

# Genetic Basis of Pulmonary Arterial Hypertension

## Current Understanding and Future Directions

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Mutations in two receptors of the transforming growth factor-beta family have recently been shown to be present in the majority of cases of inherited (familial) pulmonary arterial hypertension (PAH). Study of the biology of these receptors, bone morphogenetic protein receptor type-2 (BMPR2), and activin-like kinase type-1 (ALK-1) will certainly reveal pathogenic mechanisms of disease. Exonic mutations in BMPR2 are found in about 50% of patients with familial PAH, and ALK1 mutations are found in a minority of patients with hereditary hemorrhagic telangiectasia and co-existent PAH. Because familial PAH is highly linked to chromosome 2q33, it is likely that the remaining 50% of family cases without exonic mutations have either intronic BMPR2 abnormalities or alterations in the promoter or regulatory genes. Also, only about 10% of patients with "sporadic" idiopathic PAH have identifiable BMPR2 mutations. Mutations in BMPR2 confer a 15% to 20% chance of developing PAH in a carrier's lifetime. Thus, there must be gene-gene or gene-environment interactions that either enhance or prevent the development of the vascular disease in persons carrying a mutation, and there must be other patterns of susceptibility based on genetic makeup. To elucidate the genetic basis of PAH further, investigations are needed, including genome scanning for major and minor genes, analysis of genetic profiles of patients for candidate genes likely to modify risk for disease (e.g., serotonin transporter alleles, nitric oxide-synthases), proteomics, transgenic mice, and altered signal transduction. Advances in genetic testing, presymptomatic screening, and biomarkers should permit early detection of disease in those at risk of PAH and allow trials of preventive therapy in carriers. (J Am Coll Cardiol 2004;43:33S-39S) © 2004 by the American College of Cardiology Foundation

In this report, the state of knowledge with regard to the genetic basis of pulmonary arterial hypertension (PAH) will be summarized, and future approaches will be outlined. The genetic basis of all forms of PAH is broader than that for the disease traditionally called "familial primary pulmonary hypertension"; multiple genetic conditions associated with pulmonary hypertension are listed in Table 1. This report will be largely confined to the genetic basis of forms of PAH related to mutations in bone morphogenetic protein receptor type-2 (BMPR2) and activin-like kinase type-1 (ALK1), two receptors in the transforming growth factor (TGF)-beta family.

A familial association with primary pulmonary hypertension (PPH, now termed idiopathic PAH) was reported by Dresdale in 1954 (1) only three years after the first description of this disease (2). Subsequent case reports of other families with two or more members affected with PAH were made in American and European published data, and in 1984 contact was made with 13 previously reported families,

revealing numerous new cases in close and distant relatives (3). The pattern of inheritance was observed to be autosomal dominant with highly variable and incomplete expression among families, a female predominance, the suggestion of genetic anticipation, and a clinical course indistinguishable from nonfamilial PAH (4). In the National Institutes of Health (NIH)-funded PPH (PAH) registry, 6% of affected patients were identified as having familial disease (5).

The development of family registries and banking of deoxyribonucleic acid led to linkage studies using microsatellite markers that localized a marker for familial PAH on chromosome 2q31-32 (6,7). The possibility of other involved loci has not been systematically examined, although all families studied link disease to that region of chromosome 2 (8). Because the segment on chromosome 2 was too long for easy sequencing, a candidate gene approach led to the identification of mutations in a receptor in the TGF-beta superfamily of receptors, BMPR2, in association with disease (9,10). The strategy employed to identify the disease-causing gene is summarized in Figure 1.

Recently, a second PAH gene was found in some persons with hereditary hemorrhagic telangiectasia (HHT) whose mutation in ALK1 receptor confers susceptibility to pulmonary hypertension in addition to HHT lesions (11). This appears to be a less common cause of heritable PAH,

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**Abbreviations and Acronyms**

ALK1	= activin-like kinase type-1
BMPR2	= bone morphogenetic protein receptor type-2
HHT	= hereditary hemorrhagic telangiectasia
PAH	= pulmonary arterial hypertension
PASP	= pulmonary artery systolic pressure
PPH	= primary pulmonary hypertension (now termed idiopathic PAH)
TGF	= transforming growth factor

although as a member of the TGF-beta family, ALK1 is likely to share signaling abnormalities with mutated BMPR2 (12,13).

