British Heart Foundation

Lungs under pressure

Impact of BHF support for pulmonary hypertension research

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Contents

Relieving the pressure: Impact of BHF support for pulmonary hypertension research

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1. Introduction

Pulmonary hypertension (PH) is a potentially life-threatening condition caused by high blood pressure in the arteries of the lungs. This high pressure can be caused by several different pathophysiological factors.

Over the last 60 years our understanding of PH, its causes, pathobiology and treatment have increased dramatically. The clinical classification of PH has been guided by the World Symposia on Pulmonary Hypertension, now held every 5 years. The next symposium in 2023 will mark the 50th anniversary since the first. With input from world leaders in basic science and clinical management of PH, including BHF-funded researchers, these symposia have developed and evolved the classification of PH into five main groups. These groups describe the aetiology and physiological changes that are thought to cause the increase in pulmonary artery pressure. The five groups of PH are:

- **Group 1:** Pulmonary Arterial Hypertension (PAH) – remodelling of the pulmonary arteries in the absence of secondary causes
- **Group 2:** PH due to left heart disease, often in heart failure patients
- **Group 3:** PH due to lung diseases such as chronic obstructive pulmonary disease (COPD) and/or hypoxia
- **Group 4:** PH due to pulmonary artery obstruction such as chronic thromboembolic PH (CTEPH)
- **Group 5:** PH with unclear and/or multifactorial mechanisms

In its entirety PH is a relatively uncommon condition with over 9,000[a] people in the UK living with a diagnosis of PH. But many more people living with PH are undiagnosed, most frequently associated with heart failure (Group 2) or lung disease (Group 3).

Of the five groups, only two groups have approved treatment options: Group 1 (PAH) where the increase in pulmonary artery pressure is driven by a progressive remodelling of the small pulmonary arteries; and Group 4 (CTEPH) where blood clots block arterioles and arteries to reduce blood flow.

Although PAH and CTEPH are rare they still affect approximately 6,000[a] people in the UK and are managed by seven specialist clinical centres in the UK: Golden Jubilee Hospital, Glasgow;

[a] https://digital.nhs.uk/data-and-information/publications/statistical/national-pulmonary-hypertension-audit/12th-annual-report

The Freeman Hospital, Newcastle; The Royal Hallamshire Hospital, Sheffield; Royal Papworth Hospital, Cambridge; The Royal Free Hospital, University College London; The Royal Brompton Hospital, London and Hammersmith Hospital, Imperial College London.

PAH is now further sub-categorised based on the causes of the disease:

- Group 1.1 - Idiopathic PAH, where the cause is unknown
- Group 1.2 - Heritable PAH, where PAH is caused by genetic variations
- Group 1.3 – Drug- and Toxin- induced PAH
- Group 1.4 - Associated PAH, where PAH develops in conditions with known localization of lesions in the small pulmonary arterioles, including connective tissue diseases, HIV infection, portal hypertension, congenital heart disease and Schistosomiasis

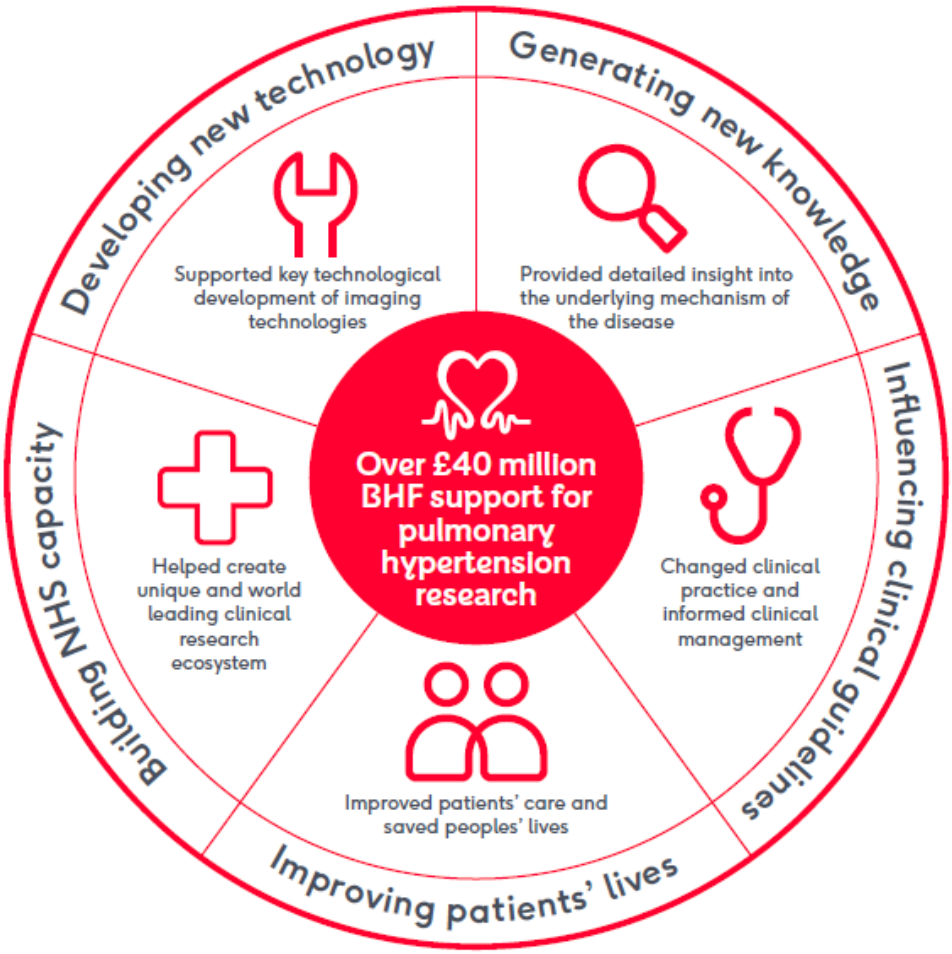
There have been substantial advances in the pharmacological treatment of PAH, CTEPH and persistent pulmonary hypertension of the newborn (PPHN) over the past 25 years. However, there is no cure other than a rarely available lung or heart-lung transplant for PAH patients, or pulmonary endarterectomy surgery for CTEPH. Most recently we have also seen the development of a pulmonary angioplasty service to broaden the options for patients.

