

## Seminar

## Primary pulmonary hypertension

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**Primary pulmonary hypertension (PPH) is a rare disorder characterised by raised pulmonary-artery pressure in the absence of secondary causes. Precapillary pulmonary arteries are affected by medial hypertrophy, intimal fibrosis, microthrombosis, and plexiform lesions. Most individuals present with dyspnoea or evidence of right heart failure. Echocardiography is the best non-invasive test to screen for suspected pulmonary hypertension. The discovery of mutations in the coding region of the gene for bone morphogenetic protein receptor 2 in patients with familial and sporadic PPH may help not only to elucidate pathogenesis but also to direct future treatment options. The pathogenesis is not completely understood, but recent investigations have revealed many possible candidate modifier genes. Without treatment, the disorder progresses in most cases to right heart failure and death. With current therapies such as epoprostenol, progression of disease is slowed, but not halted. Many promising new therapeutic options, including prostacyclin analogues, endothelin-1-receptor antagonists, and phosphodiesterase inhibitors, improve clinical function and haemodynamic measures and may prolong survival.**

Primary pulmonary hypertension (PPH) is a progressive disorder characterised by raised pulmonary-artery pressures with pathological changes in precapillary pulmonary arteries. PPH was defined in the US National Institutes of Health registry as a mean pulmonary-artery pressure of more than 25 mm Hg at rest, or 30 mm Hg with exertion, in the absence of heart disease, chronic thromboembolic disease, underlying pulmonary disorder, or other secondary causes.<sup>1</sup> Until the past decade, the disorder was rapidly progressive, leading to right heart failure and death in a median of 2.8 years from diagnosis.<sup>2,3</sup> Since the introduction of intravenous epoprostenol, a prostacyclin, in the 1990s, survival has greatly improved, and current investigations of new therapeutic options indicate a brighter outlook for patients with PPH. Furthermore, with the recent discovery of mutations in the coding region of the gene for bone morphogenetic protein receptor 2 (BMPR2) in families with PPH,<sup>4,5</sup> novel therapies may eventually be available to address the underlying pathogenesis at the molecular level.

### Historical perspective

The term PPH was coined by Dresdale in 1951.<sup>6</sup> In 1954, he reported the first documented cases of familial PPH, in a mother and son with raised pulmonary-artery pressure found on right heart catheterisation.<sup>7</sup> In 1967, an increased frequency of PPH in Europe was linked to the use of the appetite suppressant aminorex fumarate; as a result, in 1973, WHO convened an international meeting on PPH.<sup>8</sup> The US National Institutes of Health sponsored a multicentre prospective investigation of PPH starting in 1981 to clarify clinical and epidemiological features.<sup>1</sup> In 1998, WHO held another symposium on PPH, to commemorate the 25th anniversary of the first world meeting, and classified PPH as one of the causes of pulmonary arterial hypertension (PAH; panel 1).<sup>9</sup>

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### Epidemiology and risk factors

The frequency of PPH in the general population is estimated at 1–2 cases per million people,<sup>8</sup> and there are twice as many female as male patients.<sup>1,10,11</sup> The disease can present at any age, but most commonly in the third decade of life in women and the fourth decade in men, with a mean age at diagnosis of 36.4 years.<sup>1</sup> No ethnic predisposition was apparent in the National Institutes of Health registry, and the proportions by ethnic group paralleled those in the general population.<sup>1</sup> The mean time from symptom onset to diagnosis was 2 years, and the interval was shorter in patients with a family history of PPH.<sup>1</sup> The registry found a familial prevalence of 6%,<sup>1</sup> but subsequent studies that scrutinised family histories and pedigrees predicted higher proportions.<sup>12,13</sup> Analyses of large families with PPH have revealed autosomal dominant inheritance, estimated penetrance of 10–20%, and genetic anticipation (ie, the onset of the disease at an earlier age in successive generations; figure 1).<sup>10–12</sup>

Many different risk factors predispose to the development of PAH; the most publicised factor is appetite suppressants. Aminorex fumarate was linked to an epidemic of PAH in Switzerland, Germany, and Austria in the late 1960s, and withdrawal of this agent was followed by a fall in the incidence of PAH.<sup>14</sup> The recent widespread use of fenfluramine and its derivatives in Europe produced an increase in PAH cases.<sup>15</sup> In a case-control study, use of diet pills within the previous year predicted an odds ratio of 10.1 for the development of PAH; for exposure of longer than 3 months the odds ratio was 23.1.<sup>15</sup> Other factors implicated include the combination of fenfluramine and phentermine in the USA and ingestion of toxic rapeseed oil in Spain.<sup>16,17</sup> In addition, certain diseases predispose to PAH, including

### Search strategy

We searched Medline from 1966 to the present for articles with the key word "pulmonary hypertension" alone or combined with other key words. These articles were appraised on the basis of the abstract and relevance to this review. We discovered many other articles after we had read the relevant papers in depth. Highest priority was given to investigations of sound scientific merit and well-constructed clinical trials.

### Panel 1: WHO classification of pulmonary hypertension<sup>9</sup>

#### Pulmonary arterial hypertension

Primary pulmonary hypertension (PPH)

- Sporadic
- Familial

Related to:

- Collagen vascular disease
- Congenital systemic-to-pulmonary shunts
- Portal hypertension
- HIV infection
- Drugs/toxins
  - Appetite suppressants
  - Toxic rapeseed oil
- Persistent pulmonary hypertension of the neonate

#### Pulmonary venous hypertension

Left-sided atrial or ventricular heart disease

Left-sided valvular heart disease

Extrinsic compression of central pulmonary veins

- Fibrosing mediastinitis
- Adenopathy/tumours

Pulmonary veno-occlusive disease

#### Pulmonary hypertension associated with disorders of the respiratory system and/or hypoxaemia

Chronic obstructive pulmonary disease

Interstitial lung disease

Sleep-disordered breathing

Alveolar hypoventilation disorders

Chronic exposure to high altitude

Neonatal lung disease

Alveolar-capillary dysplasia

#### Pulmonary hypertension due to chronic thrombotic and/or embolic disease

Thromboembolic obstruction of proximal pulmonary arteries

Obstruction of distal pulmonary arteries

- Pulmonary embolism (thrombus, tumour, ova or parasites, foreign material)
- In-situ thrombosis
- Sickle-cell disease

#### Pulmonary hypertension due to disorders directly affecting pulmonary vasculature

Inflammatory

- Schistosomiasis
- Sarcoidosis
- Other

Pulmonary capillary haemangiomatosis

HIV infection,<sup>18</sup> cirrhosis with portal hypertension,<sup>15,19</sup> and collagen vascular diseases (panel 2).<sup>9</sup> A relation between oestrogen and PPH has been postulated given the higher prevalence in women than men,<sup>20</sup> but epidemiological data have not shown a link with use of oral contraceptives.<sup>2,15</sup>