**THE BMPR2 MUTATIONS IN FAMILIAL PAH (PPH)**

The BMPR2 gene, on chromosome 2q33, has 13 exons. Exons 1-3 encode an extracellular domain, exon 4 encodes the transmembrane domain, exons 5-11 a serine/threonine kinase domain, and exons 12 and 13 a very large intracellular C-terminus of unknown function that appears to be unique to BMPR2. Mutations in familial PPH have been reported in all exons except for 5 and 13 (9,10). Polymorphisms have been found in exons 6, 8, and 12. Each mutation is unique

**Table 1.** PAH: Mendelian Causes

- |   |   |
|---|---|
| <b>A. Idiopathic PAH: PPH</b>   |   |
| 1.  | Exonic mutations in BMPR2 (50% of known families with familial PPH)   |
| 2.  | Intronic mutations in BMPR2 (up to 15% of families currently identified)  |
| 3.  | Isolated exonic BMPR2 germline mutations (about 10% of sporadic cases)  |
| a.  | Spontaneous heritable mutation  |
| b.  | Inherited mutation in a family with no other clinically affected individuals  |
| 4.  | ALK1 mutations associated with hereditary hemorrhagic telangiectasia (rare)   |
| 5.  | Other PPH mutations near region q32 on chromosome 2, as yet unidentified?   |
| 6.  | Other Mendelian PPH genes in the genome? (unlikely but uncertain)   |
| <b>B. Pulmonary veno-occlusive disease</b>                                  |   |
| 1.  | One family identified with a BMPR2 exonic mutation. Screening of isolated cases underway (likely to be a rare association).   |
| <b>C. Sickle cell anemia</b>  |   |
| 1.  | SS and SC hemoglobinopathies leading to repeated pulmonary vascular injury and pulmonary vascular thrombosis  |
| <b>D. Gaucher's disease</b>   |   |
| 1.  | It is currently unclear what the genetic relationship is. Some Gaucher disease patients have had splenectomies, and BMPR2 polymorphisms have been found in four; significance as yet unknown. |
| <b>E. Conditions where major genes are likely to be associated with PAH</b> |   |
| 1.  | Hypercoagulable states, including PAI-1 inhibitor mutations   |
| 2.  | High-altitude pulmonary edema, disorders of ventilatory drives, hypoxic pulmonary hypertension  |
| 3.  | Collagen vascular diseases.   |

ALK1 = activin-like kinase type-1; BMPR2 = bone morphogenetic protein receptor type-2; PAH = pulmonary arterial hypertension; PAI = plasminogen activator inhibitor; PPH = primary pulmonary hypertension.

to a family and co-segregates with disease. The amino acid substitutions resulting from point mutations are in either highly conserved or functionally critical domains of the receptor, and thus are predicted to alter receptor function (14-16). The TGF-beta family of receptors is highly conserved throughout nature. Other missense mutations predict truncation of coding of the transcript. The location of the mutation in families with BMPR2-related PPH does not seem to alter the gender ratio, age of onset, or severity of disease. Locations of many of the known mutations in BMPR2 are shown in Figure 2. A mutation in BMPR2 was recently reported in a family with pulmonary hypertension due to veno-occlusive disease, suggesting that the signaling abnormality is not confined to the precapillary arterioles in all cases (17).

