Genetic and molecular mechanisms of pulmonary hypertension

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ABSTRACT – Pulmonary arterial hypertension frequently occurs secondary to common cardiopulmonary diseases, and more rarely as a primary condition. Observations in animals and man have suggested genetic influences underlying the susceptibility to pulmonary hypertension. A gene underlying many cases of familial and apparently sporadic cases of primary pulmonary hypertension has now been identified, and a genetic approach to hypoxia-induced pulmonary hypertension promises to reveal new insights into the unique nature of the pulmonary circulation. The exploration of potential candidate genes influencing susceptibility and the identification of new genes promise new therapeutic approaches to the treatment of pulmonary hypertension.

The unique nature of the pulmonary circulation

The main role of the pulmonary circulation is to deliver deoxygenated blood to the alveolar capillaries where gas exchange occurs. To achieve this vital role the pulmonary circulation has developed as a high flow, low resistance vascular bed to protect the thin blood-gas barrier from high intravascular pressures that would otherwise promote alveolar oedema formation. Consequently the mean pulmonary arterial pressure (~13mmHg) is approximately one fifth of the systemic arterial pressure, and because of the great capacity of the pulmonary circulation to recruit normally closed vessels, rarely rises above 30mmHg even at very high levels of cardiac output[1]. In contrast to the systemic circulation, the normal pulmonary circulation exhibits minimal resting tone, and appears to be almost maximally dilated under resting conditions. The maximum resistance in the pulmonary circulation lies in the precapillary arterioles, the site of some of the earliest pathological changes during the development of pulmonary hypertension. One of the intriguing features of the pulmonary circulation is vasoconstriction to hypoxia[2], whereas the systemic circulation responds by vasodilatation. This mechanism, when localised to a particular lung segment or lobe, serves to preserve arterial oxygenation by reducing blood flow to underventilated lung regions, for example in pneumonia or acute asthma[3]. However, the importance of this role should not be overestimated, since it is only about 50% efficient at preserving arterial oxygenation in man and the intensity of hypoxic pulmonary vasoconstriction varies greatly between species. Indeed, it could be argued that the mechanism is a vestigial response representing the equivalent of the amphibian reflex that diverts blood flow from the lungs to the skin, which serves as an alternative organ of gas exchange during hibernation[4]!

Hypoxia-induced pulmonary hypertension

When alveolar hypoxia involves most of the lung and is prolonged, any usefulness of acute hypoxic pulmonary vasoconstriction is offset by a rise in pulmonary arterial pressure. This is accompanied by structural changes in small peripheral pulmonary arteries, consisting of increased wall thickness of muscular arteries and the appearance of new muscle in normally non-muscular arteries[5,6]. Such changes occur in the lungs of patients with chronic obstructive airways disease and in populations living at high altitude[7].

Evidence for genetic susceptibility to hypoxia-induced pulmonary hypertension

A genetic component to hypoxia-induced pulmonary hypertension has been suggested by observations in several animal species. It was noted some years ago that imported Hereford cows reared at altitude in the high plains of the Colorado Rocky Mountains in the United States were susceptible to high altitude pulmonary hypertension[8]. This manifested as 'brisket disease' where the region at the front of the cow's chest (the brisket) becomes oedematous. This trait affected only a minority of the cows and was found to be inherited[8]. It was subsequently possible to breed the trait out of the herds by only breeding from resistant animals. In an early and elegant embryo transfer experiment, the genetic component was confirmed by transferring hypoxia-susceptible embryos to hypoxia-resistant

heifers. The calves when born retained the susceptibility to hypoxia-induced pulmonary hypertension, excluding the maternal uterine environment as an important factor[9].

Different strains of rat have different susceptibilities to hypoxia-induced pulmonary hypertension[10,11]. For example, the Fisher rat is more resistant to hypoxia-induced pulmonary hypertension than the Wistar-Kyoto rat strain (Fig 1A)[12]. This does not seem to be due to a difference in oxygen sensing because the rats exhibit a similar degree of polycythaemia in response to chronic hypoxia. Furthermore, the anatomy of their small pulmonary arteries is similar under normoxic conditions, but the Wistar-Kyoto rat develops more severe pulmonary vascular remodelling and right ventricular hypertrophy during chronic hypoxia[13]. Analysis of pulmonary artery pressures after three weeks' exposure to chronic hypoxia in the parental strains shows clear separation of the pulmonary artery pressures (Fig 1B). In a cross breeding experiment the F1 generation demonstrates intermediate values for pulmonary arterial pressures, whereas inter-sibling mating of this F1, to produce the F2 generation, results in animals with a range of pulmonary artery pressures that span the original parental values. A genomic screen using polymorphic microsatellite markers has revealed a quantitative trait locus (QTL) on rat chromosome 17 that is linked to right ventricular hypertrophy in these animals[14]. Further proof that this region is involved in the differential susceptibility will involve the production of congenic rat strains in which the QTL is introduced into the resistant Fisher strain, thereby conferring susceptibility. A search for potential candidate genes in this region may be assisted by the identification of genes differentially expressed in hypoxic rat lung.

Large differences have been reported between individuals in the magnitude of the pulmonary artery pressor response to acute hypoxia. In addition, studies in the town of Leadville (3100m above sea-level) in the Colorado Rockies showed that the degree of pulmonary hypertension measured at right heart catheterisation varied widely between subjects, with some individuals demonstrating marked elevation of pulmonary arterial pressure[15].

These observations are consistent with differing genetic susceptibilities to hypoxia-induced pulmonary hypertension. Measurements of pulmonary artery pressure in different populations living at high altitude also suggest genetic factors in the control of the pulmonary vascular response to hypoxia[16]. There is significant variation in the magnitude of pulmonary hypertension between different high altitude populations. For example, the Tibetans exhibit less pulmonary hypertension than the inhabitants of the Peruvian Andes, who in turn are less susceptible than recent immigrants to high altitude, such as Caucasian Americans, and the Han Chinese in Tibet. Interestingly the least susceptible population is the Tibetans whose ancestors have lived at altitude for more than 50,000 years[17]. It would seem from these studies of peoples living at high altitude that it is genetically advantageous to exhibit a blunted cardiovascular response to chronic hypoxia, and that the susceptibility to hypoxia-induced pulmonary hypertension is bred out over many generations.

