

## Clinical Implications of Determining BMPR2 Mutation Status in a Large Cohort of Children and Adults With Pulmonary Arterial Hypertension

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- Background:** Bone morphogenetic protein receptor type 2 (BMPR2) mutations occur in idiopathic and familial pulmonary arterial hypertension (IPAH, FPAH); however, the impact of these mutations on clinical assessment and disease severity remains unclear. We investigated the role of BMPR2 mutations on acute vasoreactivity and disease severity in IPAH/FPAH children and adults.
- Methods:** BMPR2 mutation types were determined in 147 IPAH/FPAH patients. Hemodynamics were obtained at baseline and with acute vasodilator testing.
- Results:** Of 147 patients (69 adults, 78 children; 114 with IPAH, 33 with FPAH), 124 (84%) were BMPR2 mutation-negative, and 23 (16%) were mutation-positive. BMPR2 mutation-positive patients were less likely to respond to acute vasodilator testing than mutation-negative patients (4% vs 33%;  $p < 0.003$ ;  $n = 147$ ). BMPR2 mutation-positive children also appeared less likely to respond to acute vasodilator testing than mutation-negative children. BMPR2-positive patients had lower mixed venous saturation ( $57 \pm 9\%$  vs  $62 \pm 10\%$ ;  $p < 0.05$ ) and cardiac index (CI;  $2.0 \pm 1.1$  vs  $2.4 \pm 1.5$  liters/min;  $p < 0.05$ ) than BMPR2-negative patients.
- Conclusions:** Patients with BMPR2 mutations are less likely to respond to acute vasodilator testing than mutation-negative patients and appear to have more severe disease at diagnosis. Determination of BMPR2 mutations appears to help identify IPAH/FPAH children and adults who are unlikely to respond to acute vasodilator testing and, thus, unlikely to benefit from calcium channel blockade (CCB) treatment. *J Heart Lung Transplant* 2008;27:668-74. Copyright © 2008 by the International Society for Heart and Lung Transplantation.

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by right ventricular failure and death if untreated. Bone morphogenetic protein type 2 (BMPR2) mutations have been identified in idiopathic pulmonary arterial hypertension (IPAH) and familial pulmonary arterial hypertension (FPAH).<sup>1-4</sup> Although there have been significant advances in the treatment of PAH with disease-specific targeted therapies, there is

still no cure. Furthermore, choice of "optimal" medical treatment remains challenging due to our inability to predict which agent will be most efficacious for a given patient.<sup>5,6</sup> To date, the only method to predict whether a patient will respond favorably to a given treatment is by determining their response to acute vasodilator testing during right heart cardiac catheterization.<sup>7-9</sup> Patients with a robust response to acute vasodilator testing, with no change or an increase in cardiac output, may benefit from long-term oral calcium channel blockade (CCB).<sup>10</sup> These patients, with appropriate treatment, also appear to have a slower disease progression than those without a significant acute vasodilator testing response.<sup>7,9</sup> Elliot et al<sup>11</sup> reported that IPAH and FPAH adult patients with BMPR2 mutations are less likely to respond to acute vasodilator testing than BMPR2 mutation-negative patients. However, the significance of BMPR2 mutations in IPAH and FPAH children has not yet been described. Furthermore, the impact of BMPR2 mutations and mutation type on disease severity and/or clinical course remains unclear.

The objectives of our study were to determine: (1) whether BMPR2 mutations are associated with

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Submitted December 12, 2007; revised February 8, 2008; accepted February 17, 2008.

Supported by Grant No. HL-060056 from the NIH-NHLBI (to J.H.M.).

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response to acute vasodilator testing in children as well as in adults; and (2) if mutation types are associated with disease severity in children and/or in adult patients. We also analyzed whether there were specific characteristics among this pediatric and adult cohort that could identify patients more likely to respond to acute vasodilator testing, and/or predict disease severity.

## METHODS

We studied a cohort of consecutive pediatric and adult patients referred to the New York Presbyterian Pulmonary Hypertension Center between 1991 and 2005, in whom a diagnosis of IPAH or FPAH was confirmed according to the World Health Organization's Venice 2003 Pulmonary Hypertension Symposium consensus.<sup>12</sup> Blood samples for genetic studies and detailed family histories were obtained for all patients. Patients were excluded if they did not provide a blood sample or have hemodynamic acute vasodilator testing data.

