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# Primary pulmonary hypertension: molecular basis and potential for therapy

**Nung Rudarakanchana and Nicholas W. Morrell**

**Primary pulmonary hypertension (PPH) is defined clinically by sustained elevation of pulmonary arterial pressure without a demonstrable cause, and is a progressive, often-fatal disease. PPH can be associated with ingestion of appetite suppressants, human immunodeficiency virus infection and certain autoimmune diseases. Familial PPH is known to account for 6% of all cases. Mutations in the gene encoding the bone morphogenetic protein (BMP) type II receptor have been identified in 72% of affected families and 26% of apparently sporadic cases. BMPs are members of the transforming growth factor β superfamily and affect intracellular signalling via Smads and mitogen-activated protein kinases. Evidence supports a 'two-hit' hypothesis in which PPH is triggered by accumulation of genetic and environmental insults in a susceptible individual. Elucidation of the precise molecular and cellular mechanisms underlying PPH will provide a powerful basis for the development of novel therapeutic strategies in the treatment of this devastating condition.**



The normal adult pulmonary circulation is a low-pressure, low-resistance system with little or no basal tone. An increase in pulmonary arterial pressure (PAP) can arise in a variety of clinical situations, including chronic hypoxic lung disease, chronic recurrent pulmonary embolism, elevated pulmonary blood flow and elevated pulmonary venous pressure secondary to insufficiency of the left ventricle. If a causative pathological process can be identified, an elevation in PAP is classified as secondary pulmonary hypertension. Raised PAP may also arise spontaneously in primary pulmonary hypertension (PPH), the focus of this review. At

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a recent World Health Organisation symposium (Ref. 1), experts recommended that PPH be renamed as idiopathic pulmonary arterial hypertension. However, it is not yet clear whether this new classification will be widely accepted, and therefore this article will continue to employ the current terminology of PPH.

## Primary pulmonary hypertension

### Clinical symptoms

PPH was first described clinically by Dresdale in 1951 (Ref. 2) soon after the advent of cardiac catherisation, which enabled direct measurement of circulation pressures. It is defined clinically by a sustained elevation of PAP (>25 mm Hg at rest, or >30 mm Hg during exercise) without a demonstrable cause. Symptoms are largely nonspecific but can include worsening shortness of breath, chest pain, syncope (fainting), fatigue and peripheral oedema. Patients often attribute initial symptoms to lack of fitness or being overweight, and many present with advanced disease. Clinical signs include: an accentuated pulmonary component of the second heart sound (a so-called 'loud P2'); right ventricular heave or cardiac murmur such as tricuspid regurgitation (the tricuspid valve is sited at the opening of the right auricle into the right ventricle) due to right ventricular dilatation; and signs of right ventricular failure, including increased venous pressure, oedema and hepatomegaly (enlargement of the liver) (Ref. 3).

Investigation of a patient should include echocardiography, to estimate systolic PAP and reveal right ventricular changes, and cardiac catherisation, to measure PAPs directly. Other causes of pulmonary hypertension should be excluded. Lung biopsy is rarely indicated, even though absolute confirmation of the diagnosis of PPH requires histological examination of the lung tissue.

Initially, an increase in right ventricular function compensates for the increased pulmonary resistance. However, PPH is a progressive disease and decompensated cardiac failure inevitably follows. In 1987, a multicentre study reported the mean survival from the time of diagnosis as 2.8 years (Ref. 3).

### Epidemiology

PPH is a rare disorder with an estimated incidence of 2–3 per million per year (Ref. 4). There is a female bias, with a female to male ratio of 2.3:1. The median age of diagnosis is 36 years but the disease can occur at any age. Prevalence may be increasing as a result of the advent of modern therapies and increased recognition of the disease. PPH appears to occur worldwide and insufficient information is available to determine whether specific populations have different risks for this disease.

### Cellular pathology

Pathologically, PPH is characterised by the obliteration of precapillary pulmonary arteries (Ref. 3). Histologically, small pulmonary arteries show increased wall thickness, distal extension of smooth muscle into normally nonmuscular vessels, the formation of a neointima, and plexiform lesions. The intimal lesions comprise cells of a myofibroblast phenotype, and the expression of endothelium-specific markers is confined to the cells lining the vascular lumen (Refs 5, 6) (Fig. 1). Plexiform lesions are composed of a proliferation of endothelial cells supported by a myofibroblast stroma (Refs 5, 7). Interestingly, one study has reported that endothelial cells of plexiform lesions in PPH are monoclonal, whereas those in secondary pulmonary hypertension are polyclonal in origin (Ref. 5).

### Aetiology

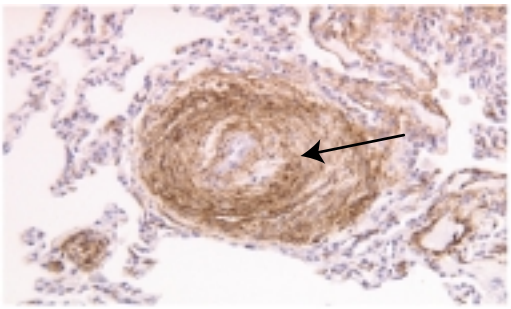
The precise molecular mechanisms underlying PPH have hitherto remained elusive. Clues to the aetiology of the disease have been suggested by environmental stimuli associated with the development of severe pulmonary hypertension. There is an acknowledged association with appetite suppressants – in particular, the fenfluramine/dexfenfluramine group (Ref. 8). PPH is also associated with the ingestion of drugs and toxins, as exemplified by the epidemic in 1981 that followed the ingestion of adulterated rape-seed oil in northern Spain (Ref. 9). Severe PPH can also follow human immunodeficiency virus (HIV)-1 infection (0.5–2% of HIV cases) (Ref. 10), and a recent report highlighted an association with human herpesvirus (HHV)-8 infection (Ref. 11). PPH is associated with autoimmune diseases, such as thyroiditis and scleroderma, and the presence of circulating anti-Ku and anti-nuclear antibodies (Ref. 12). Anecdotal associations have been reported with cocaine, amphetamines and oestrogen compounds (Ref. 12). The disease can first manifest itself during pregnancy (Ref. 13), when haemodynamic