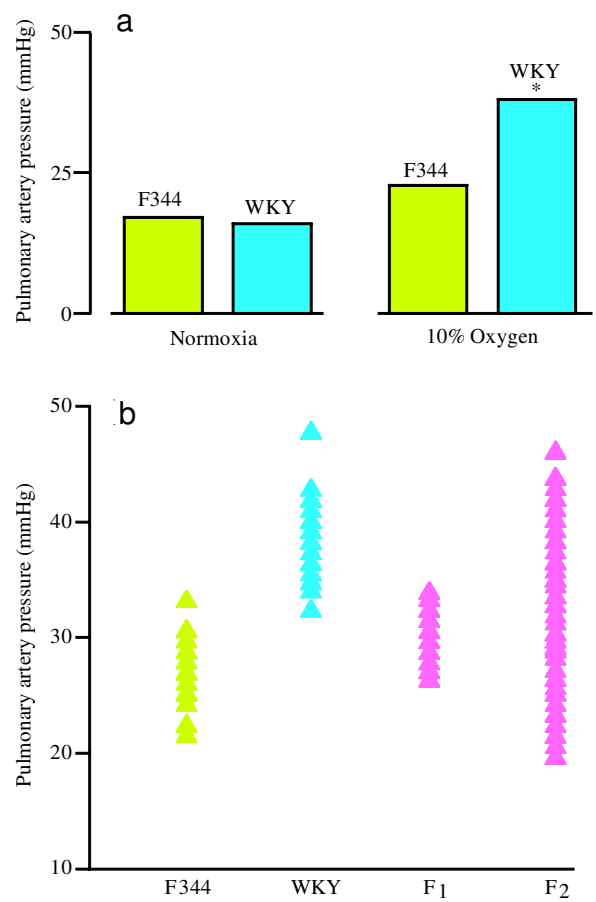


Fig 1. Pulmonary arterial pressures in two rat strains. Graphs showing the mean pulmonary arterial pressure (a) in the Fisher (F344) and Wistar-Kyoto (WKY) rat strains demonstrating the greater increase in pulmonary arterial pressure (PAP) in the WKY strain after 3 weeks of chronic hypoxia. The range of mean pulmonary arterial pressures in individual animals is shown in (b). When the two parental strains are cross-bred the resulting F1 generation has values of mean PAP intermediate between the parental strains. Inter-sibling mating generates the F2 generation with pressures that span the original parental values.

Illustration by Doig Simmonds from *Pulmonary Circulation: Basic Mechanisms to Clinical Practice*, Imperial College Press, 2001

Study of high altitude populations may provide the best means for identifying the genes that confer susceptibility to hypoxia-induced pulmonary hypertension in man. These studies are at an early stage, though an association has been reported between a common polymorphism in the gene for angiotensin converting enzyme (the I allele) and susceptibility to high altitude pulmonary hypertension in highlanders from the Kyrgyz Republic in Central Asia[18].

Mechanisms of hypoxia-induced pulmonary hypertension

Although circulating factors modulate tone in the pulmonary circulation, the capacity to constrict to hypoxia is a property that is intrinsic to the pulmonary artery smooth muscle cell. Thus smooth muscle cells isolated from peripheral pulmonary

arteries depolarise and contract when bathed in hypoxic cell culture media[19]. Acute hypoxia causes a rapid (within seconds) constriction of the pulmonary circulation which is inhibited by L-type Ca^{2+} channel blockers and enhanced by calcium channel openers such as BAY K 8644[20]. This indicates that hypoxia causes membrane depolarisation and Ca^{2+} influx via voltage-gated Ca^{2+} channels. The initial membrane depolarisation is associated with an inhibition of outward potassium current (I_K), demonstrated by whole-cell patch-clamp studies in freshly dispersed smooth muscle cells isolated from resistance arteries[21]. These electrophysiological studies have mostly indicated that the potassium channel inhibited at the initiation of acute hypoxic pulmonary vasoconstriction is of the K_{DR} subdivision of the K_V family[22]. This sequence of events is similar to that involved in O_2 chemotransduction in carotid body glomus cells.

With more prolonged hypoxia (minutes to hours) changes in gene expression occur within pulmonary vascular cells. Hypoxia can alter various cellular functions secondary to metabolic changes, but it is also clear that hypoxia has effects independent of anaerobic metabolism that seem to be a direct effect of low oxygen concentrations on gene expression. One of the best known examples of hypoxia-induced gene expression is the regulation of erythropoietin synthesis in the kidney and liver[23]. Hypoxaemia stimulates erythropoietin gene expression and the release of hormone into the blood stream. Erythropoietin stimulates red cell production from the bone marrow to increase oxygen delivery to the tissues. This results in the polycythaemia seen in some patients with chronic hypoxaemia.

The promoter regions of the erythropoietin gene and other hypoxia-inducible genes contain a consensus sequence for the binding of the transcription factor, hypoxia-inducible factor-1[24] (Fig 2)[25]. Hypoxia-inducible factor-1 (HIF-1) is a heterodimer of HIF-1α and HIF-1β, which form part of a family of basic-loop-helix DNA binding proteins. Of these two subunits it is the binding of the α subunit that is critical for the oxygen dependent response[26]. Whereas HIF-1β, which is also known as the aryl hydrocarbon receptor nuclear translocator (ARNT), can dimerize with several different basic loop helix transcription factors, HIF-1α is unique to HIF-1. HIF-1 messenger RNA and protein is widely expressed in mammalian cells and probably represents a ubiquitous mechanism for regulation of oxygen sensitive genes. It acts in concert with other transcription factors, for example the DNA binding complex termed activator protein-1 (AP-1) and nuclear factor-κβ (NF-κβ), to determine the activation of a functional transcriptional complex and the level of gene expression.

