REVIEW ARTICLE

The Genetics of Childhood Disease and Development: A Series of Review Articles

This is the fifth article in this series. Drs. Trembath and Harrison describe genetic mutations responsible for Primary Pulmonary Hypertension and their relation to other forms of genetic and acquired pulmonary vascular disease.

Alvin Zipursky
Editor-in-Chief

Insights into the Genetic and Molecular Basis of Primary Pulmonary Hypertension

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ABSTRACT

The pathogenesis of primary pulmonary hypertension (PPH) remains poorly understood. Molecular genetic studies have identified that mutations within the gene *BMPR2* on the long arm of chromosome 2 underlie familial PPH. This review explores the significance of the PPH gene identification and examines additional genetic determinants, emphasizing the immediate implications for assessment and management of patients and their relatives. (*Pediatr Res* 53: 883–888, 2003)

Abbreviations

PAH, pulmonary arterial hypertension
PPH, primary pulmonary hypertension
BMPR2, bone morphogenetic protein receptor type 2 gene
TGF- β , transforming growth factor beta
HHT, hereditary hemorrhagic telangiectasia
ALK-1, activin receptor-like kinase 1 gene

PULMONARY ARTERIAL HYPERTENSION

Pulmonary arterial hypertension (PAH) describes a spectrum of diseases defined by a sustained increase in pulmonary artery pressure (mean >25 mm Hg at rest and >30 mm Hg during exercise). PAH is typically associated with endothelial and vascular smooth muscle cell proliferation within small pulmonary arteries (Fig. 1) (1). Raised pulmonary artery pressures

lead to progressive right heart hypertrophy and eventual failure.

The diagnostic classification of PAH has recently been revised to distinguish between primary pulmonary hypertension and PAH occurring in association with other conditions including connective tissue diseases, HIV infection, portal hypertension, or use of appetite suppressants (Table 1). The classification also distinguishes between conditions for which the underlying cause of elevated pulmonary artery pressure is known and for which specific therapeutic approaches exist, such as chronic thromboembolic disease, chronic hypoxic lung disease and left sided cardiac disease.

PRIMARY PULMONARY HYPERTENSION

Primary pulmonary hypertension (PPH) is a devastating disease, the pathogenesis of which remains poorly defined.

Received May 8, 2002; accepted August 14, 2002.

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Grant support from the British Heart Foundation (RCT programme grant numbers RG/2000012) is gratefully acknowledged. Rachel Harrison is a British Heart Foundation Clinical Research Fellow.

DOI: 10.1203/01.PDR.0000061565.22500.E7

Figure 1. Histology of primary pulmonary hypertension. Photomicrographs of paraffin-embedded lung sections from a control subject (*Panel A*) and subject with pulmonary arterial hypertension (*Panel B*). Normal lung shows thin-walled peripheral pulmonary arteries and normal alveolar capillaries. In contrast, in pulmonary arterial hypertension, thick-walled peripheral pulmonary arteries are occluded by a cellular intimal proliferation. The sections were stained with hematoxylin and eosin. (Photomicrographs reproduced with the permission of Dr. Susan Stewart, Papworth Hospital).

Disease progression leads to right heart failure with median untreated survival times 2.8–3.4 y from diagnosis (2, 3, 4). It is rare, with a reported incidence of 1–2 million per year in developed countries (2, 5). Disease onset may be at any age, with the mean age of onset at 36.4 y (2).

Recent developments in treatment are likely to improve survival and quality of life for many patients yet the disease