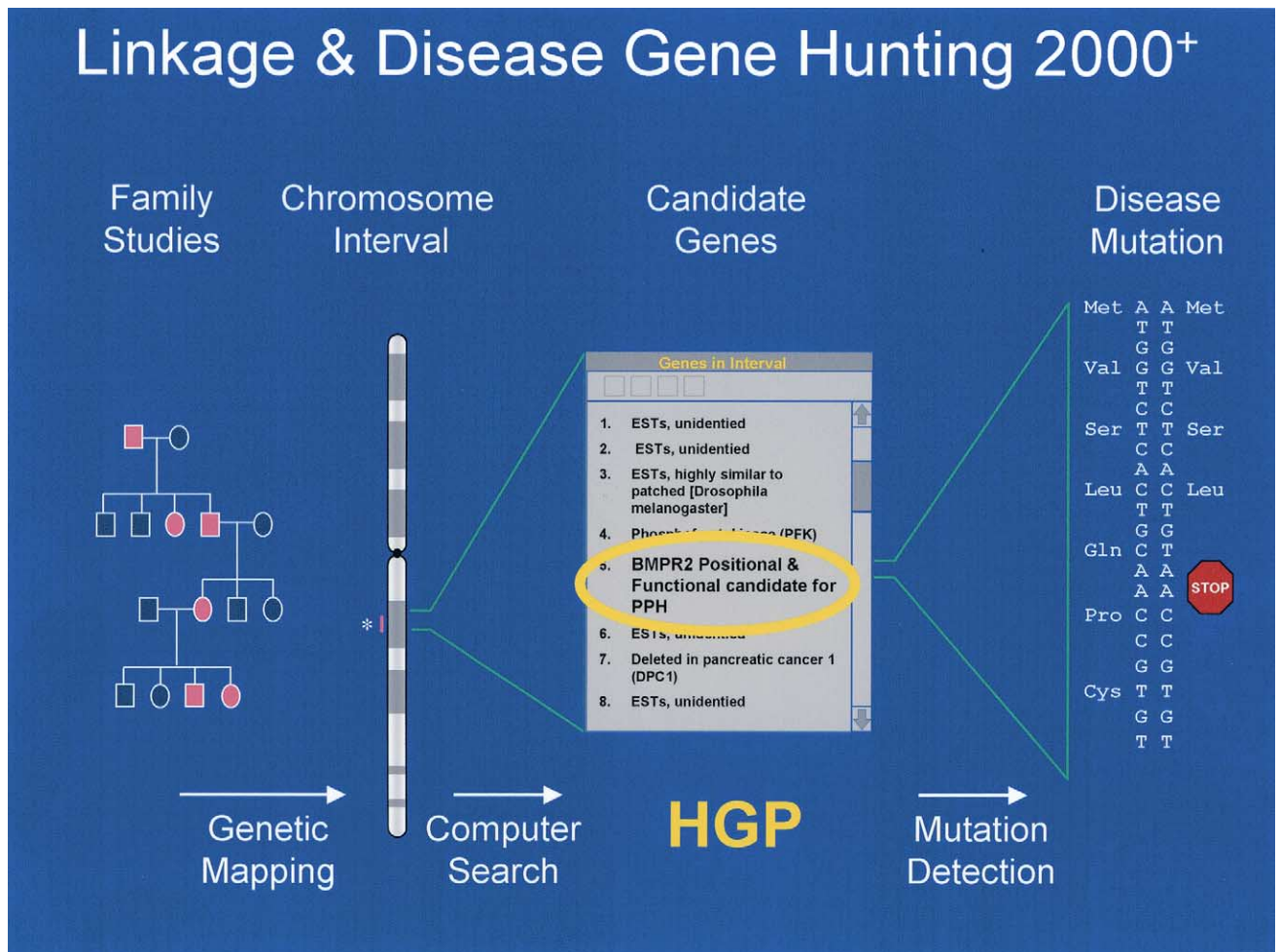
**THE BMPR2 MUTATIONS IN "SPORADIC" PAH**

About 25% of sporadic cases of PPH were initially thought to have BMPR2 mutations (18), although more recent unpublished analyses of sporadic cases by multiple groups have placed this closer to 10%. Semantic confusion arises when an apparently sporadic case of PPH is found to have an exonic BMPR2 mutation. In some cases, this is apparently a new spontaneous germline mutation, which presumably can be inherited by any offspring and carries the same 15% to 20% risk of disease. In other cases, the mutation has been found to be inherited, and thus is really a family case, albeit the first diagnosis in the family. The rate of mutations in the general population is unknown, but must be exceedingly low because of their absence in control populations. Of the remaining cases of apparently sporadic PPH, about 90%, a small number might have intronic BMPR2 mutations, ALK1, or other Mendelian causes, but the majority do not appear to have a major genetic basis for disease. It is likely that genetic predispositions exist based on normal variations in genes that may influence the pulmonary circulation (Table 2).

