

REVIEW ARTICLE

Hypoxia-induced pulmonary hypertension—Utilizing experiments of nature

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An increase in pulmonary artery pressure is a common observation in adult mammals exposed to global alveolar hypoxia. It is considered a maladaptive response that places an increased workload on the right ventricle. The mechanisms initiating and maintaining the elevated pressure are of considerable interest in understanding pulmonary vascular homeostasis. There is an expectation that identifying the key molecules in the integrated vascular response to hypoxia will inform potential drug targets. One strategy is to take advantage of experiments of nature, specifically, to understand the genetic basis for the inter-individual variation in the pulmonary vascular response to acute and chronic hypoxia. To date, detailed phenotyping of highlanders has focused on haematocrit and oxygen saturation rather than cardiovascular phenotypes. This review explores what we can learn from those studies with respect to the pulmonary circulation.

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1 | INTRODUCTION

Under physiological conditions, the adult pulmonary circulation is maintained as a high-flow, low-pressure, and low-resistance system through which the entire cardiac output (CO) must pass. Exposure to

hypoxia leads to the constriction of small resistance arteries in the lung, referred to as hypoxic pulmonary vasoconstriction (HPV) (Euler, 1946). It is a physiological mechanism to divert blood to better oxygenated lung segments and optimize gas exchange by adapting blood flow (perfusion) to alveolar ventilation (Dunham-Snary

Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; BMPR2, bone morphogenetic protein receptor type 2; CO, cardiac output; FIH, factor inhibiting hypoxia-inducible factor; HAPH, high-altitude pulmonary hypertension; HIF, hypoxia-inducible factor; HPV, hypoxic pulmonary vasoconstriction; HREs, hypoxia response elements; PAH, pulmonary arterial hypertension; PAP, pulmonary artery pressure; PASMC, pulmonary arterial smooth muscle cell; PHD, prolyl hydroxylase; PO₂, partial pressure of oxygen; PVR, pulmonary vascular resistance; ROC, receptor-operated Ca²⁺ channel; SOC, store-operated Ca²⁺ channel; VDCC, voltage-dependent L-type of Ca²⁺ channel; VEGFR2, VEGF receptor 2; VHL, von Hippel-Lindau.

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et al., 2017; Sylvester, Shimoda, Aaronson, & Ward, 2012). This is beneficial if there is regional obstruction of airflow and the beneficial effect of HPV on arterial partial pressure of oxygen (PaO_2) is maximal when the amount of the hypoxic lung is 30%–70%. If the area of vasoconstriction is small, the influence on PaO_2 is negligible. HPV is thus an autoregulatory mechanism that protects PaO_2 by decreasing the amount of shunt flow that can occur through the hypoxic lung (Benumof, 1985; Marshall & Marshall, 1980; Sylvester et al., 2012; Theissen & Meissner, 1996). In the case of *global* alveolar hypoxia, such as that occurring at high altitude, HPV can be detrimental because it leads to a sustained overall vasoconstriction and a significant increase in pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP)—see Box 1 (Dunham-Snary et al., 2017; Penaloza & Arias-Stella, 2007).

Box 1 Pulmonary haemodynamics

Pulmonary arterial pressure (PAP) is a function of cardiac output (CO) and pulmonary vascular resistance (PVR): $\text{PAP} = \text{CO} \times \text{PVR}$. An increase in CO and/or PVR will lead to the elevation of PAP. As described by Poiseuille's Law, PVR is inversely proportional to the fourth of power of radius (r) of pulmonary arteries: $\text{PVR} = (8\eta L)/(\pi r^4)$, where η is viscosity of the blood, L is the total length of pulmonary artery, π is a constant (3.14), and r is the intraluminal radius of pulmonary artery. This equation indicates that a small decrease in vessel lumen diameter (or radius) would lead to a marked increase in PVR. In patients with high-altitude pulmonary hypertension (HAPH), increased PVR is the major cause for increased PAP as CO remains largely unchanged.

Sustained exposure to hypoxia leads to structural changes in pulmonary vessels that increase vascular stiffness, decrease the luminal diameter of arteries, and increase resistance to blood flow. HPV and vascular remodelling, together with a rise in haematocrit and thus blood viscosity, comprise the components of hypoxia-induced pulmonary hypertension, which places an increased workload on the right ventricle (Julian & Moore, 2019; Penaloza & Arias-Stella, 2007). Hypoxia may contribute to the pulmonary hypertension that occurs in some patients with chronic obstructive pulmonary disease, chronic lung fibrotic diseases, and obstructive sleep apnoea (Simonneau et al., 2019). While not associated with alveolar hypoxia, hypoxia-sensitive genes are expressed in the remodelled arteries of patients with pulmonary arterial hypertension (PAH). Here, we review high-altitude pulmonary hypertension (HAPH—see Box 2) and explore the potential that studies in animals and humans exposed to chronic hypoxia might make to identifying and prioritizing pharmacological targets for PAH.

