Eur Respir J 2003; 22: 560–562
DOI: 10.1183/09031936.03.00095303
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European Respiratory Journal
ISSN 0903-1936

CASE STUDY

Primary pulmonary hypertension after amfepramone (diethylpropion) with BMPR2 mutation

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Primary pulmonary hypertension after amfepramone (diethylpropion) with BMPR2 mutation. M.J. Abramowicz, P. Van Haecke, M. Demedts, M. Delcroix. ©ERS Journals Ltd 2003.

ABSTRACT: Primary pulmonary hypertension (PPH) is characterised by sustained elevations of pulmonary arterial pressure without a demonstrable cause, leading to right ventricular failure and death. Hereditary mutations in the bone morphogenetic protein receptor type II (BMPR2) gene result in familial PPH transmitted as an autosomal dominant trait, albeit with low penetrance. The causes in cases without a BMPR2 mutation are unknown, but a syndrome of pulmonary arterial hypertension (PAH) similar to hereditary PPH is associated with systemic connective tissue disease, congenital heart disease, portal hypertension, and human immunodeficiency virus infection, or with the use of appetite-suppressant drugs.

The authors identified a BMPR2 gene mutation in a 27-yr-old female who developed PAH after a short course of the appetite-suppressant drug amfepramone (diethylpropion). This allowed molecular genetic counselling and prevention of potentially harmful drug exposure in the patient's son treated for attention deficit disorder with methylphenidate, an amphetamine-related drug. No BMPR2 mutation was found in four additional, unrelated patients with appetite suppressant-related PPH.

The findings provide strong evidence that amfepramone can trigger primary pulmonary hypertension in a bone morphogenetic protein receptor type II gene mutation carrier, and indicate that other genes are probably implicated in genetic susceptibility to appetite suppressants.

Eur Respir J 2003; 22: 560–562.

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Keywords: Appetite-suppressant drugs, bone morphogenetic protein receptor type II, primary pulmonary hypertension, serotonin

Received: October 18 2002
Accepted after revision: March 20 2003

M.J. Abramowicz is supported by the Fonds Forton/Fondation Roi Baudouin, Belgium, and by the Fonds de Recherche Clinique, Hôpital Erasme-Université Libre de Bruxelles, Belgium.

Primary pulmonary hypertension (PPH) is a syndrome of dyspnoea, fatigue, chest pain and syncope resulting from an increase in pulmonary vascular resistance leading to right ventricular failure and death [1]. An affected first-degree relative is found in $\geqslant$6% of cases. Germline, heterozygous mutations of the gene coding for the bone morphogenetic protein receptor type II (BMPR2) gene result in hereditary PPH being transmitted in families as an autosomal dominant trait [2, 3]. However, the penetrance of such mutations, *i.e.* the fraction of gene mutation carriers in whom the disease does develop, is quite reduced, to ~10–20%. The reasons for low penetrance are unknown, but it is likely that both environmental factors and other genetic factors play a role. A syndrome of pulmonary arterial hypertension (PAH) that is similar to PPH in terms of clinical course, histology, and response to treatment, occurs in association with connective tissue disease, congenital heart disease with left-to-right shunt, portal hypertension, and human immunodeficiency virus infection, or with the use of appetite-suppressant drugs [4]. Such cases are sometimes referred to as secondary pulmonary hypertension (PH). From a clinical standpoint, a diagnosis of PPH is made after exclusion of all causes of secondary PH [1]. A hereditary BMPR2 mutation is found in ~30% of apparently sporadic PPH cases [5].

The overall incidence of PPH is 1–2 cases per million people per year. After prolonged appetite-suppressant drug intake, the risk of developing PPH is increased to 1 per 10,000 [6, 7]. Epidemiologically, the appetite suppressants that are clearly associated with PPH are mainly fenfluramine, dexfenfluramine, and aminorex, which caused an epidemic of PPH during the late sixties in central Europe [8]. The implication of amphetamines, like amfepramone (diethylpropion), is less clear because of the small number of patients exclusively exposed to these drugs [6]. Why only a small fraction of appetite-suppressant drug users develop PPH is unknown, but some genetic, presumably multigenic susceptibility is likely. BMPR2 germline mutations have recently been reported in four unrelated patients with fenfluramine-associated PH [9]. However, the mechanisms by which fenfluramine triggers PPH remain unknown, and to the authors' knowledge, amfepramone has not been reported in association with a BMPR2 mutation in a PPH patient.

