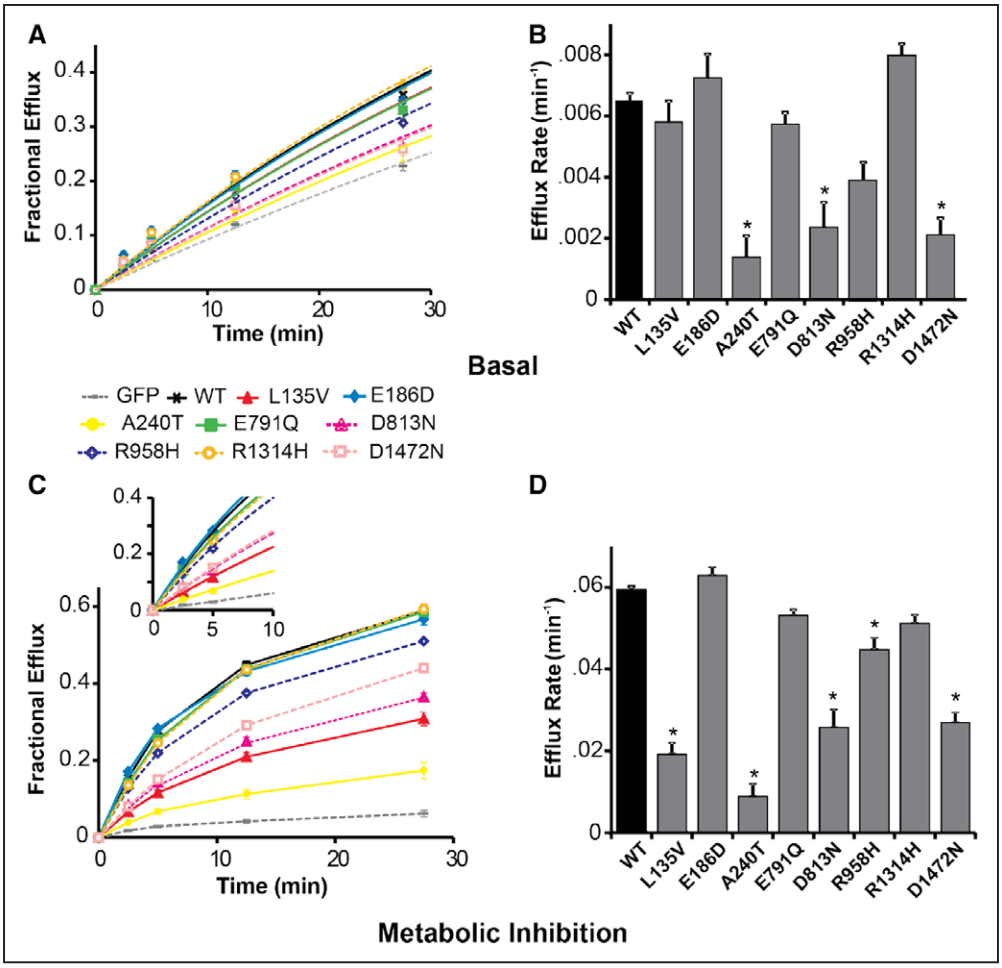

Figure 3. Functional impact of SUR1 (sulfonylurea receptor 1) mutations on macroscopic K_{ATP} (SUR1+Kir6.2) channel activity.
$^{86}Rb^+$ efflux was measured over time from COSm6 cells expressing K_{ATP} channels containing SUR1+Kir6.2. **A**, Basal efflux for wild-type (WT; black curve) vs mutant (colored curves) SUR1-containing K_{ATP} channels and GFP alone (gray curve). **B**, Mean rate constants for K_{ATP}-dependent $^{86}Rb^+$ efflux under basal conditions. **C**, 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. WT vs 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 (Table III in the Data Supplement). **D**, 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 SEs. **P*<0.05 for the comparison between WT SUR1 and each mutant, as calculated by a 1-way ANOVA and post hoc Tukey test. GFP indicates green fluorescent protein.

