Part 1

Pulmonary disease

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Primary pulmonary hypertension: pathophysiology and therapy

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Introduction

Primary pulmonary hypertension (PPH) is a progressive and fatal disease, whose pathobiology has been elusive. It is clear, however, that the initial explanations that this was a disease of vasoconstriction of the pulmonary vasculature was a gross oversimplification of the biological mechanisms that are involved [1]. With our better understanding of vascular biology and the various mediators involved in the regulation of vascular tone and growth, we now realize that a number of pathways may be involved in what we call PPH, and that many different cell types within the arteries may be implicated in the aetiology. For example, some have suggested that an abnormality in the endothelium can account for the changes that are noted in both the intima and media [2]. However, it is also possible that a primary abnormality of the pulmonary artery smooth muscle cell or the extracellular matrix may be causative in this disease. Given the heterogeneous nature of the pathological changes that have been described in patients with primary and secondary forms of pulmonary arterial hypertension, it is likely that more than one abnormality is playing a key role [3,4]. Nonetheless, over recent years in treating patients with PPH there has been considerable progress that has arisen from our attempts to understand the pathogenesis of the disease.

The genetic basis of primary pulmonary hypertension

A familial pattern of disease transmission for PPH has been well characterized. Recently, the gene for familial PPH, known as *PPH-1*, has been described [5]. It is autosomal dominant with markedly reduced penetrance, and is located on chromosome 2q33 (Chapter 2). Using a positional candidate approach it has been shown that a mutation in the gene for bone morphogenic protein receptor 2 (*BMPR2*) is responsible. BMPs fall within the super family of transforming growth factor beta (TGF-β) receptors, and have been shown to regulate a diverse number of biological processes including cell differentiation, proliferation, apoptosis and morphogenesis [6]. The precise way that this defect causes PPH remains unclear, and it may be that the presence of either an exogenous risk factor (such as the fenfluramines) or a second genetic abnormality is necessary to induce the disease. Of particular interest is that these genetic mutations have also recently been described in 25% of patients with 'sporadic' PPH [7].

The rationale behind vasodilator therapy

In nearly all pathological series on PPH, varying degrees of medial hypertrophy exist that have been interpreted to be an expression of underlying vasoconstriction [3,4,8]. Indeed, some of the earliest physiological studies in patients with PPH have shown that the administration of a vasodilator such as a acetylcholine can cause immediate and pronounced vasodilatation [9,10]. Since the vessels are characterized by marked thickening of the media, it was originally postulated that this represented uncontrolled growth and constriction of the smooth muscle cells in the pulmonary vascular bed. The fact that a wide spectrum of responsiveness has been noted in patients with PPH has been interpreted as a reflection of the chronicity as well as the severity of the underlying disease [1,11]. It has been suggested that patients who present earlier appear to be more likely to respond to an acute vasodilator

challenge, and those that are more advanced are less likely to be responsive [2,12].

Nonetheless there remains considerable uncertainty regarding the role of vasoconstriction in PPH and the favourable effects of vasodilators. Although vasoconstriction can be demonstrated in some patients with PPH, it is uncommon to demonstrate any pulmonary vasodilator response to any agent in patients who present with advanced disease, suggesting that vasoconstriction is not a feature of the disease in the late stages. A close look at the histopathology of PPH, however, would suggest that this clinical observation should be of no surprise. Patients with PPH will typically have severe concentric laminar intimal fibrosis, often with obliteration of the vessels representative of an angioproliferative vasculopathy. This suggests that a dominant problem in PPH may be uncontrolled myointimal growth, consistent with the recent genetic observations.

It has been reported that patients with PPH may also possess an abnormality in the function of the potassium channels in pulmonary artery smooth muscle cells that causes membrane depolarization and increased cytoplasmic calcium, which was not apparent in patients with secondary pulmonary hypertension [13]. This could promote pulmonary vasoconstriction, as well as pulmonary arterial smooth muscle cell proliferation and raises the possibility that some patients with PPH may have a unique mechanism for their pulmonary hypertension arising in the pulmonary smooth muscle cells. This may also explain why calcium channel blockers seem to be effective predominantly in patients with PPH, and only rarely with secondary pulmonary hypertension.

Calcium channel blockers were the first oral vasodilators that were demonstrated to have sustained, pronounced benefits in patients with PPH [14]. Published data, however, illustrated that only a minority of patients (approximately 20%) will respond to calcium channel blockers at the time that they initially present with the diagnosis of PPH [14,15]. Given the complexity, as well as reported hazards of high doses of calcium channel blockers in patients with PPH, it is widely advocated that patients be tested acutely with a short acting vasodilator to select those patients that would be most likely to respond to calcium channel blockers chronically [16–19].

