Pulmonary Arterial Hypertension
Insights from Genetic Studies

James E. Loyd[1]

[1]Vanderbilt University Medical Center, Vanderbilt Medical Center North, Nashville, Tennessee

Familial pulmonary arterial hypertension (FPAH) was described 60 years ago, but real progress in understanding its origins and pathogenesis is just beginning. Germline mutations in bone morphogenetic protein receptor type 2 (*BMPR2*) are responsible for the disease in most families, and also in many sporadic cases of idiopathic PAH. Heritable PAH refers to patients with a positive family history, or with a responsible genetic mutation, and is an autosomal dominant disease that affects females disproportionately, may occur at any age, and is characterized by reduced penetrance and variable expressivity. These characteristics suggest that other endogenous or exogenous factors modify its expression. Several different factors have recently been demonstrated to modify the clinical expression of *BMPR2* mutation, including estrogen metabolites and functional polymorphisms in transforming growth factor–β1 and CYP1B1. Furthermore, a linkage study recently identified modifier loci for *BMPR2* clinical expression, which suggests an oligogenic model. Clinical testing for *BMPR2* mutations is available for families with heritable and idiopathic PAH, and is an evolving model of personalized medicine. Variable age of onset and decreased penetrance confound genetic counseling, because the majority of carriers of a *BMPR2* mutation will never develop PAH, but often transmit the risk to their progeny.

Keywords: pulmonary; pulmonary artery; pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a progressive disease, characterized by widespread proliferative occlusion of the smallest pulmonary arteries (Figure 1). PAH was formerly known as primary pulmonary hypertension (PPH) from 1950 to 1998. As small pulmonary arteries become progressively occluded, then pulmonary vascular resistance successively increases, until it eventually causes heart failure after the right ventricle can no longer compensate the increased workload. PAH occurs at all ages, but affects women much more frequently than men. The sporadic form is now known as idiopathic PAH (IPAH), comprised 94% of all PPH in the National Institutes of Health registry natural history study in the mid-1980s (1), and is clinically and pathologically indistinguishable from the familial PAH (FPAH) form. Heritable PAH (HPAH) was recently adopted as terminology for PAH in families, and for IPAH in patients who have a responsible genetic mutation. Despite discovery of the genetic origin for most FPAH families during the last decade, it remains unknown which specific signaling pathways are critical to develop the disease, and why PAH is restricted to only this very small segment of the vasculature.

(*Received in original form July 2, 2010; accepted in final form February 15, 2011*)

Supported by the Vanderbilt Clinical and Translational Science Awards grant UL1 RR024975 from National Center for Research Resources/National Institutes of Health.

Correspondence and requests for reprints should be addressed to James E. Loyd, M.D., Vanderbilt University Medical Center, T1219 Vanderbilt Medical Center North, Nashville, TN 37232. E-mail: jim.loyd@vanderbilt.edu

Proc Am Thorac Soc Vol 8. pp 154–157, 2011
DOI: 10.1513/pats.201007-047MS
Internet address: www.atsjournals.org

SYNOPSIS OF PAH TREATMENT

Although effective therapy for PAH has been developed recently, it remains tragically inadequate since one third of all patients with PAH still die within 3 years (2). Prior to availability of effective treatment in the mid-1990s, half of all patients with PAH died within three years. Several pharmacologic therapies are now FDA approved for PAH, including prostacyclins, endothelin receptor antagonists, and phosphodiesterase inhibitors, but they are limited in overall efficacy, and some are very expensive ($175,000/year). Treatment has been shown to improve PAH symptoms and functional status, but meta-analyses (3–5) yield conflicting results about its effect on survival. Furthermore, no therapy has been shown to limit or reverse the pulmonary vascular disease that underlies PAH. Lung transplantation is a final option for some, but has many limitations itself, including a mean survival of only 5 years. In summary, a tremendous need exists to develop a highly effective PAH therapy, which should be facilitated by understanding PAH pathogenesis.