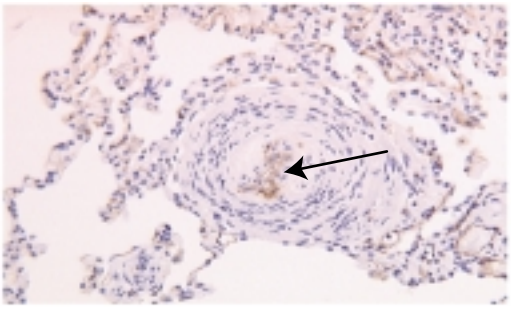
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**a** Anti-α-actin (smooth muscle)



**b** Anti-CD31 (endothelium)



Intimal lesions characteristic of primary pulmonary hypertension (PPH)

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**Figure 1. Intimal lesions characteristic of primary pulmonary hypertension (PPH).** The intimal layer of a normal blood vessel comprises an endothelial monolayer and a subcellular layer of extracellular matrix and smooth muscle cells (SMCs). Staining of lung sections from patients with PPH by antibodies directed against (a) SMC α-actin and (b) the endothelium-specific marker CD31 shows that intimal lesions comprise cells of a myofibroblast phenotype, with endothelial cells restricted to the lining of the vascular lumen. Arrows indicate the vascular lumen. Magnification, ~200×. Figure reproduced from Ref. 63, with permission from Lippincott, Williams & Wilkins (Copyright © 2002, American Heart Association, Inc.).

demands place increased stress on an already impaired cardiovascular reserve.

## Familial PPH and mutations in BMPR-II

Familial PPH was first described by Dresdale in 1954 (Ref. 14), and is now known to account for 6% of all cases of PPH (Ref. 15). Segregation analysis of affected pedigrees reveals an autosomal dominant inheritance with markedly reduced penetrance; only 10–20% of people carrying the disease gene will display the disease phenotype (Ref. 16). The disease also exhibits anticipation of age of onset, with disease onset occurring at earlier ages in subsequent generations (Ref. 16); to date no genetic basis for this phenomenon is known.

In 1997, a genome-wide screen established linkage to a region on chromosome 2q33 (Ref. 17). Several candidate genes were investigated and, in 2000, two groups reported that heterozygous mutations of the gene (*BMPR2)* encoding bone morphogenetic protein type II receptor (BMPR-II) underlie familial PPH (Refs 18, 19). To date, 72% of families analysed have shown either a *BMPR2* mutation or evidence of linkage to the *BMPR2* locus (Ref. 20). Moreover, 26% of cases of apparently sporadic PPH have been found to harbour germline *BMPR2* mutations, with documented cases of de novo mutations (Refs 20, 21).

Currently, 57 different *BMPR2* mutations have been found: these comprise 14 missense, 13 nonsense and 15 frameshift mutations, five large deletions, three splice-site mutations and seven single-nucleotide polymorphisms (Ref. 20; see http://www.le.ac.uk/genetics/pphgenes/index.html for the latest analysis). Mutations span the ligand-binding domain, as well as the transmembrane, kinase and cytoplasmic tail domains. Most nonsense and frameshift mutations are predicted to result in premature truncation of the gene transcript (58% of all mutations), with nonsense-mutation-mediated mRNA decay preventing production of the mutated BMPR-II polypeptide. Therefore, it seems likely that the molecular mechanisms for altered BMPR-II function, at least in some cases, involve haploinsufficiency. However, it has been reported that specific disease-causing mutations in the ligand-binding and kinase domains of BMPR-II exert dominant-negative effects on BMP signalling (Ref. 20). Although *BMPR2* possesses a $(GGC)_{8–16}$ trinucleotide repeat (Ref. 16), there is no evidence for expansion of trinucleotide repeats, which is known to underlie genetic anticipation in other diseases.

The true frequency of *BMPR2* mutations might be underestimated since the techniques employed so far could have failed to detect large gene deletions or rearrangements. Furthermore, the absence of *BMPR2* mutations in some families



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affected with PPH and in the majority of sporadic cases suggests that further genes, possibly related to the BMP/transforming growth factor (TGF)-β pathway, might yet be implicated in this disease. Indeed, mutations in the gene encoding endoglin (a TGF-β receptor complex accessory protein) and the gene for activin receptor-like kinase type 1 (ALK-1; the putative TGF-β type I receptor) have been identified in patients with hereditary haemorrhagic telangiectasia (HHT) type 1 and type 2, respectively (Refs 22, 23). HHT is a rare, autosomal dominant disorder characterised by vascular dysplasia and haemorrhage, and defects in ALK-1 are thought to disturb the signalling pathways that control angiogenesis. Patients with HHT can also develop severe plexiform pulmonary hypertension, which is pathologically indistinguishable from PPH (Ref. 22).

## Bone morphogenetic proteins

### BMPs and vascular remodelling

BMPs are the largest group of cytokines within the TGF-β superfamily and were originally identified as molecules regulating growth and differentiation of bone and cartilage (Ref. 24). BMPs are now known to regulate growth, differentiation and apoptosis in a diverse number of cell lines including mesenchymal and epithelial cells (Refs 24, 25), acting as instructive signals during embryogenesis and contributing to the maintenance and repair of adult tissues (Ref. 26). BMP-7 inhibits serum-stimulated and growth-factor-induced proliferation of human aortic smooth muscle cells (SMCs) (Ref. 26), and BMP-2 inhibits injury-induced initimal hyperplasia in a rat carotid artery balloon injury model (Ref. 27). Furthermore, BMPs inhibit cell proliferation of pulmonary artery SMCs (PASMCs) isolated from normal control patients but fail to inhibit growth of PASMCs isolated from PPH patients (Ref. 28). Thus, disruption of BMP signalling pathways might result in the failure of a critical antiproliferative or differentiation mechanism in the pulmonary vasculature.