### Pathology

PPH is characterised by obstruction of small pulmonary arteries in association with plexiform lesions, medial hypertrophy, concentric laminar intimal fibrosis, fibrinoid degeneration, and thrombotic lesions (figure 2).<sup>21-23</sup> Several pathology studies have classified patients as having either plexogenic or thrombotic arteriopathy.<sup>21-23</sup> These analyses tried to differentiate clinical characteristics and mortality on the basis of pathology with the assumption that the two types of arteriopathy were distinct disease processes. In a study of post-mortem samples of lung from 23 individuals from 13 families with known familial PPH, we categorised all pulmonary-artery

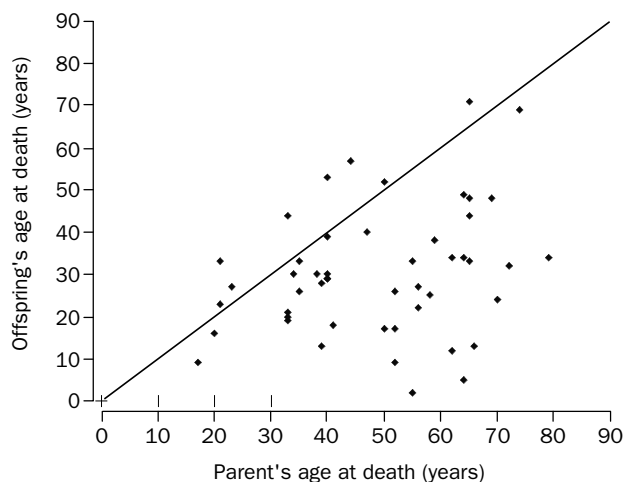


Figure 1: **Genetic anticipation in familial PPH**

Comparison of pairs of parent and offspring who both died from PPH is a method to limit statistical bias. The graph represents 48 offspring-parent deaths from our Familial PPH Registry. Data points above the line represent offspring who were older at death than their parents, and those below the line offspring who died at an earlier age than their parents.

lesions.<sup>24</sup> Organised thrombi and plexiform lesions were noted among members of the same family, which suggests that patterns of vascular pathology do not represent distinct entities but are pleiotropic manifestations of one disease.

### Panel 2: Risk factors for development of PAH

#### Drugs and toxins

Definite	Aminorex Fenfluramine Dexfenfluramine Toxic rapeseed oil
Very likely	Amphetamines L-tryptophan
Possible	Meta-amphetamines Cocaine Chemotherapeutic agents
Unlikely	Antidepressants Oral contraceptives Oestrogen therapy Cigarette smoking

#### Demographic and medical conditions

Definite	Sex (female)
Possible	Pregnancy Systemic hypertension Splenectomy
Unlikely	Obesity

#### Diseases

Definite	HIV infection
Very likely	Portal hypertension Collagen vascular diseases Congenital systemic-to-pulmonary cardiac shunts (Eisenmenger's syndrome)
Possible	Thyroid disorders Haemoglobinopathies (sickle-cell disease, thalassaemia, spherocytosis) Type Ia glycogen storage disease (von Gierke's disease) Lipid storage disorders (Gaucher's disease) Hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu disease)

From Humbert et al. *Clin Chest Med* 2001; **22**: 459-75; with permission.

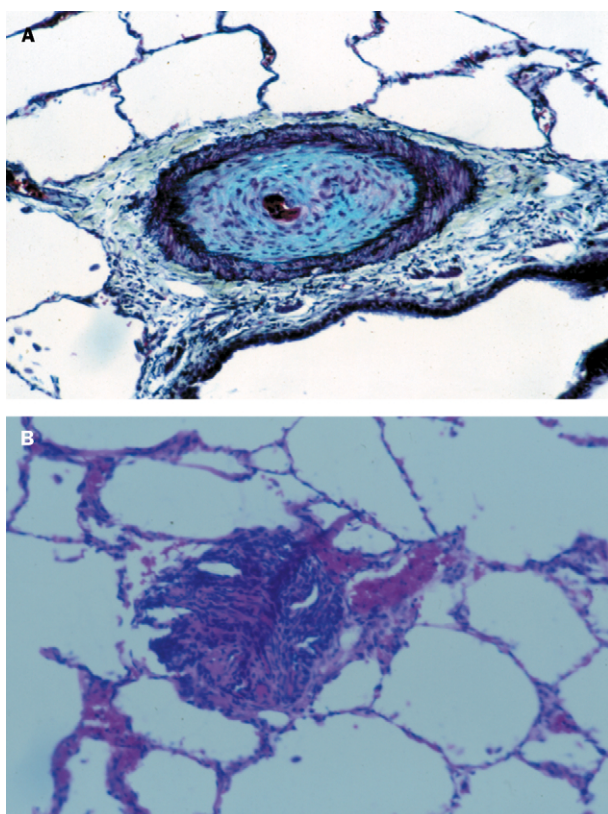


Figure 2: **Pathology of PPH**

A: Pulmonary artery from a patient with PPH, showing severe concentric lamellar intimal fibrosis, medial hypertrophy, and in-situ thrombosis of the small residual lumen. The alveolar architecture surrounding the pulmonary artery is normal. B: Plexiform lesion showing proliferative vascular lesion with several lumens.

Plexiform lesions, the classic pathological finding in PPH, are present in about one in three lung-biopsy specimens,<sup>21–23</sup> and are in most cases located near the origin of a small pulmonary artery with focal medial disruption, intimal proliferation, and aneurysmal dilatation (figure 2).<sup>22</sup> However, plexiform lesions also occur in other disorders with PAH and are therefore not pathognomonic for PPH.<sup>21,25,26</sup> Debate about the cell of origin of plexiform lesions, and thus possibly the initiator of the pathogenetic defects underlying PPH, has persisted for many years. Some investigators propose that proliferation of smooth-muscle cells and transformation into myofibroblasts are the major events in formation of plexiform lesions.<sup>25,27</sup> Others believe that endothelial cells, responding to cytokines, growth factors, or vascular injury, underlie the beginning of phenotypic disease.<sup>26,28</sup> Support for this theory comes from studies that showed monoclonality in endothelial cells from plexiform lesions in PPH in contrast to polyclonal cell populations in secondary pulmonary hypertension.<sup>29</sup> Furthermore, microsatellite instability has been observed in a DNA repair enzyme, a proapoptotic gene, and the gene for transforming growth factor  $\beta$  (TGF $\beta$ ) receptor II in plexiform endothelial cells.<sup>30</sup> These changes may provide a growth advantage. Most investigators now agree that pulmonary vascular proliferation and remodelling, and not vasoconstriction, is probably the central pathogenesis of PPH.

