# Defining the Role and Clinical Relevance of BMPR2 Mutations In Pulmonary Arterial Hypertension

**Jane H. Morse, MD**
Professor Emerita of Clinical Medicine
and Special Lecturer
Department of Medicine
College of Physicians and Surgeons
Columbia University, New York, New York

## Introduction

Recent advances in molecular genetics during the past four years have defined two genes that causally underlie the development of pulmonary arterial hypertension. The first and perhaps most important is bone morphogenetic protein (BMP) receptor II gene (BMPR2),[1,2] and the second is activin receptor kinase-like 1 (ALK1).[3,4] The specifics of the genetic relationships between ALK1 mutations and pulmonary arterial hypertension in hereditary hemorrhagic telangiectasia (HHT) are discussed in an accompanying article. Another accompanying article reviews the relationship between serotonin and the serotonin transporter in pulmonary arterial hypertension. Both BMPR2 and ALK1 are receptors in the transforming growth factor beta (TGF-beta) superfamily (**Figure 1**).[5] They participate, in ways yet to be fully elucidated, in preventing proliferation of cells in small pulmonary arterioles that can cause pulmonary arterial hypertension. Although the discovery of mutations in these two genes has provided previously unrecognized experimental approaches to elucidating the pathobiology of this disorder, ie, investigating the BMP/TGF-beta signaling pathway, the main focus of this review is on the reported BMPR2 mutations and their clinical relevance in pulmonary arterial hypertension.

## Clinical Classification

The classification of pulmonary arterial hypertension includes a heterogeneous group of clinical entities, sharing similar pathological changes in the pulmonary vasculature, that has evolved within one rubric. The framework for this discussion of BMPR2 mutations will use the Venice 2003 World Health Organization (WHO) clinical classification of the various subcategories of pulmonary arterial hypertension.[6] The subcategories are idiopathic pulmonary arterial hypertension (formerly called sporadic primary pulmonary hypertension), familial pulmonary arterial hypertension, and pulmonary arterial hypertension related to connective tissue diseases, congenital systemic to pulmonary shunts, portopulmonary hypertension, HIV infection, and drugs and toxins.

## BMPR2 Mutations in Familial Pulmonary Arterial Hypertension

**Gene discovery.** Familial pulmonary arterial hypertension, by definition, has two family members with the disease. Early pedigree studies showed that familial pulmonary arterial hypertension (formerly familial primary pulmonary hypertension) was inherited as an autosomal dominant disease with incomplete penetrance; that is, not all family members who are obligate carriers of a BMPR2 mutation will develop disease.[7] Overall penetrance is between 10% and 20%. Affected families also had an increased female to male ratio (2.7:1) and display genetic anticipation, eg, the mean age of death is younger with succeeding generations.[8] The techniques of using microsatellite markers followed by linkage analyses pinpointed the physical locus of the gene, initially called PPH1, to a large ~25 cM region on chromosome 2 q31,32 (**Figure 2**).[9,10] Fine mapping of more families reduced the initial size of the locus to 3 cM.[11,12] Subsequently, two groups working independently simultaneously reported that heterozygous germline mutations in BMPR2 caused familial pulmonary arterial hypertension.[1,2] Both groups used positional cloning, a method that sequences candidate genes at the relevant chromosomal locus, to find the disease-causing gene. Soon after, BMPR2 mutations were reported in idiopathic pulmonary arterial hypertension.[13]

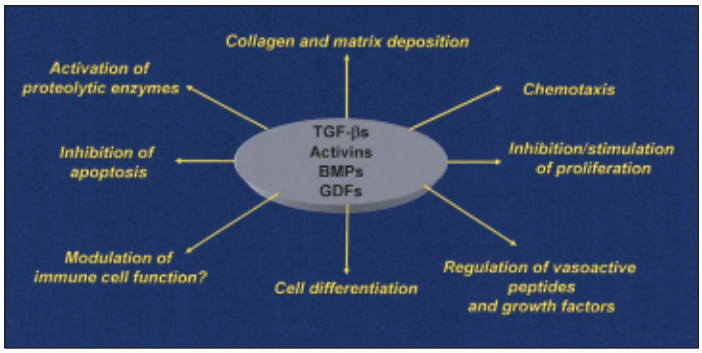


**Figure 1. Potential roles of transforming growth factor-beta (TGF-ß) superfamily in vascular remodeling. The TGF-ß superfamily has diverse roles in a wide variety of physiological processes, including cell proliferation, differentiation, immunity, and inflammation. BMP=bone morphogenetic protein; GDF=growth and differentiation factor. Reprinted with permission from Humbert et al.[5]**