Table 1. 1998 World Health Organisation classification of pulmonary hypertension

1. Pulmonary arterial hypertension
 - 1.1 Primary pulmonary hypertension
 - (a) Sporadic
 - (b) Familial
 - 1.2 Related to:
 - (a) Collagen vascular disease
 - (b) Congenital systemic to pulmonary shunts
 - (c) Portal hypertension
 - (d) Human immunodeficiency virus infection
 - (e) Drugs/toxins
 - (1) Anorexigens
 - (2) Other
 - (f) Persistent pulmonary hypertension of the newborn
 - (g) Other
2. Pulmonary venous hypertension
 - 2.1 Left sided atrial or ventricular heart disease
 - 2.2 Left sided valvular heart disease
 - 2.3 Extrinsic compression of central pulmonary veins
 - (a) Fibrosing mediastinitis
 - (b) Adenopathy/tumours
 - 2.4 Pulmonary veno-occlusive disease
 - 2.5 Other
3. Pulmonary hypertension associated with disorders of the respiratory system and/or hypoxaemia
 - 3.1 Chronic obstructive pulmonary disease
 - 3.2 Interstitial lung disease
 - 3.3 Sleep disordered breathing
 - 3.4 Alveolar hypoventilation disorders
 - 3.5 Chronic exposure to high altitude
 - 3.6 Neonatal lung disease
 - 3.7 Alveolar-capillary dysplasia
 - 3.8 Other
4. Pulmonary hypertension due to chronic thrombotic and/or embolic disease
 - 4.1 Thromboembolic obstruction of proximal pulmonary arteries
 - 4.2 Obstruction of distal pulmonary arteries
 - (a) Pulmonary embolism (thrombus, tumour, ova and/or parasites, foreign material)
 - (b) In situ thrombosis
 - (c) Sickle cell disease
5. Pulmonary hypertension due to disorders directly affecting the pulmonary vasculature
 - 5.1 Inflammatory
 - (a) Schistosomiasis
 - (b) Sarcoidosis
 - (c) Other
 - 5.2 Pulmonary capillary haemangiomatosis

remains incurable. Recommended therapies are similar for children and adults and include anticoagulation, calcium channel blockers, prostaglandins (epoprostenol and other prostacyclin analogues) and endothelin receptor antagonists. A minority of patients undergo lung or heart-lung transplant (6).

Pediatric patients present at any time throughout childhood, with signs and symptoms influenced by age of onset (7). Young children may fail to thrive, or present with syncopal or seizure like episodes, whereas older children offer similar complaints to adults, such as exertional breathlessness or chest pain.

Environmental factors such as the ingestion of toxic oil (8) or appetite suppressant drugs (5) are also associated with the development of PAH, and have caused epidemics of disease. Women are twice as likely as men to develop PPH (similar

gender ratios are observed for cases with and without a family history of disease), suggesting that hormonal factors may be important in disease development, or that genetic (X-linked) factors may influence predisposition to disease (2, 9).

PPH typically occurs as a sporadic disorder, yet figures from the National Institutes of Health registry demonstrate that at least 6% of PPH patients report a family history of the disease (2). Importantly, the natural history of PPH appears similar in both familial and sporadic forms of the disease. In families, PPH segregates as an autosomal dominant trait, the disease gene acting with markedly reduced penetrance (9) such that a PPH gene carrier may have no greater than a 10% to 20% chance of developing the clinical disorder (10). The age of onset of clinical disease in PPH gene carriers is highly variable, both within and between PPH families. Anticipation, a phenomenon by which successive generations are affected at an earlier age, is observed in some families (11).

Taken together these observations indicate that a genetic susceptibility is not sufficient to cause disease, but that diverse environmental exposures may trigger disease in genetically susceptible individuals.

SEARCH FOR A PPH GENE

In 1997, two independent groups searching for a PPH gene established linkage to the long arm of chromosome 2 at 2q 31–33 (12, 13). Analysis of over 40 independently ascertained families revealed that all affected subjects in these families shared a similar chromosomal region (14, 15). Analysis of crossover (recombination) events within families enabled further reduction of the critical interval for the PPH gene (16, 17), and sequence analysis of potential candidate genes in this region identified heterozygous mutations within the gene bone morphogenetic protein receptor-2 – *BMPR2* (14, 18).

BMPR-II AND THE TGF- β SUPERFAMILY

The *BMPR2* product (BMPR-II) is a receptor in the multi-functional transforming growth factor-beta (TGF- β) signaling pathway. The ligands for this receptor – bone morphogenetic proteins (BMPs) – are peptides required for growth and differentiation of bone and cartilage. Both TGF- β and BMPs are necessary for differentiation, proliferation and apoptosis of many cell types and have critical roles in embryogenesis (19, 20). All members of the TGF- β superfamily signal by binding to types I and II cell surface receptors and activating serine/threonine kinases through the formation of heteromeric complexes (21) (Fig. 2).