The primary analysis included 147 patients for whom we had DNA and acute vasodilator testing data. We chose to perform analyses with 147 patients, using only 1 individual per family (proband only in FPAH pedigrees;  $n = 33$ ) to prevent introducing a potential bias due to having multiple related individuals with the same mutation in the analyses. An alternative set of analyses, where data from 163 patients, including multiple individuals from the FPAH pedigrees (i.e., 49 FPAH patients identified in the 33 FPAH families), could have been performed by taking into account family membership and relatedness within the family. However, we elected to do a more conservative analysis of 1 patient per family. This was done because it was unclear whether environmental effects would co-vary in proportion with relatedness within a family, and also because of the possibility of an ascertainment bias in our sample in the collection of large pedigrees. We understood that this type of analysis with probands would only likely demonstrate an overall lower mutation frequency than previously published findings.<sup>13-17</sup> Data are presented as mean  $\pm$  SD unless otherwise stated. All patients provided written informed consent (and assent if indicated) according to a protocol approved by the institutional review board of the Columbia University College of Physicians & Surgeons, New York, New York.

## Hemodynamics and Acute Vasoreactivity Testing

Complete hemodynamic data were obtained during right heart catheterization (using standard techniques) at the time of diagnosis at rest and during acute vasodilator testing (80 ppm inhaled nitric oxide [iNO]).<sup>17-19</sup> Acute pulmonary vasoreactivity was defined using a definition that appears to best predict long-term CCB response.<sup>10</sup> Patients who were respon-

sive had a fall in mean pulmonary artery pressure (PAPm)  $\geq 10$  mm Hg to a PAPm  $\leq 40$  mm Hg, with no change or an increase in cardiac output.<sup>20</sup> For patients with PAPm  $< 40$  mm Hg, PAPm and pulmonary vascular resistance index (PVRi) had to decrease by  $\geq 20\%$  to be considered responsive to acute vasodilator testing.<sup>7</sup>

## Molecular Analysis

**Mutation analysis of *BMPR2* exons.** *BMPR2* was screened for both large deletions using multiplex ligation-dependent probe amplification (MLPA) and small deletions and point mutations using DNA sequencing. Nucleic acid sequencing was performed on all patient samples (IPAH and FPAH). Only FPAH families without a primary sequence finding were screened with MLPA: two with large deletions are described in Table 4. The DNA sequence of the 13 exons and adjacent intron/exon boundaries of *BMPR2* were screened using bidirectional nucleic acid sequencing using a cycle-sequencing kit (Big DyeR, version 1.1; Applied Biosystems [ABI], Foster City, CA) and detected with a Model ABI 3100 sequencer. DNA sequencing traces were inspected using MUTATION SURVEYOR, version 2.0 (SoftGenetics, Inc., State College, PA). For FPAH, 1 or 2 affected individuals per family were initially sequenced for mutation discovery and, once a mutation was identified, samples from all available members of the family were sequenced for the mutation to ensure cosegregation with disease transmission. For IPAH, *BMPR2* mutations were determined in the affected individual and, when available, in the parents. Patients who had the S775N polymorphism were classified as *BMPR2* mutation-negative as this polymorphism has been found in normal controls.<sup>11</sup>

MLPA was performed with 100 ng of genomic DNA using a probe set (P093 Salsa MLPA HHT/PPH1; MRC-Holland, Amsterdam, The Netherlands), according to the manufacturer's instructions.<sup>21</sup> Probe amplification products were detected on an ABI 3100 (Applied Biosystems, Foster City, CA). MLPA peaks were visualized with GENEMARKER software, version 1.3 (SoftGenetics, Inc.). Normalization and calculation of dosage ratios was performed as described at [www.muc-holland.com/MLPA%20analysis.htm](http://www.muc-holland.com/MLPA%20analysis.htm). The control value for each probe was the average of five normal control samples run at the same time as the PAH samples. Dosage ratios of  $>0.7$  and  $>1.35$  were interpreted as deletions or duplications, respectively.