PAH is characterised by a progressive narrowing and obliteration of the pulmonary arterioles and arteries due to cell proliferation, migration, apoptosis and recruitment of circulating inflammatory and progenitor cells. Unfortunately, due to its rare status and unspecific symptoms (breathlessness, lethargy, syncope), PAH is usually diagnosed late when the pulmonary vascular remodelling has progressed to substantially damage the pulmonary circulation, increasing blood pressure in the arteries of the lungs. As a result, the right side of the heart, the one pumping blood into the lungs, works harder and becomes enlarged and weaker, ultimately leading to right heart failure, a highly debilitating condition. Right heart failure is different from heart failure caused by direct damage to the heart, such as after a heart attack, where the left side of the heart is most affected.

Current drugs target three biochemical pathways (nitric oxide, endothelin, prostacyclin) to promote dilation of the pulmonary blood vessels and decrease the pulmonary blood pressure in PAH. This results in an easing of symptoms and increase in survival time but there is little evidence that they stop the progressive pulmonary vascular remodelling driven by the proliferation, migration and cell death of resident pulmonary vascular cells, and the infiltration of circulating progenitor and inflammatory cells.

Since its creation in 1961 the BHF has supported research into the pulmonary circulation and pulmonary hypertension. The very first BHF Chairholder, Professor Peter Harris, was appointed in 1966. His research on the pulmonary circulation extended to determining how animal and human pulmonary blood flow was adapted at high altitude, with expeditions to the Andes and Himalayas where low ambient oxygen levels can lead to high pulmonary artery pressures. Since then, the BHF has awarded almost 200 grants worth over £40M funding all stages of research, from discovery to clinical studies and supported the training of more than 30 PhD students, the career development of more than 20 fellows and 3 BHF Professors.

This report summarises the BHF's contribution to PH research, with a particular emphasis on PAH, in three main areas: discovery science, development of research tools and methods, and the effects of our funding on clinical guidelines, clinical practice, and patients' lives.



2. Generating new knowledge

The BHF has been funding basic science and clinical research into understanding the pathobiology and molecular mechanisms responsible for the development of PAH, critical in developing develop novel diagnostic tests and treatments to mitigate this condition.

Summary

- BHF has supported research into the pathological mechanisms involved in the development of PH since the 1960s, including funding world-renowned pioneering experts in the field - Professor Donald Heath and the very first BHF Chair Professor Peter Harris.
- BHF funding has contributed to understanding the role of the nitric oxide pathway in the pathophysiology of PH and the development of novel treatments.
- BHF funding helped in discovering the gene responsible for more than 70% of inherited forms of PAH.
- BHF-funded clinical research helped understand the pathobiology of persistent pulmonary hypertension of the newborn (PPHN).
- BHF-funded research has identified several promising targets that are under investigation for the development of future treatments.
- The BHF currently supports research into identifying novel mechanisms involved in the development of the disease.

a) Unveiling pathology

In 1956, five years before the creation of the BHF, Donald Heath[ab] (a medical student at the time) and colleagues in Sheffield were the first to demonstrate the importance of pathological changes in the small pulmonary arteries in patients with congenital heart disease and used the term "hypertensive pulmonary artery disease" to describe the condition [1]. In 1958, he published a detailed description of progressive structural changes in the small pulmonary arteries in the presence of chronic elevation of pulmonary artery blood pressure complicating congenital heart disease [2], which is still referred to and universally accepted today. His remarkable work shed light on a condition that was still almost unknown. Professor Heath also had a strong interest in PH linked to lung disease associated with breathing difficulties and a lack of oxygen (hypoxia) that occurs at high altitude. He observed structural changes in the pulmonary blood vessels that were distinct from those observed in the young PH patients with congenital heart disease and wondered whether the lack of oxygen could be involved.

The BHF funded work by BHF Professor Peter Harris[c] and Professor Donald Heath to study the lungs of people living permanently at high altitude and exposed to hypoxia [3]. Between the

[a] https://thorax.bmj.com/content/thoraxjnl/49/supplement/S1.1.full.pdf
[b] https://history.rcplondon.ac.uk/inspiring-physicians/donald-albert-heath
[c] https://history.rcplondon.ac.uk/inspiring-physicians/peter-charles-harris

1960s and the 1980s, they led 11 expeditions at high altitude in Peru, Bolivia and Nepal, with support from several funders including the BHF. They studied how both animals and native populations adapted to low oxygen and found evidence for genetic adaptation in populations of humans and animals (llamas, yaks) that have been living in those extreme conditions for generations. The lungs of yaks had adapted genetically to high altitude by eliminating the normal vasoconstrictor response (dynamic narrowing of the pulmonary arteries) due to the reduction in oxygen and therefore limiting the elevation of the pulmonary blood pressure. This confirmed the view that understanding of PH lay in a more intimate knowledge of the physiology and biochemistry of abnormalities of the pulmonary vasculature.