**Types of BMPR2 mutations, molecular location, and clinical aspects.** One initial familial pulmonary arterial hypertension study reported nine BMPR2 mutations, which segregated with disease, in 19 affected families,[1] and the International Consortium reported seven mutations in eight affected families.[2] Qualitatively, the mutations consisted of missense, nonsense, frameshift and splice-site alterations in the exons and intron/exon boundaries of BMPR2. There was no single founder and the mutations were private to each family. Although three

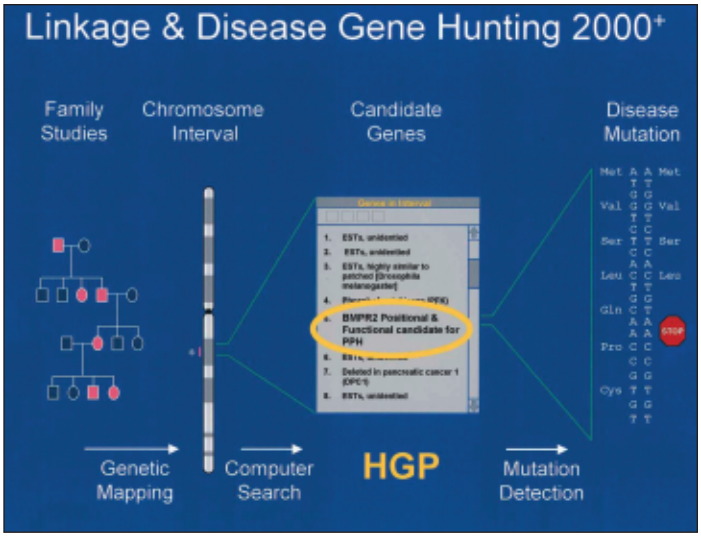


**Figure 2. The process leading to the discovery of mutations in bone morphogenetic protein receptor type-2 (BMPR2) as the cause of familial primary pulmonary hypertension is depicted. Collection of deoxyribonucleic acid from families with sufficient numbers of affected and unaffected members allowed linkage studies using microsatellite markers that led to identification of a chromosome interval on chromosome 2, at q31–32. Candidate genes known from the Human Genome Project (HGP) in the interval were then identified and tested by deoxyribonucleic acid sequencing. Point mutations in exons of the BMPR2 gene were found that co-segregated with affected individuals known from the family pedigrees. Reprinted with permission from Newman et al.[25]**

families had the same mutation, data available from microsatellite markers showed that the haplotypes, patterns of surrounding markers, were different in each family.[1] A spontaneous mutation was reported in a family with familial pulmonary arterial hypertension[1] and spontaneous mutations were subsequently reported in idiopathic pulmonary arterial hypertension.[13] Although not rigorously studied, the disease penetrance, originally estimated at 10% to 20%, varied within families and between families. There are still no published extensive genotype-phenotype correlations in familial pulmonary arterial hypertension. Consanguinity was not observed initially,[1,2] and has rarely been observed in affected families.