Studies of knockout mice reveal the critical role of the BMP pathway in vascular development. Homozygosity for a null mutation in *BMPR2* is lethal during early embryogenesis (Ref. 29), and mice deficient in Smad-5, one of the BMP-restricted Smad proteins that mediate signalling (see below), die as a result of angiogenesis defects – specifically, failure to recruit vascular smooth muscle to endothelial structures (Ref. 30).

### BMP signalling pathways

#### *BMP receptors and ligands*

TGF-β superfamily receptors are serine/threonine kinase receptors that exist either as constitutive heterodimers or are recruited to receptor complexes upon ligand stimulation (Refs 31, 32). Type II BMP receptors are distinguished from other TGF-β superfamily type II receptors by a long C-terminal sequence of unknown function following the intracellular kinase domain (Ref. 33). Both long- and short-form BMPR-II have been isolated, with the short-form splice variant lacking almost the entire exon 12 (Ref. 33). Although the short form of the receptor is widely expressed in human tissues [J.A. Flanagan (University of Leicester, Leicester, UK), pers. commun.], it is not known whether this form of the receptor serves a differential function compared with the long form. Type I receptors have short cytoplasmic domains and directly phosphorylate Smad proteins (Ref. 24). Two BMP type I receptors have been identified: BMPR-IA/ALK-3 and BMPR-IB/ALK-6 (Ref. 34).

Binding of specific ligands [BMP-2, BMP-4, BMP-7, growth differentiation factor (GDF)-5 or GDF-6] (Refs 35, 36) to the extracellular ligand-binding domain of BMPR-II results in the formation of a heterodimer receptor complex with BMPR-I (Ref. 32). Pre-formed type-I–type-II receptor complexes also appear to exist (Ref. 32). The specific signalling pathway activated by BMPR-II might depend on whether pre-formed complexes are stimulated by ligand (in a Smad-dependent pathway) or whether ligand leads to recruitment of type I and type II receptors to the signalling complex [in a mitogen-activated protein kinase (MAPK)-dependent pathway] (Fig. 2). It is likely that pre-formed complexes are heterodimers containing one type I and one type II receptor, whereas ligand-recruited complexes consist of homo-oligomerised type I receptors and one type II receptor. Thus, structural differences in the receptor complex might account for the selection of distinct signalling pathways (Ref. 32). Following ligand binding in both types of receptor complex, the type II receptor phosphorylates a glycine/serine-rich domain on the proximal intracellular portion of an associated type I receptor (Fig. 2).

By contrast to the other receptor systems of the TGF-β superfamily, BMP type I receptors are able to bind ligand with high affinity whereas the type



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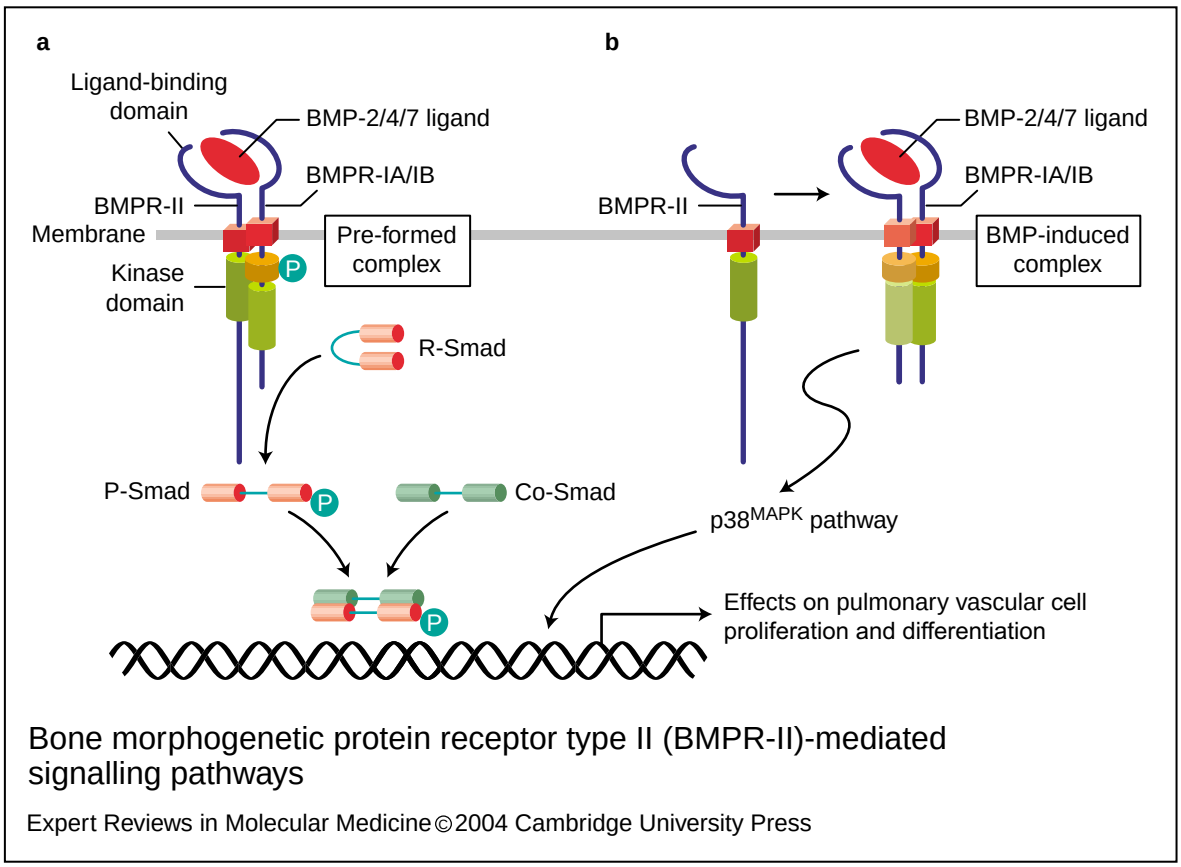