Case report

A 27-yr-old female complained of progressive dyspnoea, fatigue, and dizziness at exercise, and experienced two episodes of syncope over 10 months before medical evaluation. The patient had suffered from low blood pressure since the delivery of her second child and had been treated briefly with

etilefrine 6 months before admission. Medical history consisted of appendectomy and correction of a left double ureter.

The only risk factor identified for PAH was intake of amfepramone chlorhydrate, 75 mg per day during two cures of 4 and 5 weeks, respectively, 3.5 and 1.5 yrs before onset of symptoms, in order to resolve persistent weight gain postpartum. The patient had never used fenfluramine or dexfenfluramine. The family history was unremarkable over three generations on both parental sides. The patient had two sons, aged 4 and a half and 2 and a half yrs. The eldest suffered attention deficit disorder and was chronically treated with methylphenidate (Rilatin®, Novartis), a drug that is chemically related to amphetamine and other appetite suppressant drugs.

The diagnostic work-up confirmed PPH by ruling out other causes of PAH. The lung function was normal except for a decreased diffusion capacity for carbon monoxide of 62%. There was no evidence for left ventricular dysfunction or valve anomalies, or intracardial shunt at transoesophageal echocardiography. The ventilation/perfusion scan showed a patchy distribution pattern. Echography of the liver and portal vein, chest radiograph and high-resolution computer tomography of the thorax were normal. Testing for antinuclear antibodies was negative.

On admission, the patient scored as New York Heart Association (NYHA) class 3. Physical examination showed a blood pressure of 100 over 70 mmHg, a heart rate of 98 min^{-1}, a loud second heart sound, no oedema and a normal jugular pressure. EchoDoppler examination of the heart showed a grade 3 tricuspid regurgitation with a pressure gradient of 69 mmHg and decreased right ventricular contractility with a Tei-index of 0.87. Right heart catheterisation revealed a mean pulmonary arterial pressure of 52 mmHg, a cardiac index of 1.65 $L \cdot min^{-1} \cdot m^{-2}$, a right atrial pressure of 7 mmHg, and a wedge pressure of 5 mmHg. The pulmonary vascular resistance was 1,232 $dyn \cdot s \cdot cm^{-5}$. There was no reversibility of the PH during inhalation of nitric oxide. The 6 min walking distance was 396 m and the maximal oxygen consumption was 10.7 $mL \cdot kg^{-1} \cdot min^{-1}$.

The patient was successfully treated with the dual endothelin receptor antagonist Bosentan within the Bosentan Randomized Trial of Endothelin Antagonist Therapy-I study [10], and was anticoagulated with phenprocoumone. The patient scored as NYHA class 2 and the 6 min walking distance was 488 m 2 yrs later.

Deoxyribonucleic acid (DNA) was extracted from peripheral blood using standard methods and was studied by direct sequencing of the whole coding sequence of the BMPR2 gene. The 13 exons and intronic junctions [10] were polymerase chain reaction (PCR) amplified individually and sequenced on both strands using the Thermosequenase sequencing kit (Amersham Life Science, Little Chalfont, UK) and a Sequence Navigator Software (PE applied BioSystems, Foster City, CA, USA) running on an ABI 373 sequencer (PE applied BioSystems). Overlapping PCR fragments were produced for larger exon 12 (primers sequence available upon request).

A cytosine was found to be deleted in position 2705 of the BMPR2 complementary DNA (delC2705), at the level of codon 766. This one base pair deletion in the protein-coding sequence causes a shift in the triplet reading code and is predicted to truncate the protein product in exon 12. This mutation has not been reported previously. The patient was heterozygous for the mutation.

The same analysis was performed in four additional, unrelated patients who developed PPH after appetite-suppressant drug intake. No further BMPR2 mutation was identified. This study was approved by the ethical committee of the Hôpital Erasme-ULB.