**BMPR2.** BMPR2 is a receptor in the BMP/TGF-beta superfamily, initially cloned in 1995, that has 13 exons, 4000 base pairs, and 1038 amino acids.[14-17] Exons 1 to 3 comprise the extracellular ligand-binding domain, which usually binds ligands BMP2 and BMP4 in the lung. Exon 4 comprises the transmembrane domain, and exons 5 to 11 make up the serine-threonine kinase domain. Exons 12 and 13 comprise the cytoplasmic tail of unknown function. The initial report, and most subsequent reports of BMPR2 mutations have not studied the introns, the regulatory sequences interspersed between the exons, and have not used quantitative polymerase chain reaction (PCR) or other techniques to ensure that deletions, especially large ones, are present. BMPR2 mutations have been reported in all exons except exon 13; the spectrum of frameshift/nonsense mutations, concentrated in the extracellular and kinase domains, are found throughout the molecule whereas missense mutations have not been reported in large exon 12 (**Figure 3** and **Table 1**). A Web-based list of many but not all BMPR2 mutations is available (http://archive.uwcm.ac.uk/uwcm/mg/search/642243.html). The number of reported mutations is growing exponentially, as illustrated by the number of symbols in **Figure 3**.

Each BMP and TGF-beta ligand assembles a specific transcriptional complex of two type 1 and two type II receptors.[18,19] The type II receptor phosphorylates the type I receptor, and this complex then phosphorylates a series of intracellular mediators or SMADs specific to each pathway. The SMAD-receptor complex in turn combines with co-SMAD 4, which is transported into the nucleus where transcriptional responses are initiated (**Figure 4**). The outcome depends on the target gene to which the SMADs have gained access. Mutations in the extracellular domains of BMPR2 are predicted to interfere with heterodimer formation or ligand binding and mutations in the kinase domain to interfere with phosphorylation; mutations in the long cytoplasmic tail have an unknown function.

Haploinsufficiency, a case in which an individual who is heterozygous for a specific gene mutation, is clinically affected because a single copy of the normal gene is incapable of providing sufficient protein production for normal function, has been suggested as the molecular mechanism for pulmonary arterial hypertension in patients with a BMPR2 mutation;[20] however, a dominant negative effect, a mutation whose gene product adversely affects the normal, wild-type gene product within the same cell, has also been implicated. Two functional *in vitro* studies have supported these predictions.[21,22] In these studies, missense mutations within the extracellular and kinase domains lost their signal transduction abilities whereas constructs with mutations that caused truncation of the cytoplasmic tail retained their ability to transduce BMP signals. Since a deleterious effect, as opposed to a benign polymorphism, is harder to document in missense mutations (without functional studies), most genetic studies have shown that the missense mutations involved sites conserved in evolution and that these mutations have not been reported in large numbers of normal individuals.

**BMPR2 mutations, asymptomatic BMPR2 mutation-positive carriers, and suggested locus heterogeneity.** A German group reported that familial pulmonary arterial hypertension may be a heterogeneous disease with a second locus, more centromeric than BMPR2, on chromosome 2q31.[23] However, to date, no mutations in any other genes have been found in this region. This group of investigators also reported that the risk haplotype can be identified in family members by an abnormal pulmonary artery systolic response to exercise when compared to the response in normals.[22] Pulmonary hypertension was also diagnosed by echocardiography in several asymptomatic family members.[24] A prospective European Union-sponsored study to evaluate the response during exercise using Doppler echocardiography should provide more definitive data on the usefulness of this test.[25]

Our 5-year unpublished observations found that previously identified asymptomatic carriers in familial pulmonary arterial hypertension families developed the disease. Eight were identified as carriers by microsatellite marker haplotype determinations, five by BMPR2 mutations, and another was not tested by either method. Prospective longitudinal studies allowing comparisons between asymptomatic gene-positive family members