Defects in the TGF- β signaling pathway have been implicated in numerous disease states. Mutations in the genes for components of the TGF- β signaling pathway contribute to the pathogenesis of cancer and underlie the inherited vascular disorder hereditary hemorrhagic telangiectasia (HHT), while acquired changes in TGF- β function have been observed in a wide range of disorders including fibrotic disease of the lung and atherosclerosis (22, 23).

Figure 2. Diagram of the TGF- β signaling pathway. TGF- β or BMPs bind to type II receptors, such as BMPR-II, stimulating formation of a receptor complex with type I receptors (22). Complex formation activates the protein kinase domain of the type I receptor, resulting in activation of intracellular signaling molecules such as the Smad proteins. This results in formation of a complex of Smad proteins, which moves into the nucleus leading to cell specific transcriptional regulation. This model of the signaling mechanism may vary according to ligand and receptor type. Further “accessory” receptors such as endoglin may also be involved in the receptor complex, and other intracellular pathways may be activated. (From Thomson JR *et al.* Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF- β family. *J Med Genet* 2000;37:741–745 reproduced with permission.)

BMPR2 MUTATIONS IN FAMILIAL AND SPORADIC PPH

Over 40 unique heterozygous *BMPR2* mutations have been identified in patients with a known family history of PPH, accounting for nearly 55% (40 of 73) of families studied (14, 18) (also see www.pphgenes.net for an updated list of identified PPH associated mutations). It is important to note that these are germline mutations, represented in all cell types. Mutations are widely dispersed throughout the distinct functional domains of the gene, and appear to be unique or “private” to each family studied (Fig. 3). This variety of mutations will complicate attempts to design simple diagnostic genetic tests for the disease.

A number of explanations may account for the failure to detect mutations in a proportion of familial PPH. Deleterious mutations may occur in gene regions that have not routinely been sequenced, such as regulatory or intronic sequences. Large-scale gene rearrangements will also have been missed by direct sequencing methods. Additionally, it remains possible that other genes, yet to be identified, contribute to the genetic basis of PPH.

In a study of fifty PPH patients with no family history, germline mutations in *BMPR2* were identified in almost one third (24) with the range of mutations paralleling those seen in

Figure 3. Position of *BMPR2* mutations. Structure of *BMPR2* indicating position of identified mutations. Filled symbols denote known familial cases of PPH; open symbols denote sporadic cases.

recognized familial cases. Some of these mutations were inherited from apparently healthy parents, others arose spontaneously (*de novo*). *BMPR2* mediated genetic susceptibility to PPH is an important contributor to the etiology of this devastating disorder.

FROM GENE TO DISEASE

BMPR2 mutations described are predicted to interrupt the signaling pathways mediated by BMPR-II protein, resulting in a predisposition to proliferation rather than apoptosis of cells within the small pulmonary arteries. In support of this hypothesis, BMPs appear to suppress proliferation of pulmonary artery smooth muscle cells from healthy individuals and patients with secondary forms of pulmonary hypertension, yet fail to suppress proliferation in patients with primary pulmonary hypertension (25). Expression of BMPR-II in pulmonary vascular endothelium appears markedly reduced in PPH patients, particularly in those who have mutations in *BMPR2* (26). Such a reduction in cell surface receptors may be sufficient to disrupt normal signaling pathways. The precise molecular mechanisms by which *BMPR2* mutations predispose to the vascular changes that characterize PPH, are the subject of further intense study.

However, a mutation within *BMPR2* is clearly not sufficient to cause onset of disease. Anorexigens are known to have caused epidemics of PAH in the general population, although the vast majority of individuals taking such drugs do not develop disease. When a group of 33 patients with anorexigen-associated PAH was studied, *BMPR2* mutations were found in three patients with no family history of pulmonary hypertension (27), with an additional mutation identified in two sisters with familial PPH. These data suggest that *BMPR2* mutations may not be the major genetic susceptibility contributing to disease associated with anorexigens.