In the 1970s, the BHF funded work to replicate PAH in the lab to help investigate the molecular mechanisms involved in the pathology. We funded Professor Heath's work at the University of Liverpool and research by Professor Lynne Reid at the Royal Brompton Hospital to characterize animal models of PAH. They applied quantitative analyses to assess structural changes in the pulmonary blood vessels in rats exposed to hypoxia for different length of times, or exposed to monocrotaline, the active substance in the seeds of *Crotalaria spectabilis [4, 5]*. Both models are still commonly used to understand the pathophysiology of the disease and to test novel drug treatment. Professor Reid also applied qualitative analyses of the developing pulmonary vasculature allowing more precise determination of abnormalities in congenital heart disease and in newborn with lung disease.

b) Deciphering the molecular mechanisms of PAH:

i. Identifying the vasoconstrictive components of PAH

Blood vessels are typically made of an inner layer of endothelial cells in contact with the circulating blood, followed by layers of smooth muscular cells and an outer layer of adventitial fibroblasts. Together these cells are responsible for controlling the diameter of the blood vessels, which contract or dilate in response to a variety of signals.

- **The hypoxic pulmonary vasoconstriction response:**

The hypoxic pulmonary vasoconstriction response is a mechanism specific to the pulmonary vasculature, which allows the small pulmonary arteries to contract in response to low levels of oxygen and redirect the blood to better-oxygenated areas of the lung, thereby optimising the re-oxygenation of the blood. By contrast hypoxia dilates blood vessels outside the lung. As noted above, early work from Professor Harris identified that the hypoxic pulmonary vasoconstriction response had been eliminated by genetic selection in animals living permanently at high altitude [6].

However, even today the mechanisms underlying hypoxic pulmonary vasoconstriction remain incompletely understood. We are funding Intermediate Basic Science Research Fellow Dr Olena Rudyk at King's College London to understand how pulmonary vessels sense low oxygen levels and how this leads to blood vessel constriction and changes in the structure of the pulmonary blood vessels in PAH. She has found that a modified form of protein kinase G (PKG) 1α (oxidised form) is found in both preclinical models and samples from PAH patients, which seem to attenuate the progression of the disease, not only by promoting vasodilation but also by limiting maladaptive growth and the fibrotic (scarring) response [7]. Her research has increased our

understanding of the mechanisms leading to PAH and offers potential novel therapeutic opportunities to explore further.

- **The nitric oxide pathway: from inhaled nitric oxide to PDE5 inhibitors and sGC stimulators**

Endothelium-dependent regulation of vascular tone – the level of constriction or dilation of the vessel – was one of the key discoveries in physiology in the 1980s. In 1986, the gas nitric oxide (NO) was identified as the factor released by endothelial cells that induces relaxation of blood vessel smooth muscle. BHF Professor Andrew Henderson in Cardiff contributed significantly to the identification of this factor as NO [8]. It was subsequently discovered that NO was generated from the amino acid arginine by the enzyme nitric oxide synthase and dilates blood vessels by activating the enzyme guanylate cyclase (sGC), which in turn produces a molecule called cGMP. These discoveries were rewarded with the Nobel Prize for Medicine or Physiology in 1998.

The BHF has funded research into understanding the role of NO and cGMP in the changes in structure and function of the pulmonary circulation in PH. Professor Tim Higenbottam at the University of Cambridge was amongst the first to test the use of vasodilator drugs in PH patients. He first used a prostacyclin analogue with some success. Then, with BHF funding in the late 1980s, he demonstrated the importance of the NO pathway in pulmonary vessels [9], and then found in 1991 that this pathway was impaired in blood vessels isolated from PH patients [10]. This led Professor Higenbottam and his team to test for the first time the inhalation of NO in people with severe pulmonary hypertension [11]. This proof-of-concept study revealed that inhaled NO induced dilatation of pulmonary blood vessels, decreasing pulmonary arterial blood pressure. Moreover, they showed that it was selective to the pulmonary circulation, meaning that it didn't decrease blood pressure in the rest of the body, which could have had adverse consequences. This pioneering work revealed the potential of NO to treat PH and paved the way for the development of further treatments targeting the NO pathway.

In the late 1990s, the BHF started funding Professors Martin Wilkins and Lan Zhao at Imperial College London to study the role of cGMP in the regulation of pulmonary blood vessel structure and function. By studying blood samples from people living at high altitude in Kyrgyzstan, Professor Wilkins and his team identified a rare genetic variant in *GUCY1A3*, a gene that produces guanylate cyclase, that was associated with having lower blood pressure in the lungs, a key defence against developing PH. This work, supported throughout by the BHF, contributed to the development of a new class of drugs for PH, namely soluble guanylate cyclase stimulators, represented by the drug riociguat, which is now being used to treat people with PAH and CTEPH.

The BHF also funded Professor Wilkins and colleagues' work on the enzyme phosphodiesterase type 5 (PDE5), responsible for the degradation of cGMP. The team validated this new target in preclinical models and healthy volunteers [12]. They conducted a small proof-of-concept study in PAH patients, comparing the PDE5 inhibitor Sildenafil with Bosentan (an endothelin receptor antagonist), the only other available oral therapy for PAH at the time. Sildenafil demonstrated comparable efficacy with Bosentan, had a greater effect on reducing cardiac mass (an integrated measure of heart work), and was well tolerated [13]. Sildenafil is now the most common first line therapy for PAH patients.

- **The zinc pathway**

Adopting a similar approach to the one used for their work around the cGMP pathway, Professors Wilkins and Zhao started research to identify further gene(s) that protect against PH, which would define new pathways that could then be targeted with drugs to reproduce the effect.

