Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension


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Remillard CV, Tigno DD, Platoshyn O, Burg ED, Brevnova EE, Conger D, Nicholson A, Rana BK, Channick RN, Rubin LJ, O’Connor DT, Yuan JX-J. Function of Kv1.5 channels and genetic variations of KCNA5 in patients with idiopathic pulmonary arterial hypertension. Am J Physiol Cell Physiol 292: C1837–C1853, 2007. First published January 31, 2007; doi:10.1152/ajpcell.00405.2006.—The pore-forming α-subunit, Kv1.5, forms functional voltage-gated K+ (Kv) channels in human pulmonary artery smooth muscle cells (PASMC) and plays an important role in regulating membrane potential, vascular tone, and PASMC proliferation and apoptosis. Inhibited Kv channel expression and function have been implicated in PASMC from patients with idiopathic pulmonary arterial hypertension (IPAH). Here, we report that overexpression of the Kv1.5 channel gene (KCNA5) in human PASMC and other cell lines produced a 15-pS single channel current and a large whole cell current that was sensitive to 4-aminopyridine. Extracellular application of nicotine, bepridil, corrodeine, and endothelin-1 (ET-1) all significantly and reversibly reduced the Kv1.5 currents, while nicotine and bepridil also accelerated the inactivation kinetics of the currents. Furthermore, we sequenced KCNA5 from IPAH patients and identified 17 single-nucleotide polymorphisms (SNPs); 7 are novel SNPs. There are 12 SNPs in the upstream 5’ region, 2 of which may alter transcription factor binding sites in the promoter, 2 nonsynonymous SNPs in the coding region, 2 SNPs in the 3’-untranslated region, and 1 SNP in the 3’-flanking region. Two SNPs may correlate with the nitric oxide-mediated decrease in pulmonary arterial pressure. Allele frequency of two other SNPs in patients with a history of fenfluramine and phentermine use was significantly different from patients who have never taken the anorexigenics. These results suggest that 1) Kv1.5 channels are modulated by various agonists (e.g., nicotine and ET-1); 2) novel SNPs in KCNA5 are present in IPAH patients; and 3) SNPs in the promoter and translated regions of KCNA5 may underlie the altered expression and/or function of Kv1.5 channels in PASMC from IPAH patients.

MEMBRANE POTENTIAL plays an important role in regulating cytotoxic free Ca2+ concentration ([Ca2+]cyt) in pulmonary artery smooth muscle cells (PASMC) and thus pulmonary vascular tone by controlling Ca2+ influx through voltage-dependent Ca2+ channels (VDCC). The resting membrane potential is primarily determined by activities of Na+-K+-ATPase (Na+ pumps) and K+ channels in the plasma membrane. In human PASMC, activity of voltage-gated K+ (Kv) channels contributes to the regulation of membrane potential, [Ca2+]cyt, and, as a consequence, of excitation-contraction coupling in pulmonary vascular smooth muscle (66, 78). Inhibition of Kv channels in PASMC causes membrane depolarization, which then opens VDCC, increases [Ca2+]cyt by promoting Ca2+ influx, and induces pulmonary vasoconstriction. Inhibition of Kv channel activity is also implicated in stimulating PASMC proliferation by increasing [Ca2+]cyt (50) and in attenuating PASMC apoptosis by decelerating apoptotic volume decrease and decreasing cytoplasmic caspase activity (28). Conversely, activation of Kv channels in PASMC, such as induced by nitric oxide (NO) (80), causes membrane hyperpolarization, inhibits VDCC activity, and cause pulmonary vasodilation. Furthermore, activation of K+ channels is also involved in mediating apoptotic volume decrease, an early hallmark of apoptosis (7), and facilitating apoptosis (9).

Kv1.5 is an important pore-forming α-subunit that forms functional Kv channels in human PASMC and other smooth muscle cell types (5, 26, 44, 47, 81). Overexpression of the human Kv1.5 channel gene, KCNA5, in human PASMC and other cell lines (e.g., HEK-293 and COS cells) causes membrane hyperpolarization, accelerated apoptotic cell shrinkage, and enhanced cell apoptosis (8). Downregulation of KCNA5 expression using antisense oligonucleotides or short interfering RNA causes membrane depolarization and increases [Ca2+]cyt (5, 17), while pharmacological agents that block Kv1.5 channels [e.g., 4-aminopyridine (4-AP), corrodeine, and pergolide] stimulate PASMC contraction (5, 21), migration (56), and proliferation (50), but inhibit apoptosis (8).

Idiopathic pulmonary arterial hypertension (IPAH), previously referred to as primary pulmonary hypertension, is a fatal and progressive disease that predominantly affects young women. Increased pulmonary arterial pressure (PAP) in IPAH patients, due to heightened pulmonary vascular resistance (PVR), is mainly caused by sustained pulmonary vasconstriction, pulmonary vascular wall remodeling (e.g., medial hypertrophy), and obliteration of small arteries in association with in situ thrombosis (24). Pulmonary vascular medial hypertrophy results mainly from increased proliferation and/or decreased apoptosis of PASMC (24). Decreased K+ channel expression and activity may contribute to the excessive PASMC proliferation in IPAH patients.

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 Indeed, PASMC from IPAH patients exhibit down-regulated expression and inhibited function of Kv channel α pore-forming subunits (e.g., Kv1.5) compared with PASMC from normal subjects and secondary pulmonary hypertensive patients (77, 81). Decreased Kv currents result in membrane depolarization, promote Ca$^{2+}$ influx through VDCC, and increase [Ca$^{2+}$]$_{cyt}$. Similar phenomena have also been documented in PASMC from patients treated with appetite suppressants (e.g., aminorex and fenfluramine) (45, 69), which have been linked to the development of IPAH, as well as in PASMC from chronically hypoxic animals (52, 70, 71). Expression and function of the Kv1.5 channel are inhibited in PASMC from IPAH patients (81), in normal PASMC treated with fenfluramine (45), and in PASMC isolated from rats with chronic hypoxia-mediated pulmonary hypertension (6, 52, 55, 70, 71). Therefore, Kv1.5 channel dysfunction or downregulation may represent a predisposing factor in the development of pulmonary vasoconstriction and vascular medial hypertrophy in patients with IPAH, in conjunction with other factors and genetic defects.

The aims of this study were 1) to characterize biophysical and pharmacological properties of Kv1.5 (KCNA5) channels, 2) to define the sequence and transcription factor binding sites in the putative promoter region of the human Kv1.5 channel gene (KCNA5), and 3) to identify novel single-nucleotide polymorphisms (SNPs) in KCNA5 and its immediate upstream and downstream flanking sequences from IPAH patients.