Endothelin-1 (ET-1) is a potent vasoconstrictor, the expression of which is regulated by hypoxia[27]. Patients with pulmonary hypertension, either idiopathic or secondary to underlying cardiopulmonary disease, have elevated plasma ET-1 levels[28]. Endothelin-1 has been implicated in the development of pulmonary hypertension and acts as a comitogen to stimulate proliferation of cultured human pulmonary artery smooth muscle cells[29]. Increased ET-1 and ppET-1 mRNA levels have been reported in lungs of chronically hypoxic rats[13], and differences in lung ET-1 expression may partly explain the differences in susceptibility to hypoxia-induced pulmonary hypertension between rat strains. Immunohistochemical studies have shown that ET-1 immunoreactivity is restricted to endothelial cells and less than 10% of bronchial epithelial cells in extra-acinar airways. In contrast, in chronically hypoxic rats, the bronchial epithelium stains intensely and uniformly, and small pulmonary arterioles show enhanced staining of the endothelium[13].

Angiotensin II (ANG II) is also a powerful vasoconstrictor in the pulmonary circulation and can stimulate activation of mitogen activated protein kinases and hypertrophy of human pulmonary artery smooth muscle cells via the ANG II type 1 receptor[30]. Angiotensin converting enzyme (ACE) is the main enzyme responsible for the generation of ANG II from ANG I, though other enzymes (e.g. human heart chymase) may also lead to production of ANG II independent of ACE. In the chronically hypoxic rat the expression of ACE mRNA and protein is increased in small peripheral pulmonary arteries undergoing remodelling[31]. In addition, right ventricular ACE activity and expression is increased during chronic hypoxia, particularly in fibrotic regions of the myocardium[32].

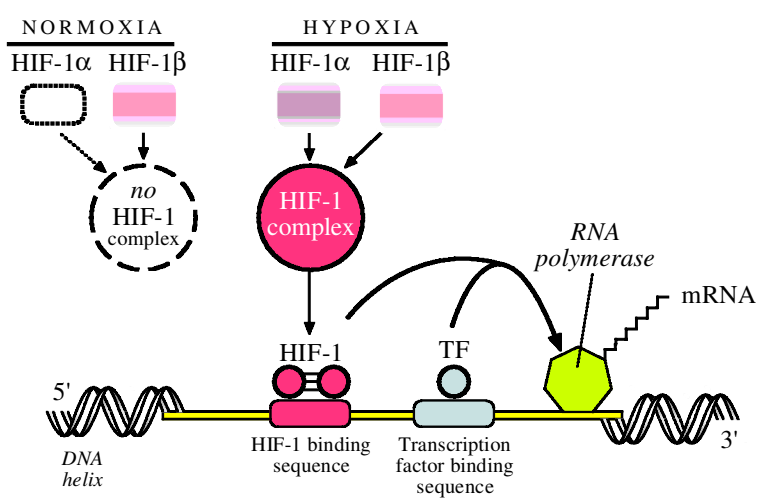


Fig 2. Schematic diagram of a gene promoter region showing the involvement of HIF-1 in hypoxia-inducible gene transcription. Hypoxia induces the formation of HIF-α, which dimerizes with the constitutuvely expressed HIF-1β to form the HIF-1 transcription factor. The presence of bound HIF-1, together with other transcription gactors (eg AP-1, NF-κβ), activates the transcriptional complex including RNA ploymerase leading to mRNA transcription.

Illustration by Doig Simmonds from *Pulmonary Circulation: Basic Mechanisms to Clinical Practice*, Imperial College Press, 2001

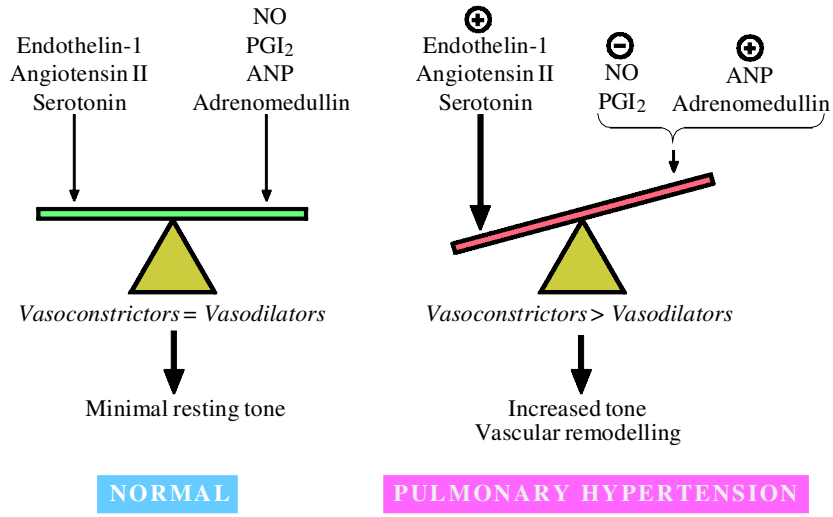


Fig 3. Vasoconstriction and vasodilatation in the pulmonary circulation. In the normal pulmonary circulation, there is a balance between the mediators of vasoconstriction and vasodilatation, maintaining minimal resting tone. In the hypertensive pulmonary circulation there is upregulation of vasoconstrictor pathways and reduced production of nitric oxide (NO) and prostacyclin (PGI_2). Although there are increased circulating levels of the vasodilators atrial natriuretic peptide (ANP) and adrenomedullin, this is insufficient to counteract the effect of vasoconstrictors, the net result being increased pulmonary vascular tone and vascular remodelling.