In 1996, Wilkins and Zhao observed that a specific strain of rat develops only very mild PH when exposed to hypoxia [14]. Following years of cross-breeding and genetic studies, susceptibility to hypoxia-induced PH was tracked to chromosome 17 [15], then progressively by region of the chromosome to a gene that encodes a zinc transporter (Zip12) and the specific changes in that gene responsible for this protection. The results published in 2015 highlighted for the first time the importance of zinc and the protein Zip12, responsible for transporting zinc into cells of the blood vessel wall, in the development of PAH [16]. They deciphered the molecular mechanisms linking Zip12 and zinc to the changes in structure and function of pulmonary blood vessels in preclinical models of PAH. In parallel, they studied the relevance of this novel pathway in patients and observed that Zip12 is increased in the pulmonary blood vessels of people with PAH and people living at high altitude while it is absent in healthy people living at low altitude.

Their next step is to find ways to target this pathway to mimic the effect of the mutation that confers protection in the rats. The team has received further funding from a venture company to fund a drug discovery programme, with potential small molecule and antibodies in pre-clinical development.

ii. Characterising Pulmonary vascular remodelling

- **The serotonin hypothesis:**

In the 1990s, the use of new diet pills such as aminorex, fenfluramine, and chlorphentermine was shown to be associated with a risk of developing PAH. All molecules are activators of the serotonin pathway which led researchers to the "serotonin hypothesis" and its potential role in the development of PAH.

For the past 20 years, the BHF has funded Professor Mandy MacLean and her team at the University of Glasgow, and more recently at the University of Strathclyde, to decipher the role of the serotonin pathway in the development of PAH. The group showed that the production of serotonin by pulmonary endothelial cells is increased in patients with PAH and in animal models. Serotonin enters pulmonary blood vessel cells by a specific transporter, and it can activate a specific receptor on the surface of pulmonary smooth muscle cells [17]. The transporter and receptor cooperate to induce both contraction and proliferation of these cells, contributing to the functional and structural changes of the pulmonary vasculature seen in PAH.

Those findings suggest that targeting the production of serotonin synthesis or its signalling pathway could be a promising approach for the development of novel therapies for PAH.

Professor MacLean's group showed that inhibition of serotonin synthesis, inhibition of serotonin receptors and inhibition of the serotonin transporter all prevent or reverse experimental PAH. This work has led to a current clinical trial of a drug that inhibits the synthesis of serotonin by the company Altavant Sciences[a] with Professor MacLean as Scientific Advisor.

[a] https://altavant.com/wp-content/uploads/2019/10/2019_CHEST_poster_FINAL.pdf

Further research by the group has also revealed the link between the serotonin pathway and the fact that PAH affects women more frequently than men (see section below).

- **The oestrogen pathway and the sex paradigm in PAH**

PAH is more prevalent in women than in men, but men have a worse prognosis. Elevated plasma oestrogen is associated with the risk and severity of idiopathic forms of PAH in both men and postmenopausal women. Since 2010, the BHF has funded Professor Maclean and her group to investigate how oestrogen influences the development of PAH, as well as studying the converging effects of the oestrogen and serotonin pathways in the disease.

Oestrogen is synthesised from testosterone via the enzyme aromatase. The team have shown that inhibition of aromatase with the drug anastrozole can reverse PAH in females in a preclinical model of the disease, recently verified in a few patients [18].

More recently the group has shown that the metabolism of oestrogen is dysfunctional in PAH patients and damaging metabolites accumulate in the blood and lungs of patients. They showed that inhibition of oestrogen metabolism reverses several models of experimental PAH.

The team have also tested the effect of metformin, a drug used for the treatment of diabetes and which has been described to have multiple actions, including the inhibition of aromatase. They showed that metformin reversed PAH in a preclinical model of the disease and this was associated with a decreased aromatase activity and reduced oestrogen synthesis [19]. Her group has also demonstrated that the diet pill dexfenfuramine causes PH via its effects on the serotonin pathway and increased oestrogen metabolism [20].

Professor Maclean and colleagues' work thus contributed to understanding the therapeutic potential of inhibiting the aromatase enzyme for the treatment of PAH, and clinical trials using anastrazole[a] and metformin[b] are currently recruiting in the USA.

- **The role of inflammation**

Inflammation and the recruitment of inflammatory cells are regarded as playing a key role in the pathogenesis of PAH though the specific role of individual immune cell populations remains unclear.

BHF Intermediate Basic Science Research Fellow Dr Mark Ormiston and colleagues in Cambridge have found that a certain type of immune cells - called Natural Killer (NK) cells - don't function properly and are reduced in number in patients with PAH, and reported the development of spontaneous PH in two independent genetic models of NK cell dysfunction [21]. This work supports an important role for NK cells in the regulation of pulmonary and systemic vascular function and the pathogenesis of PAH. Dr Ormiston is now exploring this pathway further to find out if modifying the behaviour of NK cells may be a promising target for new PAH therapies.

Recent BHF funding to Professor Allan Lawrie and his team in Sheffield has also identified a link between sex and inflammation, where male but not female macrophage deficient mice were found to develop PAH [22]. The role of inflammation and sensing of inflammatory stimuli is also currently being studied by BHF Intermediate Clinical Research Fellow Dr Roger Thompson in Sheffield, who is investigating the role of a central mechanism of innate immune defence, called

[a] https://clinicaltrials.gov/ct2/show/NCT03229499
[b] https://clinicaltrials.gov/ct2/show/NCT03629340

double stranded RNA sensing, as a mechanism and potential therapeutic avenue for PAH [23, 24].