**THE BMPR2 MUTATIONS IN OTHER COHORTS**

The population carrier frequency for BMPR2 mutations has not been measured. However, an indirect estimate suggests that it is as low as 0.001% and may be as high as 0.01% (19). In data concerning normal control cohorts already published, amounting to about 350 subjects (9,10,14,19), no exonic BMPR2 mutations have been found that would be predicted to alter function of the receptor.

**PAH with appetite-suppressant drugs.** Mutations of the BMPR2 gene have been reported in PAH associated with fenfluramine derivatives (19). Three BMPR2 mutations were found in 33 unrelated French patients (9%), and a fourth mutation in two sisters. The three single mutations were exon 2 A246C, exon 5 G545A, and exon 11 T1447C, all predicted to reduce function of the receptor. The two sisters had an exon 6 631C >T mutation, resulting in a nonsense mutation (R211X) predicted to produce a truncated protein. The mutation-positive patients had a some-



**Figure 1.** 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.

what shorter duration of fenfluramine exposure before illness than did the mutation-negative patients. The import of this data is compatible with the working hypothesis that gene-gene or gene-environmental interactions are required for the onset of PPH.

**PAH with scleroderma-spectrum disease.** Germline heterogeneous BMPR2 mutations were not found in 24 patients with PAH in the scleroderma spectrum of disease (20). However, one centromere-positive Jewish patient with localized cutaneous CREST had an exon 13 G2948A (R983Q) variant considered likely to be a polymorphism because the same mutation was also found in one of 100 normal Israeli Ashkenazi Jews. This polymorphism has not been found in 350 normal chromosomes. Another report failed to find BMPR2 mutations in 12 patients with PAH and connective tissue diseases (21).

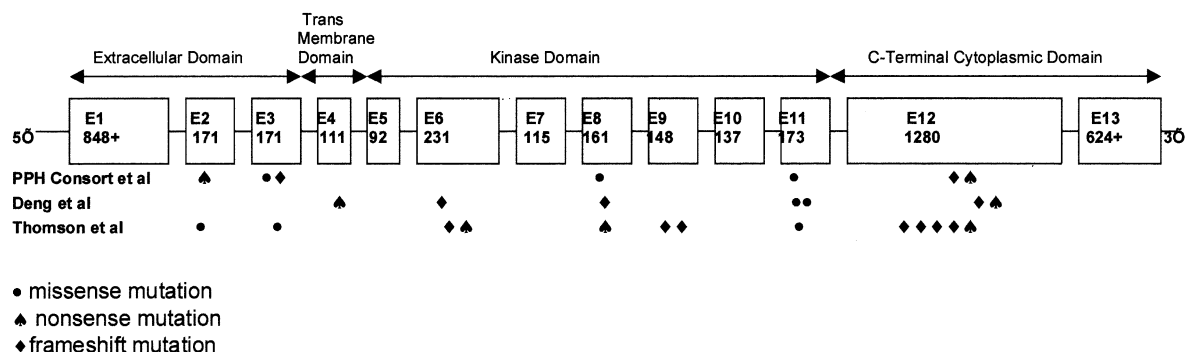
**Human immunodeficiency virus (HIV)-associated PAH. PORTAL HYPERTENSION AND CONGENITAL HEART DISEASE.** No BMPR2 mutations were found in 19 French patients with HIV-associated PAH, 11 intravenous drug

abuse, and 8 via sexual/blood contact (22). No BMPR2 mutations were found in 11 similar U.S. patients and 25 British patients (J. A. Morse and R. C. Trembath, unpublished data, June 2003). There have been no published reports of BMPR2 mutations in adults and children with PAH and congenital systemic-to-pulmonary shunts and in portopulmonary PAH.