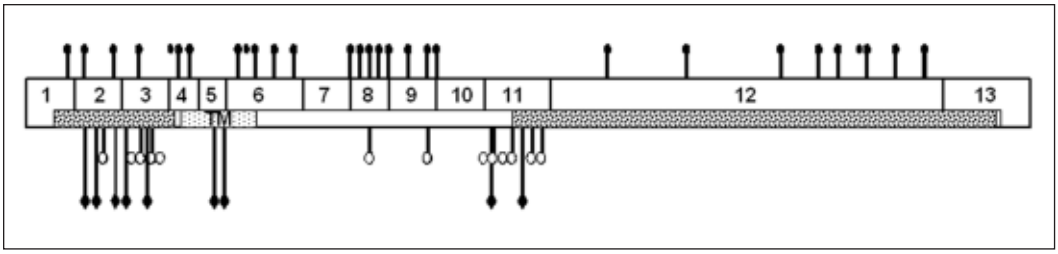


**Figure 3. BMPR2 domains and positions of reported mutations. Truncating (frameshift/nonsense) mutations reported in familial/idiopathic pulmonary arterial hypertension[1,2,13,20] are illustrated above the diagram of the BMPR2 gene by closed circles and missense mutations below the gene by open circles. Diamonds below the molecule also illustrate the missense mutations in pulmonary arterial hypertension associated with fenfluramine derivatives[33] and with congenital heart disease.[34] Exons 1 to 3 comprise the extracellular domain, exon 4 the transmembrane region, exons 5 to 11 the kinase region, and exons 12 and 13 the cytoplasmic tail. (The exon 11 C483R mutations, though illustrated as both an open circle and a diamond, are actually from a single person initially reported in idiopathic pulmonary arterial hypertension[13] but later reclassified as pulmonary arterial hypertension associated with fenfluramine derivatives.[33])**

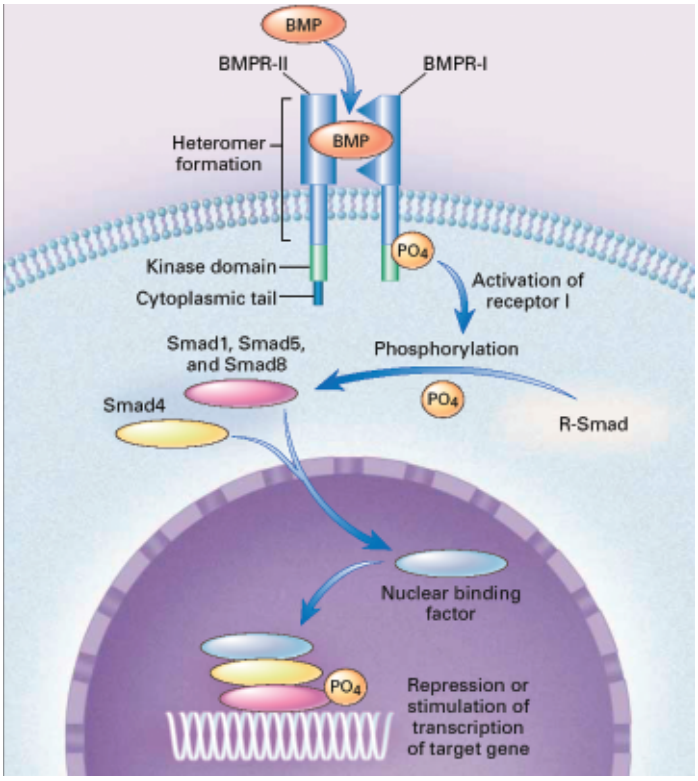


**Figure 4. Proposed mechanism of action of bone morphogenetic proteins on pulmonary circulatory cells. Bone morphogenetic protein receptors I and II (BMPR-I and BMPR-II) are adjacent on cell membranes. Bone morphogenetic protein binds to the extracellular domain (ligand binding) of BMPR-II, resulting in the formation of a heteromeric complex with BMPR-I. BMPR-II then phosphorylates the transmembrane region of BMPR-I, activating the kinase domain. The activated BMPR-I phosphorylates receptor Smad (R-Smad), thus activating one or more receptor-dependent cytoplasmic Smad proteins (Smad1, Smad5, and Smad8), which bind with Smad4 and migrate to the nucleus. The phosphorylated Smad complex attaches to a binding factor in the nucleus, and the resulting assembly either stimulates or represses gene transcription by interacting with DNA. In patients with familial primary pulmonary hypertension, changes caused by mutations have been found along the entire span of BMPR2. Reprinted with permission from Newman et al.[26]**

and those who develop disease, hopefully will provide information on the natural history of the disease, identify risk factors and gender differences, and provide data on disease penetrance. Genetic comparisons between both unaffected and affected gene-positive cohorts should aid in determining the role of candidate modifier genes as either protective or disease enhancing.

