REVIEW

Human high-altitude adaptation: forward genetics meets the HIF pathway

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Humans have adapted to the chronic hypoxia of high altitude in several locations, and recent genome-wide studies have indicated a genetic basis. In some populations, genetic signatures have been identified in the hypoxia-inducible factor (HIF) pathway, which orchestrates the transcriptional response to hypoxia. In Tibetans, they have been found in the *HIF2A* (*EPAS1*) gene, which encodes for HIF-2α, and the *prolyl hydroxylase domain protein 2* (*PHD2*, also known as *EGLN1*) gene, which encodes for one of its key regulators, PHD2. High-altitude adaptation may be due to multiple genes that act in concert with one another. Unraveling their mechanism of action can offer new therapeutic approaches toward treating common human diseases characterized by chronic hypoxia.

Hypoxia is a central feature of many widespread human diseases, including ischemic heart disease, stroke, anemia, chronic obstructive pulmonary disease, and pulmonary hypertension, among others. In fact, following various pathologic states, it can be seen in essentially any tissue of the body. If one were interested in experimentally devising a strategy for allowing an organism to survive under chronic hypoxia, one would perform the obvious forward genetic screen; namely, subject the organism to chronic hypoxia and then, after many generations, identify the phenotypic features and, ultimately, the underlying genetic changes. Such an approach, given its unbiased nature, could offer new insights and potentially novel therapeutic targets for promoting optimal organism and tissue responses to chronic hypoxia.

In this regard, the human species has undergone a dramatic experiment of nature. At varying times in human demographic history, humans colonized multiple high-altitude locales, including the Tibetan Plateau, the Andean Altiplano, and the Semien Plateau of Ethiopia (Fig. 1; Beall 2013). Today, >140 million humans live at high altitude, defined as >2500 m, as this is the elevation at which most people display a fall in oxygen saturation of hemoglobin (Niermeyer et al. 2001). Both the barometric pressure and the absolute concentration of oxygen decline as a function of elevation. For example, at 4000 m, an altitude typical of the Tibetan Plateau, the oxygen concentration is only 60% of that available at sea level. For well over a century, the unique suite of physiological adaptations to chronic hypoxia observed among long-term resident populations has been well documented (for review, see Hornbein and Schoene 2001). Studies conducted over the past decade as well as more recent genomic studies support a genetic basis for these adaptations (Simonson et al. 2012; Scheinfeldt and Tishkoff 2013). Interestingly, the patterns of genetic changes differ among the three populations. Intriguingly, genetic signatures in genes of the hypoxia-inducible factor (HIF) pathway, the central pathway that transduces changes in oxygen tension to changes in gene expression, have been identified (Kaelin and Ratcliffe 2008; Lendahl et al. 2009; Majmundar et al. 2010; Semenza 2012). This suggests that in indigenous high-altitude populations, selection for adaptation to chronic hypoxia (as opposed to cold, increased UV irradiation, or some other environmental stress experienced at high altitude) is a key component of their recent human evolution.

This review discusses findings on human adaptation to high altitude, with a particular focus on Tibetans, for whom the strongest case has been made for genetic changes in the HIF pathway being linked to adaptation. In non-human species, studies have examined how *Drosophila* has adapted to experimental hypoxia (Zhou et al. 2008, 2011). Additional studies have focused on understanding physiologic adaptations to hypoxia in a variety of organisms, ranging from Andean hummingbirds to deer mice (Natarajan et al. 2013; Projecto-Garcia et al. 2013). Other studies, including ones on snow leopards, yaks, Tibetan wild boars, Tibetan macaques, naked mole rats, and Tibetan antelopes, have undertaken genomic examination of these species in order to gain insight into their adaptation (Kim et al. 2011; Qiu et al. 2012; Cho et al. 2013; Ge et al. 2013; Li et al. 2013; Fan et al. 2014). Much of this research has been discussed in a number of excellent

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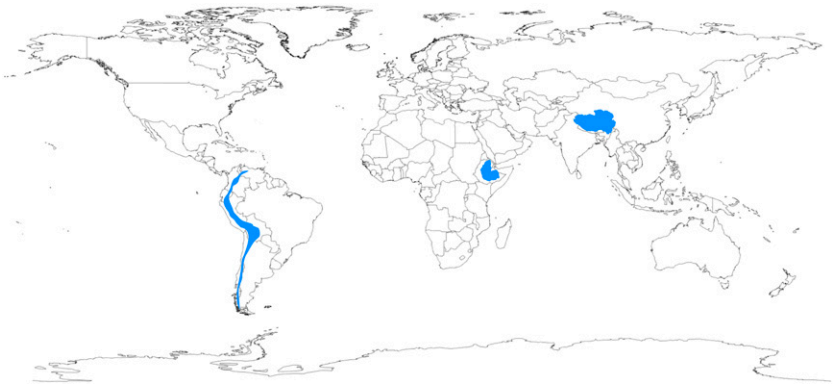

Figure 1. The geography of human adaptation to high altitude. Geographic locations where humans have adapted to life at high altitude are in blue and include (from *left* to *right*) the Andean Altiplano, the Semien Plateau, and the Tibetan Plateau. Adapted from Bigham (2008).

recent reviews (Storz et al. 2010, 2013; Zhou and Haddad 2013) and is not discussed further here.

Human populations adapted to high altitude and physiologic responses

The set of human physiological responses to hypoxia has been well documented for over a century. Beginning with the work of Francois-Gilbert Viault in the 1890s, early studies focused on the Andean pattern of physiological adaptation to high altitude (Cueto 1986). In the 1970s, research began to focus on understanding the physiological adaptations present in populations of the Tibetan Plateau. More recently, attention has turned to the Ethiopian pattern (Beall et al. 2002). Overall, this research has led to a literature documenting the suite of human physiological responses to high-altitude habitation in long-term residents of high altitude, recent low-altitude migrants to high altitude, and high-altitude sojourners (Hornbein and Schoene 2001). Each of these groups has helped us understand the extent to which the observed physiological responses to high altitude are the result of acclimatization, developmental adaptation in which trait characteristics are acquired and become fixed during the period of growth and development, or genetic adaptation. With the hypoxic challenge of increasing altitude, ambient oxygen pressure decreases, resulting in a drop in the arterial partial pressure of oxygen (PO_2) followed by a drop in arterial oxygen saturation (SaO_2). To overcome this decline in arterial oxygen content and maintain O_2 homeostasis, the body responds in various ways, including increasing ventilation over the short term and increasing red blood cell production over the long term. Long-term high-altitude populations display unique circulatory, respiratory, and hematological adaptations to life at high altitude, highlighting the range of phenotypic diversity in altitude response phenotypes (Table 1). Below, we review the major physiologic adaptations to high-altitude hypoxia among these groups.