It is important to note that calcium channel blockers may in some cases have no effect, and more importantly may have an adverse affect [20]. Concerns over calcium blockers being associated with increased cardiovascular mortality in patients with coronary disease are based on factors that may also play a role in PPH. If there is no demonstrable vasoconstriction as an underlying abnormality in a patient presenting with pulmonary hypertension, there would be no justification for the use of chronic vasodilators. In addition, a patient who presents at an advanced stage may no longer have the ability to respond to a vasodilator, given the extensive nature of the vascular changes. More importantly, however, calcium channel blockers can cause neurohormonal activation that could be very problematic in these patients [21], and many possess negative inotropic properties that could worsen underlying right ventricular dysfunction [22].

On the basis of the published data, our group makes the following recommendations regarding the use of vasodilators in PPH. First, because these drugs have the potential for serious deleterious effects, acute testing of short acting vasodilators should be done in all patients before chronic calcium channel blocker therapy is used. At the present time it does not appear that there is any superiority over using intravenous adenosine, intravenous epoprostenol (prostacyclin), or inhaled nitric oxide as the testing agent. The decision to initiate chronic therapy should be based on the demonstration of a pronounced acute pulmonary vasodilator effect. Although there is no agreement on this definition, we believe that there should be at least a reduction in the mean pulmonary artery pressure to under 30 mmHg (4 kpa) associated with an increase in cardiac output. Patients who deteriorate while on calcium channel blocker therapy should have it withdrawn, and not have it increased, as it may worsen underlying cardiac function.

The rationale behind anticoagulation

One of the most common findings in patients with PPH is the presence of eccentric intimal pads that are presumed to be caused by thrombosis in situ, as well as recanalized thrombotic lesions that are randomly scattered throughout the pulmonary vascular bed. The assumption that this represents recurrent microembolization has been abandoned, as several studies have characterized the presence of a procoagulant state in patients with PPH that would logically predispose to the development of thrombosis in situ [23]. Studies on the value of anticoagulation in these patients support the notion that ongoing thrombosis might either be causative or serve to perpetuate PPH in some patients (Figure 1.1). A retrospective study of patients with PPH followed at the Mayo Clinic, Rochester, Minnesota, demonstrated improved survival of those who received warfarin anticoagulation versus those who did not [24]. Similarly, patients who developed PPH from exposure to the diet pill aminorex fumarate seemed to have improved survival when treated with warfarin anticoagulation [25]. In the only