### Genetics

Study of families affected by PPH led to the discovery of *BMPR2* as a primary gene for familial PPH on chromosome 2q33.<sup>4,5</sup> The heterogeneous germline

mutations in *BMPR2* are present in about 50% of familial PPH cases and 26% of sporadic cases and encode frameshift, partial deletion, splice-site, non-sense, and mis-sense mutations, most of which lead to premature termination of the protein.<sup>4,5,31,32</sup> Many PPH families without a mutation in the *BMPR2* coding (exonic) region show linkage to the *BMPR2* locus.<sup>5,33</sup> In these families, the failure to find a specific mutation could be secondary to gene rearrangements, large deletions or insertions, or non-coding (intronic) mutations.<sup>32</sup> Familial PPH shows genetic anticipation—onset and death occur at an earlier age in subsequent generations. Trinucleotide-repeat-expansion disorders such as Huntington's disease share this characteristic.<sup>34</sup> Since relevant nucleotide repeats have not been found in the *BMPR2* gene, genetic anticipation in familial PPH could be the result of ascertainment bias or repeat expansions could be present in modifier genes.

*BMPR2* is a protein of the TGF $\beta$ -receptor superfamily which bind cytokines including TGF $\beta$ , bone morphogenetic protein, activin, inhibin, and growth differentiation factor.<sup>35</sup> Along with a type I bone-morphogenetic-protein receptor, *BMPR2* binds ligand in a heterodimer complex on the cell surface and propagates signal via Smad molecules (figure 3).<sup>35,36</sup> *BMPR2* has 13 exons encoding extracellular, transmembrane, kinase, and cytoplasmic tail domains (figure 4). Originally discovered in association with bone growth, bone morphogenetic proteins, the major ligands for bone-morphogenetic-

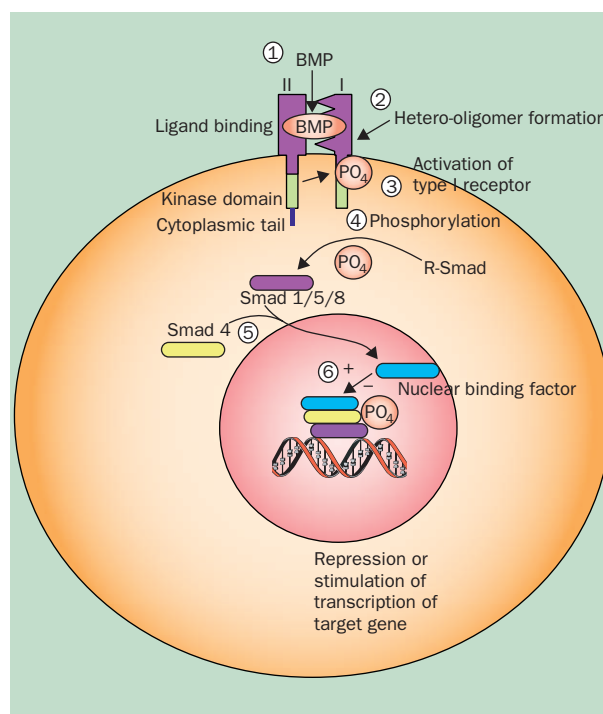


Figure 3: **Bone-morphogenetic-protein signalling pathway**

1 and 2: *BMPR1* and *BMPR2* are present on most cell surfaces as homo-dimers or hetero-oligomers. With ligand (bone morphogenetic protein; BMP) binding, a complex of ligand, two type I receptors, and two type II receptors is formed. 3: After ligand stimulation, the type II receptor phosphorylates the type I receptor in its juxtamembrane domain. 4: The activated type I receptor then phosphorylates a receptor-regulated Smad (R-Smad); thus the type I receptors determine the specificity of the signal. Smads 1, 5, and 8 are specific for BMP signalling pathway. 5: Once activated by phosphorylation, the R-Smads interact with the common mediator Smad 4 to form hetero-oligomers that are translocated to the nucleus. 6: In the nucleus, the Smad complex interacts with transcription factors and binds to DNA to induce or suppress transcription of target genes. Smads 6 and 7, inhibitory Smads (not shown), bind to activated type I receptors to prevent the phosphorylation of R-Smads.

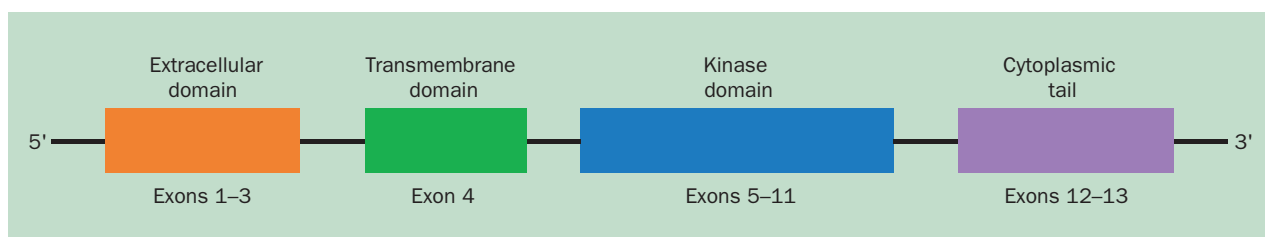


Figure 4: **Functional domains of BMPR2**

The extracellular domain of the protein binds ligand. The transmembrane domain anchors the protein in the cell membrane. After ligand binding, the kinase domain phosphorylates BMPR1. The function of the cytoplasmic tail domain is not known, but it may aid in binding of the type II receptors to type I receptors or other type II receptors.

protein receptors, are also important in embryogenesis, development, apoptosis, cell differentiation, and proliferation.<sup>37</sup>

Haploinsufficiency, in which expression of 50% of a gene product will result in disease, has been proposed as a mechanism of *BMPR2* mutations.<sup>32</sup> In recent studies, a dominant-negative effect has been shown.<sup>38,39</sup> Two groups of investigators have shown that mis-sense mutations of cysteine residues in the extracellular or kinase domain of *BMPR2* result in intracellular localisation of the mutated receptor and negligible Smad signalling.<sup>38,39</sup> Protein with non-cysteine mis-sense mutations in the kinase domain or mis-sense or non-sense mutations in the cytoplasmic tail was expressed on the cell surface with reduced Smad signalling.<sup>38,39</sup> A gain of function with increased activation of one of the mitogen-activated protein kinases, p38, was also discovered in a mouse epithelial cell line transfected with mutant *BMPR2* plasmids.<sup>38</sup> By use of immunohistochemistry on explanted lung tissue, Atkinson and colleagues found much lower endothelial-cell expression of *BMPR2* in patients with PPH than in

healthy controls, with the lowest expression in patients with known *BMPR2* mutations or linkage to the *BMPR2* locus.<sup>40</sup> Future studies should investigate how these heterogeneous mutations produce the same phenotype.

