Identification of a Novel Mutation in the Gene for Bone Morphogenetic Protein Receptor II in an Israeli Patient with Familial Primar y Pulmonary Hypertension

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Abstract

Background: Primary pulmonary hypertension is a rare disorder, characterized by progressive pulmonary hypertension and right heart failure. It may be familial or sporadic. Mutations in bone morphogenetic protein receptor II (BMPR2), a member of the transforming growth factor-beta receptor superfamily of receptors, underlie many cases of the disorder.

Objectives: To perform molecular analysis of a patient with familial PPH and provide her and her family with suitable genetic counseling.

Methods: DNA was extracted from 10 ml whole blood, and the *BMPR2* gene was screened for mutations. Individual exons were amplified by polymerase chain reaction and sequenced. Mutation confirmation and molecular characterization of additional family members was performed using restriction enzyme analysis followed by appropriate genetic counseling.

Results: We identified a novel T to C missense mutation expected to result in substitution of arginine for a conserved cysteine in the ligand-binding domain of *BMPR2*. Screening of family members demonstrated the presence of the mutation in the father and a younger asymptomatic sister of the index patient.

Conclusions: Molecular diagnosis in PPH allows for identification of at-risk family members and raises the option of earlier diagnosis and possibly instituting earlier treatment in affected individuals. However, molecular screening of asymptomatic family members raises difficult ethical questions that can only be resolved by conducting large multicenter prospective studies in *BMPR2* carriers.

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Primary pulmonary hypertension is a rare disorder, characterized by an elevation in pulmonary arterial pressures due to progressive obliteration of the pulmonary arteriolar bed. The high pulmonary blood pressures lead to progressive dyspnea and syncope, and, if left untreated, invariably result in right heart failure and death.

The incidence of PPH in Israel is 8 patients per million, with 1.4 new cases per million a year [1]. Data reported in the previous two decades indicate that the median survival after diagnosis of PPH is 2.8 years [2], although novel drug development has resulted in improved patient survival and has reduced the need for lung transplantation [3]. Pulmonary hypertension may be primary or secondary to factors such as ingestion of appetite suppressants, human immunodeficiency virus infection or portal hypertension [3–5]. Most cases of PPH are sporadic, however 6–10% of the cases represent a familial disorder with low penetrance (10–20%) [3]. PPH occurs at all ages and affects women twice as commonly as men.

Linkage of PPH to chromosome 2q31-33 was initially reported in 1997 and was found in over 70% of the familial cases [6]. An association has been found between mutations of the gene encoding the bone morphogenetic protein receptor II (BMPR2), a member of the transforming growth factor-beta superfamily of receptors, and the presence of PPH. Fifty-five percent of patients with familial PPH and 25% of sporadic PPH patients demonstrated heterozygous mutations in this gene [7–10], indicating that many cases of apparently sporadic PPH are in fact "familial" and it is only the low penetrance of the gene that makes them seem sporadic [11]. We describe a case of familial PPH in whom we identified a novel mutation in the ligand-binding domain of the *BMPR2* gene. The ethical issues relating to screening for BMPR2 mutations are discussed.

Patients and Methods

Clinical data

The proband is a 25 year old woman who was recently diagnosed with PPH. She had been asymptomatic until 6 months prior to presentation and had completed a normal full-term pregnancy and vaginal delivery 9 months prior to the appearance of symptoms. The patient complained of progressive fatigue and dyspnea and was admitted to the emergency room with severe right-sided cardiac failure. At this time, the patient was hypoxemic at rest (saturation 75–80% on room air) and was unable to get out of bed, even with assistance. Echocardiography demonstrated the presence of severe pulmonary hypertension with enlarged and poorly functioning right atrium and ventricle. Right heart catheterization showed pulmonary pressures of 80/45, mean 58 mmHg, cardiac output 3.82 L/min and pulmonary vascular resistance of 12.03 WU (normal 0.5–1.5), with

PPH = primary pulmonary hypertension

no significant improvement following vasodilators. Because of her advanced cardiac failure, she was started on treatment with intravenous prostacyclin together with oxygen, anticoagulants, diuretics and ionotropes. The patient gradually stabilized and following discharge continued to improve with a New York Heart Association grade II–III functional capacity. She has resumed her previous occupation and takes care of her 3 year old daughter with only limited assistance.