Box 2 Consensus definitions

High-altitude pulmonary hypertension (HAPH): Defined by consensus as a mean pulmonary artery pressure >30 mmHg; systolic >50 mmHg measured at the altitude of residence, right ventricular hypertrophy, heart failure, and the absence of excessive erythrocytosis (females $\text{Hb} < 19 \text{ g}\cdot\text{dl}^{-1}$; males $\text{Hb} < 21 \text{ g}\cdot\text{dl}^{-1}$) (Leon-Velarde et al., 2005).

Pulmonary arterial hypertension (PAH): Mean resting pulmonary artery pressure ≥ 25 mmHg and a wedge pressure ≤ 15 mmHg (Galie et al., 2015).

2 | MOLECULAR BASIS OF HPV

The precise mechanism of HPV remains unclear (Sylvester et al., 2012). Published studies indicate that both the sensor and effector mechanisms responsible for acute HPV are located in pulmonary arterial smooth muscle cells (PASMCs) (Bigham & Lee, 2014; Wang et al., 2006; Young, Williams, & Thompson, 2019), while endothelial cells and extracellular matrix cells play an important role in modulating HPV (Dimmeler et al., 1999; Fukuroda et al., 1994; Sakao, Tatsumi, & Voelkel, 2009; Tian, McKnight, & Russell, 1997; Xu et al., 2015). The initial event for HPV is thought to be a mitochondrial redox signal in response to low PO_2 (Dunham-Snary et al., 2017; Peng et al., 2011; Sommer et al., 2017), and then the “ O_2 sensor” signals to the “effectors”, leading to smooth muscle contraction. The canonical mechanism of HPV includes PASMC membrane depolarization due to acute hypoxia-induced reduction of K^+ channel activity. This subsequently opens **voltage-dependent L-type of Ca^{2+}** channels (VDCCs) and increases cytosolic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$) via Ca^{2+} influx through VDCC. Meanwhile, hypoxia can also directly open receptor-operated Ca^{2+} channels (ROCs) and store-operated Ca^{2+} channels (SOCs), causing an increase in $[\text{Ca}^{2+}]_{\text{cyt}}$ in PASMC (Dunham-Snary et al., 2017; Luks & Swenson, 2015; Mauban, Remillard, & Yuan, 2005; Sylvester et al., 2012). Elevated $[\text{Ca}^{2+}]_{\text{cyt}}$ is a major trigger for PASMC contraction and proliferation (He et al., 2018; Kuhr, Smith, Song, Levitan, & Yuan, 2012; Song et al., 2018). Therefore, if mitochondria or the mitochondrial respiratory chain is the O_2 sensor in PASMC, the membrane receptors and ion channels and cytosolic Ca^{2+} are the effectors for HPV.

3 | HYPOXIA-INDUCED VASCULAR REMODELLING

The initial pulmonary vascular structural changes on exposure to hypoxia include endothelial blebbing and disruption of the endothelial barrier, allowing an influx of plasma proteins, including growth factors,

across the endothelial barrier. The hallmark of chronic hypoxia-induced pulmonary vascular remodelling is the extension of vascular smooth muscle to previously un-muscularized arterioles (Arias-Stella & Saldana, 1963; Stenmark, Fagan, & Frid, 2006). Medial and adventitial thickening are also observed, the former from smooth muscle cell hypertrophy as well as accumulation of smooth muscle cells, the latter from an increase in fibroblasts and myofibroblasts and in extracellular matrix.

The remodelling witnessed with hypoxia differs from that seen with PAH in that with most species, including man, it is less severe than in PAH, and there is no occlusion of vessels. One exception is the neonatal hypoxic calf model, which can develop marked intimal thickening associated with a very high PAP (Stenmark et al., 1987). The plexiform lesion seen in PAH is not a component of hypoxia-induced pulmonary hypertension. An influx of inflammatory cells is evident in hypoxic lungs but is less pronounced than that in seen in PAH.

Moreover, the concept that hypoxia leads to an increase in PVR from remodelling that narrows the vessel lumen has been challenged, as has the idea that hypoxia leads to vascular rarefaction or “pruning.” Hypoxia stimulates angiogenesis. Studies in rats have shown that chronic hypoxia increases total pulmonary vessel length, volume, endothelial surface area, and the number of endothelial cells (Howell, Preston, & McLoughlin, 2003; Hyvelin et al., 2005). Coupled with experimental studies in rodents showing that inhibition of the anti-angiogenic factor, **angiostatin (plasminogen)**, aggravates, and overexpression of VEGF protects rats from hypoxia-induced pulmonary hypertension, the suggestion is that, at least in rodents, angiogenesis plays a significant role in the response of the pulmonary circulation to chronic hypoxia, perhaps acting to reduce the effects of HPV and structural changes elsewhere on the right ventricle.