Since TGF $\beta$  receptors are involved in cell proliferation and apoptosis, a decrease in bone-morphogenetic-protein signalling could lead to loss of antiproliferative or apoptotic mechanisms in the pulmonary circulation. Normal propagation of aortic smooth-muscle cells in response to growth factors and injury is suppressed by bone morphogenetic proteins.<sup>41,42</sup> By contrast, Morrell and co-workers found dysregulated growth inhibition of pulmonary-artery smooth-muscle cells from PPH patients when they were exposed to bone morphogenetic proteins and TGF $\beta$ .<sup>43</sup> TGF $\beta$ -receptor mutations have also been identified in clonal populations in atherosclerotic lesions and in hereditary polyposis colon cancer.<sup>44,45</sup> Similarly, monoclonality of endothelial cells has been detected in PPH plexiform lesions.<sup>29</sup> Dysplastic angiogenesis involving endothelial cells and pulmonary hypertension pathologically indistinct from PPH is

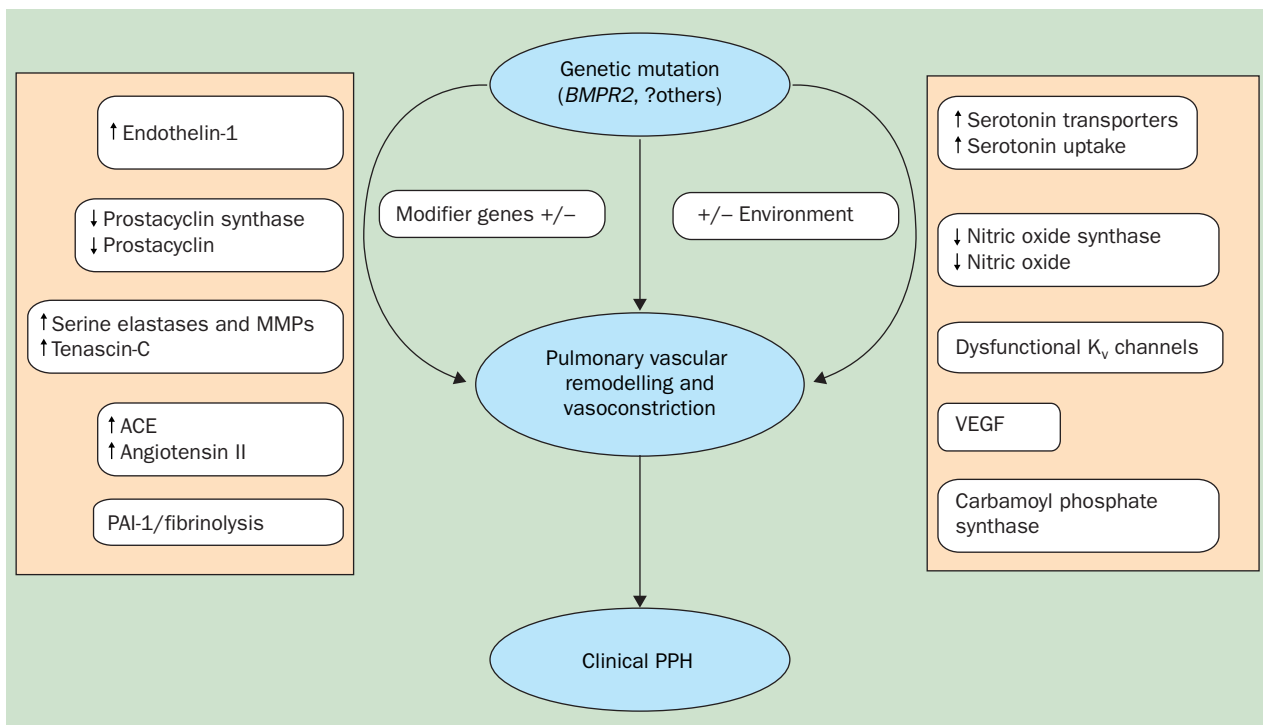


Figure 5: **Proposed pathogenesis for the development of PPH**

Genes implicated in the pathogenesis of PPH are prostacyclin synthase, serotonin transporters, nitric oxide synthase, serine elastases, and matrix metalloproteinases (MMPs), voltage-gated potassium ( $K_v$ ) channels, angiotensin-converting enzyme (ACE), vascular endothelial growth factor (VEGF), carbamoyl phosphate synthase, and plasminogen activator inhibitor type 1 (PAI-1). Endothelin-1 production adds to the vasoconstriction in PPH, but whether this is secondary to changes in the above genes, a result of endothelial dysfunction, or a primary pathogenetic event is not clear. Pulmonary vascular remodelling results from the effects of genetics, modifying genes, and environment.

occasionally present in hereditary haemorrhagic telangiectasia.<sup>46–48</sup> Mutations in endoglin and activin-receptor-like kinase 1, both TGF $\beta$  receptors, are associated with hereditary haemorrhagic telangiectasia types 1 and 2, respectively.<sup>46–48</sup> That other vascular disorders may involve mutations in TGF $\beta$  receptors is thus a distinct possibility.

Another important issue is the role of modifier genes in the pathogenesis of PPH. Because phenotypic disease occurs in only 10–20% of individuals with *BMPR2* mutations, the existence of modifier genes or environmental triggers is highly probable. Genes with evidence for implication in the pathogenesis of PPH are shown in figure 5. The number of modifying genes necessary for disease expression and whether an underlying genetic mutation such as in *BMPR2* needs to be present are currently unknown. Moreover, factors such as oestrogen are liable to influence development of overt PPH. A “two-hit” theory has been proposed in which a susceptible individual with a *BMPR2* mutation would require additional insults before manifesting disease.<sup>49</sup>

## Pathogenesis

### Endothelial dysfunction

An imbalance between vasodilators and vasoconstrictors has long been thought to have a key role in the development of PPH. An early study showed increased production of thromboxane, a vasoconstrictor, and decreased formation of prostacyclin, a vasodilator, in patients with PPH.<sup>50</sup> A subsequent study found lower expression of prostacyclin synthase in small pulmonary arteries in PPH patients than in controls.<sup>51</sup> In addition, the overexpression of prostacyclin synthase in rodent experimental models protects against development of pulmonary hypertension.<sup>52,53</sup> Nitric oxide is made by vascular endothelium, catalysed by nitric oxide synthase, and promotes vasodilation and inhibits smooth-muscle growth.<sup>54</sup> Giaid and Saleh found that endothelium from PPH patients showed negligible immunohistochemical staining for nitric oxide synthase compared with healthy controls.<sup>54</sup> Endothelin 1, a potent vasoconstrictor and mitogen for smooth-muscle cells,<sup>55,56</sup> is also produced by vascular endothelium and localises to muscular pulmonary arteries of PPH patients.<sup>55</sup>