MATERIALS AND METHODS

Cell preparation and culture. Primary cultured PASMC from transplant patients and normal human PASMC purchased from Cambrex were used for electrophysiological experiments in this study. Lung tissues were obtained from patients undergoing lung or heart transplantation. Peripheral muscular pulmonary arteries (150–300 μm diameter) isolated from the explanted lung tissues were first incubated in Hank's balanced salt solution that contained 2 mg/ml collagenase (Worthington Biochemical) for 20 min to remove adventitia with fine forceps and to remove endothelium by a surgical scalpel. The remaining smooth muscle was then digested with (in mg/ml) 2.25 collagenase, 0.5 elastase, and 1 albumin (Sigma) at 37°C to make a cell suspension of PASMC. The cells were resuspended in the smooth muscle growth medium (SmGM; Cambrex) and cultured in an incubator under a humidified atmosphere of 5% CO$_2$, 95% air at 37°C. Human PASMC from normal subjects were also cultured in SmGM and used at passages 4–6 for experimentation. The medium was changed after 24 h and every 48 h thereafter. The SmGM was composed of smooth muscle basal medium supplemented with 5% FBS, 0.5 ng/ml human epidermal growth factor, 2 ng/ml human fibroblast growth factor, and 5 μg/ml insulin. Cells were subcultured or plated onto 25-mm coverslips using trypsin-EDTA buffer (Cambrex) when 70–90% confluence was achieved.

Human embryonic kidney epithelial cells (HEK-293) and COS-7 (monkey kidney fibroblast-like cells) cells (American Type Culture Collection, and human coronary arterial smooth muscle cells (HCASMC; Cambrex) were cultured in high-glucose (4.5 g/l) DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Biofluids) and incubated in 5% CO$_2$ at 37°C in a humidified atmosphere. Rat PASSC and mesenteric artery smooth muscle cells (MASMC) were isolated from intrapulmonary arteries removed from Sprague-Dawley rats (125–250 g) according to a previously published protocol (78, 79), plated onto 25-mm coverslips in 10% FBS-DMEM, and cultured (at 37°C) in an incubator equilibrated with 5% CO$_2$. Cells were subcultured or split using 1 mg/ml trypsin (Sigma) when 70–90% confluence was achieved.

Constructs. In the KCNA5/pBK construct (kindly provided by Dr. M. Tamkun from Colorado State University), the coding sequence of the human KCNA5 gene was subcloned into XbaI and KpnI multiple cloning sites of the phagemid expression vector pBK-CMV (Stratagene). For electrophysiological experiments, a KCNA5/green fluorescent protein (GFP) construct was designed to visualize the transfected cells. In the KCNA5/GFP construct, the coding sequence of the human KCNA5 gene was subcloned into EcoRI and XbaI sites of the pCMS-EGFP mammalian expression vector (Clontech). In the pCMS-EGFP vector, the GFP gene (which encodes the enhanced green fluorescent protein, a red-shifted variant of wild-type GFP from *Aquorea victoria*) is expressed separately from the gene of interest and is used as a transfection marker.

Transfection of KCNA5. Cells were transiently transfected with the expression constructs using Lipofectamine reagent according to the manufacturer's instruction. Briefly, cells were first split and then cultured for 24 h. Transfection was performed on 40–80% confluent cells at 37°C in serum-free Opti-MEM I medium (Invitrogen) using 1.6 μg/ml DNA and 4 μl/ml of Lipofectamine reagent. After 5–7 h of exposure to the transfection medium, cells were refed with culture medium containing 10% FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Electrophysiological measurement of Kv currents in KCNA5-transfected cells. Phagom constructs containing the GFP-tagged human KCNA5 coding sequence were transfected into cells at first, as described previously (8). Whole cell and single channel Kv currents of HEK-293 and COS-7 cells and 5–15% for human PASMC, human CASMC, rat PASMC, and rat MASMC using Lipofectamine reagent.

Measurement of pulmonary hemodynamics in patients with IPAH. A flow-directed balloon-tipped Swan-Ganz catheter was positioned into the right ventricle or pulmonary artery via the internal jugular vein. PAP was measured by a pressure transducer (Nemic) connected to a Mac-Laboratory 7000 hemodynamic and electrocardiographic...
monitoring system (GE Medical System). Cardiac output (CO) was measured by a thermodilution technique and PVR was calculated according to PAP and CO by the monitoring system. PAP, PVR, and CO were compared before and after inhalation of NO during catheterization to determine pulmonary vascular reactivity.

Isolation of genomic DNA from patients. Blood samples were collected from patients via the pulmonary catheter and stored in EDTA-containing tubes. Genomic DNA was extracted from the blood samples using a DNA isolation kit (PUREGENE, Gentra Systems). DNA purity was measured as the ratio of the absorbance at 260 and 280 nm with a spectrophotometer (model DU 520, Beckman Coulter).

Sequencing of KCNA5 and identification of SNPs. SNP discovery of the KCNA5 gene was performed by Agencourt Bioscience using its proprietary SeeSNP Modeling Suite for assay design, utilizing the GenBank accession number for human chromosome 12 (NT_009759) as input. The software mapped the mRNA and promoter sequence of KCNA5 against GenBank sequence contigs where complete sequence alignment was identified. The software then designed amplification primers and developed an ampiclon tiling model spanning both coding and regulatory regions, including all intron/exon borders and the full exon sequence. Only primer pairs yielding high PCR success rates and specificity (Table 1) were passed onto high throughput PCR setup and sequencing. Genomic DNA samples were amplified against the 10 validated ampiclons spanning KCNA5, excluding a portion of exon 6, which did not amplify during optimization due to high GC content. The PCR products were sequenced using BigDye version 3.1 reactions on ABI3700 instruments. SNPs identified in the sequence traces were verified using Phred/Phrap/Consed software and compared with known SNPs deposited in the National Center for Biotechnology Information (NCBI) SNP databank.

Promoter prediction and identification of transcription factor binding sites. Gene2Promoter and PromoterInspector software (Genomatix) were used to predict and verify the sequence and placement of the KCNA5 promoter. Gene2Promoter mapped our base sequence against GenBank sequence contigs where complete sequence alignment was identified. The identified region was marked as a true positive if a transcription start site was located within or up to 200-bp downstream of the predicted promoter region. Vertebrate transcription factor binding sites were verified using MatInspector software (Genomatix).