PAH IN FAMILIES

FPAH is phenotypically identical to IPAH, and was recognized soon after the original report of PPH in the early 1950s. FPAH represented 6% of all PPH in the U.S. National Institutes of Health prospective study in the 1980s. The REVEAL registry, enrolling at 54 centers in the United States in 2006–2007, recently reported 1,166 patients with IPAH, along with 69 patients with FPAH, representing 5.6% of the combined group (6). A report from France in 2008 described patients with IPAH and those with FPAH who were seen in the French Network on Pulmonary Hypertension between January 1, 2004 and June 1, 2007, and who were tested for bone morphogenetic protein receptor (BMPR) 2 mutation (7). Among a total of 233 patients with IPAH and those with FPAH, 38 (16.6%) reported a positive family history. A later report by the French Network on Pulmonary Hypertension in 2010 (8) about PAH survival described enrolling 354 patients between October 2002 and October 2003, including 26 (7%) with a positive family history. The earliest FPAH reports in the 1950s demonstrated vertical transmission and father-to-son transmission, which implicate a single autosomal dominant gene. Mechanisms of the reduced penetrance and earlier onset in subsequent generations are not yet understood.

FPAH LINKAGE AND BMPR2 DISCOVERY

Our group began to study a Tennessee family at Vanderbilt University Medical Center (Nashville, TN) in 1980, then reviewed the FPAH literature and reported six patients in this family (9). This family has since demonstrated a genetic founder effect, as it has suffered 35 family members with PAH during 5 decades, including 29 women and 6 men (Figure 2). Our sample bank grew slowly until it was sufficient to support a gene search in 1997, when Nichols and colleagues (10) linked a region of 2q32 with a highly significant logarithm of odds score using microsatellite markers. Our team extended that progress to

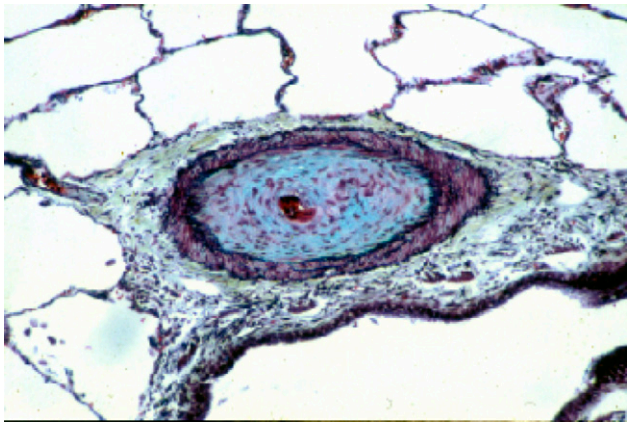

Figure 1. Histopathology of small pulmonary artery of patient with heritable pulmonary arterial hypertension (HPAH), showing total loss of vascular lumen by proliferative intimal lesion, with vascular media smooth muscle hypertrophy, in addition to small central microthrombus.

identify *BMPR2* as the responsible gene in the year 2000 (11). Simultaneous identical progress was contributed independently by Morse and colleagues (12) at Columbia Presbyterian (New York, NY), who also collected a large FPAH cohort and linked FPAH to 2q32 in 1997, then discovered mutations in *BMPR2* are responsible for FPAH in 2000 (13).

MUTATIONS IN SPORADIC IPAH

After it was discovered to be a basis for FPAH, *BMPR2* mutation became an obvious candidate gene for IPAH, as the phenotypes are identical. We tested 50 patients with IPAH and found that 13 of 50 patients (25%) carried *BMPR2* mutations (14). Subsequent studies have shown that 10–40% of IPAH cohorts carry *BMPR2* mutations (15, 16). It seems paradoxical that, in the general population overall, most *BMPR2* mutations reside within "sporadic" IPAH cases, which is a consequence of IPAH comprising the vast majority, while carrying a small, but significant, prevalence of underlying mutations. Reduced penetrance leading to successive skip generations commonly conceals the true genetic origin, and *de novo* mutations also occur.

By the mid-2000s, mutations in *BMPR2* had been identified by sequencing studies for half of the known FPAH families. Then, Cogan and colleagues (17) developed RT-PCR approaches, which were later supplemented by multiple ligation-dependent probe amplification (MLPA), to identify insertion or deletion mutations in *BMPR2* in another 25% of families. In addition, mutation in other members of the transforming growth factor (TGF)–β superfamily have been recognized to occasionally be the basis of PAH, including mutations in ACVRL1/ALK1, ENG, and SMAD 8 (18).

