MUTATION IN THE GENE FOR BONE MORPHOGENETIC PROTEIN RECEPTOR II AS A CAUSE OF PRIMARY PULMONARY HYPERTENSION IN A LARGE KINDRED

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ABSTRACT

Background Most patients with primary pulmonary hypertension are thought to have sporadic, not inherited, disease. Because clinical disease develops in only 10 to 20 percent of persons carrying the gene for familial primary pulmonary hypertension, we hypothesized that many patients with apparently sporadic primary pulmonary hypertension may actually have familial primary pulmonary hypertension.

Methods In a study conducted over 20 years, we developed a registry of 67 families affected by familial primary pulmonary hypertension. Through patient referrals, extensive family histories, and correlation of family pedigrees, we discovered shared ancestry among five subfamilies. We assessed some family members for mutations in the gene encoding bone morphogenetic protein receptor II (BMPR2), which has recently been found to cause familial primary pulmonary hypertension.

Results We linked five separately identified subfamilies that included 394 known members spanning seven generations, which were traced back to a founding couple in the mid-1800s. Familial primary pulmonary hypertension has been diagnosed in 18 family members, 12 of whom were first thought to have sporadic disease. The conditions of 7 of the 18 were initially misdiagnosed as other cardiopulmonary diseases. Six members affected with familial primary pulmonary hypertension and 6 of 10 at risk for carriage have undergone genotype analysis, and they have the same mutation in BMPR2, a transversion of thymine to guanine at position 354 in exon 3.

Conclusions Many cases of apparently sporadic primary pulmonary hypertension may be familial. The recent discovery of mutations in BMPR2 should make it possible to identify those with susceptibility to the disease. (N Engl J Med 2001;345:319-24.)

METHODS

We identified the first subfamily in 1980. 6 The propositus was a 30-year-old woman who gave a history of unexpected deaths in her immediate and extended family, including the deaths of her mother, three aunts, and a cousin. The cousin’s condition had been initially misdiagnosed in the 1960s as postpartum cardiomyopathy but was ultimately identified as primary pulmonary hypertension. Two aunts died at 23 and 24 years of age after having given birth without obstetrical complications. At autopsy, tissue from the mother, who died of undiagnosed heart failure, revealed classic concentric intimal fibrosis and plexiform lesions in the pulmonary arteries. After encountering this unusual family that included six affected members whose conditions were first misdiagnosed as other cardiopulmonary diseases. Mutational analysis of the gene encoding bone morphogenetic protein receptor II (BMPR2) in six affected members revealed that all have a T354G missense mutation in exon 3 that encodes an amino acid substitution of tryptophan for cysteine. 9
with the Prospective Registry of the National Institutes of Health, introductions through the Pulmonary Hypertension Association, and extensive researching of family histories. We have relied heavily on key persons who keep family records and maintain family contacts and who have shared crucial information that has enabled us to make connections. As our pedigree data base has expanded, we have been able to link subfamilies previously not known to be related, usually by cross-checking common surnames. We have entered all information into Cyrillic Pedigree Software (version 2.1, Cherwell Scientific Publishing, Oxford, United Kingdom) and a Microsoft Access data base.

For molecular studies, DNA was obtained from leukocytes isolated from samples of venous blood. The location of the gene associated with familial primary pulmonary hypertension was previously identified on chromosome 2q31–32 by linkage analysis of DNA microsatellite markers from affected members of six families unrelated to this kinred. Because the critical interval on 2q31–32 was large for physical mapping, we surveyed genes in the region of interest that are likely to be candidates. The sequence variations that we found cosegregated on gel electrophoresis in all affected members of the kindred that we studied, and DNA mapping revealed a mutation in exon 3, a substitution of guanine for thymine at nucleotide position 354. This is predicted to result in a substitution of tryptophan for cysteine at codon 118. DNA was taken from 5 controls who married into the kindred and from more than 60 other normal, unrelated controls.

RESULTS

The husband (born in 1835) and wife (born in 1838) of generation I had seven children, two of whom were the ancestors of all the currently known subfamilies of this kindred (Fig. 1). We do not know which parent transmitted primary pulmonary hypertension and have no information on the mother’s family. If the mother was the carrier, there is another potentially large subfamily of this kindred. We have information on seven generations, including 394 named descendants, over 200 of whom are alive and at increased risk of having the mutated gene. In the entire pedigree, there have been 23 unaffected obligate carriers of the gene, who are known because they each had an affected descendant and an ancestor who was a carrier or was affected. There were 18 members in whom familial primary pulmonary hypertension was diagnosed, giving a total of 41 members known to have the gene.