## Statistical Analysis

All IPAH and FPAH patients with *BMPR2* mutation results were included in the analysis. Comparisons between categorical data were made using chi-square tests. Continuous data were compared using un-

**Table 1.** Demographics ( $n = 147$ )

	Male/female, $n$ (%)	Ethnicity, $n$ (%)				Age <sup>a</sup> (years)
		White	Black	Hispanic	Asian	
Adults	19 (28%)/50 (72%)	55 (80%)	2 (3%)	5 (7%)	7 (10%)	42 ± 12
Children	30 (38%)/48 (62%)	66 (85%)	7 (9%)	1 (1%)	4 (5%)	8 ± 5
Total	49 (33%)/98 (67%)	121 (82%)	9 (6%)	6 (4%)	11 (8%)	

<sup>a</sup>Age at diagnostic right heart catheterization (mean ± SD).

paired *t*-tests or analysis of covariance (ANCOVA) models. Data are presented as mean ± SD. A 0.05 level of significance was used. Because these analyses were considered exploratory, a post hoc method to reduce the chances of committing a Type I error was not used. The data were analyzed using SAS/STAT software.

## RESULTS

### Patient Population

In total, 147 patients (69 adults, 78 children; 98 females [67%]) were comprised the study population, which included 114 IPAH patients (49 adults, 65 children) and 33 FPAH patients (20 adults, 13 children). Patients ≥18 years of age were classified as adults. Age at baseline catheterization was 42 ± 12 years for adults (range 20 to 66 years) and 8 ± 5 years for children (range 4 months to 17.8 years) (Table 1).

### Acute Vasoreactivity and BMPR2

Among the total population of 147 patients, 42 (29%) were acute responders and 105 (71%) were non-responders.<sup>20</sup> Eighty-four percent (124 of 147) of the total patient cohort was BMPR2-negative, and 16% (23 of 147) were BMPR2 mutation-positive.

Of the 147 patients, those with BMPR2 mutations were less likely to have a positive acute vasodilator testing response than BMPR2-negative patients (4% [ $n = 1$ ] vs 33% [ $n = 41$ ];  $p < 0.003$ ;  $n = 147$ ). Children with BMPR2 mutations also appeared less likely to respond to acute vasodilator testing than those without BMPR2 mutations (13% vs 44%;  $n = 78$ ) (Figure 1); however, a reliable statistical analysis could not be performed due to the small number of children with BMPR2 mutations. Subgroup analyses demonstrated that, overall, children were more likely to respond to acute vasodilator testing than adults ( $p < 0.002$ ;  $n = 147$ ) (Figure 2).

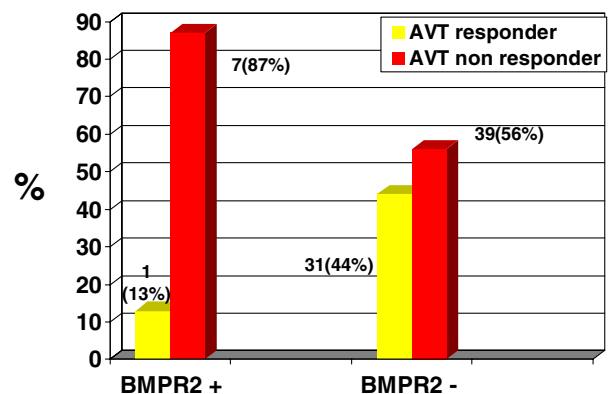
### Disease Severity and BMPR2

The PAPm for the entire cohort ( $n = 147$ ) at baseline was 60 ± 19 mm Hg, mean systolic blood pressure (SBPm) = 82 ± 14 mm Hg, mean right atrial pressure (RAPm) = 8 ± 6 mm Hg, mixed venous saturation (MvO<sub>2</sub>) = 61 ± 10%, arterial blood oxygen saturation (Sao<sub>2</sub>) = 93 ± 5%, PVRi = 22 ± 14 u·m<sup>2</sup>, systemic vascular resistance index (SVRi) =

