

BMPR2 mutations in pulmonary arterial hypertension with congenital heart disease

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ABSTRACT: The aim of the present study was to determine if patients with both pulmonary arterial hypertension (PAH), due to pulmonary vascular obstructive disease, and congenital heart defects (CHD), have mutations in the gene encoding bone morphogenetic protein receptor (BMPR)-2.

The BMPR2 gene was screened in two cohorts: 40 adults and 66 children with PAH/CHD. CHDs were patent ductus arteriosus, atrial and ventricular septal defects, partial anomalous pulmonary venous return, transposition of the great arteries, atrioventricular canal, and rare lesions with systemic-to-pulmonary shunts.

Six novel missense BMPR2 mutations were found in three out of four adults with complete type C atrioventricular canals and in three children. One child had an atrial septal defect and patent ductus arteriosus; one had an atrial septal defect, patent ductus arteriosus and partial anomalous pulmonary venous return; and one had an aortopulmonary window and a ventricular septal defect.

Bone morphogenetic protein receptor 2 mutations were found in 6% of a mixed cohort of adults and children with pulmonary arterial hypertension/congenital heart defects. The current findings compliment recent reports in mouse models implicating members of the bone morphogenetic protein/transforming growth factor- β pathway inducing cardiac anomalies analogous to human atrioventricular canals, septal defects and conotruncal congenital heart defects. The small number of patients studied and the ascertainment bias inherent in selecting for pulmonary arterial hypertension require further investigation.

Eur Respir J 2004; 24: 371–374.

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Keywords: Bone morphogenetic protein receptor 2 mutations
congenital heart defects
eisenmenger syndrome
pulmonary hypertension

Received: February 13 2004
Accepted after revision: May 12 2004

This study was supported by the Stony Wold-Herbert Foundation (K.E. Roberts), the Pulmonary Hypertension Association (W.P.K. Wong) and National Institute of Health-Heart Lung 60056 (J.H. Morse).

Pulmonary arterial hypertension (PAH) consists of a group of vascular abnormalities with elevated pulmonary arterial pressure and pulmonary vascular resistance. The clinical spectrum includes familial and sporadic idiopathic PAH (IPAH) (previously referred to as primary pulmonary hypertension), as well as PAH related to congenital heart disease (CHD), portal hypertension, connective tissue diseases, HIV-infection and appetite suppressant exposure [1]. Germline mutations of bone morphogenetic protein receptor (BMPR)-2, a member of the transforming growth factor (TGF)- β superfamily, have been found in familial and sporadic forms of IPAH [2–5], and in appetite-suppressant PAH [6], but not in PAH with HIV infection or PAH with connective tissue diseases [7, 8].

BMPR2 mutations have not been previously reported in PAH with CHD (PAH/CHD) in whom the PAH is due to pulmonary vascular obstructive disease. The natural history of CHD associated with large systemic-to-pulmonary shunts (e.g. atrial and ventricular septal defects, patent ductus arteriosus) results in pulmonary vascular obstructive disease, i.e. the Eisenmenger syndrome (ES) [9]. Approximately one third of all patients with CHD who do not undergo early "corrective" surgery, or who die from other causes, will die from pulmonary vascular disease [10]. Although the pathophysiological mechanisms, which lead to the histopathological changes seen in ES, are not completely understood, CHD repaired within the first 2 yrs of life is unlikely to lead to pulmonary vascular disease [10]. It is unclear in certain

patients with CHD whether the PAH results from increased flow, a primary pulmonary vascular abnormality or both.

Members of the TGF- β /BMP signaling pathway are particularly important in vasculogenesis and embryonic heart development [11–16]. Heterodimers of BMPR2 form a heterotetramer with type-1 receptors, BMPR1a (activin receptor-like kinase (ALK)-3) and BMPR1b (ALK6), in the presence of a BMP ligand such as BMP2 or BMP4. Mice with tissue-specific inactivation of ALK3 (BMPR1a) have abnormal endocardial cushion morphogenesis [11, 13]. BMPR2 has been implicated in abnormal septation in the mouse, resulting in a conotruncal abnormality, i.e. truncus arteriosus [15]. JIAO *et al.* [16] has reported that cardiac muscle conditional knock-out of BMP4 results in reduced atrioventricular septation.