The patient's sister, who was 2 years older, died 24 hours after childbirth 5 years earlier due to previously undiagnosed pulmonary hypertension. Additional siblings include two sisters and four brothers.

Molecular biology

Ethics committee approval for this study was obtained and the patient gave written informed consent. Whole blood, 10 ml, was collected in a tube containing EDTA. DNA was extracted using conventional methods, dissolved in TE and kept at 4°C. The 13 exons of the gene were amplified by polymerase chain reaction using specific primers. Amplified fragments were then sequenced using an ABI PRISM 310 Genetic Analyzer. DNA sequence from the patient was compared to the genomic sequence for BMPR2 found on the NCBI site (www.ncbi.nlm.nih.gov accession no: NM_001204). Mutation verification was performed using the restriction enzyme *Aci* I.

Results

A novel missense mutation in exon 2 of the *BMPR2* gene was identified in which a T residue replaces a C, expected to lead to an arginine for cysteine substitution at amino acid #66 in the ligand-binding domain of the receptor [Figure 1A].

The mutation was confirmed with restriction enzyme analysis using *Aci* I [Figure 1B]. In the mutated allele, a novel *Aci* I site is generated and, following restriction digestion, yields DNA fragments of 123 and 70 base pairs. *Aci* I does not cut the wild-type allele, which remains a single fragment of 193 base pairs.

Prior to the molecular testing, genetic counseling was provided to the patient and her family, and the implications of such testing were discussed. Although initially interested in undergoing testing, once a mutation was clearly identified in the proband and the option of genetic testing was raised again, most family members were now reluctant to undergo such testing. The patient's parents and one sister chose to undergo molecular testing, which led to the identification of the father and the sister as asymptomatic carriers [Figure 1C].

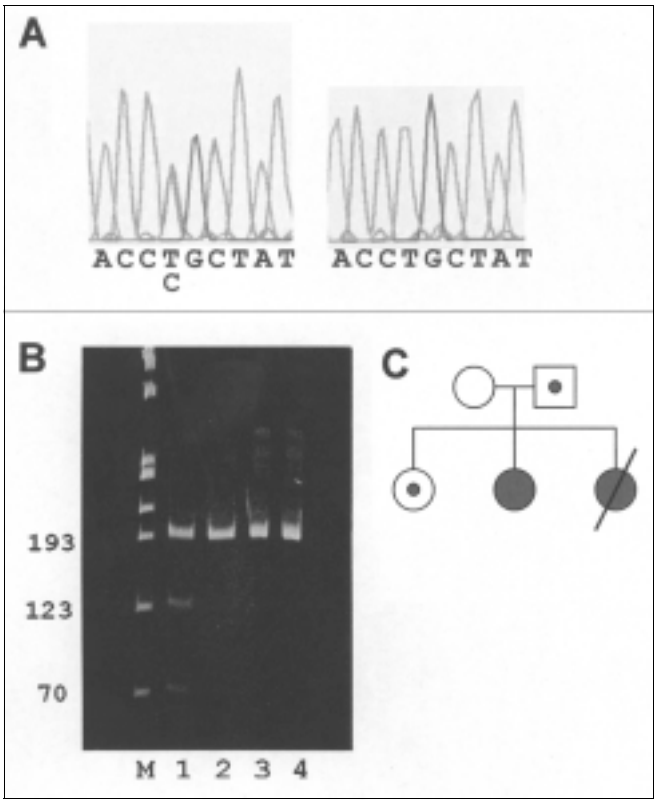

Figure 1. Results of sequence analysis. **[A]** Sequence analysis of mutant and wild-type fragment in exon 2 shows: right – normal sequence, T at position #604; left – patient's sequence, at position #604 both C and T are sequenced representing the normal and mutant alleles. **[B]** Restriction analysis of exon 2 polymerase chain reaction amplification product with *Aci I*. Control fragment is not digested, demonstrating one band on gel. The patient has three bands on gel, representing two alleles; one normal – not digested, one – mutated, digested to two fragments of 123 & 70. M = marker, 1 = patient DNA cut, 2 = patient DNA uncut, 3 = control DNA cut, 4 = control DNA uncut. **[C]** Family tree. Closed circles = PPH patients, dotted box and circle = asymptomatic carriers of BMPR2 mutations.