- **The osteoprotegerin pathway:**

Thanks to 15 years of research and continuous support from the BHF, BHF Senior Fellow Professor Allan Lawrie and his team at the University of Sheffield have identified a new pathway involved in the development of PAH. They found that the molecules osteoprotegerin (OPG) and tumour necrosis factor related apoptosis-inducing ligand (TRAIL) are increased in the blood of PAH patients and that they trigger the proliferation of pulmonary smooth muscle cells [25], a process involved in the structural changes observed in PAH. Further funding to Dr Hameed (BHF Clinical Training Fellowship) demonstrated that loss, or neutralisation of TRAIL reversed experimental PAH in rodents [26]. Further funding from the Medical Research Council (MRC) has supported the development of a human monoclonal antibody to target the OPG/TRAIL pathway. The team has recently validated these new targets in various preclinical models of PAH and demonstrated the efficacy of targeting OPG with a therapeutic antibody [27]. The team is currently looking for commercial partners to translate the human antibody into the clinic.

iii. Inherited forms of PAH: finding the gene(s)

Around the same time as Donald Heath was unveiling the pathology of PAH in the 1950s, a familial or heritable component to PAH started to be recognised, with scientific publications describing how PAH ran in families. In the same way as the identification of the vasoconstrictive aspects of PAH has led to the development of the current therapies available to patients with PAH, the identification of the genetic components driving the heritable form of PAH has led to a huge step forward in our understanding of the complex molecular mechanisms driving the pathobiology of PAH.

- **BMPR2:**

The BHF started funding Professor Richard Trembath at the University of Leicester in 1996 to identify the gene(s) causing PAH. In 1997, two teams of researchers independently identified the chromosome and specific region on that chromosome carrying the faulty gene [28]. Teaming up with the American team led by Professor William Nichols, Professor Trembath and colleagues published their results in 2000 demonstrating for the first time that mutations in the gene BMPR2 caused inherited PAH [29]. Today, we know that mutations in the gene BMPR2 are found in around 70% of familial cases and around 20% of non-heritable cases of the disease [30].

BMPR2 is a receptor for Bone Morphogenetic Proteins (BMPs), members of the Transforming Growth Factor (TGF) superfamily that regulate various cellular functions, including cell growth. In 2001, Professor Trembath and Dr Nick Morrell, now BHF Professor of Cardiopulmonary Medicine at the University of Cambridge, showed how alterations in BMPR2 cause abnormal growth responses in pulmonary artery smooth muscle cells treated with BMPs or TGF, which could contribute to the structural changes observed in PAH [31]. Since then, the BHF has supported research into further deciphering the role of the key receptor for BMPs (BMPR2) in the development of PAH and identifying ways to target this pathway. In 2015, Professor Morrell and his team showed that BMP9 treatment reversed established PAH in preclinical models of both genetic and non-genetic forms of the disease [32]. These results demonstrated the promise that direct enhancement of endothelial cell BMP signalling could be the basis for a new therapeutic strategy for PAH.

This work led to the creation of the spin-out company Morphogen-IX, which received £1.5m seed funding from Medicxi Ventures and Cambridge Innovation Capital in 2015. In 2018, the company leveraged a further £18.4m investment from Medicxi Ventures, Cambridge Innovation Capital, and Cambridge Enterprise to continue developing therapeutics for PAH. The company named their lead compound and aim to begin clinical trials in 2022.

- **Other genes:**

BHF Professor Nick Morrell, supported by MRC, NIHR and BHF funding, leads investigators across the UK to enrol patients in the UK Cohort of Idiopathic and Heritable PAH (see paragraph 3.a.) and an international consortium called PAH-ICON to identify several new rare genetic causes of PAH and CTEPH.

BHF Intermediate Basic Science Research Fellow, Dr Chris Rhodes[a] at Imperial College London, along with Professors Martin Wilkins and Nick Morrell, have combined results from the UK cohort with those from four other cohorts from Europe and North America to identify common genetic variants associated with PAH [33]. This international collaboration analysed the genome of more than 11,000 participants, making it the largest genetic study of PAH to date. They identified common variants in two genes that were associated with increased susceptibility to and severity of PAH - namely SOX17 and HLA-DPB1. Together with contemporary observations that rare genetic variation in SOX17 can cause heritable PAH, the new findings suggest that SOX17 may be more commonly involved in PAH than previously thought. The BHF is now funding Dr Rhodes to look further into the molecular mechanisms linking SOX17 to the development of PAH.

c) Paediatric PH

Before birth, the placenta supplies the baby with oxygen and the lungs are not needed. The lungs are filled with amniotic fluid and the blood vessels in the lung are constricted or closed, meaning that the pulmonary blood pressure is high. At birth, babies take their first breath, the lungs become filled with air instead of fluid and the blood vessels must dilate immediately so that the blood can get oxygenated in the lungs before being distributed to the rest of the body. The pulmonary blood pressure decreases then.

In some cases, the pulmonary blood vessels do not open properly, so the pulmonary blood pressure remains high. This is called persistent pulmonary hypertension of the newborn (PPHN). As a result, the blood cannot get properly oxygenated in the lung, meaning that not enough oxygen is getting to the heart, brain and other organs. It causes babies to look blue (blue babies or cyanosed babies) and they have difficulty breathing.

PPHN can be caused by a number of factors. The BHF funded pioneering work led by Glennis Haworth, BHF Professor at Great Ormond Street Institute of Child Health in London from 1988 to 2005, to better understand how PPHN develops with the view to better treat and prevent it.