ADDITIONAL GENETIC DETERMINANTS

Pulmonary artery smooth muscle cells from PPH patients grow faster than those from controls when stimulated by serotonin or serum, a finding that correlates with increased expression of the serotonin transporter (28). Genetic analysis of variation in the promoter region of the serotonin transporter gene (*5HTT*) is associated with overexpression of 5HTT and may also contribute to a genetic susceptibility to PPH (28). Whether *5HTT* mediated susceptibility acts independent of *BMPR2* gene variation has yet to be examined.

The acquisition of somatic mutations may also contribute to the pathogenesis of PPH. Analysis of plexiform lesions (intra-vascular lesions of disorganized endothelial cell proliferation typical of PPH) from the lungs of PPH patients showed that endothelial cell proliferation was predominantly monoclonal, whereas plexiform lesions from patients with secondary PAH were entirely polyclonal (29). Proliferating endothelial cells in plexiform lesions from PPH patients show microsatellite instability. Mutations within microsatellites are not present in germline DNA, and are associated with decreased protein expression of the TGF- β receptor II within the plexiform lesion (30). These observations indicate that somatic genetic alterations similar to those involved in neoplasia, may contribute to pulmonary arterial hypertension.

HEREDITARY HEMORRHAGIC TELANGIECTASIA AND PULMONARY HYPERTENSION

Hereditary hemorrhagic telangiectasia (HHT) is a vascular dysplasia characterized by abnormal vessel wall development, with the formation of mucocutaneous telangiectases and arteriovenous malformations within the pulmonary, hepatic or cerebral circulations. Mutations in two genes encoding TGF- β receptors, the endoglin gene (*ENG*) on chromosome 9, and the activin-receptor-like kinase 1 gene (*ALK1*) on chromosome 12

(31, 32), have been shown to underlie HHT. Patients with HHT may develop pulmonary hypertension that is clinically and pathologically indistinguishable from PPH (33, 34).

Five kindreds and one individual were identified with a family history of HHT and cases of pulmonary hypertension (35). Novel defects of *ALK1* were demonstrated in each family suggesting that these mutations may contribute to the development of PH as well as HHT. Molecular mechanisms linking the two disease processes remain to be elucidated and *ALK1* defects have not been found in families with PPH alone. However, the TGF- β cell-signaling pathway is known to have diverse effects in control of cell types within the vasculature. For example, overexpression of TGF- β simultaneously promotes both intimal growth and apoptosis of vascular endothelium (36). Elucidating such cell specific interactions will be crucial to gaining a detailed molecular understanding of the pathogenesis of PPH.

CLINICAL SCREENING AND GENETIC TESTING

Recognition of the inherited nature of a significant proportion of PPH may understandably cause concern for individuals affected by PPH. Clinical screening has been recommended for first-degree relatives of PPH patients and may include a chest x-ray, ECG, and echocardiogram. Identifying earlier stages in the progression to sustained pulmonary hypertension will be critical to determine whether treatment started at the earliest opportunity can improve outcome.

Genetic analysis of *BMPR2* may now be performed in PPH patients, and if mutations are identified, presymptomatic genetic testing offered to their relatives. This approach may have advantages in streaming clinical screening, with the prospect of potential benefit from early intervention together with avoidance of factors known to precipitate or exacerbate PPH. These include pregnancy, hypoxic environments, and anorexic drugs. Children should not be excluded from presymptomatic genetic testing as PPH may affect this age group. Clinical screening for features of PPH should therefore be considered from early years onwards. However, further research is necessary to improve understanding of the predictive value of mutation detection and a clinical screening program. For example, it is currently unclear whether different mutations predict different disease penetrance, susceptibility to particular environmental factors or response to treatment. Recent studies report that obligate gene carriers in families with PPH have elevated peak pulmonary artery pressures, when assessed by stress Doppler echocardiography during supine exercise (37). It is also important to emphasize that failure to identify a *BMPR2* mutation in an individual with PPH does not exclude a familial form of disease. It is therefore vital that genetic analysis only takes place after full genetic counseling to ensure that families understand the current limitations of genetic testing in PPH.

Acknowledgments. The authors would like to thank patients and relatives together with many colleagues for their continued support and contribution to molecular genetic studies in PPH.