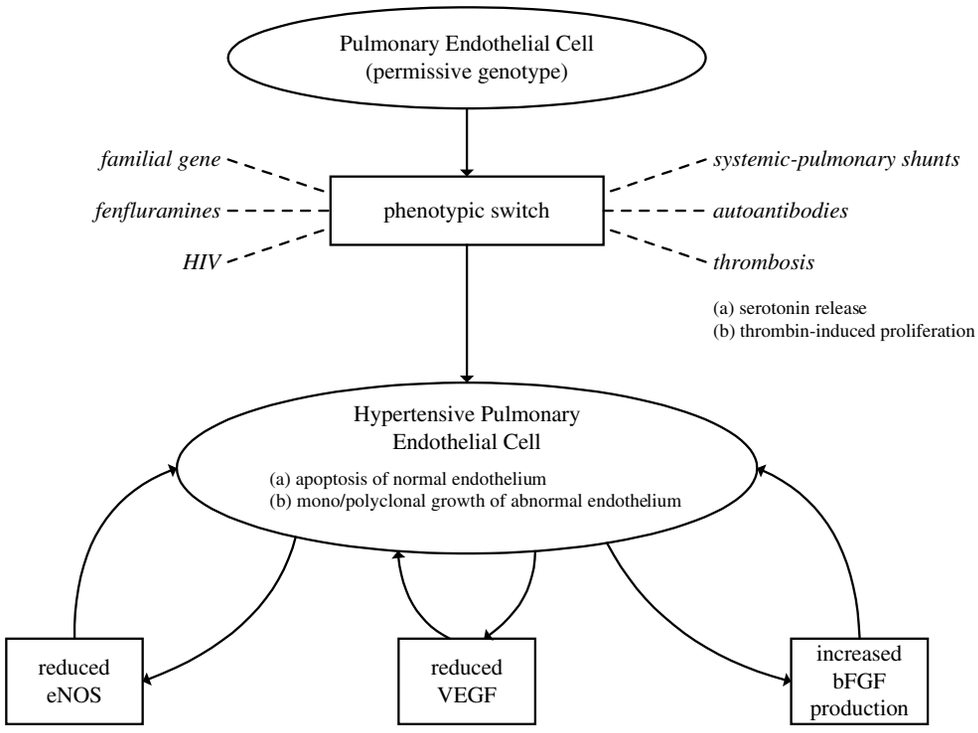

Figure 1.1 A pathway to the development of the hypertensive pulmonary endothelial cell is shown. This cell is characterized by reduced levels of endothelial nitric oxide synthase (eNOS) and increased production of basic fibroblast growth factor (bFGF). Reduced vascular endothelial growth factor (VEGF) from normal endothelial cells is also likely. HIV, human immunodeficiency virus.

prospective study to date, patients who were unresponsive to calcium channel blockers also had improved survival when treated with warfarin anticoagulation versus those who did not [15].

Whether the development of arterial thrombosis in PPH is a result of localized endothelial cell injury, circulating procoagulant factors, or platelet activation has not been fully elucidated. It is possible that any or all of these may be playing a role in these patients. Recently there have been newer antithrombotic agents that work via platelet-mediated mechanisms [26]. Whether these agents would be effective in PPH is completely unknown at this time.

On the basis of the published data, we recommend that chronic warfarin anticoagulation be used in the treatment of all patients with PPH unless there is an underlying contraindication. Although the intensity of the anticoagulant therapy has never been addressed, the common practice currently is to use a target international normalized ratio (INR) of 2.0–3.0 times control. Because of the similarities that exist, at least histologically, in patients with primary and secondary forms of pulmonary hypertension, we use warfarin anticoagulation in all patients who present with pulmonary arterial hypertension.

The rationale behind prostacyclin therapy

A wide spectrum of endothelial cell proliferation and fibrosis is extremely common in patients with PPH, as is the development of plexogenic lesions in advanced cases presumed to be derived from the endothelial cell line [27]. Whether this represents primary endothelial cell injury, endothelial cell dysfunction secondary to an extrinsic trigger, or some other process remains unknown. A multitude of factors that could adversely affect endothelial cell function and proliferation have been described. These include a reduction in circulating prostacyclin metabolites [28], which are presumably derived from the pulmonary vascular endothelium, as well as an increase in thromboxane metabolites, presumably derived from platelet activation (Figure 1.2) [27]. Marked elevations in circulating endothelin have been noted [29], which at a minimum could perpetuate endothelial cell proliferation and dysfunction. Similarly, increased expression of angiotensin converting enzyme activity in the pulmonary vascular endothelium of patients with pulmonary hypertension has now been described [30], as have reduced levels of endothelial nitric oxide synthase, implicating inadequate nitric