To ascertain whether genetic mutations either predispose to PAH/CHD in general, or to a specific cardiac abnormality, the DNA sequence of the BMPR2 gene was determined in two patient cohorts; 40 adults and 66 children with PAH/CHD.

Material and methods

Study subjects

All studies and procedures were approved by the Columbia Presbyterian Medical Center Institutional Review Board (Columbia University, New York, NY, USA) and comply

with the Declaration of Helsinki. The study group consisted of two cohorts; 66 children (aged <18 yrs) and 40 adults (aged \geq 18 yrs) with PAH/CHD. The diagnosis of each CHD was determined echocardiographically. The diagnosis of PAH due to pulmonary vascular obstructive disease was confirmed by right heart catheterisation demonstrating mean pulmonary arterial pressure >3.3 kPa (25 mmHg), mean pulmonary capillary wedge pressure <2 kPa (15 mmHg) and pulmonary vascular resistance index >3 Wood units \cdot m $^{-2}$. All patients underwent acute vasodilator drug testing during the right heart catheterisation. Evaluation and work-up excluded other causes of PAH, such as IPAH, PAH with HIV infection, connective tissue diseases or appetite-suppressant drug exposure. None of the patients reported a family history of PAH.

Mutational analysis of *BMPR2*

The 13 exons and flanking intron sequences of *BMPR2* were mutation screened by denaturing high-pressure liquid chromatography (dHPLC; Transgenomics, Inc., Omaha, NE, USA) as previously described [2]. All samples with inconclusive or mutation-suggestive dHPLC results were sequenced bidirectionally using the Big Dye[®] Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), using an ABI 3100. Mutation analysis was performed blind of a patient's diagnosis with the aid of Mutation Surveyor v2.0 (SoftGenetics Inc., State College, PA, USA).

Results

Table 1 illustrates the category of CHD, the number of defects repaired, and the presence of *BMPR2* mutations in the 40 adults and 66 children with PAH/CHD. All patients were nonresponders with acute vasodilator testing [17]. The predominant CHD in both cohorts was atrial and ventricular septal defects, with the majority being unrepaired. None of the patients had primum atrial septal defects. Table 2 illustrates the clinical and haemodynamic findings, and the type of *BMPR2* mutation present in the six mutation-positive patients. Strikingly, most of the *BMPR2* mutations observed in the adult cohort occurred in patients with atrioventricular canals (also referred to as endocardial cushion defects), and these mutations were found in three out of four (75%) such cases. One of these patients (number 1, table 2) also had Down syndrome. The range of defects was more variable in the children. One had an atrial septal defect and patent ductus arteriosus (who also had a ring 14 chromosome, number 4,

table 2); one had an atrial septal defect, patent ductus arteriosus and partial anomalous pulmonary venous return; and one had an aortopulmonary window and a ventricular septal defect. None of the six children with atrioventricular canal type C had *BMPR2* mutations. Five of these six children also had Down syndrome (not illustrated).

The six novel missense *BMPR2* mutations were in exons 2, 3, 5 and 11. Mutations in exons 2 and 3 are in the extracellular domains of *BMPR2* (hence they might interfere with heterodimer formation or ligand binding), exon 5 is in the kinase domain (responsible for phosphorylation) and exon 11 is in the long cytoplasmic tail (unknown function). Four of the changes in predicted protein sequence caused by the mutations (adults: numbers 1 and 2; children: numbers 4 and 5) alter amino acids that are conserved in evolution across human, mouse, chicken, frog and pufferfish. In contrast, the altered amino acid is conserved only in man and mouse in patients number 3 and 6 (data not shown).

The 319T>C mutation in exon 3 and the 140G>A mutation in exon 2 were spontaneous, as these two mutations were not found in either parent of the *BMPR2*-positive PAH adult with an atrioventricular canal (number 3), or the child with the rare aortopulmonary window and ventricular septal defect (number 6). DNA was not available from the parents of the other mutation-positive patients.

Discussion

This is the first report of *BMPR2* mutations in adults and children with PAH/CHD in whom the PAH is due to pulmonary vascular obstructive disease. The 6% frequency in a combined cohort of 40 adults and 66 children is similar to the 8% frequency of *BMPR2* mutations reported for PAH with fenfluramine derivatives [6]. However, it is in contrast to a 26% frequency in IPAH [5] and an \sim 50% frequency in familial PAH [2, 3]. A recent National Heart Lung Blood Institute/Office of Rare Diseases workshop suggests a 5–10% frequency for IPAH [18], as do our unpublished observations.