Professor Haworth and her team focused their research on the development of the pulmonary circulation and paediatric pulmonary hypertension about which little was known in the 1970s, with an early emphasis on structural and functional development of the pulmonary circulation in pre- and post-natal life and adaptation of the pulmonary circulation to extra-uterine life. The team identified key mechanisms, some potentially therapeutically targetable, of maladaptation to extra-uterine life [34-38]. They defined critical molecular pathways, and together with

[a] https://www.imperial.ac.uk/news/189416/new-genetic-insight-could-help-treat/

Professor Julia Polak, the importance of neuropeptides in the pulmonary circulation. Professor Haworth also identified key proteins involved in the contractile apparatus of pulmonary arterial smooth muscle cells that underlie the vasoconstriction causing PH.

The team also studied the pathobiology of pulmonary vascular disease in children with congenital heart disease, at the time a leading cause of mortality and morbidity in this patient group [39-43]. Their work led to the development of techniques used today in clinical decision making in determining the operability of congenital heart disease.

3. Developing new technology

BHF-funded research has also supported the development of methods and technology that have helped researcher and clinicians around the world to understand the pathobiology of PH and develop novel diagnostic tests.

Summary

- BHF funding has supported the development of pre-clinical models of PH to reproduce the disease in the lab, key to understanding disease processes and developing novel treatments and diagnostic tests.
- The BHF helped to build a national cohort of PH patients that is now linked to an international Consortium to create the world's largest multi-omic datasets linking clinical and research data.
- BHF-funded research has helped develop novel non-invasive imaging techniques that have the potential to replace the need for more invasive tests to diagnose and follow-up of both children and adults with PH.
- Recently, BHF funded researchers have been using new machine learning and artificial intelligence approaches to improve diagnosis and guiding clinical decision making.

a) Developing preclinical models of PAH:

Preclinical animal models are key to understanding disease process and to develop better diagnostic tests and treatments for heart and circulatory conditions.

As noted in Section 2 of this report, in 1967, Donald Heath discovered that the ingestion of seeds from *Crotalaria spectabilis* induced a form of PH in rats [4, 44]. In the 1970s, the BHF funded Professor Heath's work at the University of Liverpool and work by Professor Lynne Reid at Royal Brompton Hospital to characterize animal models of PH [45]. They measured the pulmonary blood pressure and studied the structural changes in the pulmonary blood vessels in rats exposed to hypoxia for different length of times, or exposed to monocrotaline, the active substance in the *Crotalaria spectabilis* seeds [46].

Those two models are still commonly used nowadays to understand the pathophysiology of the disease. The monocrotaline model has been described in over 1750 published papers, and the hypoxia model in over 2000 papers.

Recently, BHF-funded PhD Student Alex Ainscough, supervised by Dr Beata Wojciak-Stothard and Professor Joshua Edel at Imperial College London, has been using cutting edge technology to reduce the use of animals in the study of PAH. They have developed a pulmonary artery-on-a-chip, a tiny replica of a pulmonary artery created in the lab using pulmonary blood vessel cells isolated from people with PAH, which mimics the structure of human lung arteries affected by the disease. The team is now testing whether this can be used as a model of PAH, with the aim of providing a preclinical testing tool to assist with discovery and validation of novel therapies and to reduce the need for animal models.

b) National resources to study pulmonary hypertension

In 2013, the BHF and MRC funded Professor Nick Morrell and colleagues (a large research network including 9 hospitals and research institutions throughout the UK) to establish the UK Idiopathic/Hereditary PAH (I/HPAH[a]) cohort and create a large biobank linking clinical and research data. The cohort is still growing as new cases are identified.

The cohort enabled identification of novel genetic causes of PAH in up to 20% of patients with IPAH, most commonly BMPR2 mutations [30], and (as noted above) has contributed data to an international Consortium to identify common variants associated with PAH [33]. The BHF is currently supporting the long-term follow-up of patients in the cohort and their relatives. Importantly, this cohort also provides a platform to collect additional blood samples and investigate environmental factors involved in the development of PAH and recall for clinical studies based on genotype and phenotype.

Utilising this cohort, the Consortium has developed a large biobank with linked clinical and research data that represents one of the world's largest multi-omic datasets that now includes whole genome sequences [30], whole blood transcriptomics [47], proteomics [48], and metabolomics [49]. The study has now produced over a dozen papers in leading journals that describes five new rare genetic variants, common variants and blood molecular signatures for disease. As the cohort matures, the investigators will gather important information on the penetrance of specific mutations (i.e., how often these variant genes cause disease) and develop multi-omic signature to risk stratify patients and redefine the molecular classification of PH.

The BHF support has also contributed to the running of the Sheffield Teaching Hospitals Observational Study of Pulmonary Hypertension and other cardiovascular and respiratory diseases, which over the past 10 years has contributed samples nationally (including to the UK I/HPAH Cohort) and internationally to over 19 published studies identifying biomarkers providing new insights into disease processes.

c) Developing imaging techniques

i. Imaging children with PH

The BHF is funding Professor Vivek Muthurangu and his team at University College London who showed that quantifying a specific parameter, called septal curvature [50], on MRI scan images from children with PH could be used to accurately estimate pulmonary artery pressure. This has

[a] https://www.ipahcohort.com/

now become a clinical routine and is increasingly used worldwide, both with MR and echocardiography images. Importantly, this has reduced the need for invasive diagnostic procedures in these young patients. They were also the first to demonstrate the prognostic ability of cardiac MRI in children with pulmonary hypertension. After this study, cardiac MRI became a routine part of follow up of children with PH in their centre [51].