Hematocrit (the percent of whole blood composed of red blood cells) and hemoglobin (the oxygen-carrying metalloprotein in erythrocytes) concentration increases with rising altitude among high-altitude sojourners. This proliferation of red blood cells with increasing altitude allows for a greater oxygen-carrying capacity to overcome the low ambient oxygen tension experienced at high elevations. Like high-altitude sojourners, Andean highlanders exhibit elevated hemoglobin concentration in an altitude-dependent manner (Beall et al. 1990, 1998). In contrast, the Tibetan hemoglobin phenotype differs from the "classic" Andean model of erythrocytosis, the latter of which is also seen in individuals of low-altitude ancestry living at high altitude. Tibetan hemoglobin concentration is relatively low, thus characterized as a blunted (low) erythropoietic response to hypoxia challenge (Adams and Strang 1975; Beall and Reichsman 1984; Beall and Goldstein 1987). However, it is important to note that above 4000 m, Tibetan hemoglobin concentration increases (Beall and Goldstein 1987). Tibetans, on average, have 3.5 g/dL less hemoglobin than Andeans (Beall et al. 1998). Furthermore, concentrations of erythropoietin (EPO, a glycoprotein that controls erythropoiesis) are also slightly lower among Tibetans compared with Andeans measured at the same altitude (Winslow et al. 1989). Low hemoglobin concentration is a feature that may be adaptive because high concentrations can be associated with the hemodynamic disadvantages of hyperviscosity, its associated cardiac effects, and chronic mountain sickness (CMS). Ethiopian highlanders present yet a third hemoglobin phenotype in which their hemoglobin concentration is maintained within the ranges of sea-level populations (Beall et al. 2002).

Arterial oxygen content is a combination of the amount of oxygen bound to hemoglobin, determined by SaO_2 and hemoglobin concentration, plus the amount of oxygen dissolved in arterial blood. When measured at high altitude, individuals of low-altitude ancestry who are born and raised at high altitude do not exhibit differences in their SaO_2 levels compared with individuals of low-altitude ancestry who are born and raised at low altitude (Dempsey et al. 1971; Frisancho et al. 1995). For example, European sojourners to high altitude exhibit similar SaO_2 values compared with Europeans born and raised at high altitude (Brutsaert et al. 2000), thus suggesting that there are no developmental effects of chronic hypoxia exposure on SaO_2. Among long-term residents of high altitude, Andean highlanders display arterial oxygen levels that are ~16% higher than sea-level inhabitants residing at high altitude (Beall 2006). This is due in part to their increased SaO_2 levels, where Andeans maintain higher levels of SaO_2 at rest and during exercise compared with sea-level inhabitants measured at the same altitude (Brutsaert et al. 2000).

Table 1. *Physiologic adaptations to high-altitude hypoxia among high-altitude populations*

Phenotype	Andean	Tibetan	Ethiopian
Resting ventilation	No increase	50% higher	NR
Hypoxic ventilatory response	Blunted (low)	Similar to sea level	NR
Arterial oxygen saturation	Elevated	No increase	Elevated
Hemoglobin concentration	Elevated	Minimal increase	Minimal increase
Pulmonary arterial pressure	Elevated	Minimal increase	Elevated
Nitric oxide	Elevated	Markedly elevated	NR
Birth weight	Elevated	Elevated	NR

(NR) Not reported.

Tibetan highlanders also exhibit higher levels of SaO_2 compared with low-altitude residents measured at the same altitude (Wu and Kayser 2006), but their average SaO_2 levels at rest are not significantly different from Han Chinese born and raised at high-altitude and are lower than those observed among Andeans when measured using the same equipment and protocol (Beall et al. 1997b, 1999; Weitz and Garruto 2007). Notably, among Tibetan women, a major autosomal dominant allele for high SaO_2 has been identified in which women carrying the high oxygen saturation allele exhibit a greater offspring survival rate than women possessing the low oxygen saturation allele (Beall et al. 1994).

When acutely exposed to high altitude, lowlanders exhibit an immediate rise in ventilation known as the hypoxic ventilatory response (HVR) (Chiodi 1957). This important response to short-term high-altitude exposure is not maintained over the long-term, and resting ventilation returns to low-altitude levels after several days (Weil et al. 1971). Among indigenous high-altitude populations, two distinct pulmonary responses to continued hypoxia exposure have been observed. Andeans exhibit no increase in resting ventilation levels over low-altitude values at rest or during exercise (Chiodi 1957; Brutsaert et al. 2000) and a blunted HVR (Chiodi 1957; Beall et al. 1997a) that is commonly lower than sea-level values. Pointing to a genetic basis for this trait, the Andean HVR has been associated with Quechua ancestry (Brutsaert et al. 2005), thus suggesting an evolutionary origin. Tibetans maintain equal or higher resting ventilation compared with other acclimatized Asian and European populations measured at the same altitude (Zhuang et al. 1993; Ge et al. 1994; Beall et al. 1997a; Moore et al. 2001b), and, notably, their resting ventilation is 1.5 times higher than that observed among the Andean Aymara (Beall et al. 1997a). Furthermore, Tibetan HVR is in line with acclimatized newcomers and low-altitude populations acutely exposed to hypoxia (Zhuang et al. 1993). This suggests that Tibetans have adapted to maintain the temporary hypoxia-induced increase in ventilation observed among low-altitude native populations.

Another noteworthy pulmonary response to acute high-altitude exposure is pulmonary vasculature vasoconstriction, a subsequent consequence of which is pulmonary hypertension. Pulmonary hypertension is characteristic of several altitude-associated disorders, including acute mountain sickness (soroche) and CMS, and is a leading pathophysiological mechanism in the development of high-altitude pulmonary edema (HAPE) (Staub 1980). Long-term high-altitude residents display differences in their pulmonary vasoconstrictor response to hypoxia. For example, Tibetans display resting and exercise pulmonary arterial pressures that are in line with sea-level averages and show minimal hypoxic pulmonary hypertension (Groves et al. 1993). Furthermore, differences exist between Han and Tibetan infants in their development of pulmonary hypertension (Niermeyer et al. 1995), with subacute infantile mountain sickness (characterized by dyspnea, cyanosis, right sided-heart failure, and pulmonary hypertension) primarily observed among Han Chinese infants residing in Tibet (Sui et al. 1988). In contrast, pulmonary hypertension does exist among Andeans in both adulthood (Penaloza et al. 1963) and childhood (Sime et al. 1963), the root cause of which can be attributed to arterial structural changes that include thickening of the pulmonary arterial walls (Arias-Stella and Saldana 1962).

Traditional attention on high-altitude-adaptive phenotypes has largely focused on pulmonary and hematological factors contributing to adaptation to chronic hypoxia. However, recent research has pointed to the role of vascular factors as central components of high-altitude-adaptive phenotypes. Nitric oxide (NO) is a powerful vasodilator expressed in multiple cell types, including vascular endothelial cells, and is responsible for regulating blood flow and vascular resistance. Additionally, NO causes other cellular responses, such as arterial smooth muscle relaxation. Erzurum et al. (2007; Beall et al. 2012) showed that Tibetan highlanders exhibit greater forearm blood flow without associated hypertension or vascular resistance as well as higher levels of circulating NO compared with sea-level inhabitants. This increase in circulation could likely counteract the low levels of arterial oxygen observed in Tibetan highlanders. Other vascular changes observed among both Andean and Tibetan women include those associated with pregnancy and fetal growth. High altitude causes intrauterine growth restriction (IUGR), which results in low birth weight, an important marker of morbidity and mortality in newborns. IUGR varies with duration of altitude exposure, with multigenerational high-altitude inhabitants experiencing less reduction in birth weight than populations who have moved to high altitude more recently (Zamudio et al. 1993; Moore et al. 2001a; Julian et al. 2007). Accordingly, infants born to Andean and Tibetan women are relatively protected from altitude-associated reductions in birth weight when compared with infants born at high altitude to European and Han women,

respectively, who reside at the same altitude (for review, see Moore et al. 2011). Furthermore, the percentage of Andean ancestry is significantly correlated with birth weight, suggesting a genetic influence for this phenotype (Julian et al. 2007; Bennett et al. 2008; Soria et al. 2013).