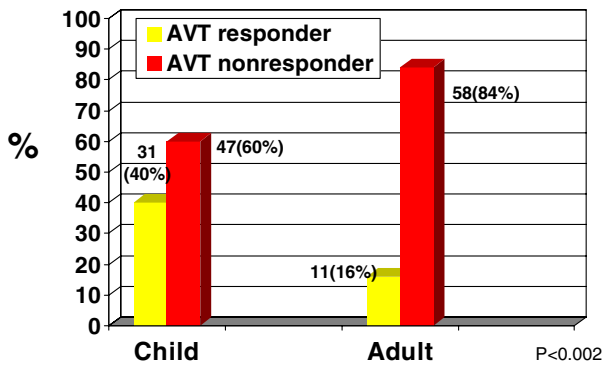
31 ± 16 u·m<sup>2</sup>, cardiac index (CI) = 2.3 ± 1.4 liters/min/m<sup>2</sup> and mean pulmonary capillary wedge pressure (PCWpm) = 8 ± 4 mm Hg. Patients with BMPR2 mutations had lower MvO<sub>2</sub> (57 ± 9% vs 62 ± 10%;  $p = 0.02$ ) and CI (2.0 ± 1.1 liters/min/m<sup>2</sup> vs 2.4 ± 1.5 liters/min/m<sup>2</sup>;  $p < 0.05$ ) than BMPR2-negative patients. The differences in RAPm (10 ± 5 mm Hg vs 7 ± 6 mm Hg;  $p = 0.07$ ) and SVRi (37 ± 16 u·m<sup>2</sup> vs 30 ± 16 u·m<sup>2</sup>;  $p = 0.06$ ) were not significantly different between BMPR2-positive and BMPR2-negative patients (Table 2).

There were several hemodynamic differences between the pediatric and adult patients. The children's systolic blood pressures were lower than those of the adult population (Table 3). However, the children had higher PAPm, MvO<sub>2</sub> and CI (62 ± 22 mm Hg vs 56 ± 13 mm Hg, 64 ± 9% vs 58 ± 10% and 2.8 ± 1.2 liters/min/m<sup>2</sup> vs 1.9 ± 1.7 liters/min/m<sup>2</sup>, respectively) and lower PVRi (20 ± 13 u·m<sup>2</sup> vs 25 ± 15 u·m<sup>2</sup>) and RAPm (5 ± 3 mm Hg vs 10 ± 7 mm Hg) than the adult patients at time of diagnosis ( $p < 0.05$  for all comparisons).

BMPR2 mutation testing identified mutations in 23 (19 FPAH, 4 IPAH) patients: 8 children (7 FPAH, 1 IPAH) and 15 adults (12 FPAH, 3 IPAH). Table 4 lists the exon, nucleic acid change, amino acid position, mutation type, and whether a mutation was being reported for the first time or the reference to the first report for each proband. There were 13 previously unreported mutations: 5 in the children (4 frameshift/splice and 1



**Figure 1.** Children with BMPR2 mutations appear to be less likely to respond to AVT than BMPR2-negative children (13% vs 44%;  $n = 78$ ).



**Figure 2.** Subgroup analyses demonstrate that, overall, children are more likely to respond to AVT than adults ( $p < 0.002$ ;  $n = 147$ ), and FPAH children are also more likely to respond to AVT than FPAH adults.

missense) and 8 in the adults (6 frameshift/splice and two nonsense). Without knowledge of exact recombination sites, the exon 4/5 deletion in the family of Patient 13 may have been identical to that previously reported.<sup>21-23</sup> Three patients (1 IPAH child, 1 IPAH adult, 1 FPAH proband) with polymorphisms previously reported in normal controls (e.g., S775N) were excluded.<sup>11,22</sup> Two children had the same mutation although their background haplotypes were different.<sup>2</sup> Four of these 23 mutations were also observed using the acute vasodilator testing analysis of Elliot et al.<sup>11</sup> Three adults had FPAH in both studies, although in our study the fourth patient had FPAH, not IPAH.