**Figure 2. Bone morphogenetic protein receptor type II (BMPR-II)-mediated signalling pathways.** (a) Following ligand binding to a pre-formed heterodimeric receptor consisting of one BMPR type II (BMPR-II) and one BMPR-I, BMPR-II phosphorylates BMPR-I; activated BMPR-I in turn phosphorylates BMP-restricted Smads (R-Smads: Smad-1, -5 and -8), which, once phosphorylated (P-Smads), form a complex with the common-partner Smad (Co-Smad: Smad-4), to translocate to the nucleus and regulate gene transcription. (b) However, if ligand binding leads to recruitment of receptors to a complex consisting of one BMPR-II and homo-oligomerised BMPR-I, subsequent BMPR-II phosphorylation of BMPR-I might lead to activation of mitogen-activated protein kinases (MAPKs), including $p38^{MAPK}$, in certain cell types.

II receptor binds with low affinity. However, the conformational changes that occur in the ligand–receptor complex when both receptor types contact ligand are required for crosslinking of the ligand to BMPR-II and the subsequent intracellular signal transduction (Ref. 33).

Despite the expanding body of research in this area, the precise structure and form of the active BMPR-II receptor complex is unresolved and demands further study.

### *Smad- versus MAPK-dependent BMP signalling*

In the presence of ligand, activated type I receptors in turn phosphorylate cytoplasmic signalling proteins known as Smads, which are responsible for TGF-β superfamily signal transduction (Refs 24, 35, 37, 38). BMPs signal via a restricted set of receptor-mediated Smads (R-Smads: Smad-1, -5 and -8), which, once phosphorylated (P-Smads), must form a complex with the common-partner Smad (Co-Smad: Smad-4), to translocate to the nucleus (Refs 24, 37) (Fig. 2). Thus, Smad proteins function in relaying signals from the cell surface to the nucleus. Target gene transcription is regulated by a variety of mechanisms, including direct binding of the Smad complex to DNA, interaction with other DNA proteins, such as AP-1 and TFE-3, and recruitment of transcriptional co-activators or co-repressors (Ref. 24). The net result of such signalling is an antiproliferative effect on the pulmonary vasculature (Ref. 28).

Although Smad signalling has long been



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recognised as the canonical BMP signalling pathway, there is mounting evidence that the MAPKs, including $p38^{MAPK}$, $p42/44^{MAPK}$ (ERK1/2) and JNK/SAPK, are modulated by BMPs and TGF-βs in certain cell types (Refs 31, 39, 40). A recent study has shown activation of $p38^{MAPK}$ by Smad-independent, BMP-activated pathways (Refs 32, 41). Evidence suggests that $p38^{MAPK}$ and $p42/44^{MAPK}$ are constitutively activated in fibroblasts derived from the remodelled pulmonary circulation of chronically hypoxic rats (Ref. 41). Inhibition of $p38^{MAPK}$ but not $p42/44^{MAPK}$ prevented the enhanced proliferative capacity of these fibroblasts. These data suggest that disruption of Smad-dependent signalling might not be the only, or indeed the most important, pathway downstream of BMPR-II and involved in the pathogenesis of PPH.

The specific pathway activated by BMPR-II might depend on whether pre-formed type-I–type-II heterodimers are stimulated by ligand (Smad-dependent pathway), or whether ligand leads to recruitment of type I and II receptors to the signalling complex (MAPK-dependent pathway) (Fig. 2). Crosstalk between the Smad and MAPK pathways has been reported, with $p42/44^{MAPK}$ activation leading to phosphorylation of the Smad-1 linker region and the subsequent inhibition of nuclear translocation of Smad-1 (Ref. 42). Thus, at least one effect of the MAPK-dependent pathway might be to antagonise the antiproliferative effects of the Smad-dependent pathway and allow vascular cell proliferation.

### *BMP signalling regulation*

The predilection of PPH for the pulmonary vessels is at first sight surprising, considering the widespread involvement of BMPs during development. However, the tissue specificity of the system is dictated by receptor and ligand expression, as well as by a wide variety of pre- and post-receptor mechanisms.

PASMCs and human pulmonary artery endothelial cells (HPAECs) express a wide range of TGF-β superfamily receptors, including BMPR-II and BMPR-IB, and bind both TGF-β and BMP-4 (Ref. 28). Interestingly, PASMCs but not HPAECs have been shown to express BMPR-IA. Activation of these receptors in PASMCs and HPAECs by BMPs leads to phosphorylation of Smad-1, $p38^{MAPK}$ and $p42/44^{MAPK}$, and induction of mRNAs for Smad-6 and Smad-7.

BMP signalling is regulated at many different levels. These include modulation through: endogenous inhibitors of BMP binding/signalling (e.g. noggin, chordin, follistatin, BAMBI and Smurf-1) (Ref. 43); the levels of expression of specific BMPs; activation of the inhibitory Smads (Smads -6 and -7) (Ref. 44); interactions with other growth signalling pathways, possibly such as those downstream of serotonin receptors; and nuclear co-activators and co-repressors. In addition, BMP signalling might be regulated by the type and density of type I and II receptors available for dimerisation, which might dictate the relative levels of pre-formed versus ligand-induced receptor complexes and therefore the differential activation of downstream signalling pathways. Such diverse levels of regulation could be responsible for the tissue specificity of BMP signalling and might, for example, underlie the pulmonary-specific pathology seen in PPH.