Illustration by Doig Simmonds from *Pulmonary Circulation: Basic Mechanisms to Clinical Practice*, Imperial College Press, 2001

Studies in knockout mice harbouring a targeted gene disruption have begun to provide important clues regarding the role of vasodilators (e.g NO, atrial natriuretic peptide) in susceptibility to pulmonary hypertension. Complete deficiency of atrial natriuretic peptide (ANP) or endothelial nitric oxide synthase (eNOS)[33] leads to a small (~20%) increase in basal pulmonary artery pressure. When mice lacking key factors such as ANP or its receptor, NPR-A[34], or eNOS[33] are exposed to a hypoxic environment for prolonged periods (>1 week) they develop more severe pulmonary hypertension, vascular remodelling and right ventricular hypertrophy than wild type mice. Thus alterations in the level of expression of these mediators (Fig 3), perhaps due to underlying genetic polymorphisms, could conceivably alter susceptibility to hypoxia-induced pulmonary hypertension in man.

Primary pulmonary hypertension

Primary pulmonary hypertension (PPH) is a rare disorder with an estimated incidence of 1–2 per million per year. It is more common in women (F:M sex ratio = 2.3:1), occurring especially in the reproductive years (mean age of onset 36 years). Primary pulmonary hypertension is a progressive, often fatal disorder with a median survival of only 2.8 years from diagnosis[35]. The disease is characterised by vascular cell proliferation and obliteration of small pulmonary arteries, leading to severe pulmonary hypertension and right ventricular failure. Typical morphological appearances include increased muscularisation of small (<200μm diameter) arteries and thickening or fibrosis of the intima (Fig 4). In severe cases dilatation of small arterioles is seen, and sometimes fibrinoid necrosis[36,37]. The term plexogenic arteriopathy is used because of the existence of plexiform lesions (200–400mm diameter) which are a tangle of capillary-like channels adjacent to a small pulmonary artery[38]. Plexiform changes may also be observed in other causes of severe pulmonary hypertension, such as that due to congenital heart disease. However, the endothelial cells comprising plexiform lesions in PPH appear to be due to a monoclonal proliferation of cells, whereas lesions in secondary PH are of polyclonal origin[39].

Several environmental factors are thought to contribute to the

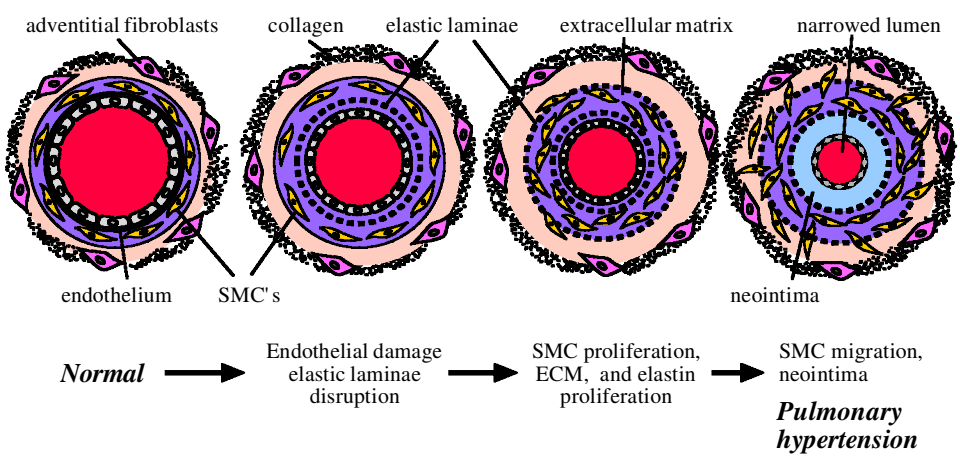


Fig 4. Vascular remodelling of pulmonary arteries. Sequence of changes in the process of vascular remodelling of muscular pulmonary arteries leading to increased thickness of the adventitia and media and formation of the neointima.

Illustration by Doig Simmonds from *Pulmonary Circulation: Basic Mechanisms to Clinical Practice*, Imperial College Press, 2001

pathogenesis of PPH. A clear association exists with the ingestion of appetite suppressant drugs, particularly of the fenfluramine/dexfenfluramine group[40–42]. There is also an association with the ingestion of Spanish toxic rape seed oil. There is an unusually high prevalence of PH (~0.5%), pathologically and clinically identical to PPH, in patients with HIV infection.

Genetics of primary pulmonary hypertension

Dresdale first described the occurrence of PPH in two sisters in 1954[43]. Its extreme rarity, and the observation that the majority of cases appear to be 'sporadic' have hampered systematic documentation of the familial incidence of this disease. In the United States, the National Institutes of Health established a National Registry for cases of PPH in the 1980s[44] and 6% of cases were clearly familial. Indeed, some cases of apparently sporadic idiopathic pulmonary hypertension were distantly related[45]. From scrutiny of family pedigrees the most likely mode of inheritance was autosomal dominant[46]. Even so, the number of affected individuals within families is less than predicted, implying incomplete penetrance of the gene. Approximately 80% of those who inherit the abnormal gene will not develop symptoms of disease.