The mutation types identified for the cohort of 23 BMPR2 mutation-positive FPAH/IPAH patients included 5 missense (22%; 4 children and 1 adult), 7 nonsense (30%; all adults) and 11 frameshift/splice/deletion (48%; 4 children and 7 adults) mutations. The splice mutations were considered deleterious as all four families with these mutations had either a splice mutation, which segregated in family members other than the

**Table 2.** Baseline Hemodynamic Parameters for BMPR2-positive vs BMPR2-negative IPAH/FPAH Patients

Parameter	BMPR2 ( $n = 147$ )		$p$ -value
	Negative mutation ( $n = 124$ )	Positive mutation ( $n = 23$ )	
PAPm (mm Hg)	59 ± 20	61 ± 13	0.73
SBPm (mm Hg)	81 ± 13	86 ± 19	0.17
RAPm (mm Hg)	7 ± 6	10 ± 5	0.07
Mvo <sub>2</sub> (%)	62 ± 10	57 ± 9	0.02
Sao <sub>2</sub> (%)	93 ± 05	93 ± 05	0.77
PVRi (u-m <sup>2</sup> )	21 ± 14	26 ± 14	0.20
SVRi (u-m <sup>2</sup> )	30 ± 16	37 ± 16	0.06
PCWp (mm Hg)	8 ± 5	9 ± 3	0.79
CI <sup>a</sup> (liters/min/m <sup>2</sup> )	2.4 ± 1.5	2.0 ± 1.1	0.03

Data expressed as mean ± SD. See text for abbreviations.

<sup>a</sup> $n = 129$ .

**Table 3.** Baseline Hemodynamic Parameters for Children vs Adults at the Time of Diagnosis

Parameter	Adult/child ( $n = 147$ )		$p$ -value
	Adult ( $n = 69$ )	Child ( $n = 78$ )	
PAPm (mm Hg)	56 ± 13	62 ± 22	0.02
SBPm (mm Hg)	90 ± 13	75 ± 12	0.0001
RAPm (mm Hg)	10 ± 7	5 ± 3	0.0001
Mvo <sub>2</sub> (%)	58 ± 10	64 ± 9	0.0003
Sao <sub>2</sub> (%)	93 ± 05	94 ± 04	0.26
PVRi (u-m <sup>2</sup> )	25 ± 15	20 ± 13	0.03
SVRi (u-m <sup>2</sup> )	39 ± 18	24 ± 11	0.0001
PCWp (mm Hg)	9 ± 5	8 ± 4	0.02
CI <sup>a</sup> (liters/min/m <sup>2</sup> )	1.9 ± 1.7	2.8 ± 1.2	0.01

Data expressed as mean ± SD. See text for abbreviations.

<sup>a</sup> $n = 129$ .

proband (Patients 1, 3 and 10), and/or had misspliced transcripts resulting in two incomplete transcripts (Patients 1, 3 and 17). When patients were categorized by BMPR2 mutation type, those with frameshift/splice/deletion mutations had higher baseline RAPm than those with missense mutations, with frameshift/splice/deletions at 13 ± 5 mm Hg and missense mutations at 5 ± 2 mm Hg ( $p = 0.01$ ;  $n = 23$ ). The RAPm difference remained statistically significant with acute vasodilator testing ( $p < 0.05$ ). There were no other significant differences in hemodynamics between mutation types.

## DISCUSSION

In a large cohort of IPAH and FPAH children and adults, those with BMPR2 mutations were less likely to respond to acute vasodilator testing than BMPR2-negative patients. Our data are consistent with results reported by Elliott et al in an adult cohort.<sup>11</sup> In addition, these findings support the potential of using genetics in selection of a medical regimen for individual PAH patients. IPAH/FPAH patients with a robust acute vasodilator testing response can often be treated with CCB and, overall, acute vasodilator testing responders appear to have a more favorable long-term prognosis than non-responders.<sup>7,9</sup> Unfortunately, one limitation of our study is that there were very few children who were positive for the BMPR2 mutation ( $n = 8$ ), and only 1 that was responsive to acute vasodilator testing (13%). Although the acute response rate for vasodilator testing for IPAH children in previous reports ranged from 20% to 40%, due to underpowering, we cannot conclude that children who are BMPR2-positive are less likely to respond to acute vasodilator testing.<sup>8,9</sup> Longer-term studies are required.