BMPR2 mutations were found in three adults with atrioventricular canal type C, and in three children with an atrial septal defect and patent ductus arteriosus, an atrial septal defect/patent ductus arteriosus and partial anomalous pulmonary venous return, and a rare conotruncal CHD. None of the atrial septal defects were primum. The present authors suspect, as larger numbers of patients with PAH/CHD are studied, *BMPR2* mutations may be found in more types of CHD. In fact, the methodology used here would miss mutations leading to large deletions and mutations affecting the promoter region of *BMPR2*. The failure to find *BMPR2*

Table 1. – Category of congenital heart disease (CHD) in 40 adults and 66 children with pulmonary arterial hypertension, number of repaired CHD, and presence of bone morphogenetic protein receptor (*BMPR*)-2 mutations

	Adults			Children		
	Total	Repaired	<i>BMPR2</i>	Total	Repaired	<i>BMPR2</i>
Patent ductus arteriosus	2	0	0	6	3	0
ASD	17	7	0	21	5	1 [#]
ASD/PAPVR	3	0	0	0	0	1 [#]
Ventricular septal defect	8	1	0	15	8	0
PAPVR	1	0	0	3	2	0
Transposition of the great vessels	3	3	0	7	3	0
Atrial ventricular canal	4	1	3	6	3	0
Rare	2	0	0	8	5	1
Total	40	12	3	66	29	3

Data are presented as n. ASD: atrial septal defect; PAPVR: partial anomalous pulmonary venous return. [#]: both CHDs had patent ductus arteriosus.

Table 2. – Clinical and haemodynamic findings and bone morphogenetic protein receptor (BMPR)-2 mutations in BMPR2-positive children and adults with pulmonary arterial hypertension/congenital heart disease

	Adult 1	Adult 2	Adult 3	Child 4	Child 5	Child 6
Age initial cat. yrs	19	57	19	3	13	3.5
Age at PAH diagnosis yrs	1	16	5	3	2	19 [#]
Age at repair yrs	NA	NA	4	NA	NA	20 [#]
Outcome/age yrs	Lost to f-u	Lost to f-u	Tx, 23	A, 16	A, 18	D, 5
Sex M/F	F	F	M	F	M	M
Ethnicity	White	White	White	White	Asian	White
Type of CHD	AVC-C	AVC-C	AVC-C	ASD/PDA	ASD/PDA/PAPVR	AW and VSD
PAP _m kPa (mmHg)	9.33 (70)	ND	10.0 (75)	8.13 (61)	9.2 (69)	10.0 (75)
RAP _m kPa (mmHg)	0.93 (7)	ND	1.06 (8)	0.53 (4)	0 (0)	0.53 (4)
CI L·min ⁻¹ ·m ⁻²	8.3	ND	4.0	2.3	3.2	4.0
PVR Wood units·m ⁻²	18	ND	23	19	10	22
SVR Wood units·m ⁻²	8	ND	13	25	21	13
MV _{Sa₂O₂} %	50	ND	52	65	60	68
Sa ₂ O ₂ %	64	ND	84	91	86	90
Genetic syndrome	Down	N	N	Ring 14	N	N
BMPR2 mutation						
Exon	2	3	3	5	11	2
Nucleic acid change	125A>G	304A>G	319T>C	556A>G	1509A>C	140G>A
Amino acid change	Q42R	T102A	S107P	M186V	E503D	G47N
Type of mutation	Missense	Missense	Missense	Missense	Missense	Missense

Cat.: catheterisation; PAH: pulmonary arterial hypertension; M: male; F: female; CHD: congenital heart defects; PAP_m: mean pulmonary artery pressure; RAP_m: mean right atrial pressure; CI: cardiac index; PVR: pulmonary vascular resistance; SVR: systemic vascular resistance; MV_{Sa₂O₂}: mixed venous oxygen saturation; Sa₂O₂: systemic oxygen saturation; NA: not applicable; f-u: follow-up; Tx: heart-lung transplant; A: alive; D: dead; AVC-C: complete atrial ventricular canal defect type C; ASD: atrial septal defect; PDA: patent ductus arteriosus; PAPVR: partial anomalous pulmonary venous return; AW: aortopulmonary window; VSD: ventricular septal defect; ND: not done; Down: Down syndrome; N: normal; Ring 14: ring chromosome 14. [#]: months.