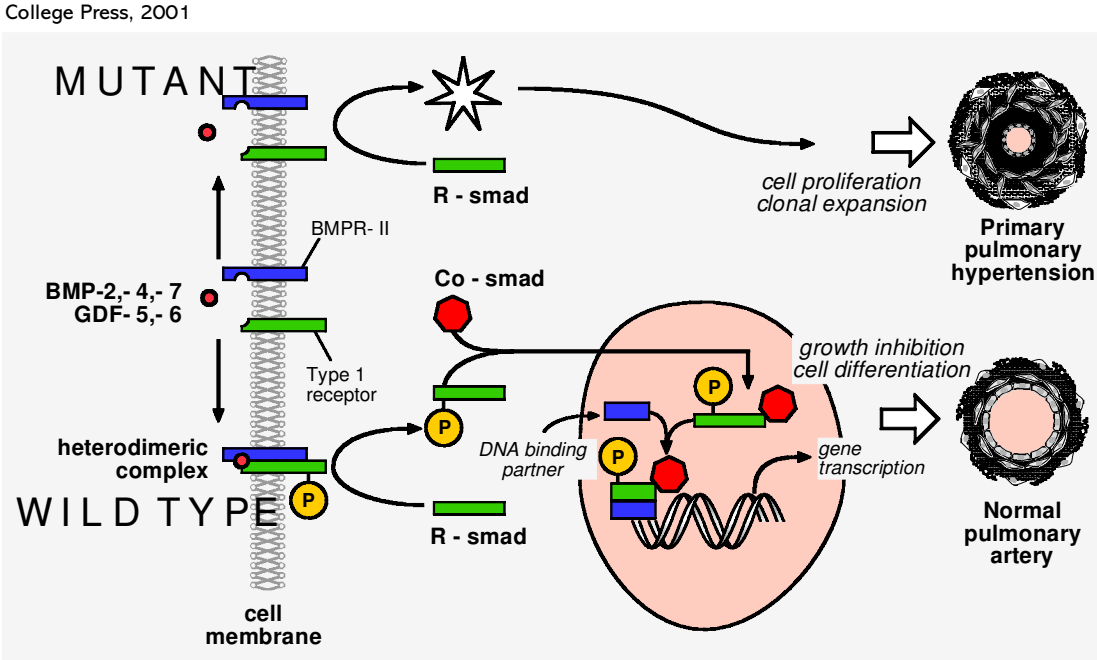
In 1997 sufficient pedigrees with familial PPH were available to allow investigators to perform a whole genome screen using polymorphic microsatellite markers. Linkage was established between familial PPH and a region on the long arm of chromosome 2 (2q33)[47,48]. Sequencing of positional candidate genes revealed heterozygous mutations involving the gene encoding the type II bone morphogenetic protein receptor (BMPR-II), a member of the transforming growth factor-β superfamily of receptors[49,50]. The heterogeneous mutations include frameshift, nonsense and missense. The frameshift and nonsense mutations predict premature truncation of the 1038 amino acid protein. Missense mutations occur at highly conserved and functionally important sites that are predicted to perturb ligand binding or disrupt the kinase domain of the receptor (Fig 5). Interestingly, the same mutations underlie 26% of apparently sporadic cases of PPH, some of which are in fact familial, the remainder arising *de novo*[51]. The techniques used to detect mutations in these studies could have missed large gene deletions or rearrangements, and may therefore underestimate the frequency of BMPR-II mutations in familial and apparently sporadic PPH.

Cellular mechanisms in the pathogenesis of primary pulmonary hypertension

Bone morphogenetic proteins (BMPs) belong to the TGF-β superfamily (TGF-βs, activin A, BMPs and growth/differentiation factors {GDFs}), and were originally identified as molecules regulating growth and differentiation of bone and cartilage[52]. However, the BMPs have been shown to be multifunctional cytokines with biological activity in a variety of cells types,

Fig 5. Role of BMPR-II in pulmonary circulation. Diagram showing the interaction of BMPR-II with type I receptors and ligands at the cell surface, and signalling via Smad proteins to regulate gene transcription. Normal BMPR-II signal transduction may contribute to the differentiation of the pulmonary vasculature during development. In the presence of the mutant receptor disrupted BMPR-II signalling presumably contributes to the cellular proliferation and hypertrophy that characterise primary pulmonary hypertension.

Illustration by Doig Simmonds, adapted from *Pulmonary Circulation: Basic Mechanisms to Clinical Practice*, Imperial College Press, 2001



including monocytes, epithelial cells, mesenchymal and neuronal cells[52]. Two BMP type I receptors (BMPRIA and BMPRIB) and a single BMP type II receptor have been identified in mammals that are both serine/threonine kinase receptors[53]. In *in vitro* cell systems the BMPR-II receptor binds BMP2, BMP4, BMP7, and GDF5 and 6, a process that is facilitated by the presence of the type I receptor[54]. The formation of heteromeric complexes with one of the 6 TGF-β superfamily type I receptors determines the specificity of ligand-receptor activation. Following ligand binding to the BMPR-II, the type II receptor complexes with and phosphorylates the type I receptor (Fig 5). This phosphorylation of a glycine-serine rich domain on the proximal intracellular portion of the type I receptor activates the type I receptor kinase domain initiating phosphorylation of cytoplasmic signalling proteins, termed Smads, which are responsible for TGF-β superfamily signal transduction[55]. BMPs signal via activation of a specific set of Smad proteins (Smad1, 5 and 8, termed R-Smads), which must complex with the co-Smad, Smad4 to function optimally[52]. The complex of R-Smad and co-Smad translocates to the nucleus where they can directly regulate gene transcription.

The identification of inactivating heterozygous mutations of the *BMPR-II* gene in familial and sporadic PPH underscores the importance of the TGF-β superfamily in the regulation of vascular development and integrity. In man, mutations in the gene encoding endoglin (a TGF-β receptor complex accessory protein) and the putative TGF-β type 1 receptor, ALK-1, have been identified in hereditary haemorrhagic telangiectasia, a condition characterised by arteriovenous malformations[56,57]. The main effects of TGF-β on vascular cells are growth inhibition, cell differentiation[58] and stimulation of collagen synthesis. BMP7 inhibits proliferation of human aortic smooth muscle cells and increases expression of smooth muscle cell differentiation markers[59], and BMP2 inhibits vascular smooth muscle cell proliferation following balloon injury in rats[60].