**Immunogenetic studies of PAH.** There are few immunogenetic studies of PPH despite earlier reports of antinuclear antibodies in this disease. Previous immunogenetic studies of PPH have reported antibody/HLA-DR, -DQ correlations in small subsets of patients (23). Anti-fibrillin-1 autoantibodies were the only ones found in high frequency; 70 of 75 adults with PPH (93%), 28 of 33 children with PPH (85%), and 12 of 18 with fenfluramine-associated PAH (67%) (22), but no significant HLA-class II associations were discovered.

A recent study of three U.S. ethnic groups with (scleroderma spectrum of disease), Hispanics, African Americans, and Caucasians, found African Americans more likely to

# Range of Mutations



- Mutations in all exons except 5
- Some obviously will be null
- Others may be dominant negative

**Figure 2.** Shown from left to right are exons 1–3 in the extracellular domain, involved in dimerization with BMPR1 upon activation by bone morphogenetic protein or other transforming growth factor-beta ligand. Exon 4 spans the transmembrane, and exons 5–11 represent the kinase domain that phosphorylates SMAD proteins. The cytoplasmic tail (exons 12–13) is an unusual feature of transforming growth factor-beta receptors. Most mutations are frameshift or nonsense changes that predict truncation of the gene transcript. PPH = primary pulmonary hypertension.

have diffuse skin involvement and pulmonary hypertension (24). Caucasians had more anticentromere antibodies and African Americans more anti-U1-ribonucleoprotein and anti-fibrillarin (U3-RNP) antibodies. Of interest, HLA-DQB1\*0301 (DQ7) was also increased in sporadic PPH with tissue plasminogen activator antibodies. Autoantibodies against B23, a nucleolar phosphoprotein, were associated with systemic sclerosis and PAH (25). Also, B23 positivity was related to anti-fibrillarin antibodies, anti-RNP antibodies, and decreased lung capacity, but HLA alleles were not assessed. An Italian group found anti-topoisomerase II $\alpha$  antibodies in SSc with pulmonary hypertension and HLA-B35, but no HLA-class II associations (26). This same group also correlated the menopause with HLA-B35 as a

risk factor for isolated pulmonary hypertension in SSc (27). It is hoped that future studies will provide the patient numbers and immunogenetic associations using the required careful ethnic matching of patient cohorts to controls.

## SCREENING OF ASYMPTOMATIC CARRIERS OF IDIOPATHIC PAH (PPH) GENES

In PPH, symptoms almost never develop until there is advanced obstruction of the vascular bed with extreme pulmonary hypertension and right heart dysfunction (28). It is hoped that earlier diagnosis and treatment of this disease may lead to better outcomes.

One recent approach to early diagnosis is the identification of gene carriers in families with heritable PPH by measurement of pulmonary arterial pressure at rest and during exercise (29). These studies suggest that 95% of family members who possess the risk haplotype or the BMPR2 mutation have an abnormal pulmonary artery systolic pressure (PASP) response to exercise (29,30). Most mutation carriers in the study were asymptomatic and had normal pulmonary vascular resistance and pulmonary artery pressures at rest and normal right heart measurements and function. In most subjects carrying the risk haplotype or the BMPR2 mutation, an abnormal increase of PASP (range, 41 to 80 mm Hg) occurred during exercise at low workloads (50 to 125 W). These pressures equaled or exceeded systolic

**Table 2.** Partial List of Candidate Genes That May Modify the Risk for PAH

Nitric oxide synthases (1 and 3)
Vasoactive intestinal polypeptide
Serotonin transporter (SERT alleles)
Urea cycle enzymes—pathway to arginine and nitric oxide
Prostacyclin receptor
Beta-adrenergic receptors
Coagulation cascade polymorphisms (in addition to PAI-1, platelet glycoproteins, others)
Immunogenetic background related to HLA and immune processing
Somatic mutations in BMPR1
Potassium channel disorders

BMPR1 = bone morphogenetic protein receptor type-1; HCA = histocompatibility locus antigen; PAH = pulmonary arterial hypertension; PAI = plasminogen activator inhibitor.

pressures achieved with extreme exercise (240 W) in athletes (31).