Research of this nature documents the potential for natural selection to act on phenotypic traits yet does not identify the genes undergirding the observed altitude-adaptive phenotypes. Genes directly involved in the molecular response to hypoxia via the HIF pathway are natural candidates for interrogation. The HIF pathway is a complex O_2-sensing system involved in embryogenesis, development, and homeostasis that activates hundreds of downstream genes in response to cellular hypoxia and is discussed in more detail below.

The HIF pathway

HIF is the master transcriptional regulator of the hypoxic response in metazoans (Semenza 1999; Giaccia et al. 2003; Wenger et al. 2005; Lendahl et al. 2009). It is a heterodimer consisting of a common β subunit (HIF-β, also known as ARNT) and one of three α subunits (HIF-1α, HIF-2α, and HIF-3α, the first two of which have been the most extensively studied). The α and β subunits contain N-terminal helix–loop–helix (HLH) and Per Arnt Sim (PAS) domains. The α subunits also contain C-terminal oxygen-dependent degradation and transcriptional activation domains. The principal means by which HIF activity is controlled in response to oxygen concentration is through site-specific prolyl hydroxylation of the α subunit (Fig. 2A; Ivan et al. 2001; Jaakkola et al. 2001; Yu et al. 2001). Under normoxic conditions, a family of three enzymes—prolyl hydroxylase domain protein 1 (PHD1, also known as EGLN2), PHD2 (EGLN1), and PHD3 (EGLN3)—hydroxylates HIF-α in its oxygen-dependent degradation domain (Bruick and McKnight 2001; Epstein et al. 2001; Ivan et al. 2002). In HIF-1α, the primary site of hydroxylation is on Pro564; in HIF-2α, it is on Pro531; and in HIF-3α, it is on Pro490 (Ivan et al. 2001; Jaakkola et al. 2001; Masson et al. 2001; Yu et al. 2001; Hirsila et al. 2003; Maynard et al. 2003). This hydroxylation provides a binding platform for the von Hippel-Lindau (VHL) protein, a component of an E3 ubiquitin ligase complex that then targets hydroxylated HIF-α for degradation by the ubiquitin–proteasome pathway. Under hypoxic conditions, this inherently oxygen-dependent modification is arrested, leading to the stabilization of HIF-α and its dimerization with HIF-β (the stability of which is not particularly regulated by oxygen concentration). In addition to being directly sensitive to changing oxygen concentrations, it must be recognized that the PHDs are also responsive to reactive oxygen species, iron and ascorbate concentrations, and Krebs cycle intermediates (Ratcliffe 2013). Therefore, the PHDs can respond to a variety of signals. Also, while hydroxylation is the key post-translational modification regulating HIF activity in response to changing oxygen concentration, it should be noted that other post-translational modifications, including acetylation and phosphorylation, can modulate HIF activity as well (Greer et al. 2012; Keith et al. 2012; Mennerich and Kietzmann 2014).

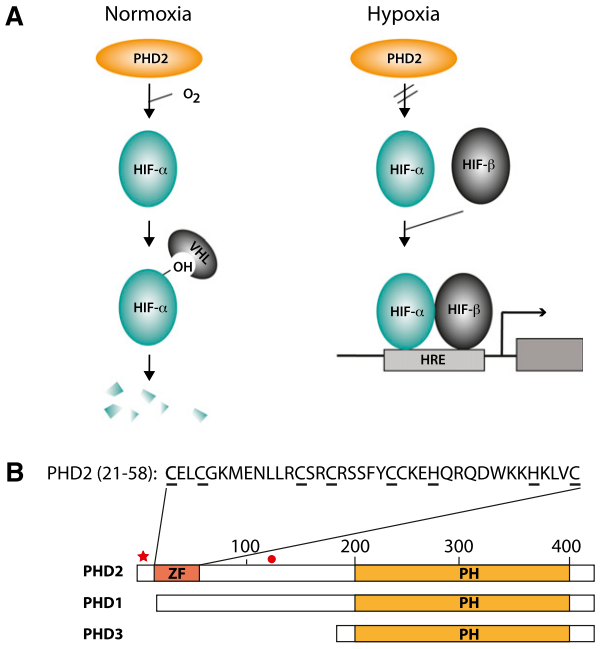

Figure 2. (*A*) The PHD2:HIF pathway. (*Left*) Under normoxic conditions, PHD2 constitutively prolyl hydroxylates HIF-α, targeting it for degradation in a VHL-dependent manner. (*Right*) Under hypoxic conditions, the hydroxylation is arrested, allowing HIF-α stabilization, dimerization with HIF-β, and binding to hypoxia response elements (HREs) that control target genes. (*B*) The three PHDs. The prolyl hydroxylase (PH) domain resides at the C-terminal end of each paralog. PHD2 is distinctive in harboring a MYND zinc finger (ZF) at its N terminus. The amino acid sequence of this zinc finger (residues 21–58) is shown. Underlines denote predicted zinc chelating residues. The positions of PHD2 Asp4 and Cys127 are indicated by a red star and a black circle, respectively.

The PHDs are members of a family of 2-oxoglutarate-dependent dioxygenases (McDonough et al. 2010). A distinct member of this family, factor inhibitor HIF (FIH), provides an additional layer of regulation of HIF-α. Specifically, FIH hydroxylates an asparagine residue in the transcriptional activation domain of HIF (Asn803 of HIF-1α and Asn851 of HIF-2α) (Hewitson et al. 2002; Lando et al. 2002a,b). Under normoxia, this modification blocks interaction with the transcriptional coactivator CBP/p300, while, under hypoxia, attenuation of this modification allows functional interaction between the two and hence transcriptional activation. In general, both FIH and the PHDs more efficiently hydroxylate long peptide/protein substrates over short peptide substrates (Koivunen et al. 2004; Ehrismann et al. 2007). Hydroxylation can therefore influence HIF activity by two distinct mechanisms: through protein stability and transcriptional activation.

Once stabilized, HIF-α dimerizes with HIF-β via their HLH and PAS domains and then activates hundreds of genes involved in systemic and cellular adaptation to hypoxia (Mole et al. 2009; Xia et al. 2009; Schodel et al. 2011). HIF-1α and HIF-2α activate overlapping as well as distinct genes and cellular responses. For example, HIF-1α is the principal paralog that up-regulates genes encod-

ing glycolytic enzymes (Semenza 1999). Moreover, HIF-1α inhibits oxidative phosphorylation and mitochondrial biogenesis (Kim et al. 2006a; Papandreou et al. 2006; Zhang et al. 2007). HIF-1α is also essential for proper maintenance of hematopoietic stem cell quiescence (Takubo et al. 2010). HIF-2α, on the other hand, is the paralog critical for regulating the *EPO* gene in specialized interstitial cells in the adult kidney (Fig. 3; Lee and Percy 2011). The product of this gene, EPO, is the central regulator of red cell mass. Studies using genetically modified mice have provided critical evidence for this, since *Hif2a* loss of function (LOF) is accompanied by anemia, whereas *Hif2a* gain of function (GOF) produces erythrocytosis (Table 2; Scortegagna et al. 2005; Kim et al. 2006b; Gruber et al. 2007; Hickey et al. 2010; Kapitsinou et al. 2010; Tan et al. 2013). In humans, important evidence for a role of HIF-2α in *EPO* regulation is provided by the identification of heterozygous GOF mutations in the *HIF2A* gene in patients with erythrocytosis (Percy et al. 2008a,b). The residues affected by these mutations cluster around the primary site of hydroxylation, Pro531, and functionally impair prolyl hydroxylation, subsequent recognition by VHL, or (most typically) both, ultimately resulting in aberrant stabilization of the HIF-2α protein (Furlow et al. 2009).