Further investigation of endothelial dysregulation has focused on vascular endothelial growth factor (VEGF), a specific endothelial growth stimulant induced by various cytokines including platelet-derived growth factor and TGF $\beta$ .<sup>57</sup> VEGF expression by endothelial cells in plexiform lesions has been established in patients with PPH and secondary PAH, but its role in pathogenesis is still speculative.<sup>58</sup> Since blockade of VEGF receptor 2 caused more severe pulmonary hypertension in hypoxic rats, VEGF may have a protective role for endothelial function and survival.<sup>59</sup> These rats showed increased endothelial cell death followed by amplified growth, probably from selection of apoptosis-resistant cells.<sup>59</sup> Further proof comes from an observed protective effect against monocrotaline-induced pulmonary hypertension in rats via delivery of smooth-muscle cells overexpressing VEGF-A.<sup>60</sup>

### Extracellular matrix

Breakdown of the extracellular matrix by vascular serine elastases and matrix metalloproteinases increases smooth-muscle-cell proliferation by release of matrix-bound mitogens and by induction of tenascin C.<sup>61</sup> This substance amplifies the response of smooth-muscle cells to growth factors, allowing sustained survival and growth.<sup>61,62</sup>

Administration of inhibitors of serine elastase and matrix metalloproteinases to monocrotaline-exposed rats lowered concentrations of tenascin C, increased myocyte apoptosis, and reduced changes of pulmonary hypertension.<sup>61,62</sup> Nitric oxide can limit serine elastase activity in smooth-muscle cells and thus aid in reducing vascular remodelling.<sup>63</sup>

### Serotonin

Stored mainly in platelets, serotonin is associated with pulmonary vasoconstriction and proliferation of smooth-muscle cells.<sup>64–66</sup> Plasma serotonin concentrations are higher than normal in PPH, possibly as a result of abnormal platelet processing and storage.<sup>67,68</sup> Even with its inhibitory action on platelet aggregation, prostacyclin infusion does not affect plasma serotonin concentrations.<sup>69</sup> Eddahibi and colleagues induced increased uptake of serotonin by rat pulmonary-artery smooth-muscle cells in response to hypoxia and were able to suppress this effect with selective serotonin-reuptake inhibitors.<sup>70</sup> Hypoxia promoted increased expression of serotonin transporters, allowing higher intracellular concentrations of serotonin and resultant smooth-muscle-cell proliferation.<sup>70</sup> Compared with controls, transgenic mice lacking the gene for serotonin transporter had less hypoxic pulmonary hypertension.<sup>71</sup> 65% of PPH patients but only 27% of normal controls were found to be homozygous for a serotonin-transporter promoter polymorphism (L-allele) linked with increased gene transcription and pulmonary-artery smooth-muscle-cell proliferation in response to serotonin exposure.<sup>72</sup> Further investigation of a relation between *BMPR2* and the serotonin pathway is under way. Appetite-suppressant drugs can serve as substrates for serotonin transporter and are transported into pulmonary-artery smooth-muscle cells.<sup>73</sup> This action could result in increased growth or other toxic actions on pulmonary-artery smooth-muscle cells and thus serve as a link between anorectic agents and development of PPH.<sup>73</sup>

### Voltage-gated potassium channels

Intracellular calcium promotes contraction of pulmonary vascular smooth-muscle cells and serves as a stimulus for smooth-muscle-cell hypertrophy.<sup>74</sup> Voltage-gated potassium channels control the cell-membrane potential and the release of calcium via sarcolemmal voltage-gated channels.<sup>74</sup> Yuan and colleagues showed that in PPH pulmonary-artery smooth-muscle cells have low mRNA for the potassium channels and low channel current, with a resultant increase in intracellular calcium compared with controls.<sup>74,75</sup> These investigators believe that dysfunctional voltage-gated potassium channels may help to explain the vasoconstriction and smooth-muscle-cell proliferation that occur in PPH. Other findings suggest that appetite-suppressant drugs may generate pulmonary hypertension by blocking voltage-gated potassium channels in pulmonary-artery smooth-muscle cells.<sup>76</sup>

### Angiotensin-converting enzyme

Increased expression of angiotensin-converting enzyme (ACE), the enzyme that brings about production of angiotensin II, is prominent in intimal and plexiform lesions.<sup>77,78</sup> Since angiotensin II activates growth of human pulmonary-artery smooth-muscle cells, high concentrations of the enzyme may contribute to vascular remodelling in distal pulmonary arteries.<sup>79</sup> Administration of an ACE inhibitor to pneumonectomised, monocrotaline-treated rats decreased development of neointimal lesions.<sup>80</sup> However, the role of ACE in the pathogenesis of pulmonary hypertension is still uncertain.

### Plasminogen activator inhibitor type 1 and impaired fibrinolysis

In-situ thrombosis is thought to have a role in PPH development by contributing to occlusion of small pulmonary arteries. Benza reported an association between PPH and a polymorphism in the plasminogen activator inhibitor type 1 promoter linked with increased transcription.<sup>81</sup> This genotype may predispose to PPH by decreasing fibrinolytic activity. Several other studies have documented a tendency towards coagulation with impaired fibrinolytic ability.<sup>82-84</sup>

### Carbamoyl phosphate synthase

A precursor for nitric oxide production, arginine, is formed in the urea cycle with carbamoyl phosphate synthase as the rate-limiting step.<sup>85</sup> Pearson and co-workers found that plasma concentrations of arginine and nitric oxide metabolites were lower in neonates with respiratory distress and pulmonary hypertension than in those with respiratory distress alone.<sup>85</sup> A polymorphism in the critical N-acetylglutamate-binding domain of carbamoyl phosphate synthase generating amplified enzymatic activity was not present homozygously in infants with pulmonary hypertension. This polymorphism may protect against pulmonary hypertension since this form of the enzyme can generate precursors for nitric oxide more quickly.

### Clinical presentation and diagnosis

The diagnosis of many cases of PPH is delayed because symptoms overlap with those of more common diseases. Dyspnoea was the initial symptom in 60% of patients in the National Institutes of Health registry, and 98% had dyspnoea at enrolment.<sup>1</sup> Less common symptoms included fatigue, chest pain, near-syncope, syncope, peripheral oedema, and palpitations.

Physical findings become more prevalent as right heart failure develops. Auscultation of heart sounds may reveal an increased pulmonary component (P<sub>2</sub>), a right-sided gallop (S<sub>3</sub> or S<sub>4</sub>), or tricuspid regurgitation. A right-ventricular lift can be transmitted along the lower left sternal border. Significant right-ventricular dysfunction can also increase jugular venous pressure with large "a" and "v" waves owing to reduced compliance of the right

ventricle and tricuspid regurgitation, respectively, along with hepatomegaly, ascites, and peripheral oedema.

Laboratory studies are non-specific; the proportion positive for low-titre antinuclear antibodies is slightly higher than in the general population.<sup>1</sup> To exclude secondary causes of PAH, tests for HIV-1 antibody, liver and thyroid function, and selected rheumatological screening antibodies should be done along with a history of use of illicit drugs.