MODIFIERS FOR CLINICAL EXPRESSION OF BMPR2 MUTATION

Reduced penetrance and variable expressivity suggest that other endogenous or exogenous factors, or both, modify HPAH clinical expression. Phillips and colleagues (19) hypothesized that functional TGF-β1 single-nucleotide polymorphisms (SNPs) could affect the balance of TGF-β/BMP signaling in *BMPR2* mutation heterozygotes to decrease the age of onset and increase the penetrance in FPAH. We assayed SNP genotypes of *BMPR2* mutation heterozygotes, their age at diagnosis, and penetrance of FPAH. We found that *BMPR2* mutation heterozygotes with the least-active TGF-β1 SNPs had greater ages at diagnosis (39.5 and 43.2 yr) than those with more active genotypes (31.6 and 33.1 yr; $P = 0.03$ and 0.02, respectively). Carriers of *BMPR2* mutation types that do not exhibit nonsense-mediated decay (NMD), and who also carry the least-, intermediate-, and most-active TGF-β1 SNP −509 genotypes, had penetrances of 33, 72, and 80%, respectively ($P = 0.003$). We also found increased expression of TGF-β1–dependent SMAD2 in lung sections of patients with FPAH. We concluded that TGF-β1 SNPs modulate penetrance and age of onset of FPAH in *BMPR2* mutation heterozygotes, probably by affecting the balance of TGF-β/BMP signaling.

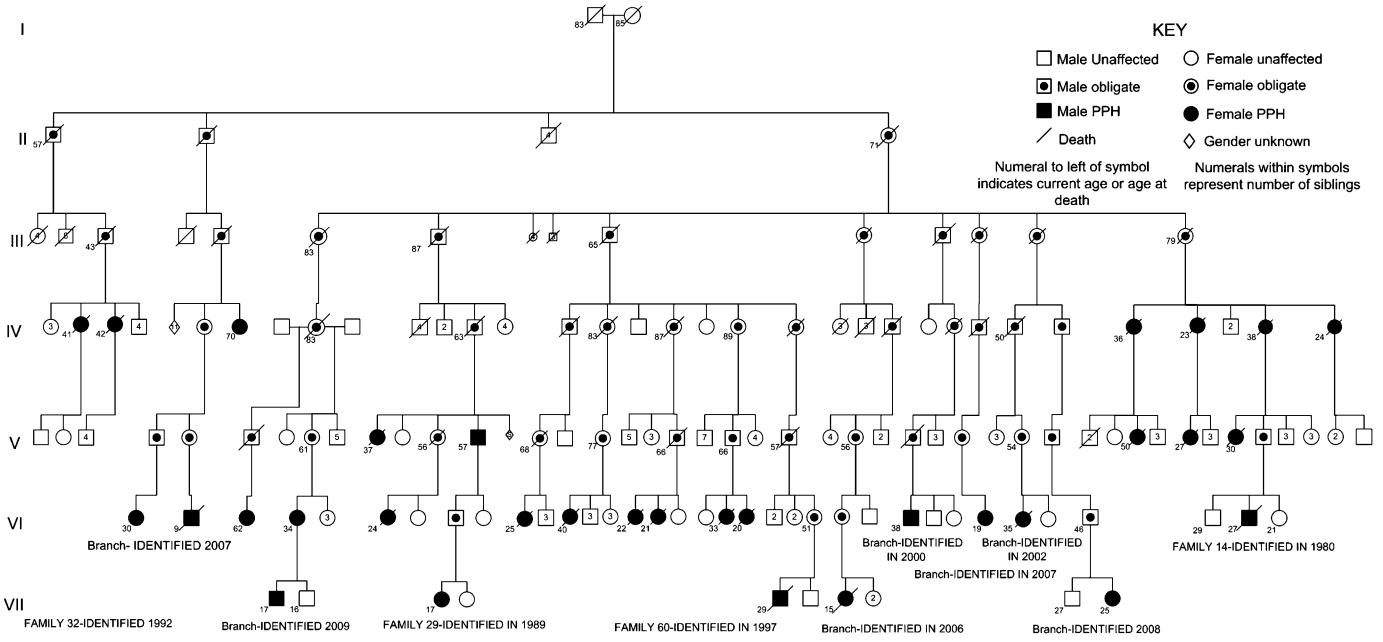

Figure 2. Pedigree of HPAH family from Tennessee with 33 patients.