Measurement of PASP using Doppler echocardiography during supine bicycle exercise might be useful to reveal genetic susceptibility to the disease. In a preliminary follow-up of 28 PPH-gene carriers, one subject with an abnormal response to exercise manifested PPH within three years. Screening by echocardiography has also resulted in the identification of PPH in several asymptomatic family members (32). Furthermore, only a small portion of asymptomatic abnormal responders will develop overt disease, and predictive biomarkers have not yet been defined.

Pulmonary systolic pressure during exercise may rise to high levels in some normal individuals, potentially leading to a false positive diagnosis. In addition, left ventricular filling dysfunction may lead to reactive pulmonary hypertension and the false diagnosis of PAH. To elucidate these important issues, a prospective, controlled European Union project to evaluate Doppler echocardiography during exercise and hypoxic challenge in PAH families and in control subjects has been started.

## GENETIC TESTING AND COUNSELING

The value of a genetic test to estimate an individual's risk for heritable disease depends on the risks and benefits of such knowledge (33). In some diseases where the penetrance is high and preventive treatment is available (e.g., multiple endocrine neoplasia), the benefits of gene testing far outweigh the risks (34). Conversely, when no effective intervention is available (e.g., Huntington's disease), the risk of genetic certainty may exceed the benefit of knowing the answer. In all genetic diseases, both pretest and posttest genetic counseling are essential for best care of subjects (35).

**Current understanding and the state of clinical testing in familial PPH.** The gene that codes for the BMPR2 receptor is large (13 exons); presently, screening for mutations is confined to persons with a known positive family history. Even in this circumstance, the sensitivity of testing for BMPR2 mutations is limited, because at least 50% of families studied to date do not have exonic mutations in BMPR2. Haplotype testing can be performed if a large enough group of affected and unaffected members of a family are available. However, tests for known BMPR2 mutations can be performed at a reasonable cost with high sensitivity and specificity. One problem is that each family has a unique mutation. The penetrance of disease for all known BMPR2 mutations is variable, as low as 15% to 20% in most families, but as high as 80% in some family groups (36). Therefore a deoxyribonucleic acid-based test for known BMPR2 mutations identifies increased risk to develop PPH but not necessarily the disease itself. Genetic testing is simply not ready for broad implementation in idiopathic PAH, but is appropriate in screening programs in families where the mutation is known and counseling is available.

Federally certified clinical laboratories are required by law to assure accuracy of results and avoidance of errors such as contamination of specimens. Genetic testing and counseling must be done solely for the benefit of the person tested. Issues of family planning, family relationships, work environment, self-image, insurability, and social comfort can only be sorted by careful personal counseling by an informed and experienced genetic counselor.

**Preliminary study of attitudes and understanding in familial PPH.** A first step in measuring and understanding the psychosocial implications of familial PPH was explored before BMPR2 was identified in association with PPH (37). Eighty-two members of a family cohort (75%) agreed to take part, and 62 completed a phone interview. More than 66% of respondents stated that they probably or definitely would have genetic testing if it became available. There was a greater interest in testing if a definite answer could be provided ( $p < 0.001$ ). The most important reason for wanting testing done was to learn about the risk to their children. Reasons given for not wanting testing included concern about the effects on family, ability to handle the results emotionally, and concern about insurance and insurability (although this factor was less prominent than in other studies). Nearly 20% of the respondents appeared to be confused about the difference between diagnostic testing and the donation of blood for research studies (37).

Numerous investigations have revealed that the expressed interest in predictive genetic testing often exceeds actual uptake. In Huntington's disease, more than 80% of people at risk expressed interest in testing, but only 10% of these individuals have chosen testing since the gene was identified (38). Factors affecting a person's decisions about testing vary, and include gender (women are more likely to be tested), perceived risk, desire to decrease uncertainty, availability of effective and acceptable medical interventions, concern about one's current and future children, and the presence of symptoms of depression (39).

## GENETIC MODIFICATION OF THE RISK FOR ACQUIRING PPH

Table 2 lists some known and potential genetic modifiers of the risk for development of PAH. The list is necessarily incomplete, and the relative importance of each or any of these genes is unknown. There may be groupings of polymorphisms that confer risk for PAH, especially if there are environmental stresses (such as fenfluramine or splenectomy). The analysis of associations of genes with the risk for disease is a complex statistical problem. Pulmonary arterial hypertension is a complex genetic disease, and the interaction of underlying major genes (such as BMPR2) and modifying genes coupled with environmental stresses will be the focus of future investigations. In addition to statistical associations, functional studies will be necessary to confirm that an associated gene product actually modifies risk.

