Lungs at high-altitude: Genomic insights into hypoxic responses

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List of Abbreviations:
ADMA Asymmetric dimethyl arginine
AHVR Acute hypoxic ventilatory response
HA High-altitude
HAPE High-altitude pulmonary edema
HIF Hypoxia-inducible factor
HPV Hypoxic pulmonary vasoconstriction
HRM Hypoxia regulated miRNAs
NO Nitric oxide
ROS Reactive oxygen species

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Abstract

Hypobaric hypoxia at high-altitude (HA) results in reduced blood arterial oxygen saturation, perfusion of organs with hypoxemic blood and direct hypoxia of lung tissues. The pulmonary complications in the cells of the pulmonary arterioles due to hypobaric hypoxia are the basis of the pathophysiological mechanisms of high-altitude pulmonary edema (HAPE). Some populations that have dwelled at HA for thousands of years have evolutionarily adapted to this environmental stress; unadapted populations may react with excessive physiological responses that impair health. Individual variations in response to hypoxia and the mechanisms of HA adaptation provide insight into physiological responses. Adaptive and maladaptive responses include alterations in pathways such as oxygen-sensing, hypoxia signaling, K⁺ and Ca²⁺-gated channels, redox balance and the renin-angiotensin-aldosterone system. Physiological imbalances are linked with genetic susceptibilities, and non-homeostatic responses in gene regulation occur by small RNAs, histone modification and DNA methylation predispose susceptible humans to these HA illnesses. Elucidation of the interaction of these factors will lead to a more comprehensive understanding of HA adaptations and mal-adaptations and will lead to new therapeutics for HA disorders related to hypoxic lungs.
Background

High mountains have a powerful attraction to mankind. Mountain ascents have remained treacherous and sometimes fatal but the risks have not deterred explorations, excursions and settlements. High-altitude (HA) results in decreased partial pressure of air and inspired PO\textsubscript{2}, and decreased blood arterial oxygen saturation (SaO\textsubscript{2}) (57, 76, 135, 137). In spite of the hypoxic threat, permanent human dwellings exist up to an altitude of 15,000 feet above sea level (91, 152). The residents at HA have survived in such an extreme environment through several adaptive features such as blunted hypoxic pulmonary vasoconstriction, increased erythropoiesis and metabolic reprogramming for the suppression of ATP turnover rates (23, 89, 91, 152). Lowland populations, on the contrary, react differentially when exposed acutely to HA. The extent of selection of genetically based trait variations is in direct correlation with adaptive physiological changes and with the number of generations lived at HA (23, 91). Understanding the impact of these variations will not only elucidate the pathophysiological mechanisms of hypoxic lungs but will also help in understanding of the adaptations of the human body to hypoxia.

Clinical features of HA disorders

The symptoms of HA disorders are often confusing, with one or more organ dysfunctions occurring simultaneously in an individual. The major disorders that inflict mankind at altitudes are acute mountain sickness (AMS) (41), sub-acute mountain sickness (SAMS) (9), high-altitude pulmonary edema (HAPE) (17), high-altitude cerebral edema (HACE) (14), high-altitude pulmonary hypertension (HAPH) (14) and Monge’s disease or chronic mountain sickness (CMS) (88). The first four disorders affect sojourners, whereas the latter two affect permanent residents.
of HA (14). Clinical features of these HA disorders are presented in Table 1. Although, the pathophysiology of these disorders is quite different, their mechanisms share features such as alveolar hypoxemia, hypoventilation, increased sympathetic nervous system output and elevated mean pulmonary arterial pressure (mPAP) compared to healthy high altitude sojourners (9, 14, 17, 41, 88). Each year many sojourners suffer from mountain sickness that may range from mild to life threatening. For these victims, investigation, prevention and treatment of HA disorders is clinical priority; however, as the focus of this review is the lung associated HA disorders such as HAPE; therefore, throughout this review more emphasis has been given to this disorder. HAPE is the patchy edema of lungs with dyspnea both at rest and during exercise, moist cough, extreme weakness, profuse perspiration and fever. The non-cardiogenic, non-inflammatory edema in HAPE is results of a mechanical process from high pressures, which could be followed by a secondary inflammatory reaction, although, the role and cause of inflammation is unclear (12, 18, 66, 132, 144). HAPE strikes the unacclimatized sojourner with oxygen saturations of below 70% (17).