RT-PCR. Total RNA was isolated from human PASMC and RT-PCR was performed according to protocols described previously (51). The sequences of sense and antisense primers (Table 2) were specifically designed from the coding regions of smooth muscle α-actin, calponin, and SM-22 (59). As a control for integrity of total RNA, primers specific for GAPDH were used. An electrophoresis documentation system (Eastman Kodak) was used to visualize the PCR product and to quantify the intensity of the PCR product bands.

Statistical analysis. Data are expressed as means ± SE. Statistical differences of hemodynamic changes were assessed using Student’s t-test. We used χ²-analyses to assess differences between genotype frequencies in drug-response groups. One-way ANOVA with post hoc analysis was performed to compare hemodynamics between different patient groups. For electrophysiological and pharmacological experiments, n refers to the number of cells used. For patient hemodynamics and SNP analysis, numbers of patients are given.

RESULTS

Biophysical properties of human Kv1.5 channels. The KCNA5 gene, located in chromosome 12p13, contains a single exon, which encodes for the Kv1.5 channel subunit, a pore-forming α-subunit that associates with other Kv channel α- and β-subunits to form native channels (Fig. 1, A–C). Topology of the translated regions of Kv1.5 channel indicates that the channel contains (1) six transmembrane (TM) domains, (2) a pore (P) region between TM5 and TM6, (3) a cytoplasmic NH₂-terminus containing a polymerization domain that is responsible for α:α and α:β association, and (4) a cytoplasmic COOH-terminus that differs between Kv channel α-subunits (Fig. 1B). Functional Kv channels are homo- or heterotetramers composed of the same number of α- and β-subunits (α₅β₄) (Fig. 1C).

In human PASMC transiently transfected with KCNA5, single channel Kv1.5 currents had a slope conductance of −14.7 pS (Fig. 1D). The slope conductance of Kv1.5 channels in human PASMC was not significantly different in KCNA5-transfected COS-7 cells, rat PASMC, Hek-293 cells, and human CASMC (14.4 pS in average) (Fig. 1E). The transfection rate of human KCNA5 was >30% in COS-7 and Hekt-293 cells and 5–15% in human PASMC, rat PASMC, and rat MASMC (Fig. 1, D and E, right and bottom images). Overexpression of KCNA5 in human PASMC increased whole cell outward K⁺ currents (recorded 24–48 h after initial transfection) by ~29 times; the amplitude of whole cell Ik(V) at +60 mV in wild-type and KCNA5-transfected human PASMC was 0.28 ± 0.01 and 8.46 ± 0.79 nA, respectively (Fig. 2A, a and b). The KCNA5 currents activated and deactivated rapidly; time constants for current activation and deactivation were 1.79 ± 0.02 and 2.12 ± 0.05 ms, respectively (Fig. 2A, d and e). The Kv1.5 currents did not show significant inactivation (Fig. 2A, a). Extracellular application of 5 mM 4-AP significantly and reversibly blocked the Kv1.5 channels; the amplitude of currents at +60 mV was reduced by ~76% (14.33 ± 0.40 vs. 3.45 ± 0.66 nA before and during treatment with 4-AP). The cultured human PASMC in which we transfected KCNA5 for electrophysiological experiments maintained the characteristics of smooth muscle cells. Extracellular application of 100 μM serotonin (5-HT) caused PASMC con-
traction (Fig. 2B). The cells highly expressed the smooth muscle markers α-smooth muscle-actin, calponin, and SM-22 (Fig. 2C).

As shown in Fig. 3, whole cell $I_{K(V)}$ recorded in KCNA5-transfected HEK-293 cells exhibit similar properties to the currents recorded in KCNA5-transfected human PASMC (see Fig. 2A). Compared with wild-type cells, transfection of an empty vector negligibly affected the endogenous outward K+ currents in HEK-293 cells, which were at the range of 300–400 pA at +60 mV, while overexpression of KCNA5 in the cells led to a 38-fold increase in $I_{K(V)}$ (Fig. 3, B and C). In KCNA5-transfected HEK-293 cells, the voltage-tail current relationship curve suggested that the currents were voltage dependent with an activation threshold at approximately −35 mV. The voltage that induced half activation ($V_{1/2}$) was approximately −5.1 mV (Fig. 4).

**Pharmacological properties of Kv1.5 channels.** To examine pharmacological properties of Kv1.5 channels, we transiently transfected the human KCNA5 gene to HEK-293 cells. Extracellular application of 4-AP, a potent Kv channel blocker, significantly and reversibly inhibited the amplitude of whole cell Kv1.5 currents (data not shown) (8, 49). 4-AP (5 mM) usually caused >70% inhibition of the amplitude of Kv1.5 currents in different transfection system (8, 49).

**Effect of correolide.** Extracellular application of correolide (1 μM), a newly synthesized compound that has been demonstrated to be a selective blocker of Kv1.x channels (16, 20), markedly reduced the amplitude of Kv1.5 currents (Fig. 5A) in HEK-293 cells. At +60 mV, correolide decreased the current amplitude by ~67.5% (from 9,449.2 ± 677.9 to 3,066.3 ± 333.4 pA; $n = 6$; $P < 0.001$). In addition to reducing the amplitude of whole cell Kv1.5 currents, correolide also decelerated the channel activation; the time constant of channel activation at +60 mV was increased from 0.696 ± 0.114 to 1.803 ± 0.422 ms ($n = 6$; $P < 0.05$) after treatment with correolide (Fig. 5, Ba and b). However, correolide did not affect the channel deactivation and inactivation (Fig. 5, B, b and C). These data suggest that correolide blocks the channel conduction by potently binding with the amino acids around the pore region (16, 19, 20) and interferes with the channel opening gating.

**Effect of ET-1.** In the pulmonary (and systemic) circulation system, ET-1 is a potent endothelium-derived contracting factor and a mitogenic factor for vascular smooth muscle cells. It has been well documented that ET-1 inhibits native Kv currents in rat and human PASMC (61). In KCNA5-transfected HEK-293 cells, acute application of ET-1 (100 nM) caused a 32% reduction of the Kv1.5 channel current (from 10,997.0 ± 1391.4 to 7,473.7 ± 1,566.1 pA, $n = 6$; $P < 0.001$) (Fig. 6A).

ET-1, however, did not affect the kinetics of channel activation, deactivation, and inactivation (Fig. 6, B and C). These results suggest that ET-1 may directly interact with Kv1.5 channels in native PASMC to reduce whole cell Kv currents. The inhibitory effect of ET-1 on Kv1.5 channels may indirectly result from activation of the endothelin receptors (e.g., ET$_A$ and ET$_B$) in the plasma membrane and the downstream signaling cascade.