Chronic hypoxia-induced vascular changes gradually reverse after returning to a normal oxygen environment (Sime, Penalzo, & Ruiz, 1971; Zhao, Hughes, & Winter, 1994). This gives hope that vascular remodelling in PAH may also be reversible. Single lung transplantation (Deb et al., 2006; Levy, Liapis, Eisenberg, Botney, & Trulock, 2001) and main pulmonary artery banding (Wagenvoort, Keutel, Mooi, & Wagenvoort, 1984) have been reported to regress occlusive lesions in PAH. Although such regression is the goal of new treatments, it remains to be demonstrated that this can be achieved pharmacologically. Haemodynamic stress appears to be a factor in the development of pulmonary vascular remodelling on exposure to hypoxia. Banding of the pulmonary artery prevented the extension of muscle into peripheral arteries and the medial hypertrophy of normally muscular arteries during chronic hypoxic exposure (Rabinovitch et al., 1983) and reversed occlusive lesions in a hypoxia-dependent rodent model of pulmonary hypertension (Abe et al., 2016).

Unlike the immediate vasoconstrictor response to hypoxia, vascular remodelling requires new protein synthesis. A panoply of factors (reviewed elsewhere, Stenmark et al., 2006; Wilkins, Ghofrani, Weissmann, Aldashev, & Zhao, 2015) have been implicated in mediating the structural changes, from vasoactive molecules (such as **endothelin**) to growth factors (e.g., **PDGF**) and cytokines (e.g., **IL-6** and **TNF- α**). Changes in the underlying response of vascular cells to

these factors are also involved. Not surprisingly, the role of **hypoxia-inducible factors (HIFs)** and their target genes are of primary interest.

4 | HIF SIGNALLING PATHWAY

HIFs belong to a group of basic-loop-helix-PER-ARNT-SIM (bHLH-PAS) proteins that function as transcription factors responding to oxygen and other stresses (Semenza, 2012; Wang, Jiang, Rue, & Semenza, 1995). HIFs function as heterodimeric transcription factors comprising a constitutively expressed β unit, HIF1 β (also known as aryl hydrocarbon receptor nuclear translocator, ARNT), and an α subunit that is sensitive to oxygen levels (Figure 1). Currently, three α subunits have been identified and include HIF1 α , HIF2 α , and HIF3 α . The α and β subunits share a high percentage of both amino acid and structural homology, the N-terminal region (bHLH) which enables DNA binding, two PAS domains (PAS-A and PAS-B) that contribute to heterodimer stability, and an oxygen-dependent degradation domain.

In an oxygen-rich environment, HIFs are instantaneously inactivated (Jewell et al., 2001) by post-transcriptional hydroxylation of conserved proline amino-acid residues within the α subunit. Hydroxylation generates a high affinity binding site for pVHL, leading to polyubiquitination and degradation by the proteasome. HIF prolyl hydroxylation is mediated by **prolyl hydroxylases (PHDs)**. PHD activity requires several co-factors including molecular oxygen; a K_M greater than 250 μ M is above the oxygen concentration typically found in arterial blood (185 μ M), and so the intracellular PO₂ will always fall below the K_M for oxygen, allowing enzyme activity to be modulated by oxygen availability over the entire physiological range (Hirsila, Koivunen, Gunzler, Kivirikko, & Myllyharju, 2003). Obviously, evolution has come up with a mechanism that constitutively synthesizes molecules that allow a very fast response to hypoxia. This seems to be a crucial adaptation. The price for this is that if low oxygen conditions do not occur, all α subunits of HIFs are degraded without any further use.

Under hypoxic conditions, the loss of hydroxylation leads to the accumulation of HIF α isoforms and the formation of heterodimers with the constitutively expressed HIF1 β (Kaelin & Ratcliffe, 2008). This HIF complex, together with the **histone acetyltransferase p300**, binds to hypoxia response elements (HREs) in DNA, initiating or enhancing transcription of target genes. There is, however, a second tier of HIF regulation through the action of an asparagine hydroxylase, known as factor inhibiting HIF (FIH). Originally found to be a negative regulator of HIF1 α , it was later shown to be an asparaginyl hydroxylase capable of hydroxylating N⁸⁰³ in the C-terminal domain of HIF1 α (Schofield & Ratcliffe, 2004). FIH has a K_M for molecular oxygen between 90 and 200 μ M. FIH hydroxylates target proteins with a higher efficiency at lower oxygen tensions than the PHDs, consequently continuing to hydroxylate target proteins in oxygen tensions below 5% (68 μ M). The efficiency of FIH hydroxylation appears to be substrate dependent, as hydroxylation of HIF1 α occurs with a greater efficiency (90%) than that of HIF2 α (<30%) in normal physiological oxygen (Bracken et al., 2006; Yan, Bartz, Mao, Li, & Kaelin, 2007). However, unlike the PHDs, FIH has a greater number of HIF-independent targets; for example, the