The present study raises the possibility that BMPR2 genotype may prove useful for decision-making in the therapeutic algorithm for adults and children with IPAH or FPAH. Based on these preliminary genotype data,

**Table 4.** Mutation Type, Exon, Nucleic Acid and Amino Acid Changes in 23 BMPR2 Mutation-positive Children and Adults With FPAH or IPAH

Patients	Gender	Age	Mutation		Nucleotide change	Amino acid change	Reference
			type	Exon			
BMPR2 mutation-positive children							
With FPAH							
1	F	12	Splice	2	c.247 + 1delGCAAGTG	p.splice (?G248fsx177)	This study
2	M	2	Frameshift	2,3	c.large deletion	p.large deletion	This study
3	F	5	Splice	3	c.-5-248delTATAGGinsAC	p.splice	This study
4	F	14	Missense	3	c.295T > C	p.C99R	Machado et al, 2006 <sup>24</sup>
5	F	11	Missense	11	c.1471C > T	p.R491W	Deng et al, 2000 <sup>2</sup>
6	F	9	Missense	11	c.1471C > T	p.R491W	Deng et al, 2000 <sup>2</sup>
7	F	11	Frameshift	12	c.2410-2413delGTCA	p.V804Pfsx1	This study
With IPAH							
8	F	12	Missense	9	c.1156G > A	p.E386Q	This study
BMPR2 mutation-positive adults							
With FPAH							
9	F	41	Nonsense	2	c.201T > G	p.Y67X	This study
10	F	46	Splice	3	c.418 + 5G > A	p.splice	This study
11	F	30	Nonsense	4	c.439C > T	p.R147X	Machado et al, 2001 <sup>25</sup>
12	F	48	Nonsense	4	c.507-510delCTTTinsAAA	p.C169X	Deng et al, 2000 <sup>2</sup>
13	F	60	Frameshift	4,5	c.large deletion	p.large deletion	This study
14	M	36	Frameshift	6	c.690-691delAGinsT	p.K230fsx21	This study
15	M	39	Nonsense	7	c.961C > T	p.R321X	Koehler et al, 2004 <sup>15</sup>
16	M	30	Nonsense	8	c.994C > T	p.R332X	Thomsen et al, 2000 <sup>4</sup>
17	F	30	Splice	9	c.1276 + 1G > A	p.splice	This study
18	F	28	Missense	11	c.1472G > A	p.R491Q	Deng et al, 2000 <sup>2</sup>
19	F	28	Frameshift	12	c.2579delT	p.N861fsx10	Deng et al, 2000 <sup>2</sup>
20	F	56	Nonsense	12	c.2617C > T	p.R873X	Deng et al, 2000 <sup>2</sup>
With IPAH							
21	F	30	Frameshift	2	c.116delC	p.P39fsx7	This study
22	M	50	Nonsense	9	c.1146T > G	p.Y382X	This study
23	F	22	Frameshift	12	c.2305delC	p.P769fsx1	This study

patients with frameshift/splice/deletion mutations had higher RAPm values than missense mutation patients. Whether BMPR2 mutation type predicts disease severity at diagnosis for individual patients requires further investigation.

The low frequency of BMPR2 mutations noted here in IPAH ( $n = 114$ , 3.5%), both for IPAH children (1.5%) and adults (6.1%), could be explained by several factors. First, the reported frequencies of BMPR2 mutations in IPAH, ~10% to 40%, pertain predominantly to adult patients.<sup>4,13,15</sup> There also may be an ethnicity or racial difference as the highest frequency has been found in Japanese patients, 40%,<sup>13</sup> and the lowest in German patients, 11%.<sup>15</sup> Our patients were predominantly white with very few Asians. The limited information available for BMPR2 mutations in children found two de novo mutations in 2 of 18 (11%) children,<sup>14</sup> whereas no mutations were found in 13 German IPAH children.<sup>16</sup> MLPA analyses were not used for our IPAH patients. This technique has resulted in an increase in the frequency of reported BMPR2 mutations in FPAH,<sup>17</sup>

whereas the technique, when applied to IPAH, found 2 of 14 (14%) IPAH adults had BMPR2 point mutations with no exon deletions/duplications and, in another report, 6 of 126 (5%) cases had gene rearrangements.<sup>17</sup>

Interestingly, during our 14-year study, 5 IPAH patients were reclassified as FPAH: 3 adults had siblings develop PAH; 1 child's mother developed PAH; and another child's identical twin and their father developed PAH. Furthermore, only relatives of probands with these deleterious mutations (frameshift/splice/deletions) developed PAH. These data support that there is greater risk with a deleterious mutation. If these 5 patients were included with the 4 IPAH patients described in Table 4, this would be 9 of 114 patients; that is, closer to 10% frequency (even without MLPA), which would identify additional mutations.