MUTATIONS WITH NMD AND BMPR2 EXPRESSION

We hypothesized that patients with missense *BMPR2* mutations have more severe disease than those with premature termination codon mutations, the transcripts of which are typically degraded by NMD. We tested for *BMPR2* mutations in 169 patients with PAH (125 with a family history of PAH and 44 with sporadic disease) (20). Of 106 patients with a detectable *BMPR2* mutation, 96 patients had lymphocytes available to measure the activity of NMD. Phenotypic characteristics were compared between *BMPR2* mutation carriers and noncarriers, and between carriers with a missense versus truncating mutation. We found a statistically significant difference in age at diagnosis between patients who carried a mutation versus those who did not, but subgroup analysis revealed this to be the case only for females. We found no difference in age at diagnosis or death, or in survival, related to the exonic location of the *BMPR2* mutation. However, patients with missense mutations had statistically significant younger age of onset and death, as well as shorter survival than those with truncating mutations. Consistent with these data, the majority of missense mutations had age of onset before 36 years, whereas the majority of truncating mutations had PAH onset after age 36. We conclude that female *BMPR2* mutation carriers have earlier onset of PAH than noncarriers, and patients with missense *BMPR2* mutations have more severe disease than those with truncating mutations. These findings suggest that specific types of mutation may lead to different strategies for new interventions.

A NOVEL BMPR2 TERMINATION MUTATION ESCAPES NMD: IMPLICATIONS FOR PAH TREATMENT

We studied a unique premature truncation codon (PTC) mutation (W13X) that did not behave in the expected manner (21). Transcripts from PTC mutations are typically degraded through NMD. We were surprised to find that this patient's cultured lymphocytes contained readily detectable levels of the PTC-containing transcript. Further analysis suggested that this transcript escaped NMD by translational reinitiation at a downstream Kozak sequence, resulting in the omission of 173 amino acids. We treated the patient cells with aminoglycoside, which decreased the levels of the truncated protein, along with a reciprocal increase in the full-length *BMPR2* protein and, importantly, BMPR-II signaling. This aminoglycoside-mediated "repair" of a *BMPR2* mutation at the protein level in patient-derived cells also has implications for novel treatment of HPAH.

FPAH PENETRANCE IS AFFECTED BY EXPRESSION OF THE NORMAL BMPR2 ALLELE

Hamid and colleagues (22) reasoned that variable levels of expression of the wild-type (WT) *BMPR2* allele might also act as a modifier to influence the penetrance of FPAH. We analyzed the WT *BMPR2* levels by real-time PCR analysis of lymphocyte cell lines derived from patients with FPAH and control subjects. We chose FPAH kindreds who carry mutations that result in activation of NMD, which leads to degradation of the mutant RNA, thus ensuring that only the WT *BMPR2* transcripts are detected in the real-time assay. We found that WT and mutant *BMPR2* levels can be reproducibly measured in patient-derived cell lines, and that unaffected mutation carrier cell lines have higher levels of WT *BMPR2* transcripts than those derived from patients with FPAH ($P < 0.005$). These findings suggest that the level of expression of WT *BMPR2* allele transcripts is important in the pathogenesis of FPAH. Work by other groups suggests that expression levels of BMPR2 may also be an underlying mechanism for other forms or models of pulmonary hypertension.

EXPRESSION ARRAYS TO IDENTIFY MODIFIERS: PATIENTS VERSUS UNAFFECTED BMPR2 MUTATION CARRIERS

In a search for genes that modify *BMPR2* clinical expression, we compared expression arrays of lymphocyte cell lines from three groups: (*1*) patients with FPAH with *BMPR2* mutations; (*2*) *BMPR2* mutation–positive family members without PAH; and (*3*) family members without mutation (23). This approach allows examination of gene expression in the absence of confounding influences from treatment or disease-related effects. We found consistent alterations in multiple pathways, including actin organization, immune function, calcium balance, cell growth, and apoptosis. One gene, CYP1B1, demonstrated 10-fold lower expression in female patients with PAH. We conclude that disease status in *BMPR2* mutation carriers correlates with alterations in proliferation, GTP signaling, and stress response pathways. The robust differential expression of the estrogen-metabolizing gene, CYP1B1, suggests that it modifies clinical expression of *BMPR2* mutation.

SEX DISPARITY AND ESTROGENS

The differential expression data implicating CYP1B1 led us to conduct studies of genetic and metabolic markers of altered estrogen metabolism in subjects who carry *BMPR2* mutation (24). Genotypes for CYP1B1 Asn453Ser (N453S) were determined for 140 *BMPR2* mutation carriers (86 females and 54 males). Nested within those subjects, a case–control study of urinary estrogen metabolite levels (2-hydroxyoestrogen [2-OHE] and 16α-hydroxyoestrone [16α-OHE1]) was conducted in females. We found fourfold higher penetrance among female subjects homozygous for the N/N genotype than for those with N/S or S/S genotypes ($P = 0.005$). Consistent with this finding, the 2-OHE/16α-OHE1 ratio was 2.3-fold lower in affected mutation carriers compared with unaffected mutation carriers ($P = 0.006$). Our findings suggest that variations in estrogen and its metabolism modify FPAH risk, and suggest new therapeutic approaches as well as mechanistic insights.