Discussion

The authors report a BMPR2 gene mutation in a patient who developed PPH after a short course of amfepramone intake. The mutation is predicted to cause a loss of gene function as it induces a frameshift, which in turn truncates the protein product in a region where other pathogenetic mutations have been reported [11]. Heterozygous (*i.e.* present in one of the two gene copies) germline loss of gene function fits the current model stating that hereditary PPH results from BMPR2 haploinsufficiency [11]. Why only 10–20% of carriers of such mutations develop clinical PPH is unknown. The patient in the current study developed PPH after two childbirths and after short courses of amfepramone intake.

There are several reports of PPH onset during pregnancy. Whether this indicates that the pre-existing disease was exacerbated by the haemodynamic changes of pregnancy or that pregnancy and hormonal changes increase the absolute risk for PPH, remains to be elucidated. A large epidemiological case-control study of PPH has shown that recent pregnancies seemed to be more frequent in PPH patients than in controls, but did not have sufficient statistical power to be conclusive [6]. The course of the disease in the patient in the current study does not suggest major peripartum deterioration, since the disease became symptomatic more than a year after the last childbirth.

Amfepramone use by the general population is rare, in the range 0.6–1.2% [6, 7], and penetrance of the BMPR2 mutation is quite low, 10–20%, making chance association of both unlikely. Furthermore, the timing of drug usage and onset of symptoms was very suggestive of a causal relationship. Therefore, the authors view amfepramone as a likely trigger of PPH in the patient.

BMPR2 mutations are believed to alter the BMP and transforming growth factor (TGF)-β1/Sma and Mothers Against Decapentaplegic (SMAD), signalling pathway in pulmonary arterial endothelium and/or smooth muscle cells, resulting in a net increase in cell proliferation [2, 3, 11]. The molecular mechanisms by which appetite-suppressant drugs trigger PPH are unknown. These drugs are indirect serotonin (5-HT) agonists *via* inhibition of the 5-HT transporter [6], and some of their metabolites produced *in vivo* are direct agonists of 5-HT_2 receptor subtypes [12]. Appetite suppressants might thus produce at least part of their pulmonary hypertensive effect through stimulation of a cross-talk between the BMP/TGF-β1/SMAD pathway and a 5-HT signaling pathway known to promote cell-cycle progression, probably *via* 5-HT_{2B} receptors [13]. Both pathways promote the cyclin D- and cyclin E-dependent transition from the G1 to the S phase of the cell-cycle [13, 14].

PPH disease progression may involve additional genetic mutations, either germinal (hereditary) or somatic (acquired). In that regard, mutation of the TGF-β receptor type II gene has been reported in endothelial plexiform lesions from sporadic PPH lungs, accompanied with a microsatellite instability-type of genomic instability and clonal cellular expansion [15]. An acquired mutation of the second BMPR2 allele in pulmonary cells might theoretically be involved in PPH disease progression. In the current case study, appropriate lung tissue was not available from the patient to test this "two-hit" hypothesis.

Predictive genetic testing in relatives who are at risk of carrying a BMPR2 mutation must be performed with caution and in the frame of careful genetic counselling focused on the low penetrance, and on the current absence of evidence that early detection of the disease can lead to reduced morbidity or mortality. In this particular family, methylphenidate treatment in the patient′s eldest son caused worry because the drug

is structurally related to amphetamines and appetite-suppressants. This motivated predictive genetic testing. The subjects chance of not carrying the mutation is 50%.

In this small series of appetite-suppressant users with PPH (n=5) the authors did not find additional cases of BMPR2 mutations. Large genomic deletion mutations or mutations in regulatory elements of the gene may have escaped the sequencing method, but it seems unlikely that BMPR2 mutations explain all cases of genetic susceptibility to appetite suppressant-associated PPH. The same conclusion is reached in another recently reported study, which found four BMPR2 mutations in 34 unrelated patients who used fenfluramine derivatives and developed PPH [9]. Interestingly, BMPR2 mutations in the latter study were significantly associated with PPH after short exposures to fenfluramines (<3 months), as in the patient in the current study (9 weeks amfepramone).

The current case study argues for amfepramone as a triggering factor for primary pulmonary hypertension in bone morphogenetic protein receptor type II mutation carriers. It blurs the distinction between primary pulmonary hypertension and pulmonary arterial hypertension secondary to the use of an appetite-suppressant drug and favours a general model integrating genetic and environmental factors in the pathogenesis of the disease.

Acknowledgements. The authors would like to thank S. Delbauve for technical help, R. Naeije for discussion and G. Vassart for support.

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