As befits factors that orchestrate the hypoxic response, HIF-1α and HIF-2α often act in the same direction. For example, both have the capacity to activate the *VEGFA* gene, which encodes for a key protein that induces angiogenesis (Keith et al. 2012). In addition, both HIF-1α and HIF-2α have been implicated in the pathogenesis of pulmonary hypertension (Fig. 3; Shimoda and Laurie 2014). For example, mice and some patients with *HIF2A* GOF mutations display pulmonary hypertension (Gale et al. 2008; Tan et al. 2013). Furthermore, in genetically engineered mouse studies, haploinsufficiency of either

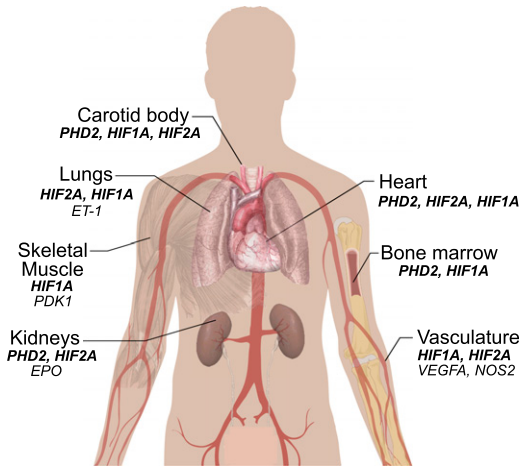

Figure 3. Potential organs involved in high-altitude adaptation, along with select HIF pathway genes (bold italics) and select HIF target genes (nonbold italics) that may be relevant. The genes shown are derived mainly from studies conducted on genetically engineered mice (see Table 2) as well as additional studies on human patients with erythrocytosis.

Hif1a or *Hif2a* delays or prevents hypoxia-induced (and, in the case of *Hif2a*, *Vhl* mutation-associated) pulmonary hypertension (Table 2; Yu et al. 1999; Brusselmans et al. 2003; Hickey et al. 2010).

However, it is also clear that HIF-1α and HIF-2α can have antagonistic activities. For example, HIF-1α promotes cell cycle arrest, whereas HIF-2α promotes progression through the cell cycle, likely in a cell context-dependent manner (Keith et al. 2012). In murine macrophages, keratinocytes, and endothelial cells, Hif-1α promotes NO production through activation of the inducible NO synthase (*Nos2*) gene, whereas Hif-2α inhibits NO production through the induction of the arginase (*Arg*) gene (Takeda et al. 2010; Branco-Price et al. 2012; Cowburn et al. 2013). Finally, $Hif1a^{+/-}$ mice display blunted respiratory responses to chronic hypoxia, while $Hif2a^{+/-}$ mice display exaggerated carotid body sensitivity to hypoxia (Fig. 3; Kline et al. 2002; Peng et al. 2011b; Yuan et al. 2013). Far less is known regarding HIF-3α targets, but current data indicate that it can act as both an activator and inhibitor of gene transcription (Makino et al. 2001; Zhang et al. 2014).

As with the HIFs, the PHDs have overlapping and distinct functions. Among the three paralogs, PHD2 has emerged as perhaps the most important. Conventional knockout of *Phd2* in mice, for example, leads to embryonic lethality, in contrast to that of *Phd1* or *Phd3*, each of which leads to viable, fertile mice (Takeda et al. 2006). *Phd2* has a broad tissue expression, and, in many transformed cell lines, knockdown of *PHD2* is sufficient to induce HIF-α stabilization (although typically not to the extent seen in hypoxia) (Berra et al. 2003; Appelhoff et al. 2004).

PHD2 also plays a distinctly critical role—through its regulation of HIF-2α—in the control of *EPO* gene transcription in interstitial cells of the kidney and hence red cell mass (Lee and Percy 2011). This was initially demonstrated by the identification of human erythrocytosis patients with heterozygous LOF mutations in the *PHD2* gene (Percy et al. 2006, 2007; Al-Sheikh et al. 2008; Ladroue et al. 2008). These mutations, it should be noted, typically affect residues that reside in the catalytic domain of the protein (Gardie et al. 2014). Subsequent studies using global conditional knockout of the *Phd2* gene demonstrated that it plays the dominant role, among the Phd paralogs, in the control of renal Epo (Minamishima et al. 2008; Takeda et al. 2008). Studies also have shown that Phd2 plays a role in regulating respiratory drive. Specifically, loss of Phd2 function is associated with increased respiration (Arsenault et al. 2013; Bishop et al. 2013). Importantly, this phenotype is not seen in either $Phd1^{-/-}$ or $Phd3^{-/-}$ mice (Bishop et al. 2013). PHD1 and PHD3 also have been studied, although not as extensively as PHD2. The *Phd3* gene is a HIF target and is a component of a negative feedback loop that down-regulates HIF activity (Minamishima et al. 2009). In the murine liver, *Phd3* also plays a vital role—via its control of Hif-2α—in regulating *Irs2* transcription and hence insulin signaling (Taniguchi et al. 2013).

The three PHDs share a common prolyl hydroxylase domain but are quite different at their N terminii (Fig. 2B; Taylor 2001). PHD2 possesses a predicted myeloid nervy

Table 2. *Select mouse lines with genetically engineered alterations in the Phd2, Hif2a, and Hif1a genes that have potential relevance to high-altitude adaptation*