NOVEL LINKAGE STUDY TO LOCATE MODIFIERS IN BMPR2 FAMILIES

Chung and colleagues (25) at Columbia Presbyterian recently described novel analytic approaches to link genetic loci that influence FPAH expression in *BMPR2* mutation carriers. They performed a genome-wide linkage scan in 15 FPAH families segregating for *BMPR2* mutations, using a dense SNP array and a novel multiscan linkage procedure. They discovered linkage in four regions: 3q22 (median LOD = 3.43), 3p12 (median LOD = 2.35), 2p22 (median LOD = 2.21), and 13q21 (median LOD = 2.09). When used in conjunction with nonparametric bootstrap, this approach provides high resolution to identify candidate gene regions containing putative *BMPR2*-interacting genes. Imputation of the disease model by LOD score maximization indicated that the 3q22 locus alone predicts most FPAH cases in *BMPR2* mutation carriers, providing strong evidence that *BMPR2* and the 3q22 locus interact epistatically. Future identification of the contributing genes in these loci is expected to contribute pivotal information about pathogenesis.

BMPR2 MUTATION MOUSE MODELS

Many investigators, including West and colleagues and others, have developed and then progressively improved relevant mouse models of *BMPR2* mutation (26). Current models recapitulate

the PAH phenotype, so that they provide real promise for further understanding. Novel interventions, such as ACE2 infusion, in the R899X *BMPR2* mutation model (26), demonstrate not only prevention of pulmonary hypertension, but, surprisingly, reverse it as well. Such models are an excellent venue for preclinical testing of novel agents, as well as for improved understanding of the pathogenetic mechanisms.

During the past decade considerable progress has been made in understanding the genetic origins and the molecular basis of the proliferative pulmonary vascular disease that characterizes PAH. However, despite discovery of the genetic origin for most FPAH families during the last decade, it remains unknown as to which specific signaling pathways are critical to develop the disease, and why PAH is restricted to a very small segment of the vasculature. Many therapies for PAH have been approved during this same time period, but none has been shown to improve the pulmonary vascular obstruction or to cure the disease in any patient. Expanding our understanding of the basic underlying mechanisms may be the best approach to direct development of effective therapies to target the vascular disease that underpins PAH.