Discussion

We describe a novel missense mutation in exon 2 of the gene encoding BMPR2 in a patient with familial PPH. Mutations of *BMPR2* have been implicated in over half (55%) the cases of familial PPH, and in 26% of sporadic cases [10].

The mechanism whereby BMPR2 mutations cause PPH is currently under investigation. The pulmonary vasculature of patients with PPH is characterized by marked thickening of arteriolar walls with luminal obliteration. BMPR2 is a member of the TGFβ superfamily of receptors and regulates cellular proliferation in response to ligand binding. In PPH there is proliferation of pulmonary arterial vascular smooth muscle cells, ultimately causing migration and proliferation of pulmonary vascular endothelial cells [12]. It has been demonstrated that in PPH, endothelial cell proliferation is monoclonal whereas in secondary disease the proliferation is polyclonal, suggesting that the plexiform lesions seen in PPH are "cancerous" due to loss of mechanisms regulating cell division [13].

BMPR2 has been found to regulate cell proliferation via two signaling mechanisms: the Smad signaling pathway, which inhibits cellular proliferation, and the $p38^{MAPK}$ signaling pathway, which enhances cell proliferation. The pathway activated depends on the mode of oligomerization of the BMPR1 and BMPR2 receptors at the cell surface [14].

TGFβ = transforming growth factor-beta

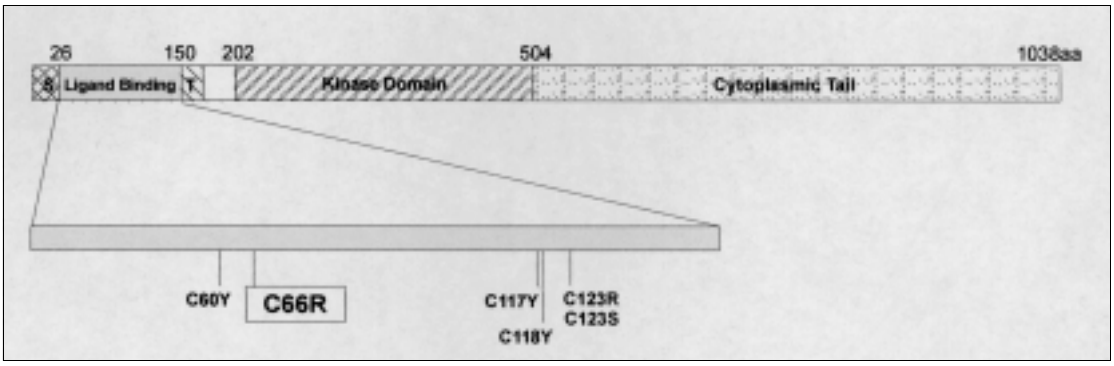

Figure 2. A map of *BMPR2* demonstrating the gene's functional regions. Listed are all known cysteine mutations in the ligand-binding domain. The mutation in our patient results in the substitution of arginine for cysteine at amino acid #66, S = signal peptide, T = transmembrane region.

The 1038 amino acid BMPR2 protein comprises ligand-binding, kinase and cytoplasmic domains [Figure 2]. Mutations have been found throughout the length of the gene, including missense, nonsense, frameshift, deletions and splice mutations [10]. The ligand-binding domain is a hydrophilic cysteine-rich domain. The domain, and specifically its cysteines, is highly conserved in the TGFβ superfamily. The cysteine in position #66 is one of the conserved cysteines in this region [Figure 2]. Five cysteine missense mutations have already been described in this region. Transfection studies of mutated BMPR2 proteins in pulmonary vascular smooth muscle cells demonstrate that substitution of cysteine residues in the ligand-binding or kinase region leads to retention of the protein in the endoplasmic reticulum and prevents receptor trafficking to the cell surface. There is thus reduced ligand binding as well as reduced ligand-induced transcriptional activity via the Smad pathway [15,16]. This finding is compatible with a dominant negative effect of these mutants on Smad signaling. It is likely that the cysteine substitution mutation found in our patient has a similar biological effect and is indeed the cause for the patient's disease.

