

Genes and Pulmonary Hypertension: Update 2006

John H. Newman, MD, James E. Loyd, MD,
Lisa Wheeler, Joy Cogan, PhD, Cindy Vnencak-Jones, PhD,
Ivan M Robbins, MD, Vickie Hannig, MS, John A. Phillips III, MD

Division of Pulmonary and Critical Care Medicine
Department of Medicine, Division of Medical Genetics
Department of Pediatrics, and Department of Pathology
Vanderbilt University School of Medicine
Nashville, Tennessee

New insights into the genetic features of pulmonary hypertension (PH) have been identified in the past year. This review summarizes what we think we know as of the summer of 2006 (**Table 1**) regarding genetics and PH. A glossary of important terminology in understanding genetics is also included.

About 50% of patients with familial PH possess identifiable exon or intron/exon boundary mutations in bone morphogenetic protein receptor type 2 (BMPR2). These mutations are not just synonymous substitutions, but include missense, nonsense, frameshift, or donor/acceptor splice mutations that can alter the structure of the receptor and damage its function.^{1,2}

These mutations are easily detected by sequencing the exons of BMPR2. There are 13 exons in BMPR2 comprising about 3000 bases of sequencing results. About 150 mutations have been found in persons with familial and idiopathic pulmonary arterial hypertension (PAH).¹ The total gene is 180,000 kilobases long, mostly introns and so sequencing the whole gene is an expensive and laborious process. When a mutation in a family has been identified, it is relatively easy to perform exon specific sequence analysis and to test other family members. However, if the mutation is not known, full sequencing of all exons is needed.

Other strategies have been developed to detect mutations. As with some other genetic diseases, about 15% to 20% of BMPR2 mutations include gross rearrangements of the gene that result in exon duplications and deletions.³ These mutations cannot be detected by routine exon-by-exon sequence analysis of BMPR2 but rather require Southern Blot analysis for detection, and BMPR2 messenger ribonucleic acid (mRNA) analysis for confirmation and specific identification of the mutation.

Trembath et al⁴ have shown that PAH is also associated with mutations in another transforming growth factor beta (TGFB) receptor family member, the activin-like kinase (Alk1) and its cofactor, endoglin.⁴ Mutations in Alk1 are the cause of hereditary-hemorrhagic telangiectasia (HHT), a vascular disease leading to abnormal arteriovenous malformations. Some patients with HHT develop PAH, and some persons with Alk1 mutations develop PAH without the classic lesions of HHT. We do not know the actual prevalence of Alk1 mutations in a population of PAH patients but think

Table 1. Current Understanding of Inheritance of PAH

- 50% of familial PAH patients have exonic BMPR2 mutations
 - 20% to 30% of BMPR2 mutations are due to larger rearrangements causing deletions or duplications
 - 15% to 25% of "sporadic" idiopathic PAH cases have BMPR2 mutations
 - Alk1/endoglin mutations (HHT) in < 5%
 - About 20% of persons with mutations get PAH
 - There must be modifying genes and other influences that trigger disease
-

BMPR2 = bone morphogenetic protein receptor, type 2; HHT = hereditary haemorrhagic telangiectasia; PAH = pulmonary arterial hypertension.

that it is less than 5%. In a full screening study of PAH populations or patients with idiopathic PAH undergoing genetic testing, mutations in Alk1 would need to be excluded to be complete.

Somatic mutations in arterial lesions in the lung of PAH patients have been found, but a recent examination by Machado et al⁵ failed to reveal loss of heterozygosity of BMPR2 in microdissected lesions in 7 PAH patients and microsatellite instability in only 1 of 37 lesions. Thus, it seems unlikely that somatic transformation of BMPR2 in the pulmonary arterioles causes PAH, although this work only excludes mismatched repair genes that cause microsatellite instability and large deletions of chromosomal material. This work also does not exclude somatic transformation of genes other than BMPR2 that might be involved in pathogenesis of PAH.