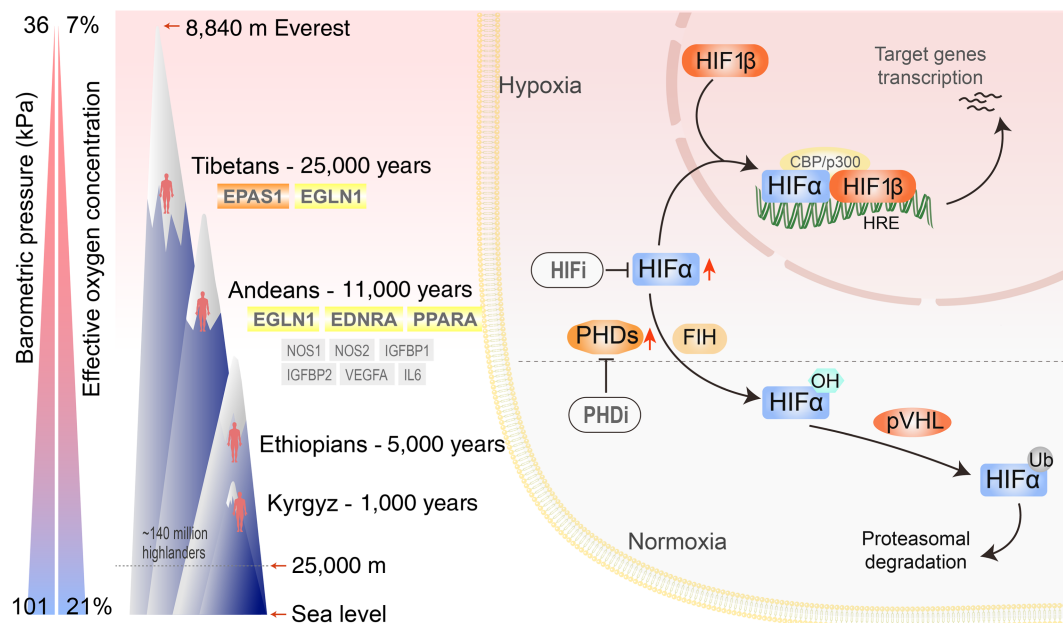


FIGURE 1 An estimated 140 million people live above 2,500 m. On the left, the barometric pressure (in kPa; 1 kPa = 7.5 mmHg or Torr) and effective oxygen concentrations (amount of oxygen molecule in a given volume of air, in %) are shown at increasing altitudes. Note that the fraction of inspired oxygen (FiO_2) in the air always stays at 21%, even at high altitude. The populations are depicted at their average residence altitude, and the time since they have occupied that region is given in years. Genetic studies in populations adapted to life at high altitudes, for example, Tibetans and Andeans, have revealed the importance of EPAS1 (which encodes hypoxia-inducible factor 2 α [HIF2 α]) and EGLN1 (encoding hypoxia-inducible factor prolyl hydroxylase 2 [PHD2]), supporting efforts to pharmacologically manipulate the HIF pathway as a treatment for pulmonary hypertension. The diagram on the right shows degradation of hydroxylated HIF α subunits in normoxia via the von Hippel–Lindau (VHL) pathway. In hypoxia, HIF α stabilization and dimerization with HIF β occurs, which together with histone acetyltransferase p300, binds to hypoxia response elements (HREs) in DNA, initiating or enhancing transcription of target genes. HIF inhibitors (HIFis) with specific activity against HIF2 α are under investigation as a potential treatment for pulmonary hypertension. FIH, factor inhibiting HIF; PHDi, prolyl hydroxylase inhibitors

efficacy for Notch hydroxylation appears to be far greater than that for HIF α (Coleman et al., 2007).

Both HIF1 α and HIF2 α isoforms have been extensively studied in pulmonary hypertension. The first direct evidence came from mice hemizygous for either HIF1 α or HIF2 α (Brusselmans et al., 2003; Shimoda, Manalo, Sham, Semenza, & Sylvester, 2001). Pulmonary disease progression following chronic hypoxia exposure was substantially delayed in these models. The aberrant stability of both HIF1 α and HIF2 α was initially reported in whole lung tissue from PAH patients, then subsequently in pulmonary endothelial and smooth muscle cells from this patient group (Ball et al., 2014; Barnes, Chen, Sedan, & Cornfield, 2017; Bonnet et al., 2006). Murine studies identified a definitive tissue-specific HIF α expression profile in the pulmonary vasculature, where HIF2 α was found to be highly expressed in the endothelium. Genetic ablation of pulmonary endothelial HIF2 α prevents the initiation and development of pulmonary vascular remodelling associated with pulmonary hypertension, whereas loss of HIF1 α in the same model system has little or no effect, highlighting the importance of pulmonary endothelial HIF2 α in this disease process (Cowburn et al., 2016). Several groups have now reported that endothelial loss of PHD2 in mice leads to the aberrant stability of HIF2 α with the development of occlusive vascular lesions and severe pulmonary hypertension (Dai, Li, Wharton, Zhu, & Zhao, 2016; Tang

et al., 2018). The concomitant genetic ablation of endothelial PHD2 and HIF2 α in this model also inhibited the phenotype, offering near complete protection from pulmonary hypertension (Dai et al., 2016; Kapitsinou et al., 2016). Mutations resulting in loss of von Hippel–Lindau (VHL) activity lead to mice with “Chuvash polycythaemia” and pulmonary hypertension, which is rescued by heterozygous deletion of HIF2 α (but not HIF1 α) (Hickey et al., 2010).