How then might dysfunctional signalling through the BMPR-II pathway, as a consequence of the heterozygous mutation, result in primary pulmonary hypertension? We know from immunohistochemical studies that BMPR-II is localised mainly to endothelial cells, but also smooth muscle cells in the distal pulmonary circulation. The heterozygous nature of the mutation would result in half the amount of normal receptor protein on the surface of pulmonary vascular cells. However, since the type II receptors must form heteromeric complexes with type I receptors in order to signal, the presence of mutant uncomplexed BMPR-II on the cell surface may act as a dominant negative, binding but not signalling in response to BMPs, and decreasing the availability of BMPs to the wild-type receptor complex. In this way the functional defect in signalling would be much greater than predicted by the heterozygous mutation alone. Failure of growth inhibitory and pro-apoptotic signals via BMPs may account for the vascular cell proliferation that characterises PPH.

Whilst it is clear that mutations in the gene encoding BMPR-II predispose to PPH, the precise genetic mechanisms leading to the pathogenesis of the disorder require further experimental investigation. For example, what is the mechanism for the low disease gene penetrance characteristic of familial PPH? And why is the process of vascular obliteration selective for the pulmonary circulation? Interestingly, acquired somatic mutations in other components of the TGF-β superfamily, for example the TGF-β type II receptor, are well recognised in pancreatic and colonic tumours[61] as well as in atherosclerotoic lesions[58]. These somatic mutations lead to loss of the growth suppressive effects of TGF-β. It is possible that the acquisition of a further somatic mutation in this or a related pathway, either spontaneously or resulting from environmental exposure, is necessary for the full clinical manifestation of the disease.

Key Points

Genetic influences in susceptibility to hypoxia-induced pulmonary hypertension are suggested by 'brisket disease' in cattle, the identification of chromosomal regions in rats that are linked to right ventricular hypertrophy, and interindividual differences in susceptibility to high altitude pulmonary hypertension in man.

The capacity of pulmonary arteries to contract in response to alveolar hypoxia is intrinsic to the pulmonary artery smooth muscle cell, and involves Ca^{2+} influx, probably as a result of membrane depolarisation following closure of voltage gated K^+ channels.

Hypoxia leads to changes in pulmonary vascular cell gene expression, partly dependent on the induction of hypoxia-inducible factor-1 (HIF-1), altering the expression of growth promoting and growth inhibiting factors.

A heterozygous mutation in the gene encoding the bone morphogenetic protein type II receptor (BMPR-II), a member of the transforming growth factor-β family of receptors, underlies many familial and apparently sporadic cases of primary pulmonary hypertension.

It is likely that a failure of the growth inhibitory and pro-apoptotic signals from bone morphogenetic proteins contributes to the vascular cell proliferation and arterial obliteration that characterise primary pulmonary hypertension.

New treatments for pulmonary hypertension

Medical treatments for PPH include anticoagulation, Ca^{2+} channel blockers and continuous intravenous, or nebulised, prostacyclin analogues, which may improve survival but are not curative[35,62,63]. Many patients require lung or heart-lung transplantation. The identification of genetic mutations or susceptibility genes underlying the pathogenesis of pulmonary hypertension may immediately suggest novel treatments for this condition, or provide a platform for the development of targeted gene therapy. Knowledge of the key mediators that regulate vascular cell tone and growth in the pulmonary circulation also suggests pharmacological targets. For example, many of the growth inhibitory effects of nitric oxide and prostacyclin rely on intracellular signalling via cyclic nucleotides[64].

Potentiation of the effects of cyclic nucleotides by inhibition of cyclic nucleotide phosphodiesterases may provide a convenient and effective way to inhibit or reverse pulmonary vascular remodelling. Novel approaches to the treatment of hypoxia-induced pulmonary hypertension would include inhibition of hypoxia-stimulated growth signals or K_V channels. The role of long-term inhibition of key vasoconstrictors and growth factors such as angio-tensin II[65,66] and endothelin-1 by specific receptor antagonists, requires urgent evaluation in patients with primary and secondary pulmonary hypertension.

Conclusions

We are entering a new era in our understanding of the pathophysiology of pulmonary hypertension, based on the identification of specific genetic mutations and susceptibility factors. In addition, a clearer understanding is emerging of the molecular mechanisms involved in the response of the cell to hypoxia. Much work remains to be done to translate these discoveries into developments in clinical practice, though already new treatments are beginning to evolve as a direct result of studies in cultured cells and animal models.