**Effect of bepridil.** As shown in Figs. 5 and 6, correolide and ET-1 predominantly reduced the amplitude of whole cell Kv1.5 currents but negligibly affected the kinetics of current deactivation and inactivation. However, in HEK-293 cells transiently transfected with KCNA5, extracellular application of bepridil (25 μM) not only reduced the current amplitude (by ~82%) but also significantly accelerated the current inactivation (Fig. 7), suggesting that different drugs may affect Kv1.5 channels by different mechanisms. The amplitude of steady-state currents (measured at 250–290 ms) in KCNA5-transfected cells was 8,582.6 ± 794.4 pA at +60 mV before treatment and 1,551.9 ± 312.0 pA after treatment with 25 μM bepridil ($n = 6$, $P < 0.001$) (Fig. 7, A, a and b). The 82% decrease in the steady-state current amplitude was associated with a significant acceleration of current inactivation, whereas bepridil did not affect the kinetics of channel activation and deactivation (Fig. 7, B and C). These data, which are in good agreement with those by Kobayashi et al. (27) in HEK-293 cells, suggest that bepridil is a classic open channel pore blocker.

**Effect of nicotine.** Acute treatment of KCNA5-transfected HEK-293 cells with nicotine (100 nM), a ligand of nicotinic acetylcholine receptors, also reduced the current amplitude by 37% (from 8,391.8 ± 442.7 to 5,191.1 ± 348.7 pA at +60 mV, $n = 6$; $P < 0.001$) (Fig. 8A). ET-1, however, did not affect the kinetics of channel activation, deactivation, and inactivation (Fig. 8, B, a and b). Acute exposure of cells to nicotine, however, slightly enhanced (or accelerated) the channel inactivation when cells were depolarized by positive potentials (e.g., +40 and +60 mV) (Fig. 8, A, a and C). Again, it is unclear whether nicotine-mediated inhibition of Kv1.5 channels is due to its direct interaction with the channel protein or to its activation of nicotinic acetylcholine receptors and downstream signaling cascade.

In human PASMC, we observed that extracellular application of nicotine caused a different effect on native Kv currents compared with intracellular dialysis of nicotine. As shown in Fig. 9, extracellular application of nicotine reduced native $I_{K(V)}$ in human PASMC, whereas introduction of nicotine into the

<table>
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<th>Standard Names (Accession No.)</th>
<th>Size, bp</th>
<th>Predicted Sense/Antisense</th>
<th>Location, nt</th>
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<tr>
<td>SM-22 (D17409.1)</td>
<td>358</td>
<td>5'-ATGGCACAACAGGTGTCGCTC-3'/5'-TTTCCGCTCCTGCTCCTCTCTC-3'</td>
<td>48–64</td>
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<tr>
<td>Calponin (NM_001299.4)</td>
<td>453</td>
<td>5'-CTTCCAAATGCTGGAACAG-3'/5'-ATGCTCCTGACATTCA-3'</td>
<td>388–405</td>
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<tr>
<td>α-Smooth muscle actin (NM_001615.3)</td>
<td>595</td>
<td>5'-ACCGACAAGGCTGGCATCTA-3'/5'-TGATCCACATCTGGCTGGAACAG-3'</td>
<td>165–183</td>
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<tr>
<td>GAPDH (BC083511.1)</td>
<td>553</td>
<td>5'-GCGGAAAGGACCTTGCGGATGTCG-3'/5'-GGAACTGTTGAGGGGAGGAGA-3'</td>
<td>599–617</td>
</tr>
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Standard names refer to the accession numbers in GenBank for the sequence used in designing the primer.

**Table 2. Oligonucleotide sequences of the primers used for RT-PCR**

For detailed analysis and results, please refer to the original article.
cytosolic space (by diffusion via the pipette) enhanced native $I_{K(V)}$ (Fig. 9, A and B). Our results suggest that nicotine may affect the channel activity via different mechanisms depending on its binding site, and the inhibitory effect of nicotine on Kv1.5 channels may be related to the development of pulmonary hypertension in chronic obstructive pulmonary disease (COPD) patients who have a long history of cigarette smoking.

As mentioned earlier, activity of Kv channels in human PASMC plays a critical role in excitation-contraction coupling of pulmonary arterial smooth muscle and in regulating PASMC proliferation and apoptosis (9, 78). The Kv1.5 (or KCNA5) channel has been demonstrated to be an important Kv channel subunit in PASMC that 1) contributes to regulating the resting membrane potential (5, 49); 2) functions as an important Kv channel subunit (or effector) to sense hypoxia and induce hypoxic pulmonary vasoconstriction (5, 49); and 3) serves as a modulator for apoptotic volume decrease and apoptosis (8). Downregulated KCNA5 expression and inhibited Kv1.5 channel function have been demonstrated to be important causes for sustained pulmonary vasoconstriction and excessive pulmonary vascular remodeling in patients with IPAH and hypoxia-mediated pulmonary hypertension (55, 70, 71, 81). Indeed, overexpression of the KCNA5 gene has been successfully used as an effective gene therapy approach in animal models (55), and opening of Kv1.5 channels is a target for many drugs (e.g., NO, prostacyclin, and sildenafil) clinically used for patients with familial and idiopathic pulmonary arterial hypertension (38, 68).

Previously, we showed that S-nitroso-N-acetyl penicillamine, an NO donor, enhanced the activity of large-conductance Ca$^{2+}$-activated $K^+$ channels and voltage-gated $K^+$ channels (29, 80). As shown in Fig. 10, extracellular application of 0.1
mM S-nitroso-N-acetyl penicillamine also enhanced Kv1.5 current amplitude in KCNA5-transfected HEK-293 cells. Therefore, KCNA5-encoded Kv channels may represent a putative target for NO and its vasodilatory action. The next set of experiments was designed to identify novel SNPs in the KCNA5 gene from patients with IPAH and to explore the potential association of the SNPs in KCNA5 with different responses to NO in these patients.

Identification of SNPs in the KCNA5 gene and its flanking sequences in IPAH patients. The average age of the participating IPAH patients was 44.6 ± 1.1 yr when the first right-heart or pulmonary catheterization was performed. Average mean PAP was 53.9 ± 1 mmHg, whereas PVR was 999.5 ± 38.9 dyn·s·cm⁻⁵. In all patients, CO was within the normal range of 2–9 l/min (4.14 ± 0.12 l/ml) (Fig. 11). IPAH predominantly affects women; 76% of the IPAH patients in our study were women. Caucasians accounted for 83% and Hispanics for 12% of the IPAH patients; there were 2 African-Americans and 1 Native American, and 5 patients who identified themselves as of “other” ethnic origin. Although the majority of IPAH patients were women, there was no significant difference in terms of age, mean PAP, PVR, or CO between men and women (Fig. 11).