3D; Table II in the Data Supplement). The E791Q and R1314H mutant fluxes were slightly reduced, whereas 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 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 (100 μM) in rubidium efflux (Figure 4A and 4B; Table III in the Data Supplement) and whole-cell patch clamp (Figure 4C and 4D; Figure IVA through IVF in the Data Supplement).

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 8 independent families, with 6 of the probands diagnosed as children in the CU-PAH study. We identified an additional 4 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.

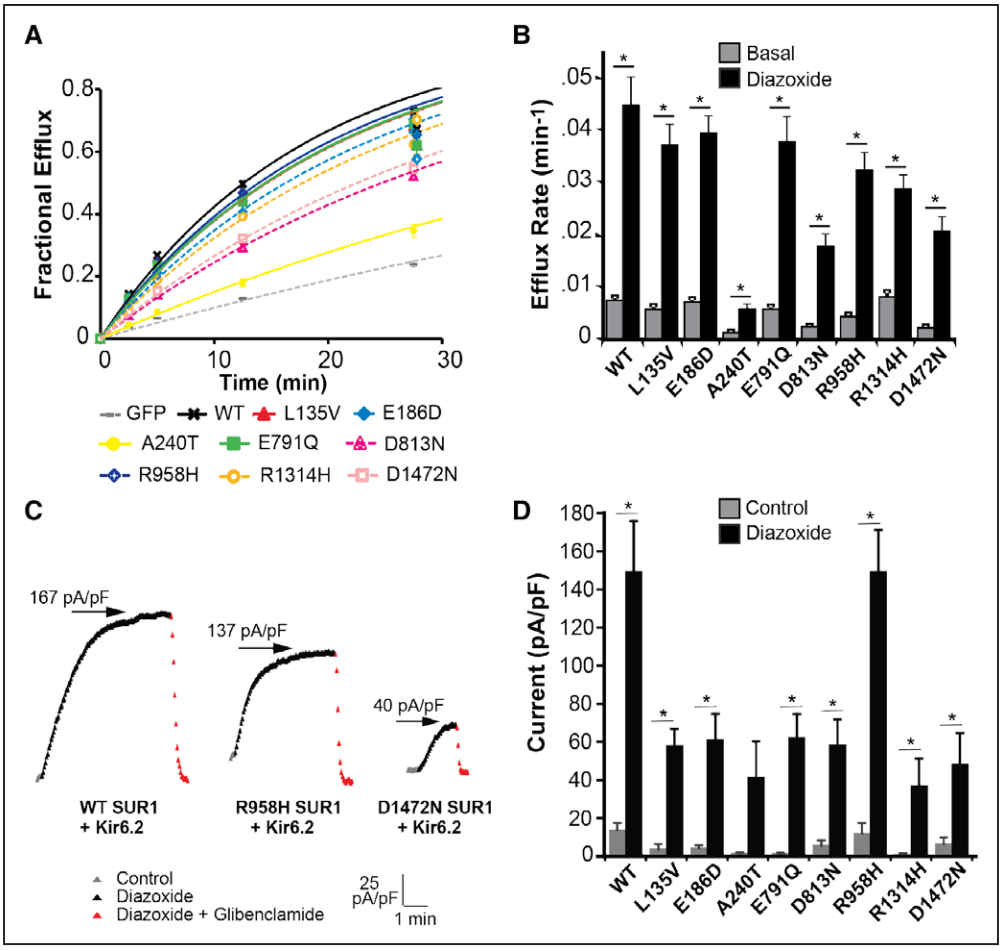

Figure 4. Pharmacological recovery of mutant K_{ATP} (SUR1 [sulfonylurea receptor 1]+Kir6.2) channels.
Diazoxide restores function of K_{ATP} channels (SUR1/Kir6.2) containing mutant SUR1. **A**, Rubidium efflux in the presence of diazoxide 100 μM for wild-type (WT) SUR1 (black curve), mutant SUR1-containing K_{ATP} channels (colored curves), and GFP alone (gray curve). **B**, Average efflux rates for WT and mutant K_{ATP} channels in basal (gray) vs diazoxide 100 μM (black) conditions. For each condition, 7 to 9 cell populations were studied. **C**, Whole-cell drug time courses of WT and selected mutant K_{ATP} channel currents with varying degrees of pharmacological activation. Time course depicts before drug application (gray, control), during diazoxide 100 μM application (black), and during coapplication of glibenclamide 10 μM with diazoxide 100 μM (red). The vertical scale is 25 pA/pF, and the horizontal scale is 1 min. Arrows indicate the maximal steady-state current density (pA/pF) achieved during diazoxide 100 μM application. **D**, Current density (pA/pF at −40 mV) for WT and each mutant SUR1-containing K_{ATP} channel, in control (gray) and diazoxide 100 μM (black) conditions; 6 to 30 cells were studied per condition. Data are shown as means; T bars indicate SEs. *P<0.05 for the comparison of basal and diazoxide (**B**), or control and diazoxide (**D**), calculated by the paired Student t test.

ABCC8 encodes the SUR1 protein—a K_{ATP} channel subunit. The 11 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] (Figures I and II in the Data Supplement). SUR1 expression has been observed in intact rat pulmonary arteries,[19] whereas 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 (Figure II in the Data Supplement), as well as SUR1 protein in both alveolar macrophages and proximal pulmonary arteries within the lung (Figure III in the Data Supplement), further studies may elucidate SUR1's primary physiological role in the pulmonary 