In sum, genetic manipulation studies with murine endothelial cells point to HIF2 α as the predominant HIF α isoform driving pulmonary hypertension. Additional support has been found in gain-of-function gene mutations. Mice with a global HIF2 α gain-of-function mutation spontaneously develop pulmonary vascular disease (Formenti et al., 2011; Tan et al., 2013). High resting PAPs have been reported on echocardiography assessment of two patients with a gain-of-function mutation in HIF2 α (Formenti et al., 2011).

5 | POPULATIONS LIVING AT HIGH ALTITUDE

Accurate up-to-date data are difficult to come by, but a commonly used statistic reports that roughly 7% of the world's population live above 1,500 m with some 140 million highlanders above 2,500 m

(Moore, Niermeyer, & Zamudio, 1998; Penalzoa & Arias-Stella, 2007) (Figure 1). Barometric pressure falls with increasing altitude. The percentage of oxygen in the atmosphere remains constant (21%), but atmospheric PO_2 reduces proportionally with barometric pressure, leading to hypobaric hypoxia. At 2,500 m above sea level, barometric pressure falls from 101 kPa (760 mmHg or Torr) at sea level to 75 kPa (562 mmHg or Torr), and effective oxygen concentration (the amount of oxygen molecules in a given volume of air) drops from 21% to 15% (see Box 3). For example, on the summit of Mount Everest (8,848 m), the barometric pressure is 36 kPa, and the % of effective (or apparent) oxygen concentration is 7% (Hopfl, Ogunshola, & Gassmann, 2003).

A recent meta-analysis has challenged the view that HAPH, as currently defined (see Box 2), is common in highlanders (Soria, Egger, Scherrer, Bender, & Rimoldi, 2016). This analysis identified 12 studies that had collected echocardiographic estimations of PAP from a total of 834 high-altitude residents, with all but one study being performed between 3,600 and 4,350 m. Mean systolic PAP was approximately 7 mmHg higher than recorded at low altitudes. It was noted that HAPH, using the consensus definition, was rare, as less than 1% of those included in the analysis had a mean PAP above 27.1 mmHg. The authors called for a reconsideration of the definition of HAPH. Interestingly, this coincides with a call to revisit the definition of pulmonary hypertension at lower altitudes, a recognition that modest elevation of PAP may have health consequences. The mean PAP in healthy individuals at rest at low altitude is approximately 14.0 ± 3.3 mmHg (Kovacs, Berghold, Scheidl, & Olschewski, 2009). Two SDs above this is 20 mmHg, suggesting that a mean PAP above this value should be considered abnormal (Simonneau et al., 2019).

Box 3 Alveolar partial pressure of oxygen (PAO_2)

PAO_2 is determined by the partial pressure of inspired O_2 (PIO_2) and alveolar (or arterial) partial pressure of CO_2 (PaCO_2). PIO_2 is determined by the fraction of inspired O_2 (FIO_2 , which is 21% in room air) and barometric pressure (P_B , or atmospheric pressure):

$\text{PIO}_2 = \text{FIO}_2 \times (P_B - P_{\text{H}_2\text{O}}) = 21\% \times (760 - 47) = 150 \text{ mmHg}$
or Torr (or 20 kPa) in sea level.

$\text{PIO}_2 = \text{FIO}_2 \times (P_B - P_{\text{H}_2\text{O}}) = 21\% \times (270 - 47) = 46 \text{ mmHg}$
or Torr (or 6 kPa) at Mt. Everest where $P_{\text{H}_2\text{O}}$ is water vapour pressure. Note that the fraction of inspired O_2 in the air always stays at 21% even at altitude.

Alveolar gas equation is used to calculate PAO_2 :

$\text{PAO}_2 = \text{PIO}_2 - (\text{PACO}_2 / \text{RQ}) = 150 - (40 / 0.8) = 100 \text{ mmHg}$
or Torr (or 13 kPa) in sea level where RQ is the respiratory quotient (≈ 0.8 , the ratio of CO_2 excretion to O_2 uptake in the lungs); PACO_2 is alveolar PCO_2 which is very close to arterial alveolar PCO_2 (PaCO_2).

While the average rise in PAP in high-altitude populations may be modest, there is significant inter-individual variability in response to hypoxia. This has been observed both between and within species. It is in this context that high-altitude regions are perhaps most useful as a natural laboratory for investigating hypoxic response mechanisms in humans (Wilkins et al., 2015). Of particular interest is the relationship of genotype to phenotype. Given that environmental hypoxia is a potent selection pressure, particularly at birth, individuals that exhibit the lowest PAP at altitude might be expected to host genetic variants associated with adaptive molecular pathways. As genetic variants linked to phenotype offer a powerful strategy for defining critical molecular pathways, studying the genetic basis of adaptive responses to hypoxia provides an important approach to elucidating major drivers of HAPH.