We compared acute vasodilator testing response rates in children vs adults, and in patients with and without BMPR2 mutations. Historically, we have observed that up to 40% of IPAH children respond to acute vasodilator testing, as compared with 7% to 10%

of adult IPAH patients.<sup>8-10</sup> This observation is confirmed by our data; that is, children with IPAH/FPAH were more likely to respond to acute vasodilator testing than adult IPAH/FPAH patients (40% vs 16%, respectively;  $p < 0.002$ ). Whether this is due to children having a different “disease” than adults, or children being diagnosed at an earlier stage of disease (i.e., prior to the development of “fixed” pulmonary vascular obstructive changes) remains unclear. However, children with BMPR2 mutations, similar to adult PAH patients, appear less likely to respond to acute vasodilator testing (Figure 1).

One limitation of this study is that the actual number of positive BMPR2 mutations was small ( $n = 23$ ; 8 children and 15 adults); thus, if separate analyses were performed on the children and adults, the power of the study would be diluted further. Another limitation of the study is that the comparison of hemodynamics between the BMPR2-positive and -negative patients may have been affected by the proportion of children and adults in each group, with a higher proportion of adults (worse baseline hemodynamics) in the BMPR2 group.

Additional limitations to our study include exclusion of patients without acute vasodilator testing or blood samples available for DNA. It is unknown whether excluding these patients or collecting the data retrospectively created a selection bias. The majority of patients had FPAH and the predominant mutation type was frameshift/splice/deletion—that is, 13 of the 23 (53%) mutations were of the frameshift/splice/deletion type. The frameshift/splice/deletion group, when compared with the missense mutation group, appeared to have worse baseline hemodynamics. These findings coincide with the belief that frameshift/splice/deletion mutations are more deleterious than missense mutations. There are several possible explanations for the altered distribution of mutation type (i.e., increased frameshift/splice/deletion and decreased missense) in our study compared with a recent summary report of 144 distinct mutations in 210 subjects, 53% and 19%, respectively vs 36% and 30%, respectively.<sup>24</sup> The increased number of missense and decreased number of frameshift mutations in the international summary could have resulted from the technical factor that mutations were predominantly determined by nucleic acid sequencing (which could have missed large exon deletions). In addition, another recent summary report<sup>23</sup> had more IPAH patients than our cohort and included mutations found in patients with PAH associated with congenital heart disease or fenfluramine derivatives. These latter mutations were more likely to have been missense mutations. In addition, although there may have been a selection bias in the distribution of mutation types reported herein, our

findings provide the first insight into a possible role of mutation type in a rare disease.

In conclusion, BMPR2-positive children and adults with IPAH or FPAH are unlikely to respond to acute vasodilator testing, and thus unlikely to respond to CCB therapy. Furthermore, pediatric IPAH/FPAH patients, regardless of BMPR2 status and/or disease type (i.e., IPAH vs FPAH), are more likely to respond to acute vasodilator testing than adult patients. Although these data support the utility of determining BMPR2 mutations in identifying patients who are unlikely to respond to CCB therapy, it is premature to recommend not performing acute vasodilator testing in patients with BMPR2 mutations. Further studies are needed for confirmation before making consensus guidelines. In addition, identifying genetic polymorphisms that may predict efficacy with various PAH therapeutic modalities may further improve treatment outcomes in PAH. Identifying specific BMPR2 mutation types may have prognostic implications that could prove useful in the timing and selection of specific PAH treatments.

The investigators thank the individuals and families with PAH for their essential role in this study. We also thank Daniela Brady, RN, and Diane Kerstein, MD, for their participation in the clinical testing for these data. Finally, we thank the genetics counselor, Helen Temple.