They also leveraged rapid imaging technologies developed by the group with BHF support, to perform scans in less than 10 minutes without sedation in children as young as 6 months old.

The team has also used real-time MRI during physical exercise in children with pulmonary hypertension [52], which suggested that poor exercise capacity can also result in skeletal muscle abnormalities rather than just cardiac dysfunction alone. These findings have opened a whole new area of possible treatment for a group of children in whom conventional therapies often fail.

ii. Imaging adults with PH / POLARIS

In 2016, the BHF, the contributed with the MRC to a £7.5M award to Professor Jim Wild and his team at the University of Sheffield for pulmonary imaging infrastructure to broaden the clinical uptake of a novel MRI technique known as hyperpolarisation MRI. The support to POLARIS (Pulmonary, Lung and Respiratory Imaging Sheffield) was specifically provided to help translate novel MR-based tools for diagnosis, prognosis and assessment of response to treatment in pulmonary and cardiac diseases to routine clinical assessment.

The group has developed hyperpolarisation MRI methods to image gas exchange in the pulmonary blood vessels. They have shown that MRI imaging can be used to diagnose PH [53], improve risk stratification of patients [54], and is an ideal surrogate end-point for clinical trials of PAH therapies predicting clinical worsening and mortality in addition to being sensitive to treatment effect and highly repeatable and thereby reducing the need for invasive testing, radiation exposure and risk to patients [55]. Over the last 15 years the group have created the world's largest registry of MR imaging data in patients with PH. They have also redefined clinical imaging practice in pulmonary vascular disease with Sheffield protocols for cardiac-pulmonary vascular MRI, now underpinning clinical examination of over 25% of the UK population of NHS patients with pulmonary hypertension. This has replaced nuclear medicine scans (requiring injection of small amounts of radioactive molecules) and this change in practice has been extended to Europe and 7 German Centres in a landmark Phase-III multi-centre diagnostic trial CHANGE-MRI[a]. Pulmonary vascular image based models have defined international guidelines for imaging in PH [56, 57].

iii. Imaging and artificial intelligence

Imaging data is particularly amenable to new machine learning and artificial intelligence approaches to improve diagnosis and guiding clinical decision making.

Professor Declan O'Regan and colleagues at Imperial College London, funded by the BHF and the MRC, developed a deep learning cardiac motion analysis program called 4D Survival. This program automatically interprets thousands of heart scans, building a detailed three-

[a] http://change-mri.de/index.php?id=373&L=1

dimensional model of the heart, so that the computer can learn to recognise the earliest and most important signs of heart failure and predict the risk of dying. So far, the team have used the technology to predict the prognosis for 302 patients, most of whom had Group 3 PH. 4D Survival outperformed doctors, being able to correctly predict a patient's prognosis 75% of the time [58]. It is hoped that the new technology will help doctors to identify patients likely to deteriorate quickly and thus have a substantial impact on clinical decision making. The team are now testing the technology's ability to help the estimated 920,000 people in the UK living with heart failure, resulting from PH or other diseases.

The group in Sheffield is also developing novel machine learning approaches that have the potential to make the diagnosis of PH less invasive. Currently, clinicians confirm the diagnosis of PH via right-heart catheterization, where a catheter or small tube is guided from the jugular vein or sometimes a large arm or leg vein to the right side of the heart and then into the pulmonary artery. This allows the measurement of the pressure inside the heart and lungs. Dr Andy Swift and colleagues have developed a machine learning tool to extract disease features from cardiac MRI scan images and automate PAH diagnosis [59]. This provides rapid and accurate diagnosis of PAH with the advantage of not being invasive.

4. Influencing clinical guidelines and practice

Because PAH is a set of diseases with different causes there are several targeted therapies available today, depending on what is causing the condition and the severity of the symptoms. Current treatments aim at slowing down the progression of the disease. In the most serious and advanced forms of PAH, lung transplant or a heart-lung transplant may be needed. If the underlying cause is identified and treated early, it may be possible to prevent permanent damage to the pulmonary blood vessels and the heart.

Summary

- BHF-funded research and researchers have contributed to several clinical guidelines for the diagnosis, treatment and management of children and adults with PH.
- BHF-funded research has contributed to the development of several drugs affecting the nitric oxide pathway and that are used in the treatment of PAH and CTEPH.
- Historic BHF funding has contributed to the refinement of heart-lung transplantation, the only cure available for the most severe forms of PAH.
- The BHF has been campaigning for years for the adoption of a new opt-out organ donation system that has been introduced in Wales in 2015, England in 2019, Scotland in 2020 and is currently being considered by Northern Ireland.

a) Targeting the NO pathway

Professor Higenbottam's pioneering work has contributed to the development of inhaled NO for the treatment of PAH and paved the way for the development of further treatments targeting

the NO pathway. BHF-funded work by Professors Martin Wilkins and Lan Zhao at Imperial College London contributed to the development of two new classes of drug acting on proteins downstream of NO:

- phosphodiesterase inhibitors, like sildenafil, are the most prescribed in the UK for the treatment of PAH, CTEPH and PPHN (including monotherapy or combination therapy)[a].
- soluble guanylate cyclase stimulators like riociguat, is increasingly being prescribed in the UK since 2015, mostly in CTEPH patients[a].

b) Diagnosis, treatment and management of paediatric PH

BHF Professor Glennis Haworth and her team's pioneering work into the pathobiology of PPHN led to early epidemiological insights and description of clinical phenotypes in children with PH and early data on safety and efficacy of targeted PH therapies in children which informed the development of clinical practice guidelines (see section 2c for more details). In 2011, Professor Haworth led the task force that defined the international consensus for the classification of paediatric PH.