**BMPR2 mutations and clinical WHO familial/idiopathic pulmonary arterial hypertension classification.** Anecdotal and published experience suggests that idiopathic pulmonary arterial hypertension, especially BMPR2 mutation-positive disease, is more frequently familial pulmonary arterial hypertension than originally appreciated.[25] Incomplete penetrance and skipped generations as well as the difficulty of obtaining complete family histories and carrying out longitudinal monitoring in our mobile society all contribute to misclassification. Two of our patients, initially classified as having idiopathic pulmonary arterial hypertension, were recently reclassified as familial when a sibling developed pulmonary arterial hypertension. Misclassification of entire families can occur as well. A superfamily consisting of five subfamilies initially felt to be unrelated was recently reported.[26] This family spans seven generations and has almost 400 members, of which 200 are at risk or obligate carriers for having a BMPR2 mutation.

Cases can also be erroneously classified as familial pulmonary arterial hypertension. For example, in families thought to have familial pulmonary arterial hypertension, the second affected member had either a previously unrecognized HIV infection, congenital cardiac defect, connective tissue disease, or other cause of pulmonary arterial hypertension, thus requiring reclassification. To illustrate this, a child with pulmonary arterial hypertension and a repaired atrial septal defect had a frameshift BMPR2 mutation whereas the mother with pulmonary arterial hypertension was mutation-negative. Genetic classifications may be more informative and appropriate as more is learned about BMPR2-positive patients and their family members.

## BMPR2 Mutations in Idiopathic Pulmonary Arterial Hypertension

Initial reports of BMPR2 mutations showed a 26% frequency in 50 unrelated patients with idiopathic pulmonary arterial hypertension.[13] BMPR2 mutations were identified in 13 patients. Although 3 patients had the same mutation, microsatellite markers surrounding the mutation indicated that the patients were not related. The patients, with no identifiable family history of familial pulmonary arterial hypertension, were recruited from the United States, the United Kingdom, and France.[13] Mutations were detected by direct nucleic acid sequencing of the exons and intron/exon boundaries of BMPR2. Two patients had *de novo* mutations and 3 had paternally derived mutations.

**Table 1. BMPR2 Mutations in Pulmonary Arterial Hypertension Associated with Congenital Heart Disease and with Fenfluramine Derivatives.**

**Congenital Heart Disease[34]**

| Patient | Sex, Age | Type of congenital heart disease/ genetic syndrome | Exon | Nucleotide change | Amino acid change | Type of mutation |
|---|---|---|---|---|---|---|
| 1 | F, Adult | AVC-C, Down syndrome | 2 | 125A>G | Q42R | Missense (spontaneous) |
| 2 | M, Child | AW and VSD | 2 | 140G>A | G47N | Missense |
| 3 | F, Adult | AVC-C | 3 | 304A>G | T102A | Missense |
| 4 | F, Adult | AVC-C | 3 | 319T>C | S107P | Missense (spontaneous) |
| 5 | F, Child | ASD/PDA, Ring 14 | 5 | 556A>G | M186V | Missense (spontaneous) |
| 6 | M, Child | ASD/PDA/PAPVR | 11 | 1509A>C | E503D | Missense |

**Fenfluramine Derivatives[33]**

| Patient | Sex, Age | Type of congenital heart disease/ genetic syndrome | Exon | Nucleotide change | Amino acid change | Type of mutation |
|---|---|---|---|---|---|---|
| 1 | F, Adult | Dexfenfluramine, 5 months | 2 | 246A>C | Q82H | Missense |
| 2 | F, Adult | Fenfluramine, 2 months | 5 | 545G>A | G182D | Missense |
| 3 | F, Adult | Fenfluramine, 1 month | 11 | 1447T>C | C483R | Missense |
| 4 | F, Adult sisters | Dexfenfluramine, 1 month and 2 months | 6 | 631C>T | R211X | Nonsense |

AVC-C, atrioventricular canal, type C; AW, aortopulmonary window; VSD, ventricular septal defect; ASD, atrial septal defect; PDA, patent ductus arteriosus; PAPVR, partial anomalous pulmonary venous return.