Author Disclosure: J.E.L. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Rich S, Dantzker DR, Ayres SM, Bergofsky, EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK, *et al.* Primary pulmonary hypertension: a national prospective study. *Ann Intern Med* 1987;107:216–223.
2. McLaughlin VV, Archer SL, Badesch DB, Barst RJ, Farber HW, Lindner JR, Mathier MA, McGoon MD, Park MH, Rosenson RS, *et al.* ACCF/AHA 2009 expert consensus document on pulmonary hypertension a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association developed in collaboration with the American College of Chest Physicians; American Thoracic Society, Inc.; and the Pulmonary Hypertension Association. *J Am Coll Cardiol* 2009;53:1573–1619.
3. Macchia A, Marchioli R, Marfisi R, Scarano M, Levantesi G, Tavazzi L, Tognoni G. A meta-analysis of trials of pulmonary hypertension: a clinical condition looking for drugs and research methodology. *Am Heart J* 2007;153:1037–1047.
4. Galie N, Manes A, Negro L, Palazzini M, Bacchi-Reggiani ML, Branzi A. A meta-analysis of randomized controlled trials in pulmonary arterial hypertension. *Eur Heart J* 2009;30:394–403.
5. Macchia A, Marchioli R, Tognoni G, Scarano M, Marfisi R, Tavazzi L, Rich S. Systematic review of trials using vasodilators in pulmonary arterial hypertension: why a new approach is needed. *Am Heart J* 2010;159:245–257.
6. Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, Barst RJ, Benaz RL, Liou TG, Turner M. *et al.* Pulmonary arterial hypertension: baseline characteristics from the REVEAL Registry. *Chest* 2010;137:376–387.
7. Sztrymf B, Coulet F, Girerd B, Yaici A, Jais X, Sitbon O, Montani D, Souza R, Simonneau G, Soubrier F, *et al.* Clinical outcomes of pulmonary arterial hypertension in carriers of BMPR2 mutation. *Am J Respir Crit Care Med* 2008;177:1377–1383.
8. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, Yaici A, Weitzenblum E, Cordier JF, Chabot F, *et al.* Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* 2010;122:156–163.
9. Loyd JE, Primm RK, Newman JH. Familial primary pulmonary hypertension: clinical patterns. *Am Rev Respir Dis* 1984;129:194–197.
10. Nichols WC, Koller DL, Slovis B, Foroud T, Terry VH, Arnold ND, Siemieniak DR, Wheeler L, Phillips JA, III, Newman JH, *et al.* Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31–32. *Nat Genet* 1997;15:277–280.
11. Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA, III, Loyd JE, Nichols WC, Trembath, RC. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. The International PPH Consortium. *Nat Genet* 2000;26:81–84.
12. Morse JH, Jones AC, Barst RJ, Hodge SE, Wilhelmsen KC, Nygaard TG. Mapping of familial primary pulmonary hypertension locus (PPH1) to chromosome 2q31-q32. *Circulation* 1997;95:2603–2606.
13. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Ventos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, *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; 67:737–744.
14. Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Milkhail G, Rogers P, *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;37:741–745.
15. Grunig E, Koehler R, Miltenberger-Miltenyi G, Zimmermann R, Gorenflo M, Mereles D, Arnold K, Naust B, Wilkens H, Benz A, *et al.* Primary pulmonary hypertension in children may have a different genetic background than in adults. *Pediatr Res* 2004;56:571–578.
16. Morisaki H, Nakanishi N, Kyotani S, Takashima A, Tomoike H, Morisaki T. BMPR2 mutations found in Japanese patients with familial and sporadic primary pulmonary hypertension. *Hum Mutat* 2004;23:632.
17. Cogan JD, Vnencak-Jones CL, Phillips JA, III, Lane KB, Wheeler LA, Robbins IM, Garrison G, Hedges LK, Loyd JE. Gross BMPR2 gene rearrangements constitute a new cause for primary pulmonary hypertension. *Genet Med* 2005;7:169–174.
18. Trembath RC, Thomson JR, Machado RD, Morgan NV, Atkinson C, Winship I, Simonneau G, Galie N, Loyd JE, Humbert M, *et al.* Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 2001;345:325–334.
19. Phillips JA III, Poling JS, Phillips CA, Stanton KC, Austin ED, Cogan JD, Wheeler L, Yu C, Newman JH, Dietz HC, *et al.* Synergistic heterozygosity for TGFbeta1 SNPs and BMPR2 mutations modulates the age at diagnosis and penetrance of familial pulmonary arterial hypertension. *Genet Med* 2008;10:359–365.
20. Austin ED, Phillips JA, III, Cogan JD, Hamid R, Yu C, Stanton KC, Phillips CA, Wheeler LA, Robbins IM, Newman JH, *et al.* Truncating and missense BMPR2 mutations differentially affect the severity of heritable pulmonary arterial hypertension. *Respir Res* 2009;10:87.
21. Hamid R, Hedges LK, Austin E, Phillips JA, III, Loyd JE, Cogan JD. Transcripts from a novel BMPR2 termination mutation escape nonsense mediated decay by downstream translation re-initiation: implications for treating pulmonary hypertension. *Clin Genet* 2010; 77:280–286.
22. Hamid R, Cogan JD, Hedges LK, Austin E, Phillips JA, III, Newman JH, Loyd JE. Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. *Hum Mutat* 2009;30:649–654.
23. West J, Cogan J, Geraci M, Robinson L, Newman J, Phillips JA, Lane K, Meyrick B, Loyd J. Gene expression in BMPR2 mutation carriers with and without evidence of pulmonary arterial hypertension suggests pathways relevant to disease penetrance. *BMC Med Genomics* 2008;1:45.
24. Austin ED, Cogan JD, West JD, Hedges LK, Hamid R, Dawson EP, Wheeler LA, Parl FF, Loyd JE, Phillips JA, III. Alterations in oestrogen metabolism: implications for higher penetrance of familial pulmonary arterial hypertension in females. *Eur Respir J* 2009;34: 1093–1099.
25. Rodriguez-Murillo L, Subaran R, Stewart WC, Pramanik S, Marathe S, Barst RJ, Chung WK, Greenberg DA. Novel loci interacting epistatically with bone morphogenetic protein receptor 2 cause familial pulmonary arterial hypertension. *J Heart Lung Transplant* 2009;29: 174–180.
26. Johnson JA, Hemnes AR, Lane KB, Robinson L, Gladson S, West J. ACE2 reverses established pulmonary arterial hypertension in BMPR2899X mice [abstract]. *Am J Respir Crit Care Med* 2010;181: A6327.