Genotype	Effect	Tissues	Phenotype	Reference
Phd2 alleles				
Phd2$^{+/-}$	LOF	Global	Mild erythrocytosis	Mazzone et al. 2009; Li et al. 2010
Phd2$^{+/-}$	LOF	Global	Increased HVR; carotid body hyperplasia	Bishop et al. 2013
Phd2$^{P294R/+}$	LOF	Global	Increased respiration under normoxia; mild erythrocytosis	Arsenault et al. 2013
Rosa26-creER; *Phd2*$^{f/f}$	LOF	Global	Marked erythrocytosis; increased vascular growth in multiple tissues	Takeda et al. 2007, 2008; Li et al. 2010
βactin-creER; *Phd2*$^{f/f}$	LOF	Global	Marked erythrocytosis; cardiomyopathy	Minamishima et al. 2008
Pax3-cre; *Phd2*$^{f/f}$	LOF	Neural crest	Marked erythrocytosis	Arsenault et al. 2013
Vav1-cre; *Phd2*$^{f/f}$	LOF	Hematopoietic	Mild erythrocytosis	Arsenault et al. 2013
CD68-cre; *Phd2*$^{f/f}$	LOF	Selected[a]	Marked erythrocytosis	Franke et al. 2013
αMHC-cre; *Phd2*$^{f/f}$	LOF	Heart	Cardiomyopathy	Moslehi et al. 2010
MLCv-cre; *Phd2*$^{f/f}$	LOF	Heart	Increased capillary area in hearts	Holscher et al. 2011
Phd2$^{gt/gt}$	LOF	Selected[b]	Increased cardiac capillary size; increased NO production following cardiac ischemia reperfusion injury	Kerkela et al. 2013
Alb-cre; *Phd2*$^{f/f}$	LOF	Liver	Activation of Hif-1α but not Hif-2α	Taniguchi et al. 2013
Hif2a alleles				
Hif2a$^{-/-}$	LOF	Global	Anemia[c]	Scortegagna et al. 2005
Ubc-cre; *Hif2a*$^{f/f}$	LOF	Global	Anemia	Gruber et al. 2007
Hif2a$^{+/-}$	LOF	Global	Resistance to hypoxia-induced pulmonary hypertension	Brusselmans et al. 2003
Hif2a$^{+/-d}$	LOF	Global	Resistance to Chuvash polycythemia-associated erythrocytosis and pulmonary hypertension	Hickey et al. 2010)
Hif2a$^{+/-}$	LOF	Global	Exaggerated HVR	Peng et al. 2011b
Pax3-cre; *Hif2a*$^{f/f}$	LOF	Neural crest	Anemia	Kapitsinou et al. 2010
LysM-cre; *Hif2a*$^{f/f}$	LOF	Macrophage	Enhanced NO production from macrophages	Takeda et al. 2010
K14-cre; *Hif2a*$^{f/f}$	LOF	Keratinocyte	Increased skin nitrate (NO derivative) levels	Cowburn et al. 2013
Alb-cre; *HIF2dPA*	GOF	Liver	Erythrocytosis	Kim et al. 2006b
Hif2a$^{G536W/+}$	GOF	Global	Erythrocytosis and pulmonary hypertension	Tan et al. 2013
αMHC-cre; *HIF2dPA*	GOF	Heart	Cardiomyopathy	Moslehi et al. 2010
Vil-cre; *Hif2a*$^{f/f}$	LOF	Intestine	Impaired iron absorption	Mastrogiannaki et al. 2009; Anderson et al. 2011
VEcad-cre; *Hif2a*$^{f/f}$	LOF	Endothelium	Defective revascularization	Skuli et al. 2012
Hif1a alleles				
Hif1a$^{+/-}$	LOF	Global	Delayed development of hypoxia-induced pulmonary hypertension	Yu et al. 1999
Hif1a$^{+/-}$	LOF	Global	Impaired HVR following chronic hypoxia	Kline et al. 2002
LysM-cre; *Hif1a*$^{f/f}$	LOF	Macrophage	Impaired NO production from macrophages	Takeda et al. 2010
Tie2-cre; *Hif1a*$^{f/f}$	LOF	Endothelium	Impaired NO production from endothelial cells	Branco-Price et al. 2012
MCK-cre; *Hif1a*$^{f/f}$	LOF	Skeletal muscle	Shift from glycolysis to oxidation	Mason et al. 2004, 2007
MLC2v; Hif1a$^{f/f}$ [e]	LOF	Heart	Protection from *Vhl* loss-induced cardiac degeneration	Lei et al. 2008

Selected aspects of phenotype with potential relevance to high-altitude adaptation are shown. Mice with genetically engineered changes in the *Vhl* gene have been reviewed elsewhere (Kapitsinou and Haase 2008). (f) Floxed; (gt) gene targeted, achieved by insertion of GeneTrap targeting vector in intron 1 of *Phd2* gene; (*HIF2dPA*) HA-HIF-2α P405A;P531A expressed from the *Rosa*26 locus in a cre-dependent manner.
[a]Cells in which deletion was observed include renal Epo-producing cells, neurons, astrocytes, and hematopoietic cells.
[b]Ninety-two percent and 85% reduction in heart and skeletal muscle, respectively, and variable reduction in other tissues.
[c]Pancytopenia also present.
[d]In a *Vhl*$^{R200W/R200W}$ genetic background.
[e]In a *MLC2v; Vhl*$^{f/f}$ genetic background.

deaf (MYND)-type zinc finger at its N terminus that is absent from the other two PHDs. This zinc finger is separated by >100 amino acids from the catalytic domain. Unlike certain MYND-containing proteins, such as the SMYD methyltransferases, where the MYND finger interacts with the catalytic domain of the protein (Ferguson et al. 2011), evidence has yet to be presented that the MYND zinc finger of PHD2 physically interacts with the catalytic domain. The zinc finger of PHD2 is strongly conserved across species and indeed is found in the single Phd ortholog from the simplest metazoan, *Trichoplax adhaerens* (Loenarz et al. 2011). Hence, PHD2 is the human PHD paralog most closely related to the ancestral Phd (Loenarz et al. 2011; Rytkonen et al. 2011). *Caenorhabditis elegans* also possesses a single Phd ortholog that harbors a zinc finger, and, interestingly, a mutant *C. elegans* with a *Phd* lacking this zinc finger displays normal Hif regulation (Shao et al. 2009). A property of this zinc finger in human PHD2 is that it binds to a Pro–Xaa–Leu–Glu (PXLE) motif (Song et al. 2013). This motif is found in select HSP90 cochaperones, such as p23 and FKBP38, as well as HSP90 itself (both HSP90α and HSP90β paralogs) (Song et al. 2013, 2014). Therefore, the zinc finger of PHD2 is a module that binds to peptides, which is consistent with known functions of other MYND zinc fingers (Matthews et al. 2009).

Differing functions for the zinc finger of PHD2 have been proposed. First, it has been proposed that the zinc finger inhibits the catalytic activity of PHD2 (Choi et al. 2005). This would predict that loss of zinc finger function would lead to augmented PHD2 activity and hence impaired HIF activation. Second, it has been observed that the interaction of PHD2 with FKBP38 promotes PHD2 degradation (Barth et al. 2007). This would predict that loss of this interaction would also lead to augmented PHD2 activity and impaired HIF activation. These first two proposals receive support from studies in *Droshophila*. *Drosphila* harbors a single Phd isoform called *fatiga* that is orthologous to PHD2, and a *fatiga* variant isoform lacking the zinc finger displays higher activity when transgenically expressed than one that contains it (Acevedo et al. 2010). A third proposal for the function of the zinc finger of PHD2 is that it facilitates HIF hydroxylation (Song et al. 2013). HIF-α is known to be an HSP90 client (Minet et al. 1999; Isaacs et al. 2002; Katschinski et al. 2002); hence, the interaction of PHD2 with the PXLE motif contained within an HSP90 cochaperone (such as p23) or HSP90 itself could allow recruitment of PHD2 to the HSP90 pathway to facilitate HIF-α hydroxylation. In contrast to the first two models, this would predict that loss of zinc finger function would lead to diminished PHD2 activity and hence augmented HIF activation.