The serotonin transporter is the gene and product repre-

sending the best-studied modifier of pulmonary hypertensive states. Cultured pulmonary artery-smooth muscle cells from patients with PPH have an abnormally strong proliferative response to serotonin or serum (40). This abnormal response is due to overexpression of the serotonin transporter (5-HTT), owing in part to a functional polymorphism located on the 5-HTT gene promoter: homozygosity for the (L) allele, the long gene promoter variant associated with a high level of gene transcription, is found in 65% to 75% of patients with PPH as compared to 25% to 30% of controls (41). Recent results showing that 5-HTT gene polymorphism determines the severity of pulmonary hypertension (PH) in hypoxemic patients with chronic obstructive lung disease support a major role for LL-genotype-driven 5-HTT overexpression in the pathophysiology of various forms of PH. Thus, 5-HTT gene polymorphism may be either an important modifier of the PH phenotype or a factor conveying susceptibility to PH in some individuals (42).

**Transgenic models of PAH.** Several groups are studying mouse models that carry *BMPR2* mutations. The first of these, developed by Beppu et al. (43), is a true null. Mice that are homozygous for this mutant *BMPR2* die very early in development during gastrulation. Delot et al. (44) have also developed a mouse that carries an in-frame deletion of exon 2 resulting in loss of the ligand binding portion of the extracellular domain. Mice homozygous for the hypomorphic allele also die in utero, but not until day 12.5. Pathologic analyses of these mice have shown persistence of the truncous arteriosus of the heart, demonstrating the importance of functioning *BMPR-II* for normal heart development.

Recently, Beppu et al. (43) have reported analyses of mice that carry the null *BMPR2* allele in the heterozygous state. Though these mice did not spontaneously develop PH, after three weeks of chronic hypoxia, both pulmonary vascular resistance and right ventricle/left ventricle + septum were greater in the heterozygous mice than in the homozygous wild-type mice. Overexpression of 5-lipoxygenase (5-LO) in the lungs of the *BMPR2* null heterozygous mice using a replication-deficient adenovirus led to an increase in PA pressure as compared to homozygous wild-type mice. Thus, 5-LO may modify the susceptibility of *BMPR2* null heterozygous mice to the development of PPH.

Genetic manipulation of the laboratory mouse has become a very powerful tool for the study of human disease. Simple transgenics and knockouts can potentially be useful for the "quick" determination as to whether a specific gene, when overexpressed or downregulated, can play a role in the susceptibility to the development of PH. The transgenic and knockout models can be crossed to produce mice carrying mutations in more than one gene to examine the synergistic effects of certain combinations of mutations to determine modifier gene effects. Unfortunately, tissue specificity may play an important role in development of the disease, and these studies will then require animal models for which gene

expression in specific tissues can be switched on and off. The transgenic approach to the study of PPH is clearly in its infancy. There are many mouse models yet to be generated, and only time will tell as to their usefulness for the unraveling of the pathogenesis of the disorder.

**Summary.** Future studies will focus on the search for modifiers, both environmental and genetic, that determine the initiation and perpetuation of the disease in individuals harboring mutations in genes conferring increased risk for this devastating condition. These studies will benefit from recent technological advances that enable tissue and cell-specific profiles of both the deoxyribonucleic acid transcript and the proteomic product together with the generation of targeted animal models of the disease. The search for and subsequent investigation of potential modifier genes will require assessment in patient and family cohorts sufficiently powered to generate statistically robust outcomes. Studies of the functional consequences of such mutations on cellular signaling will be necessary to understand the biology of disease. Recent advances have established the need to develop protocols for presymptomatic screening of at-risk family members as a prelude to the initiation of clinical trials of preventive therapy. Therapy aimed at either prevention or actual reversal of the vascular disease process will be the ultimate goal for the betterment of these patients and their families.

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