Using the primers shown in Table 1, a ~7,000-bp sequence corresponding to the human KCNA5 gene and its ±2,000-bp flanking sequence was sequenced in genomic DNA samples from IPAH patients. We identified 17 SNPs in these samples (Fig. 12A), 7 of which have not been reported in the NCBI SNP database or in the literature (Table 3). Twelve of the SNPs (5 novel) were in the 5'-upstream region, two (1 novel) in the translated region, two (one novel) in the 3'-untranslated (UTR) region, and one downstream of KCNA5 (Fig. 12A).

Two nonsynonymous SNPs (nos. 13 and 14) were located in the heteromerization or polymerization domain of KCNA5 channel; SNP no. 13 (G773a) changed the hydrophobic glycine to a basic arginine (G182R), while SNP no. 14 (G861c, NCBI ID: rs35853292) incurred a glutamate-to-aspartate change (E211D), both of which are negatively charged and hydrophilic.
KCNA SNPs in IPAH Patients

SNPs nos. 1–11 are located in regions upstream of the KCNA5 gene, which includes its putative promoter (Fig. 12, A and B). We determined that part of the putative KCNA5 promoter is located in a 600-bp sequence between residues −500 and +100. Sequence analysis of this region highlighted many transcription factor binding sites and motifs (Fig. 12B), such as two c-Myb binding sequences (nt. −449 and +15), an NF-κB consensus binding site (nt. −269), a SP1-VGF/SP1-Erk1 site (nt. −216), an E2A/E box binding motif (nt. −158), a CreA site (nt. −149), two c/EBP-apoB-intron enhancer binding sites (nt. −105 and −94), a CAP site (nt. −23), two AP-2 binding sites (nt. −362 and nt. +19), a CACCC box (nt. −139), a CREB/c-Jun consensus site (nt. +64), and an interferon-γ response element (γ-IRE) binding site (nt. +62). SNP no. 12 (C62r) coincides with the loss of the γ-IRE binding site. SNP no. 10 (C−136t) did not alter the CACCC box sequence (nt. −139). Regions upstream from the putative promoter also contained numerous binding sites: a TATA box (nt. −1669), a myoD-MCK binding site (nt. −1087), a CAP site (nt. −828), a c-Myc responsive region (nt. −805), and a number of AP-1 consensus sites (nt. −1767, −652, and −546) (data not shown). SNP no. 2 (G−140Gs) introduced a δ-repressor element (that may alter NF-κB function) binding site, and SNP no. 3 (C−1087t) caused the loss of the myoD-MCK binding site. The presence of other upstream SNPs did not delete or introduce other transcription factor binding sites.

Minor allele frequencies of the SNPs varied greatly (Table 3). SNPs within the KCNA5 gene had a relatively low allele frequency except for SNP no. 15 (A2578r), which had an allele frequency of 0.266. For SNP no. 15, 14% of the patients exhibited a heterozygous (AA/AT) genotype. A similar case could be made for SNP no. 12 (C62r), with an allele frequency of 0.425. Upstream SNPs nos. 1 (G−1951s), no. 3 (C−1087t), and no. 10 (C−136t) also occurred frequently (allele frequencies: 0.583, 0.347, and 0.900, respectively). SNP no. 1 may not interfere greatly with Kv1.5 expression and function because it is so far upstream of the KCNA5 gene, and it does not appear to alter any transcription factor binding sites. However, as we stated above, SNPs nos. 3 and no. 10 may alter a myoD-MCK binding site.
Fig. 5. Inhibition of Kv1.5 currents by corroleolide (CRL). A: representative Kv1.5 currents (a) elicited by step depolarizations (−60 to +60 mV, holding potential of −80 mV) before (Cont), during (CRL), and after (Wash) application of 1 μM CRL. Summarized (means ± SE) I-V (b, left) and conductance-voltage (b, right) relationship curves from KCNA5-transfected HEK-293 cells (n = 6) before (C), during (E), and after (W) treatment with CRL. Bars show normalized currents (I/Imax) and PVR during pulmonary catheterization in patients before and after inhalation of NO. There were 42 patients who responded to inhaled NO (20% increase in PAP), whereas 37 patients did not (−2% to +2% change in PAP). Acute inhalation of NO reduced PAP by 17.4 ± 1.8% in the 42 responders but negligibly changed PAP by 0.2 ± 0.2% in the 37 nonresponders (Fig. 13A). NO-induced PAP changes paralleled PVR in both groups (Fig. 13, B and C). The allele frequency of SNP no. 4 (T−937a) was significantly different (P = 0.01) between responders and nonresponders; 7% of NO responders were the −937T genotype, while −34% of nonresponders were the −937A genotype. The allele frequency of SNP no. 17 (G2870a) was also significantly different (P = 0.049) between responders and nonresponders (Fig. 13C).

To examine the possibility that the different response to NO in IPAH patients is due to disease severity, we compared the values of mean PAP, PVR, and CO in responders and nonresponders before treatment. As shown in Fig. 14, the averaged
PAP was comparable between NO responders and nonresponders, whereas the averaged PVR was significantly higher in nonresponders than in responders. Furthermore, the averaged CO was lower in nonresponders than in responders. These results seem to be consistent with the data reported by Sitbon et al. (64) showing that PVR in nonresponders is significantly higher than in responders (15.3 ± 1.1 vs. 4.5 ± 1.4 l/min; P < 0.001). In their study, the IPAH patients were tested by acute intravenous application of epoprostenol or inhalation of NO (65).

Among all IPAH patients that participated in this study, 37 patients had a history of taking fenfluramine and phentermine (Fen-Phen) for >3 mo. Averaged PAP in patients who had never taken Fen-Phen was ~7 mmHg higher than for Fen-Phen users (Fig. 15A), while NO-mediated changes in PAP were comparable between non-Fen-Phen patients and Fen-Phen users (Fig. 15B). Having previously reported that treatment of human PASMC with fenfluramine downregulated KCNA5 expression and decreased I\(_{K(V)}\), we compared the allele frequency of the SNPs in KCNA5 between IPAH patients with or without history of Fen-Phen use. SNP no. 5 (C-828T) and no. 10 (C-1367T) showed significantly different allele frequencies (P = 0.027 and 0.043, respectively) between these two groups of IPAH patients. SNP no. 5 was completely absent in patients who used Fen-Phen, while it was present in 12% of the patients with no history of Fen-Phen use. SNP no. 10, although it possessed a high allele frequency in both groups, was less prominent in the Fen-Phen users than in the non-Fen-Phen users (Fig. 15C).