Andean and Ethiopian adaptation to high altitude and genetic analyses

Genomic analysis of Andean populations has revealed at least 40 HIF pathway and hypoxia-related genes as candidates for natural selection to high altitude (Table 3A; Bigham et al. 2009, 2010). One hypoxia-related gene in particular, the α-1 catalytic subunit of adenosine monophosphate-activated protein kinase *(PRKAA1*, also known as *AMPKα*1), may be influential in achieving genetic adaptation to high altitude by affecting physiological responses to pregnancy that are instrumental for fetal growth (Bigham et al. 2014). Furthermore, whole-genome sequencing of an Andean CMS cohort implicated sentrin-specific peptidase 1 (*SENP1)*, an enzyme implicated in erythropoiesis, and acidic leucine-rich nuclear phosphoprotein 32 family member D (*ANP32D*), an oncogene, in the development of CMS (Zhou et al. 2013).

Genomic signatures of natural selection among Ethiopian highlanders implicate hypoxia-related as well as non-hypoxia-related genes in adaptation to high altitude. Candidate genes include basic HLH family member e41 (*BHLHE41* also known as *DEC2* or *SHARP1*), mitochondrial calcium uptake 1 (*MICU*, also known as *CBARA1*), vav 3 guanine nucleotide exchange factor (*VAV3*), aryl-hydrocarbon receptor nuclear translocator 2 (*ARNT2*), and thyroid hormone receptor β (*THRB*) as well as a gene-rich region on chromosome 19 containing several genes that are involved in vascular physiology (*CXCL17* and *PAFAH1B3)* or have been linked to hypoxia (*LIPE*) (Scheinfeldt et al. 2012; Huerta-Sanchez et al. 2013; Udpa et al. 2014). Two genes in particular, *ARNT2* and *THRB*, show suggestive relationships with hemoglobin concentration; for example, Ethiopian Amhara with two copies of the *THRB* rs826216 C allele display hemoglobin levels that are higher than those in individuals with two copies of the T allele (Scheinfeldt et al. 2012). Recent research also has identified HIF pathway candidate genes showing signatures of natural selection in other human populations, including Sherpa, Indians, and Mongolians (Aggarwal et al. 2010; Hanaoka et al. 2012; Kang et al. 2013; Xing et al. 2013; Jeong et al. 2014).

Table 3. *HIF pathway and hypoxia-related genes with genetic signatures in Andean and Tibetan populations*

(A) Andean selection-nominated genes (n = 40)[a]				
ADRA1B	*EDNRA*	*IL1B*	*NOTCH1*	*SATB1*
ARNT2	*EDNRB*	*IL6*	*NRP1*	*SNAI3*
ATP1A1	*EGLN1*	*KCNMA1*	*NRP2*	*SPRY2*
ATP1A2	*EGLN2*	*MDM2*	*PIK3CA*	*TF*
CDH1	*ELF2*	*MMP2*	*POLR2A*	*TGFA*
COPS5	*IGFBP1*	*MTOR*	*PRKAA1*	*TNC*
CXCR4	*IGFBP2*	*NOS1*	*PRKAA2*	*TNF*
EDN1	*IL1A*	*NOS2*	*PSMC3*	*VEGFA*
(B) Tibetan selection-nominated genes (n = 39)[b]				
ADRA1B	*EGLN3*	*HMOX2*	*NOS2*	*POLR2A*
ARNT	*EP300*	*IGFBP1*	*NRP1*	*PPARA*
ANGPTL4	*EPAS1*	*IGFBP2*	*NRP2*	*RBX1*
CASR	*EPO*	*IL1A*	*PDGF2*	*TNC*
COPS5	*FLT1*	*IL1B*	*PGF*	*TNF*
EDN1	*HBB*	*IL6*	*PIK3CB*	*VEGFA*
EDNRA	*HBG2*	*MDM2*	*PIK3CG*	*VEGFC*
EGLN1	*HIF1A*	*NOS1*	*PKLR*	

[a]Data are from Bigham et al. (2009, 2010).
[b]Data are from Bigham et al. (2010), Peng et al. (2011a), Simonson et al. (2010), and Yi et al. (2010).

Tibetan adaptation to high altitude and genetic analyses

Several studies have used genomic scans for positive selection among Tibetan highlanders to identify natural selection candidate loci (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011a; Wang et al. 2011; Xu et al. 2011). Importantly, studies have shown evidence of natural selection among HIF pathway and hypoxia-related genes (Table 3B). Two genes are particularly noteworthy because of the consistency with which they have been observed in these studies (Simonson et al. 2012; Petousi and Robbins 2014). One is *HIF2A*, which shows evidence of positive directional selection (selection for advantageous mutations that increase fitness of carriers) in all of these genome-wide analyses (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011a; Wang et al. 2011; Xu et al. 2011), and both *HIF2A* SNP genotypes and haplotypes significantly associate with low hemoglobin concentration in Tibetans (Beall et al. 2010; Yi et al. 2010). Intriguingly, a recent study suggests that introgression from Denisovan, a *Homo* species identified from remains found at Denisova Cave in the Altai Mountains of Siberia, or Denisova-related individuals is responsible for the Tibetan pattern of variation observed at this locus (Huerta-Sanchez et al. 2014), suggesting that ancient admixture may have contributed to potentially functionally important adaptive changes in the Tibetan genome.

A second HIF pathway candidate gene showing evidence of positive selection among Tibetans in multiple studies is *PHD2* (Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011a; Wang et al. 2011; Xu et al. 2011). Of note, the candidate genes identified for Andeans and Tibetans are largely distinct from one another, but *PHD2* has emerged as a candidate for both populations. Further analysis reveals that among Tibetans, variants in this gene are associated with hemoglobin concentration, while among Andeans, they are not (Simonson et al. 2010; Bigham et al. 2013; Xiang et al. 2013). In the Tibetan population, two nonsynonymous coding variants located in exon1—rs12097901 (C127S) and rs186996510 (D4E)—have been observed at high frequency. The former is present at a frequency of 0.8–0.9 in Tibetans, as compared with ~0.4–0.5 in a control Han Chinese population (Xiang et al. 2013; Lorenzo et al. 2014; Petousi et al. 2014). The latter SNP displays an even more pronounced divergence, with a frequency of 0.6–0.9 in Tibetans, and one of ~0.01 in Han Chinese (Xiang et al. 2013; Lorenzo et al. 2014; Petousi et al. 2014). Accordingly, this SNP (rs186996510) displays an F_{ST} value between Tibetans and Han Chinese of 0.709, ~70 times higher than that of the average of the chromosome in which it resides (Xiang et al. 2013). Two reports found that the two SNPs are in linkage disequilibrium (Lorenzo et al. 2014; Petousi et al. 2014), while a third study did not find them to be tightly linked (Xiang et al. 2013).