The low penetrance of the familial disorder indicates an important role for environmental factors, and/or additional activity of modifier genes, triggering the disease in carriers of BMPR2 mutations. Recently, Humbert et al. [17] showed that 9% of patients with fenfluramine derivative-induced pulmonary hypertension have mutations in the *BMPR2* gene as compared to none of 130 screened normal controls. Patients carrying the mutation developed PPH following a shorter exposure time to the drug than those who did not, thus demonstrating their higher predisposition for the disease. No increase in prevalence of *BMPR2* mutations has been found for other forms of secondary pulmonary hypertension. Other genetic factors that may be associated with pulmonary hypertension include mutations of the activin receptor-like kinase 1 gene (*ALK-1*), which causes hereditary hemorrhagic telangiectasia and may manifest as classical isolated PPH [18] and polymorphisms of the serotonin transporter gene [3,19]. Recently, a possible additional locus for PPH has been mapped to chromosome 2q31, 15 to 19 centimorgans proximal to BMPR2 [20,21]. The expression of angiopoietin-1 and its receptor TIE 2 are enhanced in primary and secondary forms of pulmonary hypertension, and levels correlate with disease severity [22]. Angiopoietin-1 down-regulates expression of BMPR1A, a co-receptor molecule for BMPR2, and provides evidence linking the BMPR signaling system to all forms of pulmonary hypertension.

Our patient and her family effectively demonstrate the ethical difficulties that stem from genetic screening for PPH. In contrast to other genetic pulmonary diseases, particularly cystic fibrosis where screening provides family members with relatively clear information regarding risk of developing disease, this is not the case for PPH. PPH shows an autosomal dominant form of inheritance, with disease penetrance estimated to be only 15–20% and with a variable age of onset. Thus, identifying a BMPR2 mutation in a healthy subject is a poor predictor for the development of clinical disease. On the other hand, identification of a mutation may have far-reaching implications such as an adverse effect on self-esteem, social and family status, as well as insurability, and may affect decisions regarding family planning.

There are at present no clearly defined medical recommendations regarding screening for asymptomatic carriers of BMPR2 mutations. The development of a screening test that would allow us to identify those carriers who are more likely to develop disease would make screening more meaningful for the patient and the physician. Attempts to identify such markers are ongoing: echocardiography following exercise or exposure to hypoxia have been shown to identify patients with early preclinical PPH [23]; and biochemical markers such as brain natriuretic peptide may also be of value [24]. Long-term prospective follow-up of asymptomatic BMPR2 carriers will be necessary to further clarify the value of such markers.

PPH is usually diagnosed late and thus has an overall poor prognosis. While it seems likely that the identification and treatment of patients at high risk for developing disease or those with early symptoms will improve outcome, there are as yet no data to support this premise. If so, why screen for a disease for which there is no evidence that earlier treatment can modify outcome?

Screening for BMPR2 mutations (as is the case for other genetic disorders) has implications for family members. Identification of one family member as a BMPR2 mutation carrier increases the risk that children and siblings carry the affected gene. Family members may not necessarily wish to be made aware of increased risk status in the absence of clearly defined clinical solutions.

Now that progress in understanding the genetics of PPH has been made, serious consideration must be given to the social and ethical questions that accompany genetic screening. Many of these

questions do not have clear solutions. However, they emphasize the need to better identify coexistent predisposing factors for the development of PPH and possible treatment modalities and behavior modification that may improve disease outcome. It is clear that the risk of psychosocial harm and stigmatization must be considered when offering genetic testing to patients' family members, and the implications of such testing should be extensively discussed.

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