The high-altitude populations for which most data are available are Tibetans, Andeans, Ethiopians, and, somewhat less, the Kyrgyz (Figure 1). Humans have occupied the Tibetan plateau for over 25,000 years, and those we refer to as Tibetans today split from Han Chinese, the usual control group in comparator studies, around 2,750–5,500 years ago (Yang et al., 2017). Human inhabitation of the Andean Altiplano began around 11,000 years ago, the Semian Plateau in Ethiopia around 5,000 years ago, and the Tien-Shen mountains in Kyrgyzstan only in the last 1,000 years. Given their longer history at altitude, Tibetans have had more time to adapt.

The most robustly quantitative traits studied at altitude are haemoglobin (Hb) levels (Gassmann et al., 2019) and O_2 -carrying capacity and, here, there is agreement. Tibetans have a higher resting ventilation but lower arterial O_2 content, than Andean or Han Chinese migrants and are arguably the most hypoxic of the high-altitude populations commonly studied. They also run lower Hb concentrations, by around 0.1 g.l^{-1} compared to Han Chinese at the same elevation.

Accurate cardiopulmonary phenotyping in the field is more challenging, and detailed studies are few. A widely held view is that Tibetans are more resistant to HAPH and there is a small but persuasive body of data in support of this. An early study of five Tibetans who underwent direct cardiac catheterization at $\geq 3,600$ m reported PAP measurements in the same range as those measured in populations at sea level and only a minimal change in PVR when those subjects were exposed to greater hypoxia (Groves et al., 1993). Related to this, histology of lung specimens from deceased Himalayan residents shows no remodelling of small pulmonary arteries (Gupta, Rao, Anand, Banerjee, & Boparai, 1992). More recent studies of ethnic Tibetans in a UK laboratory using Doppler echocardiography found a blunted pulmonary vascular response to both acute (minutes) and sustained (8 h) hypoxia compared to Han Chinese (Petousi et al., 2014). Also supportive is the low prevalence of chronic mountain sickness in Tibetans, compared with Han Chinese or South American Quechuas and Aymaras. At odds with these observations is the previously mentioned meta-analysis where the echocardiography-derived PAP pressures in Tibetans living between 3,600 and 4,350 m were similar to those of Andeans and Caucasians at the same elevation (Soria et al., 2016). That Tibetans maintain a similar resting

PAP to, say, Andeans despite lower arterial oxygen levels supports the concept that they are more resistant, but the marked overlap in distribution of measurements in the different populations puts that concept in context. The relative resistance of the Tibetan pulmonary vascular bed to a hypoxia-induced rise in PAP may be more pronounced at higher altitudes (e.g., >5,000 m), but there is limited opportunity to investigate this.

6 | GENETIC STUDIES IN HUMANS AT HIGH ALTITUDE

Excellent overviews of the population studies comparing the genomes of highlanders with lowlanders, and different populations living at altitude, have been provided by Witt and Huerta-Sanchez (2019) and Bigham and Lee (2014) (Brutsaert et al., 2019). As these authors note, the majority of studies focus on differences in genomic structure rather than investigating functional changes. In addition, the majority of variants identified are in non-coding regions.

The genes nominated in these studies as showing selection pressure in Tibetans and in Andeans are distinct but show some overlap. One might anticipate that each population would exhibit a different adaptive response reflecting differences in the genetic backgrounds of the original settler populations; any convergence on a common adaptation would serve to highlight its importance. In that context, genes encoding proteins in the HIF signalling cascade dominate both lists and speak to the possibility that selection for adaptation to chronic hypoxia, as opposed to some other environmental pressure, has been most important in permitting survival at altitude (Bigham & Lee, 2014; Brutsaert et al., 2019).

The two genes most consistently identified in studies of Tibetans are *EPAS1* and *EGLN1* (Beall et al., 2010; Bigham et al., 2010; Peng et al., 2011; Simonson et al., 2010; Wang et al., 2011; Xu et al., 2011; Yi et al., 2010) (Figure 1). *EPAS1* encodes HIF2 α while *EGLN1* encodes PHD2. The strongest genotype–phenotype association has been the linkage of *EPAS1* to lower Hb concentrations (Beall et al., 2010; Yi et al., 2010), which given the contribution of blood viscosity to PVR can be seen as contributing to cardiopulmonary adaptation to hypoxia. An association between two *EPAS1* variants (rs149594770 and rs73926265) and lower PAP measurements in Tibetans living at 4,700 m has been reported, but the extent to which the lower Hb levels account for this is not clear (Peng et al., 2017). The variants reported in *EPAS1* are in non-coding space, but, in support of reduced function, *EPAS1* expression is reduced in endothelial cells derived from umbilical cords (Peng et al., 2017) and lymphocytes (Petousi et al., 2014) from Tibetan subjects.