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 represents possible pathogenic mechanisms in PAH and pharmacological 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] whereas 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 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 antihypertensive and antihyperinsulinism 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. Although 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.

ARTICLE INFORMATION

Received January 24, 2018; accepted August 1, 2018.

The Data Supplement is available at https://www.ahajournals.org/doi/suppl/10.1161/CIRCGEN.118.002087.

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Acknowledgments

We thank the patients and their families for their generous contribution. Robyn Barst and Jane Morse were critical members of the team to enroll and clinically characterize patients. Patricia Lanzano provided oversight of the Columbia biorepository. Anthony Marcketta provided bioinformatics support. Jenny Rao assisted with molecular biology and cell culture preparations. Charles Stanley provided helpful discussion. We thank Ly Tu and Raphaël Thuillet for their assistance with confocal image processing and analysis. The following research nurses, study coordinators, and data managers provided invaluable support to

the NIHR (National Institute of Health Research) BioResource—Rare Diseases PAH study: Amanda Creaser-Myers, Rosa DaCosta, Margaret Day, Natalie Dormand, Dipa Ghedia, Alan Greenhalgh, Mark Grover, Anna Huis in't Veld, Val Irvine, Fiona Kennedy, Shokri Othman, Alice Parker, Gary Polwarth, Stephen Roney, Gwen Schotte, Debbie Shipley, Della Stokes, Yvonne Tan, Sara Walker, and Bath Respiratory Nurse Specialists.

Sources of Funding

Funding support was provided by National Heart, Lung, and Blood Institute (NHLBI) HL060056 (to Dr Chung), HL129656 (to Dr Bohnen), and HL45742 (to Dr Gonzaga-Jauregui). Funding for the Pulmonary Hypertension Breakthrough Initiative (PHBI) is provided under an NHLBI R24 grant, No. R24HL123767, and by the Cardiovascular Medical Research and Education Fund (CMREF). Additional funding was provided by a Medical Research Council Experimental Challenge Award MR/K020919/1 (to Dr Morrell); a British Heart Foundation Special Project Grant SP/12/12/29836 (to Dr Morrell); the National Institute for Health Research BioResource–Rare Diseases, and the National Institute for Health Research/Wellcome Trust Imperial Clinical Research Facility, at Imperial College Healthcare National Health Service Trust, London, United Kingdom.

Disclosures

None.

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