**DISCUSSION**

Kv channel function plays an important role in the regulation of excitation-contraction coupling in vascular smooth muscle.
Blockade of Kv channels causes membrane depolarization and triggers pulmonary vasoconstriction by opening VDCC and elevating $[Ca^{2+}]_{cyt}$ (54, 79), whereas activation of Kv channels causes membrane hyperpolarization and vasodilation by closing VDCC (80). Kv1.5 channel is a pore-forming $\beta$-subunit that participates in forming native Kv channels in PASMC (6, 55). Downregulated KCNA5 channel expression and decreased $I_{K(V)}$ have been demonstrated in PASMC from patients with IPAH (77, 81) and animals with chronic hypoxia-mediated pulmonary hypertension (52, 70). The resultant membrane depolarization and $[Ca^{2+}]_{cyt}$ elevation in PASMC contribute to causing sustained pulmonary vasoconstriction (54, 78, 79) and stimulating PASMC proliferation (50, 52, 77).

Another consequence of attenuated Kv channel activity is a decrease in PASMC apoptosis due to decelerated apoptotic volume decrease and inhibited cytoplasmic caspase activity (28). Overexpression of human KCNA5 in vitro not only increases $I_{K(V)}$ and causes membrane hyperpolarization but also enhances PASMC apoptosis (8). Furthermore, in vivo transfer of KCNA5, by increasing $I_{K(V)}$ in PASMC, causes significant regression of pulmonary vascular medial hypertrophy and reduction of PAP and PVR in chronically hypoxic rats (4, 55). These observations provide compelling evidence that 1) normal KCNA5 expression and Kv1.5 function are necessary for maintaining the resting membrane potential in PASMC and regulating pulmonary vascular tone (78), 2) KCNA5 gene downregulation and/or Kv1.5 channel dysfunction in PASMC are involved in the development of PAH (77), and 3) restored KCNA5 expression and/or Kv1.5 function may be a useful therapeutic approach for treatment of pulmonary artery hypertension (55).

![Fig. 9. Differential effect of extracellular and intracellular application of nicotine on native Kv currents in human PASMC.](image)

Fig. 9. Differential effect of extracellular and intracellular application of nicotine on native Kv currents in human PASMC. A: representative currents (a) and summarized I-V relationships (n = 5 cells, b) of $I_{K(V)}$ recorded from human PASMC before (control), during (nicotine), and after (washout) extracellular application of 100 nM nicotine. Currents were elicited by step depolarizations ranging between −60 and +80 mV from a holding potential of −70 mV. Time course (c) of the changes in $I_{K(V)}$ at +80 mV before, during, and after extracellular application of nicotine indicates that the effect of nicotine occurs within 2 min of exposure. B: representative currents (a) and summarized I-V relationships (n = 5 cells, b) of $I_{K(V)}$ recorded from human PASMC immediately after breaking in (0 min) and 26 min after intracellular dialysis of 100 nM nicotine (via pipette). Currents were elicited by a step protocol identical to that in A.

![Fig. 10. Nitric oxide (NO) activates Kv1.5 channels.](image)

Fig. 10. Nitric oxide (NO) activates Kv1.5 channels. A: phase-contrast and fluorescent images of a patched HEK-293 cell transfected with KCNA5-EGFP. B: representative (a) and summarized (n = 8 cells, b) currents recorded from KCNA5-transfected HEK 293 cells before and after treatment with 0.1 mM S-nitroso-N-acetyl penicillamine (SNAP). Currents were elicited by a step depolarization to potentials ranging between −60 and +80 mV from a holding potential of −70 mV. Current amplitudes were significantly greater at all membrane potentials, including −60 mV (c). However, relative current enhancement ($I_{SNAP}/I_{Control}$) did no vary significantly with test potential (d). *P < 0.05 vs. control.
Biophysical and pharmacological properties of Kv1.5 channels. Native Kv channels in human PASMC are homotetramers and heterotetramers composed of the same or different Kv channel α-subunits, respectively. It has been demonstrated that Kv1.5 not only forms homotetrameric channels but also forms heteromeric channels with other Kv1 family members (e.g., Kv1.2 and Kv1.4) (2, 14, 23). In addition to four pore-forming α-subunits, the native Kv channels also contain four regulatory β-subunits in rat atrial cells, inhibiting channel inactivation is known to occur by a mechanism similar to the “ball-and-chain” theory in which an inactivating domain (e.g., a cytoplasmic NH2-terminus of the pore-forming subunit, a cytoplasmic regulatory β-subunit, or a drug like bepridil) physically occludes into the channel pore in the cytoplasmic site. Such a mechanism for inhibition would be consistent with the accelerated inactivation kinetics during bepridil treatment.

Interestingly, our data also demonstrate that extracellular application of ET-1 and nicotine significantly reduced the amplitude of currents generated by K+ efflux through homomeric Kv1.5 channels. ET-1 is an important endothelium-derived constricting factor and mitogenic factor which can cause sustained pulmonary vasoconstriction and pulmonary vascular remodeling (10). The inhibitory effect of ET-1 on Kv1.5 channels shown in this study provides convincing evidence that the contractile and mitogenic effect of ET-1 may partially result from its inhibition of Kv1.5 channels in human PASMC (61). How ET-1 inhibits Kv1.5 channel activity remains unclear, but the potential mechanisms may relate to the following: 1) a direct interaction of the peptide with the extracellular pore region of the channel protein, 2) phosphorylation of the channel protein (via the cytoplasmic PKC-binding domain) mediated by increased protein kinases upon activation of ET receptors, (ETλ and/or ETβ) (61), and 3) indirect inhibition of the channel activity by Ca2+ mobilization from intracellular stores (53). As far as nicotine is concerned, studies have shown that direct infusion of nicotine may reduce NO-mediated vasorelaxation, despite the fact that inhaled nicotine may maintain circulating nitric oxide in humans (33, 35, 39). In addition to inhibiting Kv1.5 channels, as shown in this study, nicotine has also been demonstrated to inhibit Ca2+-activated K+ channels in human umbilical vein endothelial cells (30), HERG channels expressed in Xenopus oocytes (74), and ATP-sensitive K+ channels in vascular smooth muscle cells (34). Nicotine may also decrease Kv1.5 current by altering KCNA5 subunit expression. Shin et al. (62) reported such a finding with Kv1.1 subunits in rat gastric mucosal epithelial cells. The inhibitory effect of nicotine on ATP-sensitive K+ channels may result partially from nicotine-mediated production of reactive oxygen species (34). Although Kv channels are modulated by reactive oxygen species in PASMC (40, 72), it remains unclear whether nicotine-mediated inhibition of Kv1.5 channels is related to the production of reactive oxygen species.
Interestingly, our study also demonstrated that extracellular application of nicotine and intracellular dialysis of nicotine had divergent effects on native Kv channels in PASMC. The inhibitory effect of nicotine, applied externally, on $I_{K(V)}$ is likely due to activation of nicotinic acetylcholine receptors on the surface membrane. However, it is unclear why nicotine, applied internally, augments $I_{K(V)}$ and what mechanisms are involved in this effect. One of the possibilities is that nicotine, when applied internally, may bind with the cytoplasmic regulatory $\beta$-subunits to decelerate inactivation of the pore-forming $\alpha$-subunits.