More recent observations, albeit mostly unpublished, suggest that the frequency of BMPR2 mutations in idiopathic pulmonary arterial hypertension is more realistically between 5% and 10%.[25,27,28] A German group found 11 of 99 (11%) adults with idiopathic pulmonary arterial hypertension had BMPR2 mutations[27] and found no mutations in 13 children with idiopathic disease.[28] In a subset of children with presumed idiopathic disease, there was an abnormal pulmonary artery systolic response to exercise in a parent and/or other family members.[28] The authors hypothesized that idiopathic pulmonary arterial hypertension in children may have a different genetic background than that in adults. However, this is not the case in American families where BMPR2 mutation-positive children with pulmonary arterial hypertension are present in many familial pulmonary arterial hypertension families.[1,2]

A more recent study reported BMPR2 mutations in 4 of 66 (6%) adults and in 1 of 75 (1%) children with idiopathic pulmonary arterial hypertension.[29] The adults had two frameshift and two nonsense mutations and the child had a missense mutation. All five mutations were found in patients with thyroid disease. Sixteen of the 66 adults (24%) had thyroid disease when compared to a 5% to 8% prevalence of thyroid disease in the normal population. The frequency of thyroid disease in the children with idiopathic pulmonary arterial hypertension did not differ significantly from that of the normal population. The association of thyroid disease, especially autoimmune thyroiditis, with idiopathic pulmonary arterial hypertension is well described[30] and not yet well understood; whether BMPR2 mutations predispose to thyroid disease is unknown.

## BMPR2 Mutations in Cases Associated with Fenfluramine Derivatives

Use of appetite suppressants, such as the fenfluramine derivatives, has been associated with the development of pulmonary hypertension[31] and with cardiac valvular disease.[32] A single study reported BMPR2 mutations in 3 of 33 (9%) unrelated patients and two sisters with pulmonary arterial hypertension, all from France, who used appetite suppressants.[33] **Table 1** illustrates the three different missense BMPR2 mutations reported in the 33 patients with idiopathic pulmonary arterial hypertension and the fourth nonsense mutation in both sisters. BMPR2 mutations were not found in a control population of 130 normal individuals from France. Unfortunately, the authors did not have a cohort of patients available for mutation analysis that used these drugs but did not develop pulmonary hypertension. The duration of appetite suppressant use for each patient is included in **Table 1**. Of interest, the mutation-positive patients had a significantly shorter duration of drug exposure before the onset of disease than the mutation-negative patients. The difference in exposure time suggested that these drugs could provide an additional risk factor for pulmonary arterial hypertension in those patients with mutations. The authors concluded that the onset of disease required "two events," namely the presence of a heterozygous germline mutation followed by the use of a fenfluramine derivative. Since BMPR2 mutations were found in only 9% of patients with pulmonary arterial hypertension related to fenfluramines, other genes and mechanisms are more likely to be associated with the development of pulmonary arterial hypertension in patients with a history of appetite suppressant use.

## BMPR2 Mutations in Cases Associated with Congenital Heart Disease

This category pertains to patients whose pulmonary arterial hypertension is due to abnormal systemic to pulmonary shunts as a result of congenital heart disease. A single study reported a 6% frequency of BMPR2 mutations in a mixed cohort of 40

adults and 66 children with pulmonary arterial hypertension/congenital heart disease.[34] The predominant defects in both cohorts were atrial and ventricular septal defects but included a number of patients with more complex congenital heart disease. The congenital heart disease was determined echocardiographically and pulmonary arterial hypertension was confirmed by right heart catheterization.

**Table 1** illustrates the six novel missense BMPR2 mutations reported in 3 of 4 adults with complete atrioventricular canal defects and in 3 children, with complex congenital heart disease.[35] The finding of BMPR2 mutations in 3 of the 4 adults with atrioventricular canal defects contrasted with the failure to find mutations in six children with such defects. One BMPR2 mutation-positive adult with an atrioventricular canal defect also had Down syndrome. Three of the six mutations were de novo, as they were not found in either parent (**Table 1**). The 6% frequency of BMPR2 mutations in this combined cohort is significantly less than the 26% frequency reported for idiopathic pulmonary arterial hypertension,[13] but similar to the 9% frequency of BMPR2 mutations reported for pulmonary arterial hypertension associated with appetite suppressants.[33] This study requires replication and evaluation of BMPR2 mutations in patients with these types of congenital heart disease without the presence of pulmonary hypertension.