## TGF-β superfamily receptors and pulmonary vascular disease

The effects of TGF-β superfamily signalling on vascular growth and structure are complex and highly context specific, depending on the ligand, heteromeric receptor complexes, cell type, downstream signals and transcriptional programme. The identification of *BMPR2* mutations in PPH provides further evidence for the crucial role of the TGF-β superfamily in pulmonary vascular disease.

### TGF-βR-II

Microsatellite instability leading to spontaneous somatic mutation in the TGF-β type II receptor gene (*TGF-βR2*) and genes involved in the regulation of apoptosis have been identified more frequently in plexiform lesions from patients with PPH than in plexiform lesions of patients with other forms of pulmonary hypertension (Ref. 45). In approximately 30% of plexiform lesions there is a somatic frameshift mutation in *TGF-βR2*, encoding a premature stop codon. Furthermore, in 90% of plexiform lesions, TGF-βR-II protein is not produced, in contrast to the abundant expression in endothelial cells outside these lesions (Ref. 45). Thus, it is possible that monoclonal cell expansion of endothelial cells within the plexiform lesions might be associated with this mutation.

### TGF-β ligand

Several studies have implicated TGF-β ligand



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in the pathogenesis of PPH. In PASMCs from PPH patients, TGF-β1 enhances cell proliferation, in contrast to the growth-inhibitory effect observed in normal PASMCs (Ref. 28). This is not due to alterations in TGF-β1 receptor ratios or downregulation of the TGF-β1 type II receptor (Ref. 28). In addition, elevated levels of TGF-β2 and TGF-β3, but not TGF-β1, are found in remodelled PPH arteries (Ref. 46).

### Endoglin and ALK-1

As mentioned above, mutations in the genes encoding endoglin and ALK-1 underlie HHT types 1 and 2, respectively (Ref. 47) – conditions frequently complicated by pulmonary arteriovenous malformation; in addition, ALK-1 mutations might lead to the development of PPH in some families with HHT (Ref. 22). At first sight, it appears paradoxical that mutations in similar pathways can lead to vascular dilation lesions (arteriovenous malformations) on the one hand and vascular obstruction (PPH) on the other. It appears that the effects of TGF-β on vascular cell differentiation and proliferation are complex, and depend both on the local vascular context and interactions with additional environmental or genetic factors.

## Serotonin and PPH

Serotonin (5-hydroxytryptamine or 5-HT) is implicated in the molecular pathogenesis of PPH. PASMCs from patients with PPH display a greater proliferative response to 5-HT compared with PASMCs derived from normal controls (Ref. 48), and patients with PPH have a sustained increase in circulating plasma levels of 5-HT (Ref. 49). Furthermore, appetite suppressants are known to inhibit 5-HT uptake by transporters and further augment circulating 5-HT levels by triggering indoleamine release. Ingestion of appetite suppressants such as anorexigens of the fenfluramine group for a period of three months or longer is reported to increase the risk of developing PPH by a factor of 3.7- to 23-fold in various studies (Refs 8, 50).

There is much debate as to whether the 5-HT cell-surface receptors or the 5-HT transporter (5-HTT) play a pivotal role in 5-HT-induced cell proliferation. Inactivation of $5\text{-HT}_{2B}$ receptors in mice protects against the development of hypoxic pulmonary hypertension (Ref. 51); in addition, in both humans and mice, pulmonary hypertension is associated with increased levels of $5\text{-HT}_{2B}$ receptor expression (Ref. 51). A number of observations also support a role for 5-HTT in PPH. It has been suggested that several anorexigens might act as substrates for 5-HTT and, following transport into the cell, might effect mitogenic events similar to those arising from 5-HT (Ref. 52). Transgenic mice lacking the gene for 5-HTT are less susceptible to the development of hypoxic pulmonary hypertension compared with control animals (Ref. 53). Conversely, mice that overexpress 5-HTT exhibit exaggerated hypoxia-induced pulmonary hypertension (Ref. 54). Lung tissue taken from PPH patients displays increased expression of 5-HTT (Ref. 48). Homozygosity for a 5-HTT promoter polymorphism (L-allele) is significantly increased in a PPH patient population compared with a control population (65% versus 27%); this polymorphism is associated with increased gene transcription and 5-HT-induced PASMC proliferation (Ref. 48). Thus, although the precise molecular mechanism whereby 5-HT contributes to the pathogenesis of PPH is yet to be fully resolved, there is no doubt as to its importance in understanding the development of this disease.

## Other potential contributors to pathogenesis

PASMCs derived from PPH patients have low mRNA levels for a voltage-gated potassium channel ($K_V$ channel) and decreased channel current, together with a corresponding increase in intracellular calcium levels, compared with controls (Refs 55, 56). Intracellular calcium is a widely recognised stimulant for PASMC contraction and hypertrophy. The '$K_V$ channel hypothesis' for PPH is strengthened by the finding that some anorexigens block these channels in PASMCs (Ref. 57).

Patients with PPH produce increased levels of the vasoconstrictors thromboxane (Ref. 58) and endothelin 1 (Ref. 59), and decreased levels of the catalyases of vasodilator formation prostacyclin (Ref. 58) and nitric oxide synthase (Ref. 60). It is possible that an imbalance between vasodilators and vasoconstrictors is important in the pathogenesis of PPH; certainly, these observations have been crucial to the development of current medical therapy.

Two recent studies have focused attention on the possible role of angiopoietin 1 in PPH. Intriguingly, the two studies appear to reach



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conflicting conclusions as to whether the growth factor is causative or curative with respect to the disease. One study showed that administration of angiopoietin 1 has dramatic protective effects in the rat model of monocrotaline-induced pulmonary hypertension, and hypothesised that this was due to the rescue of endothelial cells from apoptosis (Ref. 61). A separate study reported elevated levels of angiopoietin 1 in lung tissue from PPH patients and went on to demonstrate an associated decrease in BMPR-IA levels (Ref. 62). The authors suggest that angiopoietin 1 promotes SMC hyperplasia through downregulation of BMP signalling pathways. Additional studies are required to resolve this conflict and define the precise role of angiopoietin 1 in PPH.