In addition to patients with idiopathic and thromboembolic pulmonary hypertension, patients with COPD and emphysema may also develop pulmonary hypertension as a result of sustained pulmonary vasoconstriction, increased stiffness (or decreased compliance) of pulmonary vascular wall, deposition of extracellular matrix proteins around the vessels, and hypoxia-mediated pulmonary vascular remodeling (41). Many COPD patients with secondary pulmonary hypertension have a long history of cigarette smoking. The nicotine-mediated inhibition of Kv1.5 channels, as shown in this study, may serve as an additional mechanism for the development of pulmonary hypertension in COPD patients with history of smoking.

Possible impact of SNPs in KCNA5 on channel expression and function in IPAH patients. Forty-four SNPs are listed in the NCBI SNP database for the human KCNA5 gene and its 2,000-bp 5' and 3'-flanking sequences. The KCNA5 gene itself contains 23 of these SNPs, with only 5 SNPs within the coding region (i.e., exon 1). Three recent studies have identified another 12 low-frequency SNPs in various populations, with possible linkage to long QT syndrome and drug resistance. Iwasa et al. (25) reported a synonymous S3-S4 loop G383G mutation in Japanese citizens. Simard et al. (63) reported five synonymous and six nonsynonymous (allele frequency 7%) in the cardiac KCNA5 of 190 individuals from three ethnic groups (Caucasian, Asian, and African-American), including the G383G mutation; four SNPs in the NH2 terminus, one in the TM-1 domain, two in the TM-1/TM-2 loop, one in the TM-3/TM-4 loop, one in the TM-6 domain, and two in the COOH-terminus. Expression of the five nonsynonymous SNPs and four nonsynonymous SNPs in Chinese hamster ovary cells did not alter KCNA5 gating properties, whereas the two COOH-terminal variants (P532L and R578K) altered current sensitivity to quinidine and propafenone, both clinically used antiarrythmic drugs. Plante et al. (48) identified 3 SNPs in a population of 96 French-Canadians; two of the SNPs (A251T and P307S) had been identified previously in the study by Simard et al. (63). When expressed in Chinese hamster ovary cells, two of the SNPs altered channel gating (decreased amplitude, slowed inactivation, accelerated opening); these effects led to slight prolongation of the action potential. Their data also suggested that changes in channel gating associated
with these SNPs required the presence of the regulatory Kv β-subunit. From the latter two studies, it is possible that SNPs in KCNA5 may have serious physiological implications in cardiovascular disease.

As mentioned earlier, decreased Kv channel expression and function are implicated in PASMC from IPAH patients and in PASMC from animals with hypoxia-mediated pulmonary hypertension. IPAH is a rare and fatal disease; the incidence of IPAH is ~1–2 per million of general population (18). Taking advantage of our access to blood and DNA samples from of patients with this rare disease, we also aimed to identify novel SNPs in the KCNA5 gene in the patients with IPAH. We identified 17 SNPs, 7 of which have not been previously reported (Table 2). While the bulk of the SNPs occurred in the upstream region, two nonsynonymous SNPs within the KCNA5 coding region were identified. Because the SNPs (G773a and G861c) are located in the heteromerization or polymerization domain where Kv channel α- and β-subunits interacts with each other, they may affect assembly of functional tetrameric Kv channels (60, 76). We are currently evaluating the impact of these two SNPs on Kv1.5 currents. The other two SNPs (A2578r and T2806r/g) in the 3’-UTR may alter the regulatory effects of protein kinases on channel activity or may be involved in posttranslational regulation of Kv1.5 channel expression.

Although we identified a putative KCNA5 promoter in the 5’-upstream sequence, the transcriptional regulation of the gene is not necessarily limited to the transcription factor binding sites in this 600-bp region. It is possible that transcription factors may modulate KCNA5 transcription by binding to sequences farther upstream of our predicted promoter region. In analyzing the putative 600-bp promoter region and the region farther upstream, we identified multiple transcription factor binding sites which suggest that KCNA5 transcription may be regulated by NF-κB, CREB, c/EBP, c-Myb, Erk1, and AP-2, all of which have been implicated in the regulation of vascular smooth muscle proliferation and apoptosis and, potentially, in the development of PAH (22, 32, 75). SNP no. 12 (C62r) itself may result in loss of a γ-IRE binding site. Since the γ-IRE is involved in urokinase plasminogen activator-mediated cell migration and activation of STAT-1 (15), it is possible that loss of the γ-IRE binding site in KCNA5 promoter influences thrombosis-mediated PASMC proliferation. Although most of the SNPs upstream of KCNA5 do not directly alter the known binding sequences, they may still affect transcriptional regulation of the gene by indirectly altering the tertiary structure of the promoter and the binding of transcription factors to the promoter region. While we identified several SNPs in the putative promoter region of KCNA5 gene, it is, however, still unknown whether these SNPs are functionally involved in the transcriptional regulation of the gene. Another

Table 3. SNPs in the KCNA5 gene identified in IPAH patients

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<td>90% (G)/10% (t)</td>
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<td>3741930</td>
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<td>27</td>
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<td>49% (C)/51% (t)</td>
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<tr>
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<td>G773a</td>
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<td>E211D</td>
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<td>2</td>
<td>0</td>
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<td>99% (C)/1% (g)</td>
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<td>GGAAGAAG (A/t)GCTGTCGCG</td>
<td>177</td>
<td>70</td>
<td>12</td>
<td>0.266</td>
<td>80% (A)/20% (t)</td>
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<tr>
<td>16</td>
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<td>TTGAGGACTA (A/t-g)CTTCTGATA</td>
<td>181</td>
<td>2 (A/g)</td>
<td>1 (TT)</td>
<td>0.006*</td>
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<tr>
<td>4625555</td>
<td>17</td>
<td>G2870a</td>
<td>TTAGGGAATCA (g/a)TCTTATTTTT</td>
<td>179</td>
<td>15</td>
<td>1</td>
<td>0.047</td>
<td>1% (A)/99% (g)</td>
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NA, not applicable. ID Ref. refers to the NCBI SNP identification number (rs-); nucleotide change is the nucleotide number relative to the start of the KCNA5 gene. ΔAA is nonsynonymous single-nucleotide polymorphisms (SNPs) that lead to change of amino acids. Data from the final column are based on the NCBI and HapMap Project databases; the allele frequency of the SNPs in the general population is shown as major allele frequency (%)/minor allele frequency (%).