The TGF-beta/BMP signaling pathway is very important in vasculogenesis and in both embryonic heart and lung development.[35,36] The types of congenital heart disease found in the BMPR2-positive patients are analogous to those reported in murine models, particularly those with defects in embryonic heart development. Homozygous BMPR2 knock-out mice die at gastrulation whereas no abnormalities have been reported for the heterozygous mouse[37] unless there is a second insult such as hypoxia. A mouse with a truncated extracellular domain of BMPR2 had absence of the septation of the outflow tract and aortic arch interruption, the anatomic correlate of human persistent truncus arteriosus type 4A.[38] Inactivation or knock-out of other members of the BMP signaling pathway also leads to cardiac defects. Mice with tissue-specific inactivation of ALK3 (BMPR1a) have abnormal endocardial cushion morphogenesis.[35,39] Finally, a cardiac muscle BMP4 conditional knock-out mouse model resulted in reduced atrioventricular septation and endocardial cushion formation.[40]

### BMPR2 Mutations in Cases Associated with Connective Tissue Disease

Published studies have not identified BMPR2 mutations in two small cohorts of patients with pulmonary arterial hypertension associated with connective tissue disease.[41,42] One report studied a mixed cohort of 12 patients with pulmonary arterial hypertension associated with connective tissue disease that included nine with systemic sclerosis, two with lupus, and one with mixed connective tissue disease.[41] Patients with thromboembolic disease and pulmonary fibrosis were excluded. A single nucleotide repeat polymorphism in exon 12 was identified but its frequency was the same in both patients and in controls. The second report determined BMPR2 mutations, lung involvement, and ANA/autoantibodies in 24 adults and included 17 patients with systemic sclerosis with limited cutaneous involvement, 6 with diffuse cutaneous involvement, and one with mixed connective tissue disease.[42] A single BMPR2 change in exon 13, 2948G>A [R983Q], was found in a patient with limited cutaneous systemic sclerosis. However, it was considered a polymorphism since the patient was a 59-year-old Ashkenazi Jew, and this same change in BMPR2 has been found in Ashkenazi Jews. Despite technical limitations and the small sample sizes of these two reports, it appears that other genes and/or mechanisms remain to be characterized in pulmonary arterial hypertension associated with the scleroderma spectrum of disease as well as other connective tissue diseases.

### BMPR2 Mutations in Cases Associated with HIV Infection and Portal Hypertension

BMPR2 mutations were not found in a small cohort of 19 patients with pulmonary arterial hypertension associated with HIV infection.[43] Viral transmission was mainly intravenous, but also via sexual contact and pregnancy. There are no reported studies of BMPR2 mutations in pulmonary arterial hypertension associated with portal hypertension.

### BMPR2 Mutations in Cases with Significant Venous or Capillary Involvement

This category of pulmonary arterial hypertension includes two diseases, pulmonary veno-occlusive disease and pulmonary capillary hemangiomatosis. There are no reports of BMPR2 mutations in pulmonary capillary hemangiomatosis. A BMPR2 mutation in pulmonary veno-occlusive disease has been reported. A proband with pulmonary veno-occlusive disease had a heterozygous frameshift exon 1, del44C mutation, also present in her asymptomatic sister, which was inferred to be inherited from her deceased mother.[44] Examination of the mutation bearing haplotype in the pedigree suggested that the inferred mutation in the mother was spontaneous and unlikely to be present in her parents. However, without tissue, the type of pulmonary hypertension in the mother could not be documented. This report suggests that BMPR2 mutations may predispose to pulmonary venous as well as pulmonary arterial disease.