Chest radiography shows typical findings of pulmonary hypertension with enlarged main pulmonary arteries, right atrial and ventricular dilatation, and clear lung fields (figure 6).<sup>1,86</sup> Ventilation/perfusion lung scintigraphy is the most reliable way to discriminate between PPH and the other major diagnosis in the differential, chronic thromboembolic pulmonary hypertension. Lung perfusion patterns are normal or mottled in the former, whereas major defects are generally present in the latter.<sup>86,87</sup> If the diagnosis is still uncertain, pulmonary angiography can be safely done even in the presence of stable right heart failure.<sup>1,88</sup> Computed tomography can be helpful if underlying parenchymal lung disease or mediastinal fibrosis is suspected.

Electrocardiography commonly reveals right axis deviation, prominent P waves in inferior leads, R waves greater than S waves in lead V<sub>1</sub>, and right-ventricular strain pattern. However, doppler echocardiography is the best non-invasive test for screening and follow-up of PPH by providing assessment of right-ventricular function and size, cardiac output, and the pulmonary-artery systolic pressure by use of the tricuspid regurgitant jet velocity. Valvular, congenital, and left-sided heart disease can all be detected by echocardiography.

Pulmonary-function tests aid in excluding parenchymal lung disorders. The results are normal in many cases of PPH but may reveal slightly reduced lung volumes and mildly to moderately reduced diffusing capacity for carbon monoxide.<sup>1</sup> Sampling of arterial blood can show a respiratory alkalosis with hypoxaemia due to a right-to-left shunt or ventilation/perfusion mismatch.<sup>8</sup> Formal cardiopulmonary exercise testing will show decreased exercise tolerance and maximum oxygen consumption, early attainment of anaerobic threshold, and possible desaturation.<sup>89</sup> The 6 min walk test is a standard method

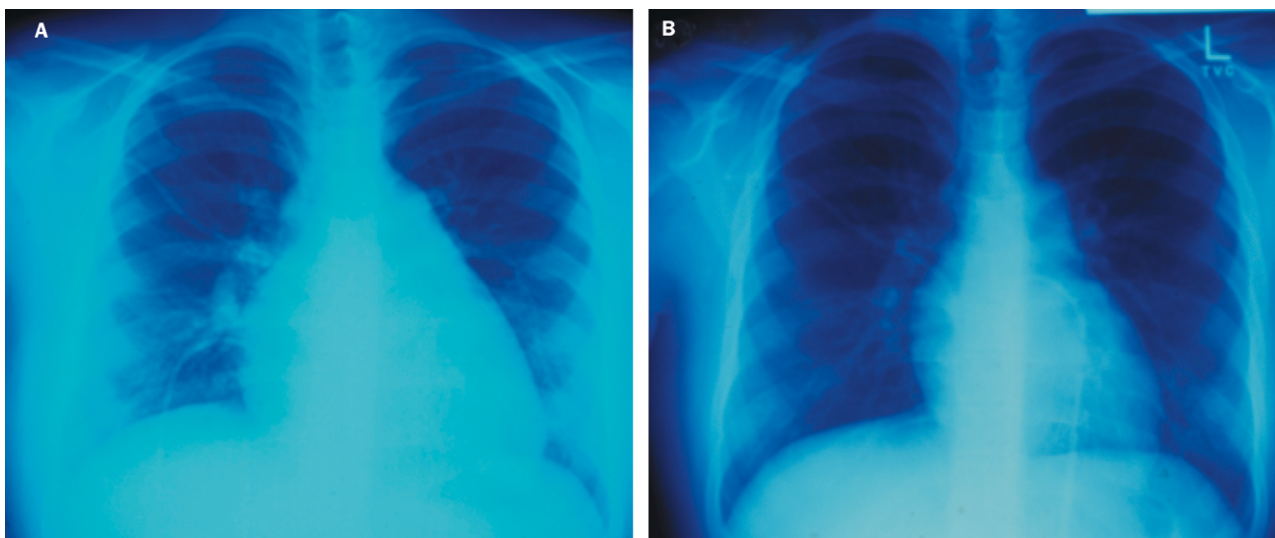


Figure 6: Chest radiographs from a patient with PPH before and after epoprostenol therapy

A: Before treatment: there is cardiomegaly from enlargement of the right atrium and ventricle along with prominent pulmonary arteries, the lung fields are clear. B: After treatment (3 months): the right atrial and ventricular enlargement has resolved. An indwelling catheter for infusion of epoprostenol is present in the right subclavian vein.

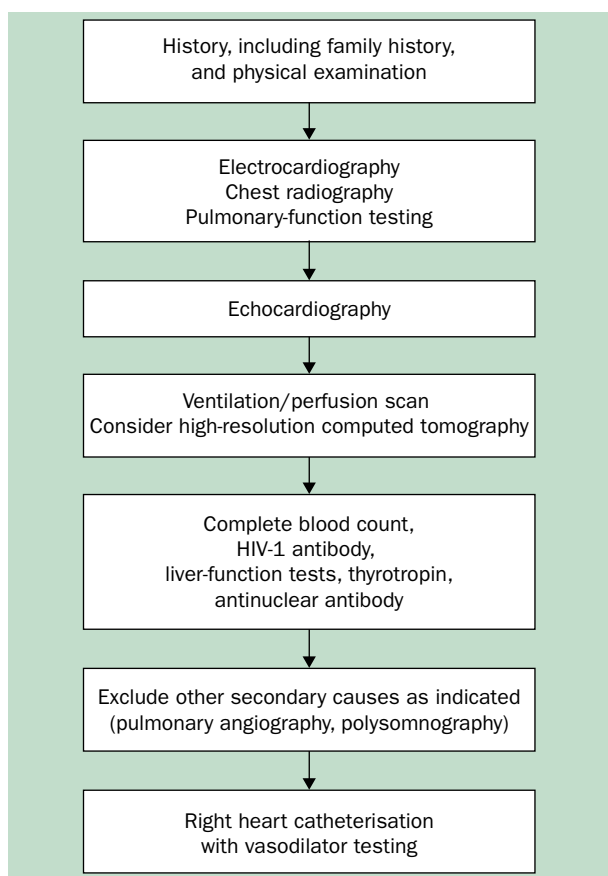


Figure 7: **Suggested investigation for pulmonary hypertension**  
Vasodilator testing=short-acting agents such as nitric oxide, prostacyclin, and adenosine.

of assessing exercise performance and response to therapy, and predicting prognosis.<sup>90</sup> For patients suspected of having sleep apnoea, polysomnography is indicated.