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References

1 Hughes JMB. Distribution of pulmonary blood flow. In: Crystal RG, West JB, Weibel ER, Barnes PJ (eds). *The lung: scientific foundations*. Philadelphia: Lippincott-Raven, 1997:1523–36.
2 von Euler US, Liljestrand G. Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol Scand* 1946;**12**:301–20.
3 Morrell NW, Nijran KS, Biggs T, Seed WA. Regional matching of ventilation and perfusion during lobar bronchial obstruction in man. *Clin Sci* 1995;**88**:179–84.
4 Boutilier RG, Glass ML, Heisler N. The relative distribution of pulmocutaneous blood flow in Rana catesbeiana: effects of pulmonary or cutaneous hypoxia. *J Exp Biol* 1986;**126**:33–9.
5 Hislop A, Reid L. New findings in pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. *Br J Exp Pathol* 1976;**57**:542–54.
6 Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, Reid L. Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. *Am J Physiol* 1979;**236**:H818–27.
7 Heath D. Pulmonary vascular disease. In: Hasleton PS (ed). *Spencer's pathology of the lung*. London: McGraw-Hill, 1996:649–93.
8 Weir EK, Tucker A, Reeves JT, Will DH, Grover RF. The genetic factor influencing pulmonary hypertension in cattle at high altitude. *Cardiovasc Res* 1974;**8**:745–9.
9 Cruz JC, Reeves JT, Russell BE, Alexander AF, Will DH. Embryo transplanted calves: the pulmonary hypertensive trait is genetically transmitted. *Proc Soc Exp Biol Med* 1980;**164**:142–5.
10 Langleben D, Jones RC, Aronovitz MJ, Hill NS, *et al*. Pulmonary artery structural changes in two colonies of rats with different sensitivity to chronic hypoxia. *Am J Pathol* 1987;**128**:61–6.
11 Sato K, Webb S, Tucker A, Rabinovitch M, *et al*. Factors influencing the idiopathic development of pulmonary hypertension in the fawn hooded rat. *Am Rev Respir Dis* 1992;**145**:793–7.
12 Wilkins MR, Zhao L, Al-Tubuly R. The regulation of pulmonary vascular tone. *Br J Clin Pharmac* 1996;**42**:127–31.
13 Aguirre JI, Morrell NW, Long L, Clift P, *et al*. Vascular remodeling and ET-1 expression in rat strains with different responses to chronic hypoxia. *Am J Physiol* 2000;**278**:L981–7.
14 Zhao L, Sebkhi A, Nunez DJ, Long L, *et al*. Right ventricular hypertrophy secondary to pulmonary hypertension is linked to rat chromosome 17: evaluation of cardiac ryanodine Ryr2 receptor as a candidate. *Circulation* 2001;**103**:442–7.
15 Vogel HJK, Weaver WF, Rose LD. Pulmonary hypertension on exertion in normal man living at 10,150 feet (Leadville, Colorado). In: Grover RF (ed). *Normal and abnormal pulmonary circulation*. Basel: S. Karger, 1963:289–91.
16 Reeves JT, Durmowicz AG. High altitude and the pulmonary circulation. In: Peacock AJ (ed). *Pulmonary circulation: a handbook for physicians*. London: Chapman and Hall, 1996:262–72.
17 Groves B, Droma T, Sutton JR, McCullough RG, *et al*. Minimal hypoxic pulmonary hypertension in normal Tibetans at 3,658m. *J Appl Physiol* 1993;**74**:312–8.
18 Morrell NW, Sarybaev AS, Alikhan A, Mirrakhimov MM, Aldashev A. ACE genotype and risk of high altitude pulmonary hypertension in Kyrghyz highlanders. *Lancet* 1999;**353**:814.
19 Madden JA, Vadula MS, Kurup VP. Effects of hypoxia and other vasoactive agents on pulmonary and cerebral artery smooth muscle cells. *Am J Physiol* 1992;**263**:L384–93.
20 McMurtry IF, Davidson AB, Reeves JT, Grover RF. Inhibition of hypoxic pulmonary vasoconstriction by calcium antagonists in isolated rat lungs. *Circ Res* 1976;**38**:99–104.
21 Weir EK, Reeve HL, Cornfield DN, Tristani FM, *et al*. Diversity of response in vascular smooth muscle cells to changes in oxygen tension. *Kidney Int* 1997;**51**:462–6.
22 Weir EK, Reeve HL, Peterson DA, Michelakis ED, Nelson DP, Archer SL. Pulmonary vasoconstriction, oxygen sensing, and the role of ion channels: Thomas A. Neff lecture. *Chest* 1998;**114**(1 Suppl):17S–22S.
23 Maxwell PH, Pugh CW, Ratcliffe PJ. Inducible operation of the erythropoietin 3' enhancer in multiple cell lines: evidence for a widespread oxygen sensing mechanism. *Proc Nat Acad Sci USA* 1993;**90**:2423–7.
24 Wang GL, Semenza GL. General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc Natl Acad Sci USA* 1993;**90**:4304–8.
25 Ratcliffe PJ. Hypoxically inducible gene expression. In: Crystal RG, West JB, Weibel ER, Barnes PJ (eds). *The lung: scientific foundations*. Philadelphia: Lippincott-Raven, 1997:319–32.
26 Pugh CW, O'Rourke JF, Nagao M, Gleadle JM, Ratcliffe PJ. Activation of hypoxia-inducible factor-1;definition of regulatory domains within the á subunit. *J Biol Chem* 1997;**272**:11205–14.
27 Kourembanas S, Marsden PA, McQuillan LP, Faller DV. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest* 1991;**88**:1054–7.
28 Stewart DJ, Levy RD, Cernacek P, Langleben D. Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease? *Ann Intern Med* 1991;**114**:464–9.
29 Zamora MA, Dempsey EC, Walchak SJ, Stelzner TJ. BQ123, an ETA receptor antagonist, inhibits endothelin-1-mediated proliferation of human pulmonary artery smooth muscle cells. *Am J Respir Cell Mol Biol* 1993;**9**:429–33.
30 Morrell NW, Upton PD, Kotecha S, Huntley A, *et al*. Angiotensin II activates MAPK and stimulates growth of human pulmonary artery smooth msucle via AT1 receptors. *Am J Physiol* 1999;**277**:L440–8.
31 Morrell NW, Atochina EN, Morris KG, Danilov SG, Stenmark KR. Angiotensin converting enzyme expression is increased in small pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. *J Clin Invest* 1995;**96**:1823–33.

32 Morrell NW, Danilov SM, Satyan KB, Morris KG, Stenmark KR. Right ventricular angiotensin converting enzyme activity and expression is increased during hypoxic pulmonary hypertension. *Cardiovasc Res* 1997;**34**:393–403.

33 Fagan KA, Fouty BW, Tyler RC, Morris KG, *et al*. The pulmonary circulation of homozygous or heterozygous eNOS-null mice is hyperresponsive to mild hypoxia. *J Clin Invest* 1999;**103**:291–9.

34 Zhao L, Long L, Morrell NW, Wilkins MR. NPR-A-deficient mice show increased susceptibility to hypoxia-induced pulmonary hypertension. *Circulation* 1999;**99**:605–7.

35 Rubin LJ. Primary pulmonary hypertension. *New Engl J Med* 1997;**336**:111–7.

36 Pietra GG, Edwards WD, Kay JM, Rich S, *et al*. Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. *Circulation* 1989;**80**:1198–1206.

37 Palevsky HI, Schloo B, Pietra GG, Weber KT, *et al*. Primary pulmonary hypertension: vascular structure, morphometry, and responsiveness to vasodilator agents. *Circulation* 1989;**80**:1207–21.