## REFERENCES

1. Atkinson C, Stewart S, Upton PD, et al. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation* 2002;105:1672-8.
2. Deng Z, Morse JH, Slager SL, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000; 67:737-44.
3. Lane KB, Machado RD, Pauciulo MW, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 2000;26:81-4.
4. Thomson JR, Machado RD, Pauciulo MW, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. *J Med Genet* 2000;37:741-5.
5. Benza RL, Park MH, Keogh A, Girgis RE. Management of pulmonary arterial hypertension with a focus on combination therapies. *J Heart Lung Transplant* 2007;26:437-46.
6. Keogh AM, McNeil KD, Wlodarczyk J, Gabbay E, Williams TJ. Quality of life in pulmonary arterial hypertension: improvement and maintenance with bosentan. *J Heart Lung Transplant* 2007;26:181-7.
7. Rich S, Kaufmann E, Levy PS. The effect of high doses of calcium channel blockers on survival in primary pulmonary hypertension. *N Engl J Med* 1992;327:76-81.
8. Barst RJ, Maislin G, Fishman AP. Vasodilator therapy for primary pulmonary hypertension in children. *Circulation* 1999;99:1197-208.

9. Yung D, Widlitz AC, Rosenzweig EB, et al. Outcomes in children with idiopathic pulmonary arterial hypertension. *Circulation* 2004;10:110:660-5.
10. Sitbon O, Humbert M, Jais X, et al. Long-term response to calcium channel blockers in idiopathic pulmonary arterial hypertension. *Circulation* 2005;111:3105-11.
11. Elliott CG, Glissmeyer EW, Havlena GT, et al. Relationship of BMPR2 mutations to vasoreactivity in pulmonary arterial hypertension. *Circulation* 2006;113:2509-15.
12. Proceedings of the Third World Symposium on Pulmonary Arterial Hypertension, Venice, Italy, June 23-25, 2003. *J Am Coll Cardiol* 2004;43(suppl S):1S-90.
13. Morisaki H, Nakanishi N, Kyotani S, et al. BMPR2 mutations found in Japanese patients with familial and sporadic primary pulmonary hypertension. *Hum Mutat* 2004;23:632.
14. Harrison RE, Berger R, Haworth SG, et al. Transforming growth factor- $\beta$  receptor mutations and pulmonary arterial hypertension in childhood. *Circulation* 2005;111:435-41.
15. Koehler R, Grunig E, Pauciulo MW, et al. Low frequency of BMPR2 mutations in a German cohort of patients with sporadic idiopathic pulmonary arterial hypertension. *J Med Genet* 2004;41:e127 (<http://www.jmedgenet.com/cgi/content/full/41/e127>).
16. Grunig E, Koehler R, Miltenberger-Miltenyi G, et al. Primary pulmonary hypertension in children may have a different genetic background than in adults. *Pediatr Res* 2004;56:571-8.
17. Aldred MA, Vijaykrishnan J, James V, et al. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *Hum Mutat* 2006;27:212-3.
18. Sitbon O, Humbert M, Jagot JL, et al. Inhaled nitric oxide as a screening agent for safely identifying responders to oral calcium-channel blockers in primary pulmonary hypertension. *Eur Respir J* 1998;12:265-70.
19. Barst RJ. Pharmacologically induced pulmonary vasodilatation in children and young adults with primary pulmonary hypertension. *Chest* 1986;89:497-503.
20. Barst RJ, McGoon M, Torbicki A, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;43(suppl):40S-7.
21. Shouten JP, McElgunn CJ, Waaijer R, et al. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucl Acids Res* 2002;30:e57.
22. Elliot CG. Genetics of pulmonary arterial hypertension: current and future implications. *Semin Respir Crit Care Med* 2005;26:365-71.
23. Cogan JD, Pauciulo MW, Batchman AP, et al. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2006;174:590-8.
24. Machado RD, Aldred MA, James V, et al. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* 2006;27:121-32.
25. Machado RD, Pauciulo MW, Thomson JR, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *Am J Hum Genet* 2001;68:92-102.