## Research in progress and outstanding research questions

### Towards a model of PPH

With the discovery of the pivotal role of BMPR-II mutations in PPH, one can begin to construct models of pathogenesis.

#### *BMPs fail to suppress proliferation of cells from PPH patients*

It has been shown that BMPR-II mRNA and protein is expressed predominantly by endothelial cells, and to a lesser extent by SMCs and myofibroblasts, in the pulmonary circulation (Ref. 63). Recently, it was found that BMP-2, -4 and -7 inhibit the proliferation of SMCs derived from normal pulmonary arteries and from patients with secondary pulmonary hypertension, but fail to suppress proliferation of cells from patients with PPH, including cells obtained from PPH cases in which no specific *BMPR2* mutations have been identified (Ref. 28). Thus, it is hypothesised that the failure of growth-inhibitory effects of BMPs in PPH cells contributes to the vascular obliteration and remodelling that characterise the condition.

#### *BMPR-II expression is reduced in PPH patients*

Although familial PPH is characterised by low gene penetrance, a recent study has demonstrated that apparently unaffected carriers of the disease gene have abnormally high increases in pulmonary pressures on exercise (Ref. 64). Furthermore, a recent study has shown that lung BMPR-II mRNA and protein expression is dramatically reduced in patients harbouring a mutation in *BMPR2* that is predicted to result in truncation of the protein (two partial deletions and one exon 3 frameshift mutation) (Ref. 63). Moreover, receptor protein expression was markedly reduced in PPH cases in which no mutation in *BMPR2* has been identified. A small but significant reduction was also observed in cases of secondary pulmonary hypertension. Since the severity of pulmonary hypertension in primary and secondary cases was similar, and the pathology of all groups demonstrated severe plexiform vasculopathy, it is likely that the dramatic reduction in BMPR-II protein expression in PPH cases is not merely a consequence of elevated PAP. Rather, it suggests that markedly reduced expression of BMPR-II is a specific feature of PPH and that lesser reductions in BMPR-II expression might contribute to other forms of pulmonary hypertension. These findings stress the importance of understanding how other environmental and genetic factors regulate the expression of BMPR-II in the lung.

#### *The 'two-hit' hypothesis*

The low penetrance of the disease, together with the existence of pathological changes indistinguishable from PPH arising from injury caused by external agents such as appetite suppressants (Ref. 8), supports a 'two-hit' hypothesis in which the vascular abnormalities characteristic of PPH are triggered by the accumulation of genetic and/or environmental insults in a susceptible individual (Fig. 3). For example, a germline *BMPR2* mutation in combination with a somatic mutation in the BMP pathway or one of the related pathways regulating cell growth and apoptosis could be sufficient to generate clinical disease. Environmental injury, such as the ingestion of appetite suppressants resulting in an increase in 5-HT-activated signalling pathways, might also impose an additional burden predisposing to disease. It is of interest to note that acquired somatic mutations in the TGF-β type II receptor and Smad-4 are well-recognised associations with certain gastrointestinal cancers, a disease process in which such a two-hit paradigm has been advocated (Ref. 31).

### Molecular analysis of *BMPR2* mutations

The exact mechanism by which *BMPR2* mutations disrupt BMP signalling has begun to be elucidated. Two recent studies have



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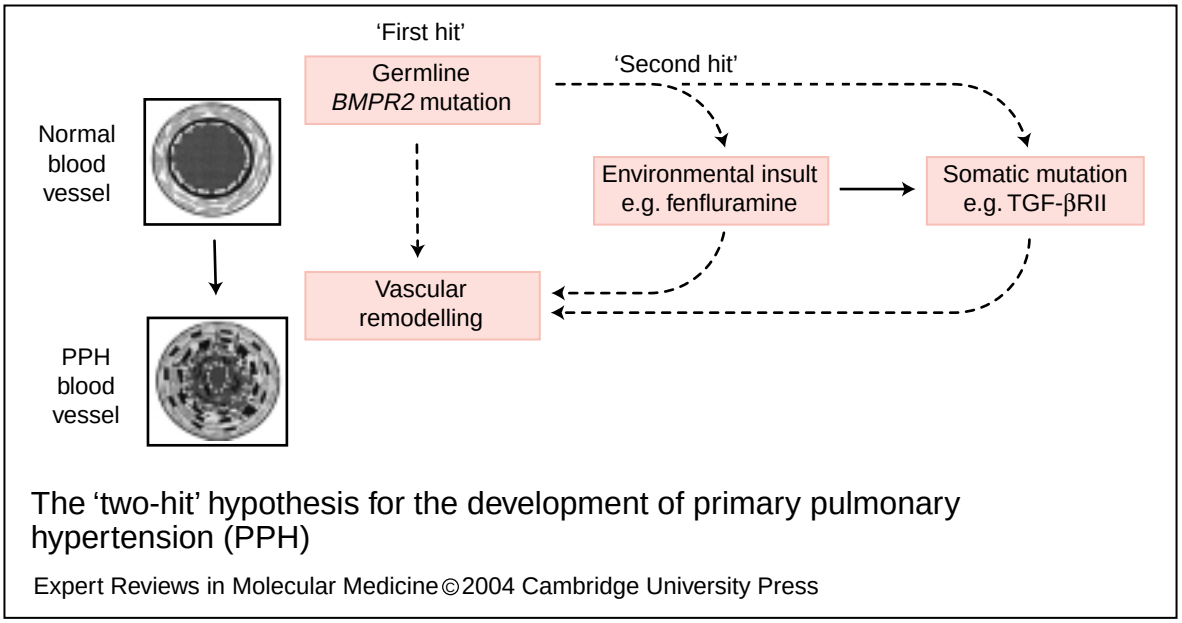