REFERENCES

1. Tuder RM, Lee SD, Cool CC 1998 Histopathology of pulmonary hypertension. *Chest* 114:1S–6S
2. Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Koerner SK 1987 Primary pulmonary hypertension. A national prospective study. *Ann Intern Med* 107:216–223
3. D'Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, Fishman AP, Goldring RM, Groves BM, Kernis JT 1991 Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med* 115:343–349
4. Oakley CW 1994 Primary pulmonary hypertension: case series from the UK. *Chest* 105(suppl):S29–32
5. Abenham L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Begaud B 1996 Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group *N Engl J Med* 335:609–616
6. British Cardiac Society G, Medical Practice C, approved by the British Thoracic S, the British Society of R 2001 Recommendations on the management of pulmonary hypertension in clinical practice. *Heart* 86:11–13
7. Haworth SG 1998 Primary pulmonary hypertension in childhood. *Arch Dis Child* 79:452–455
8. Gomez-Sanchez MA, Saenz de la Calzada C, Gomez-Pajuelo C, Martinez-Tello FJ, Mestre de Juan MJ, James TN 1991 Clinical and pathologic manifestations of pulmonary vascular disease in the toxic oil syndrome. *J Am Coll Cardiol* 18:1539–1545
9. Loyd JE, Primm RK, Newman JH 1984 Familial primary pulmonary hypertension: clinical patterns. *Am Rev Respir Dis* 129:194–197
10. Rich S Executive summary 1998 Genetics of pulmonary hypertension. World symposium on primary pulmonary hypertension, Evian, France
11. Loyd JE, Butler MG, Foroud TM, Conneally PM, Phillips JA, Newman JH 1995 Genetic anticipation and abnormal gender ratio at birth in familial primary pulmonary hypertension. *Am J Respir Crit Care Med* 152:93–97
12. Nichols WC, Koller DL, Slovis B, Foroud T, Terry VH, Arnold ND, Siemieniak DR, Wheeler L, Phillips JA, Newman JH, Conneally PM, Ginsburg D, Loyd JE 1997 Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31–32. *Nat Genet* 15:277–280
13. Morse JH, Jones AC, Barst RJ, Hodge SE, Wilhelmsen KC, Nygaard TG 1997 Mapping of familial primary pulmonary hypertension locus (PPH1) to chromosome 2q31–q32. *Circulation* 95:2603–2606
14. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, Kalachikov S, Cayanis E, Fischer SG, Barst RJ, Hodge SE, Knowles JA 2000 Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 67:737–744
15. Machado RD, Pauciulo MW, Thomson JR, Lane KB, Morgan NV, Wheeler L, Phillips JA, 3rd, Newman J, Williams D, Galie N, Manes A, McNeil K, Yacoub M, Mikhail G, Rogers P, Corris P, Humbert M, Donnai D, Martensson G, Tranebjærg L, Loyd JE, Trembath RC, Nichols WC 2001 *BMPR2* haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension *Am J Hum Genet* 68:92–102
16. Machado RD, Pauciulo MW, Fretwell N, Veal C, Thomson JR, Vilarino Guell C, Aldred M, Brannon CA, Trembath RC, Nichols WC 2000 A physical and transcript map based upon refinement of the critical interval for PPH1, a gene for familial primary pulmonary hypertension. *International PPH Consortium Genomics* 68:220–228
17. Deng Z, Haghighi F, Helleby L, Vanterpool K, Horn EM, Barst RJ, Hodge SE, Morse JH, Knowles JA 2000 Fine mapping of PPH1, a gene for familial primary pulmonary hypertension, to a 3-cM region on chromosome 2q33. *Am J Respir Crit Care Med* 161:1055–1059
18. Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA 3rd, Loyd JE, Nichols WC, Trembath RC 2000 Heterozygous germline mutations in *BMPR2*, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *International PPH Consortium Nat Genet* 26:81–84
19. Massague J, Wotton D 2000 Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J* 19:1745–1754
20. Ducy P, Karsenty G 2000 The family of bone morphogenetic proteins. *Kidney Int* 57:2207–2214
21. Miyazono K, Kusanagi K, Inoue H 2001 Divergence and convergence of TGF-beta/BMP signaling. *J Cell Physiol* 187:265–276
22. Massague J 1998 TGF-beta signal transduction. *Ann Rev Biochem* 67:753–791
23. Blobel GC, Schiemann WP, Lodish HF 2000 Role of transforming growth factor beta in human disease. *N Engl J Med* 342:1350–1358
24. Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs JS, Egan J, Crozier A, Peacock A, Alcock R, Corris P, Loyd JE, Trembath RC, Nichols WC 2000 Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding *BMPR-II*, a receptor member of the TGF-beta family. *J Med Genet* 37:741–745
25. Morrell NW, Yang X, Upton PD, Jourdan KB, Morgan N, Sheares KK, Trembath RC 2001 Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-beta(1) and bone morphogenetic proteins. *Circulation* 104:790–795
26. Atkinson CWS Upton PD, Shackleton S, Thomson JR, Trembath RC, Morrell NW 2002 Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of *BMPR-II*. *Circulation* 105:1672–1678