Professor Vivek Muthurangu and his team at University College London have developed several imaging techniques to help diagnose and follow-up young patients with PH without the need for more invasive techniques (see section 3c). Those novel methods have now become a clinical routine in UK Paediatric Centres and are increasingly used worldwide. Due to their extensive work in PH imaging in children, Professor Muthurangu and his team were heavily involved in developing an expert consensus document on the use of cardiac MR and CT for diagnosis of paediatric PH [60].

c) Heart-lung transplantation

The BHF has supported extensively the field of heart and heart-lung transplantation research. Advances in this field will discussed in another review focused on the impact of BHF support for cardiovascular surgery research.

5. Improving patients' lives

Major improvements in patient care require the support of multiple funding bodies and are achieved through international research efforts. BHF-funded research into PH has contributed significantly to improve the survival and quality of life of people with PH in the UK and worldwide.

Summary

- Research supported by the BHF has led to replacing higher risk invasive diagnostic cardiac catheterization in young patients with PH.
- BHF-funded research has supported the development of novel therapies that have increased the survival and quality of life of people with PAH and CTEPH.

[a] https://digital.nhs.uk/data-and-information/publications/statistical/national-pulmonary-hypertension-audit/2019

Several BHF funded studies have had a significant impact on the management of young patients with PH. Early studies into the utility of MRI in children with PH have resulted in this test now being routinely used these patients across the UK to replace higher risk invasive diagnostic cardiac catheterization. In addition, BHF-funded research helped to develop MR guided and augmented cardiac catheterization. This technique is used for example to measure the resistance in the lungs in children and adults with congenital heart disease. The benefits of this technique are more accurate measurements and no exposure to harmful ionizing radiation. As a result of the work carried out by BHF-funded researchers this technique is now being disseminated to the wider community and is on the cusp of becoming clinical routine.

PH is a life-threatening condition, and without effective and appropriate treatment the five-year survival is only 27% for those with severe disease, which has been increased to 54% with certain targeted therapies[a].

In 2017, the Pulmonary Hypertension Association UK ran a survey among PH patients to find out 'What it means to live with PH today'[b]. They found that:

- 87% of people with PH said their PH treatment and management had improved their overall quality of life (45% said it had improved it 'a lot')

- 78% said it had improved their general mental and emotional wellbeing (24% said it had improved it 'a lot')

- 73% said it had improved their relationships (49% said it had a 'major impact')

The patients surveyed also shared how their treatment had changed their day-to-day lives:

"*I am able to do a lot more than I could before treatment, but I now know my limitations. I'm alive to see my beautiful grandchildren, which before diagnosis I didn't think I would be.*"

"*Over the past few years, I have felt in total control of my illness and the medication I am prescribed, but if my condition goes downhill, I know the doctors will deliver the appropriate care for me.*"

"*After 7 years on triple therapy, I am able to do what I did before*"

"[When my treatment started] *I no longer needed my mobility scooter, came off the transplant list, and went back to the job I trained for after office work during my very ill years.*"

[a] https://www.nice.org.uk/guidance/gid-tag382/documents/pulmonary-arterial-hypertension-association-uk4
[b] https://d1w4rdyew1npvx.cloudfront.net/app/uploads/2019/08/What-it-means-to-live-with-PH-today.pdf

6. Building NHS capacity

In 2002, BHF Professor Glennis Haworth CBE founded the UK Pulmonary Hypertension Service for Children, which she led for many years. This clinical network, then the first of its kind in the world, helped to identify the best treatments for children with pulmonary hypertension, and still cares for children throughout the UK with the condition. Professor Haworth's work has been instrumental in improving the survival rates of these children.

PAH and CTEPH are managed by seven specialist clinical centres in the UK. Research into pulmonary hypertension in the UK is well embedded in this network of hospitals, which allows clinician and researchers to establish cohorts of patients and create large biobanks, key to finding new ways to treat pulmonary hypertension. This environment creates an integrated roadmap across the translational continuum of discovery science towards clinical application.

7. Conclusion

The BHF has supported world-leading research and researchers that helped improve the lives of many people with PH. Their contribution to the field is highlighted by Professor Marlene Rabinovitch, a world leader in PH research at Stanford University, and Dr Iain Armstrong, Chair of the Pulmonary Hypertension Association UK:

"*The BHF contributes a large amount to improving understanding of pulmonary hypertension and outcomes for patients. Their funding of research, and genetic links in particular, has a huge impact. The National Cohort Study of Idiopathic and Heritable PAH is the largest national longitudinal study of PAH and has been supported for its eight-year tenure by the BHF. The outcomes of the study have real potential to impact diagnosis times and treatment pathways by understanding more about what triggers the disease. PH is a rare disease, so its visibility on the BHF website is appreciated. And affecting both the lungs and heart means PH sits in a 'no man's land' between cardiology and respiratory, so the work the BHF is doing is really important in encouraging understanding and raising awareness of the condition.*"
Dr Iain Armstrong

"This report highlights the leadership and foresight of the British Heart Foundation in funding major breakthroughs of historical significance that have been essential to our current understanding of pulmonary hypertension and to the development of treatments used around the world, and that pave the way for finding a cure for this devastating condition."
Professor Marlene Rabinovitch

However, there is currently no cure and an imperfect understanding of the causes of this condition. More research is needed, and the world-leading expertise in PH research in the UK still needs the BHF's support. We confidently expect that new ground-breaking science and technical advances, building on previous BHF-funded research, will lead to further successes in combatting PH.

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