A key question now is how these genetic changes translate into adaptation. Lowlanders ordinarily maintain low levels of HIF-1α and HIF-2α through constitutive hydroxylation and degradation (Fig. 4, model A). Upon ascent to high altitude, attenuated PHD2 hydroxylase activity due to hypoxia leads to increased levels of HIF-1α and HIF-2α (and presumably HIF-3α) (Fig. 4, model B). In

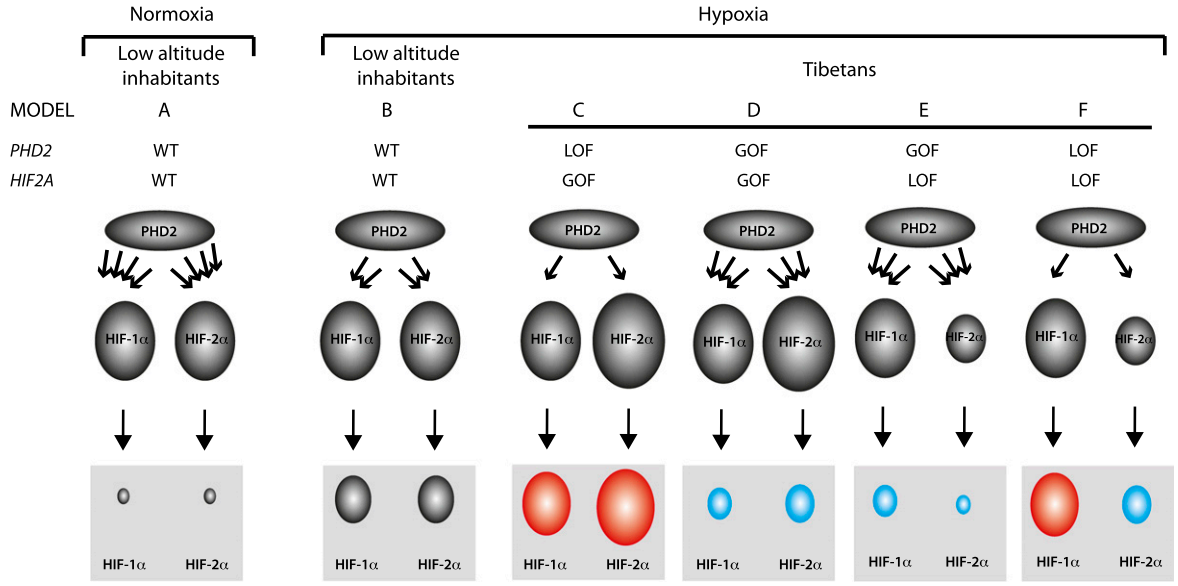

Figure 4. Models for high-altitude adaptation. (Model A) In lowlanders at low altitude, PHD2 constitutively hydroxylates (four arrows between PHD2 and HIF-α) HIF-α, leading to low levels of HIF-α (gray box). (Model B) In lowlanders who ascend to high altitude, PHD2 activity decreases (two arrows), leading to increased HIF-α levels (gray box). Models C–F show various combinations of GOF and LOF *PHD2* and *HIF2A* alleles to potentially explain Tibetan adaptation. The number of arrows emanating from PHD2 indicates hydroxylase activity (one arrow: weak; three arrows: strong), and, immediately *below* it, the size of HIF-2α indicates whether it is a GOF (larger) or LOF (smaller). The gray boxes at the *bottom* show the ultimate strength of HIF-α activation resulting from the combined PHD2 and HIF-α activity, with red denoting increased HIF-α activity relative to model B, and blue denoting decreased HIF-α activity relative to model B.

simple terms, one could posit that the Tibetan *PHD2* allele is either a GOF (enhanced hydroxylase) or LOF (diminished hydroxylase) allele and likewise that the Tibetan *HIF2A* allele is either a GOF (enhanced activity) or LOF (diminished activity) allele. This in turn leads to four models for Tibetan adaptation based on the *PHD2: HIF* axis (Fig. 4, models C–F). The *PHD2* LOF:*HIF2A* GOF combination would be predicted to lead to enhanced HIF-2α activity (Fig. 4, model C). A priori, this model seems unlikely given that this would be predicted to lead to elevated hemoglobin levels, the exact opposite of what is observed among Tibetans (Beall 2007). The remaining three models all could conceivably lead to lowered HIF-2α activity.

For example, a *PHD2* GOF:*HIF2A* GOF combination is possible if the *PHD2* allele is a relatively stronger GOF allele than the *HIF2A* allele is a GOF allele (Fig. 4, model D). Several observations, however, would seem to suggest that the *HIF2A* allele is more likely to be a LOF allele. (1) The Tibetan *HIF2A* allele, as mentioned previously, is correlated with low hemoglobin in several studies (Beall et al. 2010; Yi et al. 2010). (2) Lymphocytes isolated from Tibetans display low *HIF2A* mRNA levels (Petousi et al. 2014). If, indeed, the *HIF2A* allele was a LOF allele, it could provide an explanation for protection from pulmonary hypertension observed in Tibetans, since mice with haploinsufficiency at the *Hif2a* locus are protected from the pulmonary hypertension induced by hypoxia or the Chuvash LOF *Vhl* mutation as well as the erythrocytosis induced by the latter (Brusselmans et al. 2003; Hickey et al. 2010).

It cannot be overemphasized, however, that firm conclusions regarding the nature of this allele, including whether it is LOF or GOF, must await its molecular characterization. SNPs with high-frequency divergence between Tibetans and Han Chinese have been observed and all reside in noncoding (as opposed to coding) regions of the *HIF2A* gene (Yi et al. 2010; Peng et al. 2011a; Lorenzo et al. 2014). For example, intronic SNP rs150877473 has a frequency that rises from 0.09 in Han Chinese to 0.87 in Tibetans (Yi et al. 2010). Interestingly, this SNP changes a nucleotide in the vicinity of the 3′ splice site adjacent to exon 6 of the *HIF2A* gene and hence may functionally affect normal splicing of this gene. That being said, it should be noted that other groups have not identified Tibetan sequence variants at exon–intron boundaries; it should also be recognized that numerous other SNPs, located within introns or upstream of the *HIF2A* gene, display comparable degrees of divergence between Han Chinese and Tibetan populations (Peng et al. 2011a; Huerta-Sanchez et al. 2014; Lorenzo et al. 2014). It is conceivable that one or more of these is responsible for, or contributes to, the phenotype.

With regard to the *PHD2* allele, a number of observations support the possibility that it is a GOF allele (Fig. 4, model E). (1) Several studies have examined potential associations between the Tibetan *PHD2* allele and low hemoglobin concentration (Simonson et al. 2010; Xiang et al. 2013; Petousi et al. 2014), and correlations have been found in some, although not all, studies. (2) At low altitude, the Tibetan *PHD2* allele is correlated with blunted plasma EPO response to hypoxia (Petousi et al. 2014). (3) Lymphocytes isolated from Tibetans display lowered hypoxic induction of select HIF-1α and HIF-2α target genes (Petousi et al. 2014). (4) Erythroid progenitors that carry the Tibetan *PHD2* allele display impaired proliferation under hypoxic conditions (Lorenzo et al. 2014). Mechanistically, a GOF allele could arise from increased enzymatic activity of PHD2 toward HIF-α, and, in support of this, it has been observed that Tibetan (D4E/C127S) PHD2 displays a lower K_m for oxygen than wild-type PHD2 (Lorenzo et al. 2014). A GOF *PHD2* allele, in conjunction with the LOF of the *HIF2A* allele, would be predicted to lead to hypoactivation of HIF-α and could readily provide an explanation for protection against not only erythrocytosis but also pulmonary hypertension, since both HIF-1α and HIF-2α have been implicated in the pathogenesis of the latter (Shimoda and Laurie 2014).