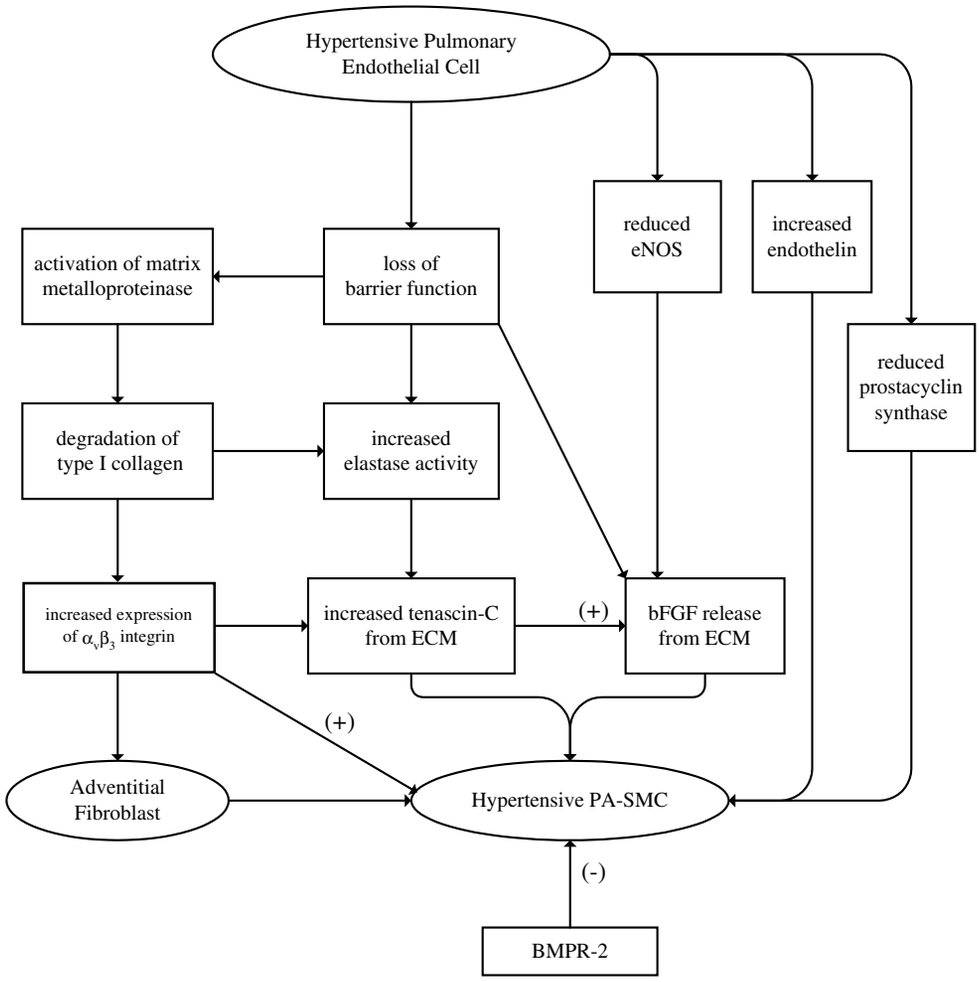

Figure 1.2 Multiple pathways to the development of the hypertensive pulmonary artery smooth muscle cell (PA-SMC) are shown. Pathways on the left have been elucidated from animal models, whereas pathways on the right are from human studies. BMPR-2, bone morphogenic protein receptor 2; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; bFGF, basic fibroblast growth factor; +, increase; −, decrease.

oxide production [31]. All of these observations make for exciting possibilities of selected therapies targeted at reversing these abnormalities.

To date, the only long-term studies of a therapy that has the potential to correct these abnormalities have focused on the use of chronic intravenous epoprostenol (prostacyclin). Prostacyclin has a number of biochemical properties, which include vasodilatation, platelet inactivation, and antiproliferative effects [32]. Several studies on the acute and chronic effectiveness of epoprostenol in PPH have all shown that it is associated with increased exercise tolerance, improved haemodynamics, and improved survival [33,34]. A recent study, demonstrating that chronic haemodynamic changes attributed to epoprostenol appear to go beyond acute vasodilatation suggests that the compound may also initiate reversal of the vascular remodelling that is part of PPH [35].

On the basis of the published data, our group believes that intravenous epoprostenol is indicated for all patients with advanced PPH that remain symptomatic in spite of conventional therapy. Indeed, this would be the ideal drug to use in all patients with PPH were it not that its current availability is very limited because of its expense, and because of the requirement for a chronic indwelling central venous catheter, which carries substantial associated morbidity. Clearly, analogues of prostacyclin that may be given through subcutaneous, inhaled, or oral routes need to be evaluated [36,37]. In addition, the mechanisms by which epoprostenol produces favourable effects in these patients need to be better understood.

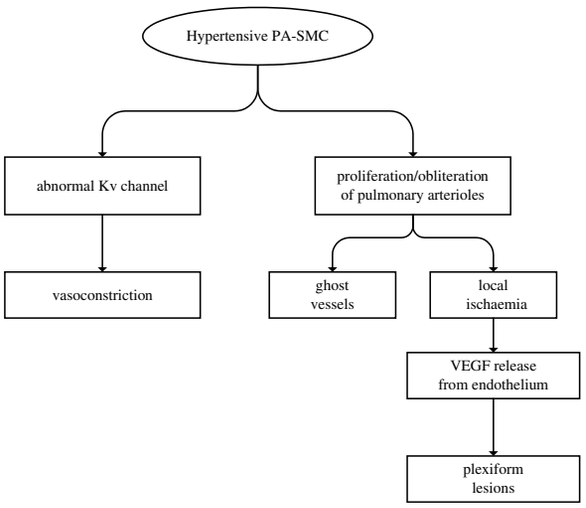

Figure 1.3 Pathways illustrating the pathobiological expression of the hypertensive pulmonary artery smooth muscle cell (PA-SMC) are shown. Vasoconstriction is an early phenomenon, whereas plexiform lesions represent chronic severe disease. VEGF, vascular endothelial growth factor.