*Same allele frequency for both A/g and A/t mutations.

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Fig. 13. Correlation of the SNPs in KCNA5 with hemodynamic responses to inhaled NO. A: summarized mean PAP (A) and PVR (B) in NO responders (Resp, n = 42 patients) and nonresponders (Non-Resp, n = 37 patients) before (Cont, open bars) and after (NO, solid bars) inhalation of NO. ***P < 0.001 vs. Cont. Allele frequencies of SNP no. 4 and no. 17 were significantly different between NO responders and nonresponders (C).
limitation of this study is that we are unable to correlate the SNPs identified from blood samples with Kv channel activity in PASMC and to define how the SNPs lead to a decrease in whole-cell $I_{K(V)}$.

In our previous studies, we have shown that 1) the amplitude of endogenous $I_{K(V)}$ is decreased, the inactivation kinetics of whole cell $I_{K(V)}$ is accelerated, and the mRNA expression of Kv1.5 (KCNA5) channels is downregulated in PASMC from IPAH patients (77, 81); 2) the basal apoptosis and staurosporine/BMP-2-induced apoptosis are both inhibited in PASMC from IPAH patients compared with PASMC from normal subjects and normotensive patients (82); 3) overexpression of KCNA5 in PASMC increases $I_{K(V)}$ and causes membrane hyperpolarization (49); and 4) overexpression of KCNA5 in PASMC and other cell types (e.g., HEK-293 and COS-7 cells) accelerates apoptotic volume decrease and enhances apoptosis (8). These observations suggest that the expression and function of Kv1.5 (KCNA5) channels are associated with the degree of pulmonary vascular remodeling in IPAH patients. Further study is needed to define whether and how the SNPs identified in KCNA5 gene from IPAH patients affect the transcription, function, and modulation of Kv1.5 channels in PASMC.

Implication of SNP occurrence in KCNA5 with drug response in IPAH patients. Ion channels, such as Kv channels, represent one of the cellular targets for NO-mediated vasodilatory, antiproliferative, and/or proapoptotic effects in the pulmonary vasculature. NO can cause pulmonary vasodilation 1) by activating $Ca^{2+}$-activated $K^+$ channels and Kv channels in PASMC, causing membrane hyperpolarization and closure of VDCC (3, 29), and 2) by directly blocking VDCC (12, 13). NO also enhances PASMC apoptosis by activating K$^+$ channels, accelerating apoptotic cell shrinkage and increasing cytoplasmic caspase activity, which may potentially cause regression of pulmonary medial hypertrophy (29). The higher allele frequency of the SNP no. 4 (T-937a) in KCNA5 in IPAH patients who do not respond to NO suggests that variations in KCNA5 transcriptional regulation may affect pulmonary vascular reactivity to vasodilators in IPAH patients. However, whether SNP nos. 4 (T-937a) and 17 (G2870a) can be used as a genetic indicator for NO responsiveness to guide therapeutic choices for IPAH patients is uncertain and needs further study in a larger group of patients.

As indicated by the hemodynamic data (Fig. 14), 1) mean PAP (before inhalation of NO) is comparable between responders and nonresponders, 2) basal PVR is higher in nonresponders than in responders, and 3) CO is lower in nonresponders than in responders. These data are similar to the observations in IPAH patients by Sitbon et al. (64). Therefore, the possibility exists that the different response to NO in IPAH patients might also be due to differences in disease severity rather than solely due to the SNPs identified in KCNA5 gene.

![Fig. 14. Distribution and comparison of hemodynamics between NO responders and nonresponders in IPAH patients. Histograms show distribution of mean PAP (A), PVR (B), and CO (C) in IPAH patients whose PAP/PVR were reduced by inhalation of NO (responders) and patients whose PAP/PVR were not changed by NO (nonresponders). Averaged values (means ± SE) of mean PAP, PVR, and CO for responders (Resp) and nonresponders (Non-Resp) are shown at right. **P < 0.01 vs. responders.](http://ajpcell.physiology.org/)

![Fig. 15. Correlation of the SNPs in KCNA5 with use of fenfluramine and phentermine (Fen-Phen). A and B: mean PAP (A) and NO-mediated changes in PAP (B) in IPAH patients with (+) or without (−) a history of taking Fen-Phen. Allele frequencies of SNP nos. 5 and 10 were significantly different in (+) and (−) Fen-Phen patients (C).](http://ajpcell.physiology.org/)
Anorexigen, which have been linked to the development of IPAH (1), also inhibit the expression and function of Kv channels (e.g., Kv1.5) in PASMC (45, 69). We identified SNP nos. 3 and 10 whose minor allele frequencies were significantly decreased in IPAH patients with Fen-Phen use. Unfortunately, because of the low number of Fen-Phen users in our study, we were unable to establish a definite correlation between SNP occurrence and Fen-Phen-related IPAH development.

In summary, Kv1.5 channel is one of the important Kv channel subunits that are functionally expressed in human PASMC (14, 21, 36, 81). Kv1.5 channel can form homotetramer meric channels that are sensitive to 4-AP, bepridil, and coroloeilde. 4-AP and coroloeilde decrease Kv1.5 currents predominantly by reducing the channel conductance, whereas bepridil decreases the currents largely by conferring (or accelerating) inactivation on the noninactivating Kv1.5 channels (i.e., bepridil acts as an open channel blocker for Kv1.5 channels). Furthermore, the inhibitory effect of ET-1 and nicotine on Kv1.5 channels suggest that blockade of Kv1.5 in PASMC may play an important role in ET-1 (and nicotine)-mediated pulmonary vasoconstriction and vascular medial hypertrophy. Because of its complex etiology, IPAH is generally considered as a multifactorial disease. In addition to genetic factors, other factors such as environmental factors, hormones, and cytokines may also contribute to the development of IPAH. The genetic factors that have been implicated in the pathogenesis of IPAH include single-nucleotide polymorphisms (SNPs) in four genes encoding cardiac ion channels: KCNQ1 (encoding Kv7.5), KCNQ4 (encoding Kv7.4), KCNJ2 (encoding Kir2.6), and KCNJ18 (encoding Kir2.18).

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