An alternative model is that the Tibetan *PHD2* allele is a LOF allele, which is supported by the observations that (1) erythroid progenitors that are homozygous for the Tibetan *PHD2* allele display EPO hypersensitivity under normoxic conditions, and (2) granulocytes isolated from Tibetans display increased expression of select HIF target genes (Lorenzo et al. 2014). A Tibetan *PHD2* LOF allele would necessarily imply that the *HIF2A* allele is a strong LOF allele, leading ultimately to activation of HIF-1α and inhibition of HIF-2α (Fig. 4, model F). It has been observed that the Tibetan (D4E/C127S) PHD2 is markedly defective in its interaction with p23 (Song et al. 2014), which would support this model. Interestingly, the defective interaction depends on both the D4E and C127S substitutions (Song et al. 2014) and therefore is consistent with the linkage disequilibrium that has been observed for the two SNPs that encode these substitutions (Lorenzo et al. 2014; Petousi et al. 2014). This model could conceivably provide an explanation for the increased respiratory drive observed in Tibetans. For example, it has been observed that mice that are heterozygous for either a knockout *Phd2* allele or a knock-in LOF (P294R) *Phd2* allele display increased respiratory drive (Arsenault et al. 2013; Bishop et al. 2013). Conversely, mice that are haploinsufficient at the *Hif1a* locus display a blunted HVR following chronic hypoxia (Kline et al. 2002; Peng et al. 2006). Taken together, this suggests an important role for the PHD2: HIF-1α axis in controlling respiratory drive. A LOF *PHD2* allele also may contribute to the increased NO levels, since *NOS2* (*iNOS*) is a well-characterized HIF-1α target gene (Takeda et al. 2010).

At the same time, however, a LOF allele might be predicted to predispose to pulmonary hypertension and exacerbate the erythrocytosis that can be seen at high altitude. The following considerations might mitigate against this. First, patients with heterozygous LOF PHD2 mutations do not typically present with pulmonary hypertension, nor do the mice with the heterozygous LOF knock-in (P294R) mutation at the *Phd2* locus (Lee and Percy 2011; Arsenault et al. 2013). This might be contrasted with humans and mice with GOF mutations in the *HIF2A* gene, in which heterozygosity is sufficient to induce pulmonary hypertension (Gale et al. 2008; Tan et al. 2013). Second and

importantly, Tibetans may be protected from both erythrocytosis and pulmonary hypertension by the high-frequency Tibetan *HIF2A* allele previously discussed.

With regard to the latter, the timing of the appearance of the Tibetan *HIF2A* and *PHD2* alleles becomes an issue of importance. Estimates for the emergence of the *HIF2A* allele have been controversial. One study proposed that it may have arisen as recently as 3000 years ago (Yi et al. 2010). However, another group estimated that it arose ~18,000 years ago (Peng et al. 2011a), and this same group proposed that the *PHD2* allele arose subsequently ~8000 years ago; this latter estimate is also consistent with an independent study that placed its emergence at 8000 years (Lorenzo et al. 2014). If, indeed, the Tibetan *HIF2A* allele preceded the *PHD2* allele, then potentially deleterious effects of the latter may have been blunted by a preexisting high-frequency protective *HIF2A* allele.

Beyond these considerations, there are yet other factors that may introduce additional complexities into the models presented in Figure 4. First, the Tibetan PHD2 variant may be differentially defective in the capacity to promote degradation of HIF-1α versus HIF-2α. Second, the function of the zinc finger in PHD2 may vary in importance in a tissue-specific manner. Third, the zinc finger of PHD2 may differentially use the PXLE motifs of p23, FKBP38, and HSP90; in this regard, it should be noted that the D4E/C127S substitution maintains zinc finger interaction with FKBP38 and HSP90, albeit somewhat more weakly (Song et al. 2014). Fourth, PHD2 is deployed in a tissue-specific manner, and the presence of two other PHD paralogs with potentially redundant activity with PHD2 will undoubtedly modulate the impact of PHD2 LOF or GOF. For example, loss of murine *Phd2* alone in the Epo-producing cells of the kidney, hematopoietic precursors, cardiomyocytes, or hepatocytes is sufficient to produce a phenotype (erythrocytosis in the first two cases, cardiomyopathy in the third case, and activation of Hif-1α in the last), indicating that the other two PHD paralogs cannot suppress Hif-α to normal levels in these tissues (Table 2; Minamishima et al. 2008; Takeda et al. 2008; Moslehi et al. 2010; Arsenault et al. 2013; Taniguchi et al. 2013). On the other hand, studies also indicate that loss of *Phd2* alone in the liver or in osteoblasts does not produce erythrocytosis, whereas concurrent loss of *Phd1* and *Phd3* does, indicating that all three Phd paralogs contribute to normal Hif-2α suppression in these tissues (Minamishima and Kaelin 2010; Rankin et al. 2012; Duan et al. 2014). Taken together, these observations indicate that the phenotypic consequences of PHD2 LOF will manifest in a tissue-specific manner. It is likely that the same would hold for PHD2 GOF mutations. It will clearly be of interest to determine whether any of the aforementioned mechanisms, or perhaps some other mechanism, accounts for the Tibetan *PHD2* haplotype.

Perspectives

It should be apparent from this discussion that the mechanisms of high-altitude adaptation in different human populations are distinct and undoubtedly complex. Furthermore, mounting evidence suggests that the observed physiological adaptations are controlled by interactions among multiple genes, especially those that are part of the HIF pathway. The Tibetan population shows the strongest support that multiple genes in the HIF pathway have been reconfigured in response to chronic hypoxia. This interaction of genes determining high-altitude-adaptive phenotypes may be contrasted with other well-known examples of recent human evolution in which selection appears to have acted on single genes. Examples of this would include the selection of a *Lactase* (*LCT*) gene variant to allow lactase persistence in European populations and the selection of an *EDAR* variant to produce a constellation of findings related to ectodermal development in Asian populations (Kamberov et al. 2013; Scheinfeldt and Tishkoff 2013). High-altitude adaptation may be more like skin color determination, in which multiple interacting loci determine phenotype (Sturm 2009).

The continued study of high-altitude adaptation may provide the basis for new therapies for diseases characterized by ischemia and chronic hypoxia. In the Tibetan population, the changes in the *PHD2* and *HIF2A* genes were selected over the course of thousands of years, providing proof of principle of their efficacy. However, challenges remain if we are to extrapolate the high-altitude findings to low-altitude, hypoxic disease states. For example, it may be necessary to target both PHD2 and HIF-2α in order to phenocopy Tibetan adaptation. This is within the realm of possibility, since there are many examples of drug combinations that concurrently target enzymes/proteins in the same pathway to achieve a beneficial effect (e.g., amoxicillin/clavulinic acid). Moreover, while it is likely that selection of HIF pathway genes in Tibetans is mainly a reflection of adaptation to chronic hypoxia, genetic changes in this population as well as other high-altitude groups may reflect adaptation to other environmental stresses present at high altitude, such as low temperature and increased UV exposure.

Human high-altitude adaptation has drawn the attention of molecular biologists, anthropologists, geneticists, and physiologists alike. It is an excellent natural experiment design in which to study the evolutionary process. By understanding how similar environmental pressures can result in either the same or different genetic adaptations, we will be better situated to understand the molecular basis for convergent human adaptations. Continued study is paramount in order to elucidate genotype–phenotype correlations and provide molecular explanations for high-altitude adaptation. We are at an early stage of this research, and much remains to be learned from these remarkable experiments of nature.

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