### Genetic Testing and Counseling

To date, genetic testing for BMPR2 mutations has been performed within research studies, mostly at specialized pulmonary hypertension centers. Hence, individual results were not available to the participants or to their physicians. However, Clinical Laboratory Improvement Act–approved genetic testing for BMPR2 mutations will become available in 2005 at selected pulmonary hypertension centers. Patients must receive genetic counseling. Patients, family members, and physicians have expressed an interest in obtaining results of BMPR2 testing. Most family members in a study of attitudes and understanding of familial pulmonary arterial hypertension wished to know individual test results.[45] DNA-based testing in a familial pulmonary arterial hypertension family with a known BMPR2 mutation is feasible and can identify individuals at risk for developing disease as well as those who are not at increased risk. DNA-based testing in a BMPR2 mutation-unknown person or family would require more expensive testing for all 13 exons of BMPR2. In this instance, the failure to find a BMPR2 mutation would provide limited information, as the technology currently used for doing the test would not detect all BMPR2

mutations and there may be other genes for familial pulmonary arterial hypertension susceptibility. Better technological approaches for identifying most, if not all, BMPR2 mutations and other familial pulmonary arterial hypertension genes are in progress.

The pros and cons of genetic testing for BMPR2 have been well presented in the genetic section of the Venice 2003 WHO Symposium on Pulmonary Arterial Hypertension.[25] This group of experts advised that genetic testing was not ready for broad implementation in idiopathic pulmonary arterial hypertension but was appropriate in families with familial pulmonary arterial hypertension where the mutation is known and genetic counseling is available.[25] The limitations are that BMPR2 mutations have been unique to each family and haplotype testing is rarely done except within research studies. Also, the variability in disease penetrance in familial pulmonary arterial hypertension can be as low as 10% to 20% but as high as 80%. The 5% to 26% reported frequencies of BMPR2 mutation in nonfamilial pulmonary arterial hypertension suggest that genetic testing has broader applications than just in familial pulmonary arterial hypertension. The number of mutations commercially being tested is growing exponentially. Hopefully, the participants and physicians obtaining the results of commercially available tests will share them with researchers as there is much to learn about the genetic aspects of each mutation, the specific risk factors, whether environmental or genetic, and the pathobiology and pharmacogenetics of pulmonary arterial hypertension.

## Conclusions

The past 4 years have seen the discovery of BMPR2 mutations in familial and idiopathic pulmonary arterial hypertension and in pulmonary arterial hypertension associated with fenfluramine derivatives and with congenital heart diseases, as well as one case of pulmonary veno-occlusive disease, but not in pulmonary arterial hypertension associated with connective tissue disease or with HIV infection. BMPR2 mutations have not been reported in the other categories of diseases associated with pulmonary arterial hypertension. Qualitatively, the reported truncating mutations have been located throughout the gene in familial/idiopathic pulmonary arterial hypertension,[1,2,13,20] whereas the missense mutations have not been found in the cytoplasmic tail in familial/idiopathic pulmonary arterial hypertension or in pulmonary arterial hypertension associated with fenfluramine derivatives[32] or with congenital heart diseases.[33] The "two hit" theory of disease, originally proposed in cancer, has been advanced to explain the incomplete penetrance of the disease.[46] The two hits can result from either genetic or environmental factors, or both. In the near future, investigators should elucidate both the currently identified and unrecognized genetic and environmental causes required for disease, provide a genetic classification, and identify biomarkers for early disease onset. These findings may allow clinicians to initiate preventative therapies and should provide new therapies and new, currently unrecognized pathobiological approaches.

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## Profile *(continued from page 4)*

Yet so far, gender seems to be the only variable that is known to modify clinical expression. In one family from Tennessee, for example, with 22 PPH patients, 19 of them are women. No one has been able to pinpoint why women are at much greater risk than men, however.

When asked if a patient's genetic or familial predisposition could be related to the pathways widely recognized in recent years as contributors to the pathophysiology of PPH, Dr Loyd noted that this is one of the areas investigators are exploring. As he described the potential for possibly associating a genetic link with these pathways, he suggested that the link may be "within a pathway that is not even yet recognized, or in one that is known, but which is not known to be related to pulmonary hypertension." Such a circumstance, however, is not new to Dr Loyd. This quandary was similar in many respects to the position he was in when he first began searching for the gene itself 25 years ago when so little was known about a familial connection and a comparable mystery was waiting to be solved. ■