Right heart catheterisation with selective pulmonary vasodilator testing is indicated to confirm suspected PPH (figure 7). Although the procedure is generally well tolerated, severe complications including hypotension and death have occurred rarely.<sup>91</sup> Right heart catheterisation provides appraisal of disease severity and prognosis because significant right heart failure is associated with poor survival.<sup>2,92</sup> New York Heart Association functional class III or IV and a low mixed venous oxygen saturation have also been associated with a poor prognosis.<sup>2</sup>

## Standard therapies

### Anticoagulation

Anticoagulant therapy has been widely used for PPH, but the evidence supporting this approach has not come from rigorous, well-designed trials. In a retrospective, single-centre study, Fuster and colleagues found that 78 PPH patients given coumadin had better survival than 37 patients not given anticoagulant.<sup>3</sup> In a trial designed to assess calcium-channel blockers in PPH, Rich and co-workers found that patients taking coumadin had better survival, but in that study anticoagulants were recommended on the basis of non-uniformity of pulmonary blood flow on lung scan, which could have led to subgroup bias.<sup>93</sup> These uncontrolled data suggest that anticoagulation may be beneficial in PPH, but better information is needed. Since survival is improving, there is now longer opportunity for anticoagulant-related complications.

### Calcium-channel blockers

Long-term therapy with calcium-channel blockers has sustained haemodynamic improvement and survival in a small subset (<25%) of PPH patients who respond to acute challenge with these agents.<sup>93,94</sup> In a study assessing survival, responders were patients experiencing a 20% decrease in mean pulmonary-artery pressure and pulmonary vascular resistance with calcium-channel blockers.<sup>93</sup> Current standard therapy includes a trial of therapy with calcium-channel blockers for patients who respond to acute vasodilator tests with nitric oxide, adenosine, or prostacyclin; a small proportion have a sustained, favourable outcome. Vasodilator testing should be done with short-acting agents only, so any adverse effects will pass quickly when the agent is withdrawn.

### Prostacyclin

Prostacyclin is produced by both endothelial and smooth-muscle cells in the vasculature.<sup>95</sup> It causes vasodilation and strongly inhibits platelet aggregation by increasing platelet cAMP concentrations through activation of adenylate cyclase.<sup>95</sup> Many studies have documented improvement in exercise tolerance, haemodynamic measures, and survival in PPH patients given intravenous epoprostenol.<sup>96-98</sup> Barst and co-workers reported 1-year, 3-year, and 5-year survival with epoprostenol of 87%, 63%, and 54%, respectively, compared with 77%, 41%, and 27% in historical controls.<sup>97</sup> Epoprostenol is also effective for treatment of PAH from scleroderma but does not give a survival advantage.<sup>99</sup> Smaller series suggest it may help patients with PAH from systemic lupus erythematosus,<sup>100</sup> congenital heart disease,<sup>101</sup> HIV infection,<sup>102</sup> connective-tissue disorders, sarcoid, and portopulmonary hypertension.<sup>103</sup> Common side-effects include jaw pain, headaches, flushing, diarrhoea, and nausea.<sup>97,98</sup> Currently, epoprostenol is considered the most effective therapy for PPH with favourable responses in the vast majority.

Because epoprostenol has a short half-life, continuous intravenous delivery and placement of a long-dwelling catheter are necessary. Complications from the delivery system include exit-site infections and bleeding, paradoxical embolism, bacteraemia or sepsis, and delivery malfunction that may result in sudden, in some cases fatal, decompensation.<sup>97,98,104</sup> In addition, epoprostenol therapy in patients with pulmonary veno-occlusive disease has been associated with acute, overwhelming pulmonary oedema.<sup>105</sup>

Even patients who do not respond to acute vasodilator testing gain long-term benefit from epoprostenol, possibly through platelet inhibition and effects on pulmonary vascular remodelling.<sup>98,104</sup> Friedman and colleagues showed that epoprostenol therapy decreased abnormal platelet aggregation and concentrations of factor VIII, von Willebrand antigen, and ristocetin cofactor.<sup>106</sup> Prostacyclin may also decrease production of endothelin 1,<sup>107</sup> increase VEGF production,<sup>108</sup> diminish smooth-muscle-cell proliferation,<sup>109</sup> and improve pressure-flow response to exercise.<sup>110</sup>

### Lung transplantation

First undertaken successfully in 1982, transplantation is the only curative therapy for PPH at this time.<sup>111</sup> Indications for transplantation include New York Heart Association functional class III/IV despite optimum medical therapy, cardiac index less than 2 L min<sup>-1</sup> m<sup>-2</sup>, right-atrial pressure greater than 15 mm Hg, and mean pulmonary-artery pressure greater than 55 mm Hg.<sup>112</sup> Single lung transplants have similar survival outcome and improvement of haemodynamic function to bilateral lung

transplants<sup>113,114</sup> but may be associated with more ventilation/perfusion mismatching and graft-related mortality.<sup>115,116</sup> The development of obliterative bronchiolitis (chronic rejection) occurs earlier and more frequently in patients given transplants for PPH than for other disorders.<sup>117</sup> With the emergence of new and more effective therapies for PPH, the decision on and timing of referral for lung transplantation may become more difficult until favourable predictors for individual long-term survival with these agents are known.

#### Atrial septostomy

After Rozkovec and colleagues reported that PPH patients with a patent foramen ovale had better survival than those without this feature,<sup>118</sup> atrial septostomy (ie, the creation of a small perforation in the atrial septum) has been used for patients with disabling right heart failure. Amelioration of syncope and better exercise performance have been reported.<sup>119,120</sup> By creating or increasing right-to-left shunt, atrial septostomy may lower mean right-atrial pressure and increase left-ventricular preload, thus unloading the right ventricle and improving cardiac output.<sup>119,120</sup> Besides the possible occurrence of life-threatening arterial hypoxaemia, the procedure-related mortality is high in patients with severe right heart failure; therefore the procedure should be considered only by experienced operators in exceptional circumstances when other treatment options are not available or as a bridge to transplantation.<sup>119,121</sup>

#### Emerging therapies Prostacyclin analogues

Owing to the inherent problems with epoprostenol, new prostacyclin analogues have been sought for the treatment of PPH. Beraprost, an oral analogue, in preliminary studies acutely improved haemodynamic measures in patients with PAH and had additive effects to inhaled nitric oxide.<sup>122,123</sup> In a retrospective, non-randomised trial, beraprost given to PPH patients maintained improvement in exercise tolerance and haemodynamic profile along with a survival benefit at 1, 2, and 3 years compared with controls.<sup>124</sup> Adverse events were similar to those with epoprostenol. A prospective, randomised 12-week trial of beraprost versus placebo for PAH patients with functional class II/III showed improved exercise tolerance and dyspnoea in the PPH subgroup.<sup>125</sup>