Growth inhibitors: a new approach

From advances made in our understanding of the molecular basis of vascular growth and the response to stimuli one can assemble a molecular pathway that may explain the pathobiology of primary pulmonary hypertension (Figures 1.1 to 1.3). Most of these pathways have been derived from the hypoxic- and monocrotaline-induced pulmonary hypertensive rat, and from immunohistochemical studies of human lung tissue. What becomes apparent is that PPH appears to represent an angiogenic-obliterative pulmonary vasculopathy as a result of abnormal or uncontrolled pulmonary vascular arterial growth. It is likely that there are multiple and redundant pathways responsible for this disease.

Recent observations provide evidence that the pulmonary hypertension initiates a positive feedback loop that serves to sustain the clinical disease entity. In an interesting study using the monocrotaline rat model, it was shown that, when a pulmonary hypertensive lung is removed and transplanted into a normal rat, the lung will reverse its disease [38]. This has been attributed to the reduction in mean pulmonary pressure that exists in the animal receiving the transplant, and subsequent absence of growth factors necessary to sustain the disease process. Interestingly, a case report of a patient who underwent single lung transplantation for PPH provides a look at the reverse experiment [39]. In this instance, a diseased patient received one normal lung, which not only resulted in a reduction in the pulmonary artery pressure but, over the ensuing years, reversed the severity of the disease process on the native lung. Taken together these observations suggest that the body may be able to produce growth inhibitors to reverse the vascular changes of advanced pulmonary hypertension. Two types of inhibitor currently being evaluated clinically are the endothelin receptor blockers and nitric oxide.

Endothelin (ET) is a constrictor of human pulmonary arteries through its action on smooth muscle ETA receptors, but can also induce vasodilatation through endothelial ETB receptors [40]. In addition to its vasomotor actions, it has been implicated in vascular remodelling in a number of animal models. ET, for example, is overexpressed in rats developing hypoxic pulmonary hypertension; this can be prevented and reversed using an ET receptor antagonist [41]. A central role for endothelin in the pathogenesis of PPH has been proposed because plasma levels are increased and there is evidence of local production in the lung [42]. Thus, if effective, endothelin receptor antagonists could produce favourable short-term haemodynamic changes by virtue of their vasodilator properties, and perhaps longer-term favourable changes by growth inhibition and potentially reversal of the chronic myointimal proliferation that exists with the disease.

Chronic elevations in endothelin levels in patients with PPH have been described by several investigators [42]. Although the exact role that endothelin is playing in this disease remains undefined, it is appealing to believe that inhibiting this potent mitogen might have beneficial effects in arresting the disease process, reversing the disease, or improving cardiac function. Two pilot clinical trials have been conducted, with favourable results of endothelin receptor blockers in patients with PPH. Their role in the spectrum of treatments for chronic pulmonary arterial hypertension remains to be defined.

Endothelial-derived nitric oxide is a mediator that controls vascular remodelling by acting as a negative regulator of vascular smooth muscle proliferation in response to a remodelling stimulus [43]. In the absence of nitric oxide, luminal remodelling is impaired and vessel wall thickness increases due to the proliferation of vascular smooth muscle cells. The mechanism by which nitric oxide works is under investigation. It may, for example, stimulate an inhibitor of vascular smooth muscle proliferation such as TGF-β or counteract the actions of known smooth muscle mitogens such as fibroblast growth factor [44]. In experimental models, high levels of nitric oxide can be both cytotoxic and apoptosis promoting.

Several strategies are under study to increase nitric oxide in patients. For example, nitric oxide can be inhaled and has been reported to be clinically effective in treating PPH [45].

Table 1.1. Possible futuristic therapy of pulmonary hypertension

	Target	Treatment
Phase I	Increase nitric oxide levels	Phosphodiesterase inhibitor
	Reverse local prostacyclin/thromboxane imbalance	Epoprostenol (prostacyclin)
Phase II	Maintain local nitric oxide production	L-arginine
	Reverse vasoconstriction	Calcium channel blockers
	or	
Phase I	Reversal of intimal proliferation	Epoprostenol (prostacyclin)
	Blockade of neurohormonal activation	Angiotensin converting enzyme inhibitor
Phase II	Increase endogenous nitric oxide production	Nitric oxide synthase gene transfer
	Reduce vascular smooth muscle cell hypertrophy	Endothelin receptor blocker

The use of nitric oxide precursors, such as L-arginine, has been employed to increase nitric oxide production by the endothelial cell [46]. The cholesterol-lowering statin drugs can increase levels of endothelial nitric oxide synthase in vessels with impaired endothelium [47]. The use of phosphodiesterase inhibitors, which prolong the activity of nitric oxide, is currently being studied in a response to case reports of patients with PPH responding to oral sildenafil [48]. Finally, cell-based gene transfer of endothelial nitric oxide synthase has been effective in inhibiting monocrotaline-induced pulmonary hypertension and may provide another strategy worthy of clinical pursuit [49].