Finally, because BMPR2 mutations only exhibit about a 20% penetrance—80% of persons with a mutation will remain clinically normal—there must be other environmental factors or modifying genes that confer either protection or risk for disease.⁶ The search for such genes is in its infancy, and none has been clearly identified. The best-studied gene that is likely to be involved in disease is the serotonin transporter, which regulates cellular uptake of serotonin, a known vasoconstrictor and mitogenic protein.⁷ Multiple

Table 2. BMPR2 Mutations in Idiopathic PAH

- 13/50 (26%) in idiopathic PAH patients with no family history
 - 11 mutations, 3 identical
 - 2 de novo; 3 parental transmission
- 3/33 (9%) idiopathic PAH patients with anorexigen use
- 11/99 (11%) German idiopathic PAH patients
- Prevalence in idiopathic PAH is uncertain
- 12/40 (40%) Japanese idiopathic PAH patients (2 de novo)
- All studies sequenced entire coding region of 13 exons

PAH = pulmonary arterial hypertension.

potential approaches to detection of modifying genes including gene expression microarray, proteomics, genetic association studies in PAH patients and whole genome microsatellite searches may help to identify shared haplotypes in PAH patients in the future.

A difficult problem to grasp is the prevalence and role of BMPR2 mutations in idiopathic PAH (**Table 2**). Idiopathic PAH is genetically defined as an isolated occurrence within a family with no known other affected members. The term “idiopathic” is more certain when a search for known PAH genes is negative in an individual with idiopathic PAH. It seems contradictory that up to 25% of patients with so-called idiopathic PAH have been found to have BMPR2 mutations when tested.⁸ Why are more members of this person's family not affected?

In the minority of cases the answer is that some of the mutations in BMPR2 are de novo, and thus the patient is the first person in a new potential family with familial PAH. De novo mutations comprise about 25% of the group of idiopathic PAH patients that are found to be BMPR2 positive. The second explanation has to do with the relatively low penetrance (20%) of clinical disease in persons with BMPR2 mutations. If a person with a BMPR2 mutation has a child, the a priori risk that the child will inherit the mutated allele is 50%. Thus, there is only a 0.5 X 0.2 or a 10% chance of the offspring developing clinical disease. If this offspring were to have children, then the likelihood of clinical disease would be 5% in each child. Thus, the statistical likelihood that a BMPR2 mutation will become detected clinically in a family over several generations is estimated to be only about 20% to 40%, depending on how many children and how many generations of children are counted. Thus, some patients with idiopathic PAH may actually belong to families with mutations in BMPR2 but with very low penetrance of disease.

Interestingly, BMPR2 mutations are not limited strictly to patients with PAH (**Table 3**). BMPR2 mutations have been found in about 6% of subjects with congenital heart disease and in several families with pulmonary veno-occlusive disease. This highlights the importance of the BMPR2 receptor in fetal cardiac development and the knowledge that the pulmonary veins can be affected by a similar disease

Table 3. BMPR2 Mutations in Other Forms of PAH

- CHD⁹
6/106 (6%) children and adults with CHD due to multiple causes
All mutations found in patients with complex CHD
De novo in the 2 patients with parental DNA
- PAH associated disease
0/24 patients with scleroderma
0/19 patients with HIV infection
- Mutations in 2 patients with PVOD now identified in exon 1 and exon 5
- One case familial, the other sporadic
- Shows that BMPR2 mutations may lead to both pulmonary arterial and venous disease

BMPR2 = bone morphogenetic protein receptor, CHD = congenital heart disease; DNA = Deoxyribonucleic acid; PAH = pulmonary arterial hypertension; HIV = Human immunodeficiency virus; PVOD = peripheral vascular occlusive disease.

process as the arterioles.