Of the 18 with familial primary pulmonary hypertension, 16 were female and 2 were male. Of the carriers, 12 were female and 11 were male. As in previous studies, the presence of male-to-male transmission (three instances) excluded X linkage. Of the 18 members with familial primary pulmonary hypertension, 7 (39 percent) were initially given a misdiagnosis, and 12 (67 percent) were originally thought to have sporadic disease, until familial disease was discovered. Misdiagnoses included postpartum cardiomyopathy, cardiomyopathy associated with hypothyroidism, pericardial tamponade, “heart attack,” atrial septal defect, ventricular septal defect, and pulmonic-valve insufficiency. Because of the high proportion of asymptomatic carriers, there are at least 200 offspring with some increased risk of having inherited the gene associated with familial primary pulmonary hypertension. Thus, the number of future descendants at risk should increase geometrically.

In this large kindred, there were originally five subfamilies that were considered to be separate entities with regard to primary pulmonary hypertension. We briefly summarize the threads of evidence that connected them in Figure 1. In the early 1980s, we developed an extended family history and examined public records in our attempt to document fully the incidence of primary pulmonary hypertension in Subfamily 14, the first family we studied. Several years later, one of us saw a patient in consultation (from Subfamily 29) who was thought to have sporadic primary pulmonary hypertension but had a dead grandparent with the same surname as an ancestor in Subfamily 14. We could not initially link the two subfamilies because of lack of information due to migration of ancestors and geographical separation. We had known about Subfamily 32 since 1992. A communication from Subfamily 60 in 1997 revealed that the ancestral name of Subfamily 14 was in the lineage of Subfamily 60, and this subfamily also had a surname in common with generation I and Subfamily 32. Subfamily 29 and Subfamily 96 were easily linked from simple family histories, which revealed that subjects in generation IV shared the same great-grandparents. The patient in generation VII of Subfamily 29 was recently referred to Vanderbilt University Medical Center for management of suspected congenital heart disease.

Figure 1 (facing page). Abbreviated Pedigree of a Large Kindred Comprising Five Subfamilies over Seven Generations and 394 Known Descendants of Generation I.

The propositus (arrow), a woman in generation V of Subfamily 14 who died at the age of 30, received her diagnosis from one of us in 1980. Details of the discovery and linkage of these subfamilies are given in the Methods and Results sections. There are at least 200 descendants at varying degrees of risk for primary pulmonary hypertension. Familial primary pulmonary hypertension has been diagnosed in 18 members (16 women and 2 men), and at least 23 (12 women and 11 men, 20 of whom are shown in the figure) are known to carry the gene for the disease. Open symbols indicate unaffected members, solid symbols members with primary pulmonary hypertension, symbols with dots carriers, squares male family members, circles female family members, and slashes deceased members. Numbers inside the symbols indicate the number of members of that sex; numbers under the symbols indicate the age at death or at the time of this writing.
The 30-year-old man in generation VI of Subfamily 96 was identified as a member of the kindred when he was asked to counsel one of our new patients about prostacyclin therapy. This new patient happened to be a member of one of the subfamilies we had studied, and the two discovered that they had an ancestor with the same surname. Subfamily 14 was part of our recent report identifying the genetic mutation associated with familial primary pulmonary hypertension.

We recently reported molecular data on six affected members of this kindred in our study identifying the mutated BMPR2 gene in multiple subfamilies affected by familial primary pulmonary hypertension. Each had a T354G transversion in exon 3 of the BMPR2 gene on chromosome 2. On the basis of current knowledge, this particular mutation is unique to this kindred. Of the six affected members, four are receiving therapy for primary pulmonary hypertension and two have died. We have determined the genotypes of 10 additional members in the bloodline (who have about a 50 percent risk of inheriting the mutation) and have found the same mutation in 6; none of these 10 members have clinical symptoms. Five controls who married into the family did not have the BMPR2 mutation.

DISCUSSION

The discovery of this very large kindred affected by familial primary pulmonary hypertension confirms and extends the hypothesis that many cases of presumed sporadic primary pulmonary hypertension are in fact genetic. Two thirds of the patients in this kindred (12 of 18) were thought to have sporadic disease. Recognition of familial disease, or the absence of it, has important implications with regard to surveillance for disease, early diagnosis and management, and personal and family counseling. The search for the mutation or mutations responsible for familial primary pulmonary hypertension has culminated in the discovery of more than 25 mutations in BMPR2. Each mutation tracks disease with fidelity in families. In addition, we have recently found that about 25 percent of patients with “sporadic” primary pulmonary hypertension also have mutations in BMPR2. Thus, it appears that a significant proportion of all cases of primary pulmonary hypertension may be caused primarily by a defect in BMPR2.