EGLN1 is a candidate gene for selection in both Tibetans and Andeans but only linked to Hb levels in Tibetans (Bigham et al., 2013; Simonson et al., 2010; Xiang et al., 2013). Two non-synonymous coding variants are more common in Tibetans than Han Chinese, who are less well adapted to high altitude. The assumption is that these variants are associated with an increase in PHD2 activity. While *EGLN1* transcript levels are not altered in lymphocytes of Tibetans at low

altitude (Petousi et al., 2014), the PHD2 [Asp4Glu; Cys127Ser] variant enriched in Tibetans exhibits a lower K_M for oxygen than wild-type PHD2 (Lorenzo et al., 2014).

Interestingly, another candidate gene for selection common to both Tibetans and Andeans is *EDNRA*, which encodes the **endothelin ET_A receptor**, a validated drug target for the treatment of pulmonary hypertension. The effects of the variant in *EDNRA* on protein expression or function have not been resolved and it has not been phenotypically linked with a pulmonary vascular phenotype in these indigenous populations. Other biologically plausible candidates for a role in the pulmonary vascular response to hypoxia common to both Tibetan and Andean studies include *NOS1* and *NOS2*, *IGFBP1* and *IGFBP2*, *VEGFA*, and *IL6*. That is not to say that other candidates identified, such as *PPARA*, encoding the nuclear receptor **PPAR α** , **PRKAA1**, and *BRINP3*, are not relevant to pulmonary vascular homeostasis. But distinguishing their direct contribution, as opposed to adaptation through energy metabolism and muscle function (Horscroft et al., 2017), is important if any are to be exploited as pharmacological targets for pulmonary hypertension.

7 | GENETIC STUDIES IN HYPOXIC ANIMALS

Studies of animals living at high altitude (Witt & Huerta-Sanchez, 2019) support the role of *EPAS1* in adaptation to hypoxia and have suggested a number of other candidates, but apart from studies in the yak and cow, little has been reported that links genetic variants to pulmonary vascular homeostasis.

An advantage of animal studies is the potential to cross-breed strains to isolate the gene of interest to understand its relationship with phenotype. This strategy was used successfully to understand the relative resistance of the rat F344 strain to pulmonary hypertension from chronic hypoxia when compared with the WKY strain (Zhao et al., 2015). Successive backcrossing of offspring from a F344xWKY cross onto the more sensitive WKY parental strain resulted in the introgression of DNA from the F344 strain (carrying the resistance gene) onto the WKY background. Sequencing the introgressed DNA demonstrated a stop-codon in *SLC39A12*, which encodes the **zinc transporter ZIP12**. Mutation of this gene in the WKY strain rendered the WKY strain more resistant to hypoxia-induced pulmonary hypertension. ZIP12 is overexpressed in lungs from patients with PAH, and it remains to be seen whether pharmacological inhibition of ZIP12 is a therapeutic strategy for these patients. Studies are ongoing to develop pharmacological tools for further experimental studies.

8 | GENETIC STUDIES IN PATIENTS WITH PAH

Insight into the genetic basis of heritable PAH was first provided through careful family studies that led to the identification of mutations in bone morphogenetic protein receptor type 2 (**BMPR2**) (Deng

et al., 2000; International PPHC et al., 2000). More recent studies of carefully curated cohorts of patients with idiopathic PAH have confirmed that *BMPR2* is the most commonly affected gene associated with PAH but also identified rare variants in a number of other genes that appear to be pathogenic (Southgate, Machado, Graf, & Morrell, 2020; Zhu et al., 2019). Several converge on the **TGF- β** signalling pathway, but other novel pathways are also implicated, including deleterious changes in genes encoding potassium channels (**KCNK3** and **ABCC8**) and transcription factors (*TBX4* and *SOX17*) (Southgate et al., 2020). These genetic insights are informing drug development, and studies to evaluate restoring *BMPR2* signalling or the imbalance in BMP-TGF- β signalling are in progress.

Notably, pathogenic variants affecting HIF2 α or VHL have not emerged from genomic studies of PAH cohorts. This is not surprising; although raised PAP is a feature, the dominant phenotype associated with dysfunction of these genes is excessive erythrocytosis, which is not a feature of PAH. Comparison of the list of variants from genomic studies of PAH cohorts with the list of genes nominated as selection targets in studies of highlanders also show no overlap. Pertinent to this, of course, is that the main genotype–phenotypes investigated in high-altitude studies so far, have investigated haematocrit and oxygen saturation rather than cardiopulmonary haemodynamics.