Future treatment strategies

Because it is not clear whether PPH is a result of an abnormality of a single cell type or a pathophysiological abnormality, we should begin looking at combination therapies designed to correct as many of the abnormalities associated with PPH as possible (see Table 1.1). Indeed, the combined use of warfarin anticoagulation with calcium channel blockers has been the first step in this approach. One lesson that has been learned in treating PPH, similar to the treatment of congestive heart failure, is that acute haemodynamic changes need not occur for drugs to produce chronic effectiveness. Finally, we should also learn from our oncology colleagues that staged therapy should also be considered. If, in fact, epoprostenol has the ability to reverse vascular remodelling of PPH, then it may be possible to administer it over the short term for patients with PPH, as part of initial therapy, in order to allow the pulmonary vascular bed to recover so that medication may be replaced with another therapy, such as oral calcium channel blockers, which might not otherwise have been effective.

Although the basis of primary pulmonary hypertension still remains unclear, the advances that have been made in our understanding of the basis of the disease have already led to exciting improvements in patient responsiveness and survival. Indeed, we anticipate that the next 10 years will continue to hold promise for the rapid development of new, effective therapies.

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2

Genetics of primary pulmonary hypertension

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Introduction

Primary pulmonary hypertension (PPH) is typically described as a sporadic disorder. However, patients with at least one affected relative have been increasingly recognized since early reports of the disorder over 50 years ago [1]. Interestingly, the natural history of PPH appears similar in both familial and sporadic forms of the disease. Recent advances in understanding the genetic basis of familial PPH are therefore likely to shed light on the pathogenic processes common to all forms of the disease.

Molecular genetic studies have identified that mutations within the gene *BMPR2* on the long arm of chromosome 2 underlie familial PPH [2,3]. This chapter describes the studies that led to these discoveries, explores the prospects for future research into the molecular mechanisms involved in the disease process and emphasizes the immediate implications for assessment and management of patients and their relatives, as a consequence of the identification of the gene associated with PPH.

Patterns of inheritance

Figures from the American National Institute of Health Registry demonstrate that at least 6% of patients with PPH have a family history of the disease [4]. However, familial cases may be difficult to detect due to delayed or missed diagnosis, inadequate case finding or the markedly reduced penetrance by which the disease gene acts. Hence, PPH individuals in families may inherit and transmit the disease gene without developing overt clinical features of the disease. This observation may explain the recognition of remote common ancestry occasionally observed in patients with apparently sporadic PPH [5].

Families with PPH demonstrate an autosomal dominant mode of inheritance, yet because the disease gene acts with reduced penetrance [6] any individual harbouring a PPH gene defect is estimated to have no greater than 10–20% chance of developing the disease [7]. This suggests that, although the gene confers a susceptibility to develop PPH, additional genetic and/or environmental factors are required for the initiation or progression of the disease. Women are twice as likely as men to develop the disease, suggesting hormonal factors may be particularly important. Of interest, a similar sex ratio is observed for cases with or without a family history of the disease. The age of onset of clinical disease is highly variable both within and between PPH families. Anticipation, a phenomenon by which successive generations are affected at an earlier age [8], has been observed.

Approach to finding the gene – positional cloning (Figure 2.1)

By 1997, two groups working independently had performed genome wide searches for the location of putative PPH genes. Linkage was established on the long arm of chromosome 2, within a broad interval of 25 cM [9,10]. Of interest, in linkage studies performed on over 40 independently ascertained kindreds, all appear to share the same chromosomal region [3,11], suggesting this to be the location of the major genetic determinant for inherited PPH. By analysis of cross-over (recombination) events, the critical interval for the PPH gene was reduced to a 4.8 cM region, flanked by polymorphic *D2S115* (centromeric) and *D2S1384* (telomeric) [12].

The region at 2q31–33 included a large number of genes, each a potential candidate for the putative PPH gene by virtue of its position. These included the apoptosis related