Bone morphogenetic proteins were first identified as cellular products found in normal bone that promote ectopic bone formation and the healing of fractures. These proteins are members of the TGF-β superfamily of circulating proteins that regulate growth and repair of tissue in all organs. Bone morphogenetic proteins exert their effects through the activation of receptors I and II, which are expressed adjacent to each other on cell surfaces and transduce intracellular signaling (Fig. 2). Ligand binding of bone morphogenetic protein with the extracellular domain of bone morphogenetic protein receptor II leads to phosphorylation of bone morphogenetic protein receptor I and activation of the intracellular serine–threonine kinase domain of bone morphogenetic protein receptor I. The activated receptor I then phosphorylates the cytoplasmic signaling protein called response Smad5, which binds with Smad4 in the cytosol and migrates to the nucleus, where they regulate DNA transcription in concert with nuclear binding factors.

The effect of activation of bone morphogenetic protein receptors depends on the cell and the circumstances and can result in either promotion or inhibition of growth. Because the likelihood that clinical primary pulmonary hypertension will develop is only 10 to 20 percent in known carriers of BMPR2 mutations, we speculate that gene modifiers such as environmental factors, estrogens, or other mutations in unknown regulatory genes may be necessary for clinical expression of the disease. The mutations in BMPR2 that have already been identified are likely to cause impairment in receptor function; thus, the normal function of bone morphogenetic protein receptor II in the pulmonary circulation may be antiproliferative. If two functioning alleles are necessary for this inhibitory function, then haploinsufficiency of BMPR2 in heterozygotes may be the mechanism of vascular dysregulation that leads to familial primary pulmonary hypertension.

Genetic testing and genetic counseling will soon become important issues to address for patients with primary pulmonary hypertension and their families, and some of the current uncertainties about transmission and the risk of disease will be resolved. Currently, there is no certified clinical laboratory protocol to test for the presence of mutations in BMPR2, but such a protocol may become available in the near future. Family history will become increasingly important as methods of identifying those at risk are developed. The family history should be extensively reviewed for all patients with presumed sporadic primary pulmonary hypertension, and surnames of ancestors should be ascertained. It is only through documentation of pedigrees that these relationships will be discovered.

Surveillance for the onset of disease in subjects at risk for familial primary pulmonary hypertension will also become increasingly important as improved therapies, such as oral or inhaled vasodilators, are developed. Presumably, early presymptomatic diagnosis will ultimately lead to prevention of disease progression and prolonged survival. Personal and family counseling is dependent on accurate diagnosis. A child of a patient or carrier has a 50 percent chance of inheriting the mutated gene. We know that in approximately 10 to 20 percent of persons carrying the gene for familial primary pulmonary hypertension, overt clinical disease will eventually develop, but we currently have no predictive test or measure of the onset of preclinical disease. Investigations are in progress to obtain
measurements of preclinical markers of vascular disease, including endothelin, TGF-β, and thromboxanes, in patients at risk and to develop information about the sensitivity and specificity of these markers. Levels of each of these endogenous mediators have been found to be elevated in patients with established primary pulmonary hypertension. Each is associated with vascular remodeling, and endothelin and thromboxanes also cause vasoconstriction, a feature of some cases of primary pulmonary hypertension.

Echocardiography with Doppler assessment of pulmonary arterial systolic pressure is the best noninvasive method for the detection of presymptomatic pulmonary hypertension. In patients known to have the gene for familial primary pulmonary hypertension, serial evaluations of selected circulating mediators and serial echocardiography may eventually be the best tools to screen for the development of disease. Until we are better able to assess pathophysiology, it will be very difficult to give susceptible persons reliable guid-

Figure 2. 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. In the kindred discussed in this report, there is a single point mutation in the kinase domain. The cells in which the mutation causes primary pulmonary hypertension have not been identified, although endothelial cells, smooth-muscle cells, and fibroblasts are likely candidates.
ance about decisions related to lifestyle, including exercise, birth control, and childbearing. It is obviously wise to advocate avoidance of smoking, diet drugs, and decongestant medications that cause vasoconstriction.

In summary, we have identified an extensive, growing cohort of related persons at risk for familial primary pulmonary hypertension, representing what is to our knowledge the largest group of persons with the same mutated gene associated with this disease. The existence of this kindred provides us with the opportunity to gain insight into the genetic transmission and expression of familial primary pulmonary hypertension and its diagnosis and misdiagnosis. Its existence also challenges us to develop better methods of detection of preclinical disease and prediction of disease expression. Finally, genetic and family counseling have become issues of growing importance for this kindred and other families whose members require better insights and information from medical scientists, so that those affected can plan their lives with as much certainty as possible.

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REFERENCES