Iloprost can be administered by inhalation with more potent acute pulmonary haemodynamic effects than inhaled nitric oxide.<sup>126</sup> Its efficacy is more pronounced during exercise than at rest.<sup>127</sup> Iloprost has been effective in severe PAH and even in one patient with circulatory shock.<sup>128,129</sup> Hoeper and colleagues documented improvement in exercise capacity and haemodynamics at 1 year in PPH patients.<sup>130</sup> However, administration of iloprost required six to eight inhalations daily, given every 2–3 h while the patient was awake. Flushing, headache, jaw pain, cough, and symptomless, slight decline in mean systemic arterial pressure and vascular resistance were reported. A multicentre, randomised trial in PAH patients of New York Heart Association class III/IV showed that 12 weeks of iloprost improved 6 min walking distance, dyspnoea, and functional class, and maintained haemodynamics compared with placebo.<sup>131</sup>

Another prostacyclin analogue, treprostinil, was recently approved for continuous subcutaneous infusion. In a randomised, placebo-controlled, multicentre trial of 470 patients with PAH, Simonneau and colleagues found improvement at 12 weeks in the 6 min walking distance, dyspnoea, and haemodynamic profile in the treprostinil

group.<sup>132</sup> The improvement in exercise capacity was dose related and more pronounced in those with advanced disease. However, 85% of patients had infusion-site pain (8% discontinued therapy), 24% experienced pump malfunctions, and three had gastrointestinal bleeding. The benefits with treprostinil have been maintained up to 18 months.<sup>133</sup> In patients with life-threatening complications with epoprostenol delivery, conversion from epoprostenol to treprostinil infusion has been safely and successfully achieved in an inpatient setting.<sup>134</sup>

Since only one retrospective study to date has shown a survival benefit with these prostacyclin analogues,<sup>124</sup> and since no trial has compared these agents with epoprostenol, no absolute recommendations on their use can be made. Nevertheless, all the new agents significantly improve exercise tolerance and haemodynamic measures, and they should be considered for PPH patients in New York Heart Association class II or early, stable class III.<sup>135</sup>

#### Endothelin-1 receptor antagonists

Since increased expression of endothelin 1, a potent vasoconstrictor and mitogen for smooth-muscle cells, in the pulmonary arteries of PPH patients seems to be related to disease severity and survival,<sup>55,136</sup> bosentan, an oral antagonist of endothelin-1 receptors A and B, has been examined. Two multicentre, randomised, double-blind, placebo-controlled trials involving patients with PAH have documented the benefits for exercise capacity, dyspnoea, and the WHO functional class for 3–4 months.<sup>137,138</sup> Adverse effects on liver function necessitated discontinuation in 2%.<sup>138</sup> A dose of 125 mg twice daily with monitoring of liver function monthly was recommended.<sup>138,139</sup> A pilot study with sitaxsentan, a specific antagonist of endothelin-1 receptor A, showed promising results in 20 PAH patients except for two cases of acute hepatitis, one fatal.<sup>140</sup> At present, bosentan is indicated for stable functional class III or IV patients with PAH, and a current study with epoprostenol should show whether additive effects are possible.<sup>139</sup>

#### Phosphodiesterase inhibitors

By activating guanylate cyclase, nitric oxide exerts its vasodilatory effects through the second messenger cGMP.<sup>141</sup> Urinary excretion of cGMP is high in PPH individuals and correlates with disease severity, suggesting that it may be a protective, compensatory response to pulmonary hypertension.<sup>141</sup> Sildenafil is a selective inhibitor of cGMP-specific phosphodiesterase type 5, the main phosphodiesterase in the pulmonary vasculature.<sup>142</sup> Sildenafil attenuated experimentally induced pulmonary hypertension in animals and acute hypoxic effects in human beings.<sup>142,143</sup> Anecdotal reports of improvement in exercise capacity and haemodynamics have been reported for PPH and HIV-associated PAH.<sup>144,145</sup> Acutely, oral sildenafil produces a reduction in mean pulmonary-artery pressure in patients with PPH, and additive effects are seen with inhaled iloprost.<sup>146</sup> Long-term studies with sildenafil alone and in combination with prostacyclin analogues and endothelin-1 receptor antagonists are needed to clarify its role in treatment.

#### L-arginine

L-arginine is essential in the production of nitric oxide<sup>147</sup> and could therefore have a therapeutic role in PAH. Mehta and co-workers<sup>148</sup> reported a decrease in mean pulmonary-artery pressure and pulmonary vascular resistance in patients with PAH given L-arginine, but other investigators could not reproduce this finding.<sup>149,150</sup>

Daily injections of L-arginine ameliorated hypoxic and monocrotaline-provoked pulmonary hypertension in rats.<sup>151</sup> In a placebo-controlled trial in 19 patients with PAH, administration of L-arginine for 1 week raised concentrations of L-arginine and L-citrulline (a byproduct of nitric oxide synthase metabolism of L-arginine) and the maximum oxygen consumption during exercise.<sup>147</sup> An extended study of L-arginine supplementation is under way. Investigations combining L-arginine with other agents used to treat PAH are also needed.

### Experimental therapies

An established drug class, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (statins), may also be relevant to PPH therapy. These drugs prevent hypoxia-mediated downregulation of endothelial nitric oxide synthase by stabilising its mRNA,<sup>152</sup> repress vascular smooth-muscle-cell proliferation in response to platelet-derived growth factor and vascular injury,<sup>153,154</sup> and attenuate hypoxic pulmonary hypertension in rats.<sup>155</sup> We are aware of no reported trials as yet involving these agents in patients with PPH.

### Screening for disease

With the advent of many effective therapies, identification of asymptomatic carriers of *BMP2* mutations could lead to trials to prevent disease onset or progression. Exercise echocardiography to identify asymptomatic gene carriers was reported to have a sensitivity of 87.5% and specificity of 100% in two large German families with PPH.<sup>156</sup> This approach and other non-invasive tests need independent confirmation. Another issue that needs to be addressed is requests for genetic testing by relatives of affected PPH individuals. Various approved genetic methods to screen for mutation carriers are becoming available.

### Future directions

The pathogenetic mechanisms that result from *BMP2* mutations need to be recognised and elucidated. The identification of environmental contributors and modifying genes also requires further investigation. Once these issues are resolved, novel therapies could be developed to counteract the underlying pathogenesis perhaps at its source. In addition, screening to identify those individuals at risk of disease should allow studies to find out whether phenotypic expression can be prevented. The contribution of TGF $\beta$  receptors to pulmonary hypertension in other disorders should also be addressed. The effects of the new prostacyclin analogues, endothelin-1-receptor antagonists, and phosphodiesterase inhibitors on survival in early and severe PPH need more clarification. Combination trials with these agents and epoprostenol or L-arginine should also be considered. In conclusion, the next decade should see the emergence of substantial advances in the diagnosis, treatment, and outlook of PPH.

#### Conflict of interest statement

We have no financial or personal relationships with other people or organisations to report that caused a conflict of interest in writing this seminar.

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