**Figure 3. The 'two-hit' hypothesis for the development of primary pulmonary hypertension (PPH).** According to this hypothesis, the vascular abnormalities characteristic of PPH are triggered by the accumulation of genetic and/or environmental insults in a susceptible individual. For example, to generate clinical disease, the combination of a germline *BMPR2* mutation (the 'first hit') with either (1) a somatic mutation in the BMP pathway, or one of the related pathways regulating cell growth and apoptosis, or (2) an environmental insult such as the ingestion of appetite suppressants (the 'second hit') might be required for vascular remodelling. Abbreviations: BMP, bone morphogenetic protein; BMPR, BMP receptor; TGF-βRII, transforming growth factor β receptor II.

shown that the mechanism by which mutated BMPR-II disrupts BMP/Smad signalling is both heterogeneous and mutation-specific (Refs 65, 66). Substitution of cysteine residues within the ligand-binding or kinase domain of BMPR-II leads to failure of trafficking of the mutant protein to the cell surface, a process that interferes with BMP type I receptor trafficking (Fig. 4). By contrast, noncysteine mutations within the kinase domain reach the cell surface but fail to activate Smad-responsive luciferase reporter genes as a result of an inability to phosphorylate BMP type I receptors (Fig. 4). All mutations studied in the ligand-binding and kinase domains exhibit a dominant-negative effect on wild-type receptor function in terms of Smad signalling. Interestingly, BMPR-II mutants with missense mutations involving the cytoplasmic tail are able to traffic to the cell surface and are capable of activating Smad-responsive luciferase reporter genes (Fig. 4). However, a feature common to mutants affecting all three receptor domains is the ligand-independent activation of $p38^{MAPK}$. Furthermore, normal mouse epithelial cells transfected with mutant receptors show enhanced serum-induced proliferation compared with wild-type-transfected cells (Ref. 65), and this abnormal proliferation is completely abolished by treatment with a selective $p38^{MAPK}$ inhibitor, SB203580 (Ref. 65). This latter result suggests that the enhanced proliferative rates seen in cells transfected with mutant BMPR-II receptors are dependent on activation of the $p38^{MAPK}$ pathway.

On the basis of these studies, it is hypothesised that reduced cell-surface expression of BMPR-II favours ligand-induced receptor complexes and the activation of $p38^{MAPK}$-dependent pro-proliferative pathways, while inhibiting Smad-dependent signalling in a mutation-specific manner. Since familial PPH is a condition associated with low penetrance, this gain of function in $p38^{MAPK}$ correlates better with clinical genetic findings than the dominant-negative effect exerted by some BMPR-II mutants on BMP/Smad signalling, which would be expected to be fully penetrant.

Further studies will be necessary to determine whether similar abnormalities can be found in more-relevant cell lines and in in vivo animal models, using knockout and transgenic mice. In



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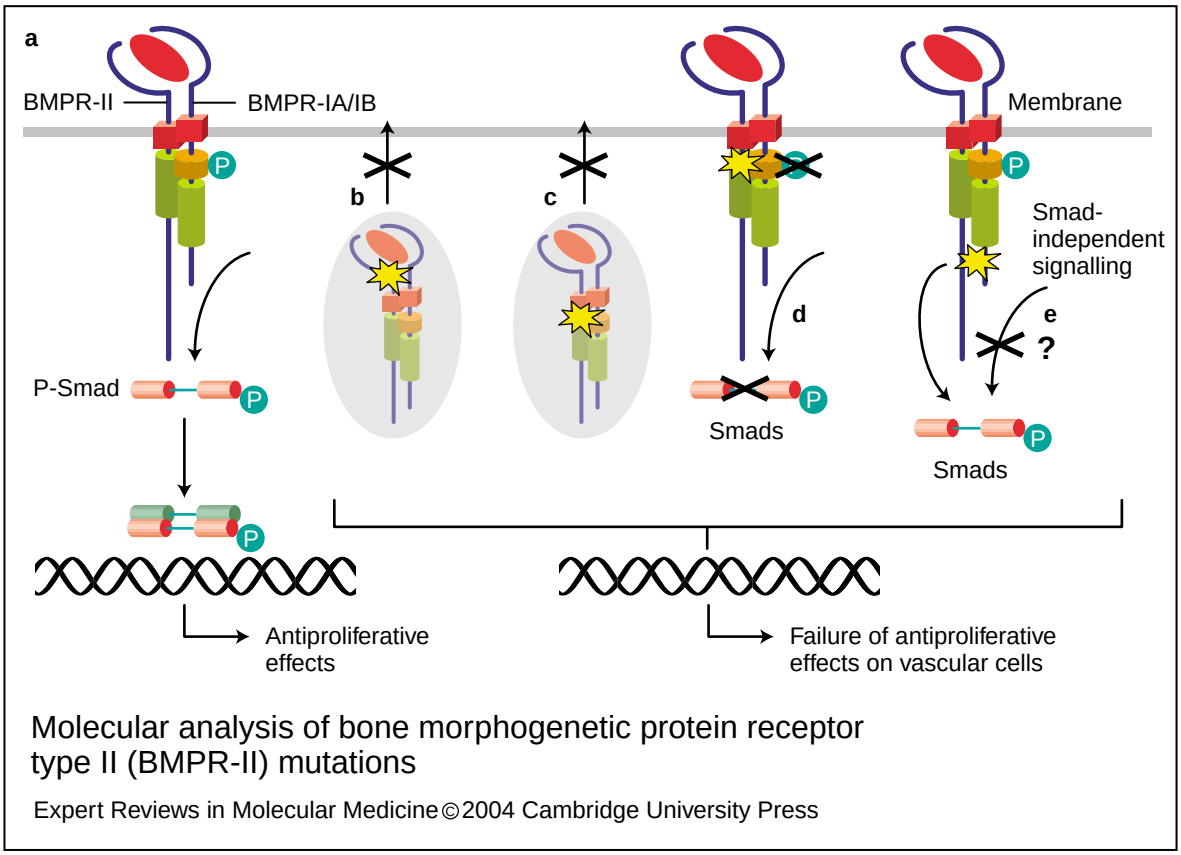