If the genotypes of highlanders are to inform PAH, then there may be more merit in comparing the effects on molecular pathways. In that respect, a recent report of predicted deleterious heterozygous mutations in *KDR*, which encodes **VEGF receptor 2 (VEGFR2)**, in two families with PAH, is of interest (Eyries et al., 2020). *VEGFA*, which encodes the ligand for **VEGFR2**, has been identified in both Tibetans and Andeans as a gene showing evidence of selection pressure (Bigam & Lee, 2014; Brutsaert et al., 2019). *VEGFR2* is critical to normal lung vasculature development and repair. Pulmonary hypertension was observed in only three out of seven mutation carriers, suggesting that a second insult is required to express the phenotype. Interestingly, environmental hypoxia is required for rats treated with a *VEGFR2* antagonist (**SU5416**) to develop pulmonary hypertension (Taraseviciene-Stewart et al., 2001). Detailed cardiopulmonary phenotyping of highlanders with respect to *VEGFA* associated variants would lead to a better understanding of this signalling pathway in pulmonary hypertension and perhaps provide insight into how it might be manipulated pharmacologically for therapeutic benefit.

9 | PHARMACOLOGICAL EXPLOITATION OF THE HIF SIGNALLING PATHWAY AS A PARADIGM

Genetic mutations that operate from birth may invoke developmental influences that contribute to a protective phenotype. Nonetheless, the enrichment of genes for *EPAS1* and *EGLN1* in adapted highlanders has given added weight to evaluating HIF signalling as a target for pulmonary hypertension. Bigam and Lee (2014) discuss the possible combinations of loss-of-function and gain-of-function *EPAS1* and *EGLN1* alleles to explain Tibetan adaptation and suggest that it may be

necessary to target both HIF2 α and PHD2 to reproduce the Tibetan phenotype (Figure 1). To date, most progress has been made with exploring inhibition of HIF2 α as a drug target for pulmonary hypertension.

As a transcription factor that activates gene expression through protein–protein interactions, HIF2 α was generally regarded as intractable for inhibition by low MW compounds. However, biophysical studies have shown that the inner core of the PAS-B domain of HIF2 α possesses a hydrophobic cavity that can bind low MW compounds that allosterically disrupt its dimerization to ARNT and thereby block transcriptional activity (Scheuermann et al., 2009). A cellular screening approach for HIF2 α inhibitors has identified Compound 76 (C76) as a useful tool compound (Zimmer et al., 2008). C76 inhibits HIF2 α translation by binding to the iron regulatory protein-1 that enables association with the IRE in the 5'UTR, even during hypoxia exposure. This association continuously represses HIF2 α translation. C76 has shown promising results in both the prevention and treatment of pulmonary hypertension in rodent models (Dai et al., 2018), including non-hypoxic models, but demonstrates only micromolar potency in vitro, and is not orally bioavailable.

A series of highly selective, orally bioavailable HIF2 α inhibitors with a favourable safety and tolerability profile have been developed, with reported efficacy in rodent cancer models and metastatic clear cell renal cell carcinoma (ccRCC) patients (Chen et al., 2016; Cho et al., 2016; Courtney et al., 2018; Wallace et al., 2016; Wehn et al., 2018). Preventative administration of such an inhibitor, PT2567, to chronically hypoxic rats attenuated many of the haemodynamic parameters associated with pulmonary hypertension and reduced the initial accumulation of inflammatory cells in the lung (Hu et al., 2019). These studies are consistent with previous murine genetic manipulation models.

However, the murine mechanistic investigations that target endothelial PHD2 expression to drive spontaneous HIF2 α -dependent pulmonary hypertension should raise a note of caution for the current use of PHD inhibitors currently in clinical trials to treat anaemia in patients with chronic kidney disease (Gupta & Wish, 2017; Sakashita, Tanaka, & Nangaku, 2019). Initial clinical trials have not reported any adverse pulmonary effects, but these were all short-term exposures. Further detailed pulmonary function analysis should be considered during continued chronic use.

10 | CONCLUSIONS

Exposure of the adult mammalian pulmonary circulation to alveolar hypoxia triggers rapid onset vasoconstriction and subsequent structural remodelling, co-ordinated through an integrated series of molecular events. Genetic studies in humans and animals coupled with genetically manipulated animal models have the potential to understand the conductors of this “orchestra.” Progress with elucidating the contributions of HIF2 α (encoded by *EPAS1*), PHD2 (encoded by *EGLN1*), and zinc transporter (encoded by *ZIP12* or *SLC39A12*) provide proof-of-concept for this strategy. At present, the published

population studies of humans living at altitude have their limitations. They have been directed towards genomic structure rather than function, are biased towards genes in the HIF pathway, and have not included detailed cardiopulmonary phenotyping. Arguably, these studies have acted to support concepts, such as the role of HIF, rather than reveal new pathways. Although such studies are still useful, if genotype-phenotype studies at altitude are to provide new insights into pulmonary vascular homeostasis, then there is a need to genotype a cohort of highlanders that have had more extensive cardiopulmonary investigations.

10.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos et al., 2019; Alexander, Cidlowski et al., 2019; Alexander, Fabbro et al., 2019a, 2019b; Alexander, Kelly et al., 2019a, 2019b; Alexander, Mathie et al., 2019).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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