mutations in the children with atrioventricular canal type C defects may be due to the small sample size/and or the association with Down syndrome. Five of the six mutation-negative children and one of the mutation-positive adults had Down syndrome. Atrioventricular canal defects are one of the most frequent CHDs that occur with trisomy 21. Therefore, these defects may result from a different genetic mechanism than those that occur without a recognised chromosomal genetic syndrome. The child with atrial septal defect and patent ductus arteriosus (number 4) also had a ring chromosome 14, a rare abnormality associated with CHD, mental retardation and seizures. The onset of disease in individuals with PAH is thought to require a combination of two or more genetic or environmental factors, as in cancer [19]. To speculate, the interplay between a congenital syndrome, a CHD and a BMPR2 mutation could provide the required two or more "hits".

The six novel missense BMPR2 mutations in exons 2, 3, 5 and 11 have the potential to be deleterious by changing the protein sequence at evolutionary conserved amino acids and, hence, altering BMPR2 function. It is also formally possible that these DNA sequence variations could be nonpathogenic polymorphisms. The current authors think this is unlikely, as they have not been reported in the literature [4], nor observed in >196 healthy individuals and >1,000 other PAH chromosomes that have been previously screened by the authors. Four of the six missense mutations were at sites conserved in evolution from man to the pufferfish, whereas the other two sites were conserved only in man and the mouse. Spontaneous BMPR2 mutations have also been reported previously in familial PAH [2] and in sporadic IPAH [5]. Unfortunately, parental DNA was not available for all patients to determine if spontaneous mutations are a universal finding in this category of PAH/CHD.

The types of CHD found in the BMPR2-positive patients are in accordance with reports regarding the role of the BMP pathway in embryonic cardiac development. Homozygous BMPR2 knockout mice die at gastrulation, whereas no

abnormalities have been reported for the heterozygous mouse [20]. Recently, JIAO *et al.* [16], using a Cre/loxP recombination conditional knockout, demonstrated that BMP-4 contributes in a dose-related fashion to normal atrioventricular septation and endocardial cushion formation. In the presence of low or no BMP-4, mice had atrioventricular canals and outflow tract abnormalities. DELOT *et al.* [15] have created a mouse with a truncated extracellular domain of BMPR2, documenting embryonic lethality at E12, and absence of the septation of the outflow tract and aortic arch interruption, the anatomical correlate of persistent truncus arteriosus type 4A in humans [15]. BMP ligands bind to a heterodimer comprised of BMPR2, with BMPR1a and/or BMPR1b to initiate signaling, therefore, mutations of either a ligand or of BMPR2 could be predicted to interfere with signalling.

The present study was initiated to provide a preliminary catalogue of BMPR2 mutations in patients with PAH/CHD in whom the PAH was due to pulmonary vascular obstructive disease. As BMPR2 mutations have not been previously investigated in either children or adults with CHD without PAH, it is presently impossible to differentiate the role of increased flow *versus* genetic mutations in predisposing to pulmonary vascular disease. Although the definition of IPAH requires the exclusion of other causes, small anatomical congenital systemic-to-pulmonary shunts may be found. Whether these represent unrelated phenomena or genetic predisposition for IPAH with a haemodynamically insignificant congenital systemic-to-pulmonary shunt triggering the onset of the pulmonary vascular disease remains uncertain [21–23]. Although pulmonary vascular disease associated with congenital systemic-to-pulmonary shunts usually follows a period of increased pulmonary blood flow, it may occur in patients who never manifested a large left-to-right shunt. Support for this comes from the observation of severe progressive PAH following repair of atrial septal defects in two children whose mothers died from IPAH, but by definition classifiable as familial PAH [24]. Extrapolation

from the presence of BMPR2 mutations in familial PAH and sporadic IPAH suggests BMPR2 mutations may be a risk factor for PAH/CHD.

It is anticipated that further functional investigations of specific members of the human bone morphogenetic protein/transforming growth factor- β pathway, aided by conditional murine knockouts, will increase knowledge of the cause(s) and interrelationship(s) between various congenital heart defects, pulmonary arterial hypertension, and the development of pulmonary vascular obstructive disease.

Acknowledgements. The authors would like to thank the patients and their physicians for their participation in these studies. They also acknowledge the generosity of the Linnen family for the gift of an HPLC apparatus.

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