**Figure 4. Molecular analysis of bone morphogenetic protein receptor type II (BMPR-II) mutations.** (a) Binding of BMP to wild-type BMPR-II initiates a signalling pathway that results in the inhibition of cell proliferation in pulmonary artery smooth muscle cells. Substitution of cysteine residues within the ligand-binding (b) or kinase (c) domain of BMPR-II leads to failure of trafficking of the mutant protein to the cell surface, a process that interferes with BMP receptor type I (BMPR-I) trafficking. (d) By contrast, noncysteine mutations within the kinase domain reach the cell surface but fail to activate Smads as a result of an inability to phosphorylate BMPR-I. (e) Interestingly, BMPR-II mutants with missense mutations involving the cytoplasmic tail are able to traffic to the cell surface and signal via Smads; we therefore hypothesise that such cytoplasmic tail mutants might be defective in terms of activating mitogen-activated protein kinase (MAPK) signalling. Indeed, this defect might involve a gain of function in terms of enhanced activation of MAPK pathways, as shown by studies in which normal mouse epithelial cells were transfected with mutant receptors (Ref. 65). The disruption of BMP signalling pathways that results from mutations in BMPR-II appears to cause the failure of the antiproliferative/differentiation mechanism in the pulmonary vasculature. Mutations are indicated throughout by a flash symbol.

addition, it is now imperative to elucidate the possible interactions between BMP signalling pathways and other molecular factors implicated in this disease, such as serotonin signalling pathways and $K_V$ channels.

## Clinical implications

### Screening

The discovery of *BMPR2* mutations in familial PPH has raised the possibility of screening for the disease among relatives of patients with a positive family history and a known mutation. The value of screening in relatives of patients without a defined mutation requires further investigation. It has been postulated that recording responses to an exercise tolerance test could be used to screen patient relatives and to identify so-called 'latent PPH' (Ref. 64), a concept that demands closer interrogation.

### Treatment

Current medical treatment for PPH includes the use of anticoagulants, calcium-channel blockers, continuous intravenous or nebulised prostacyclin analogues, and endothelin receptor antagonists (Ref. 67). Although anticoagulants have been



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utilised in the treatment of PPH for many years, there have been no rigorous, properly controlled clinical trails to assess their efficacy. Furthermore, with the increasing survival rates of patients receiving combined therapy, the proven side effects of long-term anticoagulant therapy must be taken into account. In routine treatment, patient response to an acute vasodilator challenge such as nitric oxide or prostacyclin administration is tested, and positive responders are given a trial of calcium-channel blockers. Fewer than 15% of PPH patients respond positively to the initial trial of calcium-channel blockers; however, in these patients, such agents have been proven to result in sustained improvement in exercise tolerance and haemodynamic profile, and increased survival (Refs 68, 69). Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation. Intravenous epoprostenol has been shown to improve haemodynamics and survival in a large percentage of PPH patients, including patients who do not respond to acute vasodilator challenge (Refs 70, 71, 72). However, treatment requires continuous intravenous delivery, and patients can suffer complications from long-dwelling catheterisation. Other reported side effects include jaw pain, headache, flushing, diaorrhoea and nausea (Ref. 71). Prostacyclin therapy is contra-indicated in pulmonary veno-occlusive disease because of the risk of developing pulmonary oedema (Ref. 73).

Future advances in medical therapy, as well as novel therapies such as the specific phosphodiesterase inhibitors that are currently being clinically assessed (Ref. 74), might serve to improve survival, but lung transplantation remains the only curative treatment for PPH. However, reports show an accelerated and increased frequency of chronic rejection in transplanted PPH patients, as compared with other patient groups (Ref. 75). Atrial septostomy, aimed to reduce right cardiac load, is used only as a last resort in treatment or as a bridge to transplantation.

Thus, it seems certain that elucidation of the molecular and cellular mechanisms underlying PPH will provide a powerful basis for the development of novel therapeutic strategies in the treatment of this devastating condition.

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**Reviews**

Overview of the pulmonary circulation:

Hughes, J.M.B. and Morrell, N.W. (2001) Pulmonary Circulation: From Basic Mechanisms to Clinical Practice, Imperial College Press

Summary of risk factors for the development of pulmonary arterial hypertension:

Humbert, M. et al. (2001) Risk factors for pulmonary arterial hypertension. Clin Chest Med 22, 459-475, PubMed: 11590841

A recent review:

Runo, J.R. and Loyd, J.E. (2003) Primary pulmonary hypertension. Lancet 361, 1533-1544, PubMed: 12737878

**Websites**

WHO classification of pulmonary hypertension (World Health Organisation 3rd World Symposium for Pulmonary Arterial Hypertension, Venice, 2003):

http://www.who.int

Pulmonary Hypertension Association and Phcentral are patient support organisations:

http://www.phassociation.org/
http://www.phcentral.org/

A database run by Rajiv Machado at the University of Leicester, UK, gives up-to-date details of PPH-related mutations of *BMPR2*:

http://www.le.ac.uk/genetics/pphgenes/index.html

OMIM entry for PPH:

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=178600

OMIM entry for *BMPR2*:

http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=600799

## Features associated with this article

Figure 1. Intimal lesions characteristic of primary pulmonary hypertension (PPH).
Figure 2. Bone morphogenetic protein receptor type II (BMPR-II)-mediated signalling pathways.
Figure 3. The ‘two-hit’ hypothesis for the development of primary pulmonary hypertension (PPH).
Figure 4. Molecular analysis of bone morphogenetic protein receptor type II (BMPR-II) mutations.

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