In summary, progress has been significant in the past year toward delineating the role of BMPR2 mutations in familial and idiopathic PAH, in congenital heart disease, and pulmonary veno-occlusive disease. The extent of BMPR2 mutations in the total population of patients with PAH is likely to be very large, because of underestimates of prevalence due to low penetrance. Genetic testing is now available at a few centers, and because the realm of BMPR2 mutations is a mix of exonic mutations and gross rearrangements, this testing is necessarily complex in order to completely assess for heritable disease traits. Future work will include studies of the whole genome and large populations of patients for detection of relevant modifying genes.

The authors acknowledge the following support: NIH PO 072058, and GRCC RR095.

Glossary of Words and Terms Used in this Paper

Alk1/Endoglin: A receptor complex of the TGF β family that, when mutated, results in hereditary hemorrhagic telangiectasia and, occasionally, PAH.

BMPR2: A receptor that is part of the TGF β (see below) family of receptors involved in growth and repair.

Exon: A segment of DNA that is transcribed to mRNA and ultimately translated into a protein product. Most genes have multiple exons.

Gene microarray: A technique for measuring the products of transcription of deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) and potentially, therefore, translation to final protein of a cell or tissue.

Haplotype: When 2 or more genes are close together on a chromosome they tend to travel together during the process of making the genome of the egg or sperm and, therefore, are inherited together.

Intron: A segment of DNA that is not transcribed. Formerly thought to be inactive, introns are involved in the orderly sequence of gene transcription.

Microsatellite: A sequence of DNA composed of repeating nucleotides, such as cytosine-adenine-cytosine. These can be used as genetic markers because they are inherited intact. When the repeats become longer in certain genes they can be associated with disease.

Missense, nonsense, and frameshift mutations: Missense causes a different amino acid to be substituted in a protein, nonsense causes the gene product to be truncated, and frameshift involves making all subsequent transcription wrong by changing the reading of all subsequent DNA code in a gene.

Penetrance: The mathematical likelihood that a mutation will result in clinical disease.

Somatic mutations: These occur in organ cells rather than sex cells. In gastrointestinal tumors, somatic mutations are very important for transformation from normal to disease.

Southern blot: A technique to look at all the DNA of an area of interest including exons and introns.

Splice mutations: At the borders between exons and introns are DNA sequences that allow the mRNA that is transcribed

from exons to be spliced together properly. Splice mutations can result in exons being deleted, duplicated, or put in the wrong order.

TGF β : A secreted protein that is involved in growth and repair of tissues. It is part of a large family of proteins.

References

1. Machado RD, Aldred MA, Harrison JV, et al. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat.* 2006;27:121-132.
2. Wong WK, Morse JH, Knowles JA. Evolutionary conservation and mutational spectrum of BMPR2 gene. *Gene.* 2006;368:84-93.
3. Cogan JD, Vnencak-Jones CL, Phillips JA, et al. Gross gene rearrangements constitute a new cause for primary pulmonary hypertension. *Genet Med.* 2005;3:169-174.
4. Trembath RC, Thomson JR, Machado RD, et al. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med.* 2001;345:325-334.
5. Machado RD, Southwood JV, Harrison RE, et al. Investigation of second genetic hits at the BMPR2 locus as a modulator of disease progression in familial pulmonary arterial hypertension. *Circulation.* 2005;111:607-613.
6. Newman JH, Trembath JRC, Morse JA, et al. Genetic basis of pulmonary arterial hypertension: current understanding and future directions. *J Am Coll Cardiol.* 2004;43:33S-39S.
7. Guignabert C, Izikki M, Tu LI, et al. Transgenic mice overexpressing the 5HT transporter gene in smooth muscle develop pulmonary hypertension. *Circ Res.* 2006;98:1323-1330.
8. Thomson JR, Machado RD, Pauciulo MW, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR2. *J Med Genet.* 2000;37:741-745.
9. Roberts KE, McElroy JJ, Wong WP, et al. BMPR2 mutations in adults and children with idiopathic pulmonary arterial hypertension with congenital heart disease. *Eur Respir J.* 2004;24:371-374.