27. Humbert M, Deng Z, Simonneau G, Barst RJ, Sitbon O, Wolf M, Cuervo N, Moore KJ, Hodge SE, Knowles JA, Morse JH 2002 BMPR2 germline mutations in pulmonary hypertension associated with fenfluramine derivatives. *Eur Respir J* 20:518–523
28. Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, Simonneau G, Darteville P, Hamon M, Adnot S 2001 Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J Clin Invest* 108:1141–1150
29. Lee SD, Shroyer KR, Markham NE, Cool CD, Voelkel NF, Tudor RM 1998 Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. *J Clin Invest* 101:927–934
30. Yeager ME, Halley GR, Golpon HA, Voelkel NF, Tudor RM 2001 Microsatellite instability of endothelial cell growth and apoptosis genes within plexiform lesions in primary pulmonary hypertension. *Circ Res* 88:E2–E11
31. McAllister KA, Grogg KM, Johnson DW, Gallione CJ, Baldwin MA, Jackson CE, Helmbold EA, Markel DS, McKinnon WC, Murrell J 1994 Endoglin, a TGF-beta binding protein of endothelial cells, is the gene for hereditary haemorrhagic telangiectasia type 1. *Nat Genet* 8:345–351
32. Johnson DW, Berg JN, Baldwin MA, Gallione CJ, Marondel I, Yoon SJ, Stenzel TT, Speer M, Pericak-Vance MA, Diamond A, Gutmacher AE, Jackson CE, Attisano L, Kucherlapati R, Porteous ME, Marchuk DA 1996 Mutations in the activin receptor-like kinase 1 gene in hereditary haemorrhagic telangiectasia type 2. *Nat Genet* 13:189–195
33. Sapru RP HD, Hall JI 1969 Pulmonary hypertension in patients with pulmonary arteriovenous fistulae. *Br Heart J* 31:559–569
34. Trell EJB, Linell F, Pipa J 1972 Familial pulmonary hypertension and multiple abnormalities of large systemic arteries in Osler's disease. *Am J Med* 53:50–63
35. Trembath RC, Thomson JR, Machado RD, Morgan NV, Atkinson C, Winship I, Simonneau G, Galie N, Loyd JE, Humbert M, Nichols WC, Morrell NW, Berg J, Manes A, McGaughan J, Pauciulo M, Wheeler L 2001 Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *N Engl J Med* 345:325–334
36. Schulick AH, Taylor AJ, Zuo W, Qiu CB, Dong G, Woodward RN, Agah R, Roberts AB, Virmani R, Dichek DA 1998 Overexpression of transforming growth factor beta1 in arterial endothelium causes hyperplasia, apoptosis, and cartilaginous metaplasia. *Proc Natl Acad Sci USA* 95:6983–6988
37. Grunig E, Janssen B, Mereles D, Barth U, Borst MM, Vogt IR, Fischer C, Olschewski H, Kuecherer HF, Kubler W 2000 Abnormal pulmonary artery pressure response in asymptomatic carriers of primary pulmonary hypertension gene. *Circulation* 102:1145–1150