38 Cool CD, Stewart JS, Werahera P, Miller GJ, *et al*. Three-dimensional reconstruction of pulmonary arteries in plexiform pulmonary hypertension using cell-specific markers. *Am J Pathol* 1999;**155**:411–9.

39 Lee S-D, Shroyer KR, Markham NE, Cool CD, *et al*. Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. *J Clin Invest* 1998;**101**:927–34.

40 Abenhaim L, Moride Y, Brenot F, Rich S, *et al*. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. *New Engl J Med* 1996;**335**:609–16.

41 Polak J, Oakley CM, Yacoub M, Mikhail G, Taylor-Robinson SD. Primary pulmonary hypertension: pathologist as patient. *J R Coll Physicians Lond* 1998;**32**:225–30.

42 Haworth SG. Primary pulmonary hypertension. *J R Coll Physicians Lond* 1998;**32**:187–90.

43 Dresdale DT, Michtom RJ, Schultz M. Recent studies in primary pulmonary hypertension including pharmacodynamic observations on pulmonary vascular resistance. *Bull N Y Acad Sci* 1954;**30**:195–207.

44 Rich S, Dantzker DR, Ayres SM, Bergofsky EH, *et al*. Primary pulmonary hypertension. A national prospective study. *Ann Intern Med* 1987;**107**:216–23.

45 Elliot G, Alexander G, Leppert M, Yeates S, Kerber R. Coancestry in apparently sporadic primary pulmonary hypertension. *Chest* 1995; **108**:973–7.

46 Loyd JE, Primm RK, Newman JH. Familial primary pulmonary hypertension: clinical patterns. *Am Rev Respir Dis* 1984;**129**:194–7.

47 Morse JH, Jones AC, Barst RJ, Hodge SE, *et al*. Familial primary pulmonary hypertension locus mapped to chromosome 2q31-q32. *Chest* 1998;**114**(1 Suppl):57S–58S.

48 Nicholls WC, Koller DL, Slovis B, Foroud T, *et al*. Localisation of the gene for familial primary pulmonary hypertension to chromosome 2q31-32. *Nature Genet* 1997;**15**:277–80.

49 The International PPH Consortium, Lane KB, Machado RD, Pauciulo MW, *et al*. Heterozygous germ-line mutations in BMPR2, encoding a TGF-â receptor, cause familial primary pulmonary hypertension. *Nature Genet* 2000;**26**:81–4.

50 Deng Z, Morse JH, Slager SL, Cuervo N, *et al*. Familial primary pulmonary hypertension (Gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000;**67**: 737–44.

51 Thomson JR, Machado RD, Pauciulo MW, Morgan NV, *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**:741–5.

52 Kawabata M, Imamura T, Miyazano K. Signal transduction by bone morphogenetic proteins. *Cytokine Growth Factor Rev* 1998;**9**:49–61.

53 Rosenzweig BL, Imamura T, Okadome T, Cox GN, *et al*. Cloning and characterization of a human type II receptor for bone morphogenetic proteins. *Proc Natl Acad Sci USA* 1995;**92**:7632–6.

54 Massague J, Chen Y-G. Controlling TGF-β signaling. *Genes Dev* 2000; **14**:627–44.

55 Raftery LA, Sutherland DJ. TGF-β family signal transduction in *Drosophila* development: from *Mad* to Smads. *Dev Biol* 1999;**210**: 251–68.

56 Shovlin CL, Hughes JM, Scott J, Seidman CE, Seidman JG. Characterization of endoglin and identification of novel mutations in hereditary hemorrhagic telangiectasia. *Am J Hum Genet* 1997;**61**:68–79.

57 Shovlin CL, Hughes JM, Tuddenham EG, Temperley I, *et al*. A gene for hereditary haemorrhagic telangiectasia maps to chromosome 9q3. *Nature Genet* 1994;**6**:205–9.

58 McCaffrey TA. TGF-βs and TGF-β receptors in atherosclerosis. *Cytokine Growth Factor Rev* 2000;**11**:103–14.

59 Dorai H, Vukicevic S, Sampath TK. Bone morphogenetic protein-7 (OP-1) inhibits smooth muscle cell proliferation and stimulates the expression of markers that are characteristic of SMC phenotype in vitro. *J Cell Physiol* 2000;**184**:37–45.

60 Nakaoka T, Gonda K, Ogita T, Otawara-Hamamoto Y, *et al*. Inhibition of rat vascular smooth muscle proliferation in vitro and in vivo by bone morphogenetic protein-2. *J Clin Invest* 1997;**100**:2824–32.

61 Lu SL, Kawabata M, Imamura T, Akiyama Y, *et al*. HNPCC associated with germline mutation in the TGF-beta type II receptor gene {letter}. *Nature Genet* 1998;**19**:17–8.

62 Barst RJ, Rubin LJ, McGoon MD, Caldwell EJ, *et al*. Survival in primary pulmonary hypertension with long-term continuous intravenous prostacyclin. *Ann Intern Med* 1994;**121**:409–15.

63 Langleben D, Barst RJ, Badesch D, Groves BM, *et al*. Continuous infusion of epoprostenol improves the net balance between pulmonary endothelin-1 clearance and release in primary pulmonary hypertension. *Circulation* 1999;**99**:3266–71.

64 Wharton J, Davie N, Upton PD, Yacoub MH, *et al*. Prostacyclin analogues differentially inhibit the growth of distal and proximal human pulmonary artery smooth muscle cells. *Circulation* 2000;**102**:3130–6.

65 Morrell NW, Morris KG, Stenmark KR. Role of angiotensin converting enzyme and angiotensin II in development of hypoxic pulmonary hypertension. *Am J Physiol* 1995;**269**:H1186–94.

66 Orte C, Polak JM, Haworth SG, Yacoub MH, Morrell NW. Expression of pulmonary vascular angiotensin-converting enzyme in primary and secondary plexiform pulmonary hypertension. *J Pathol* 2000;**192**: 379–84.

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