Pulmonary complications of HA exposure

Hypoxia is detected physiologically as the change in partial pressure of blood O₂ by peripheral chemoreceptors in the carotid bodies (115). The glomus cells of these carotid bodies detect and transduce the change of partial pressure resulting into depolarization, which induces calcium-dependent release of neurotransmitters to initiate acute hypoxic ventilatory responses (AHVR) (114). AHVR presages the sequence of physiological changes including hypocapnea and hypoxic pulmonary vasoconstriction (HPV) in pulmonary arterioles (PAs) of the lungs (90, 96). Local HPV diverts blood flow from poorly oxygenated areas to better ventilated areas of lung to
maintain ventilation/perfusion balance but diffuse HPV raises PA pressure and over time remodeling accentuates pulmonary hypertension (PH) (96, 109). Interestingly, some permanent HA dwellers have thin walled PAs with weak or absent HPV and low mPAP (8, 50, 109). The attenuation of HPV and thin-walled PAs in them seems in accord with genetic adaptation, which is acquired through the process of natural selection. However, the relatively newer settlements of HA like Andean, Kyrgyz and Han-Chinese do not have thin-walled PAs, rather have the arrangement of elastic tissue similar to the one found in fetal pulmonary trunk and aorta (36, 109). Perhaps they still will require a greater number of generations, over thousands of years, to adapt to HA environment. Most lowland individuals living for considerable time at HA develop characteristic morphological features such as muscularization of arteries extending to alveolar ducts and walls (36, 47). Likewise, lowland animals like indigenous cattle (Bos taurus), when exposed to HA, develop severe medial hypertrophy in the distal end of small PAs with crenation of the elastic laminae. These morphological changes result in higher PAP and arterial resistance that may lead to right heart failure and Brisket disease (10). The Susceptible humans and animals have exaggeration of these physiological responses that may result in HA illnesses.

Most of the pathophysiological mechanisms in HAPE occur in the pulmonary endothelial (ECs) and vascular smooth muscle cells (VSMCs) (Figure 1a and b) (36, 47). In a healthy lung, pulmonary vascular tone is regulated by a balance between the effects of vasodilators/antiproliferative and vasoconstrictors/co-mitogenic agents (10, 129). However, in HAPE, increased vascular tone might be a result of decreased levels of cyclic guanine monophosphate (cGMP) and cyclic adenine monophosphate (cAMP) caused by increased phosphodiesterase activity (129), depolarization by inactivation of K⁺ channels and increased levels of endogenous vasoconstrictors and reactive oxygen species (ROS) (10, 95, 129).
Dysfunction of ECs results in exaggeration of pulmonary vascular tone and structural remodeling. VSMCs and ECs, under pathophysiological conditions, exhibit greater heterogeneity and their phenotypes vary with the size and location of PAs (101). Hypoxia exposure also induces an increase in the proliferation of VSMCs and adventitial fibroblasts as shown in figure 1a (52, 74). This structural remodeling is then followed by increased pulmonary vascular resistance and pulmonary arterial hypertension, the hallmark of HAPE (17). Thus, the numerous biomolecules/modulators, that differentially interact to increase vascular tone, stimulate physiological as well as pathophysiological processes under hypobaric hypoxia.

**Oxygen modulated transcription factors**

The O$_2$-sensing molecules are important at HA as they maintain cellular and systemic O$_2$ homeostasis of the body. Among the several molecules, Hypoxia-inducible factor (HIF), HIF-prolyl hydroxylase 2 (EGLN1) and HIF-1, alpha subunit inhibitor (HIF1AN) are the most crucial O$_2$-sensing mechanism of the body (135). A schematic presentation, in Figure 2, describes the homeostatic functioning of this pathway under normoxic/hypoxic state. HIF is made up of two differentially regulated subunits, the alpha subunit (HIF1α, HIF2α and HIF3α) and aryl hydrocarbon receptor nuclear translocator subunit (135). The alpha subunit of HIF contains several regulatory domains. Among them, the C-terminal O$_2$-dependent degradation (ODD) domain is responsible for the protein stability and the C-terminal transactivation domain (CTAD) mediates its transcriptional response (28). EGLN1, a member of ubiquitous Fe(II) and 2-oxoglutarate-dependent oxygenase superfamily, under normoxic conditions, catalyzes hydroxylation of proline residues at positions 402 and 564 of ODD domain of HIF-1α so that HIF-1α gets recognised by von Hippel-Lindau ubiquination complex to stimulate
polyubiquitination and proteasomal degradation (43). Concurrently, HIF1AN hydroxylates asparagine residue at position 803 of CTAD of HIF-1α thereby blocking the interaction of this molecule with the coactivators CREB-binding protein/E1A binding protein p300 (CBP/p300), thus preventing the functional transactivation of HIF-signaling pathway (75). However, under hypoxia, the EGLN1 and HIF1AN activities are inhibited, which paves the way for normal functioning of HIF (Figure 2) to transcribe downstream target genes that maintain cellular and systemic O₂ homeostasis (82). Under this condition, AHVR, characterized by a progressive increase in baseline ventilation ensures adequate oxygen supply. Here, carotid bodies, the principle sensory organ for detecting arterial blood O₂ levels seems to play a major role (115). A substantial body of evidence suggests that carotid body chemosensory reflex is critical for AHVR (114, 117). Similarly, the erythropoietic response of HIF-1 is yet another environmentally induced response to increase red blood cell mass for enhancing O₂ delivery to the cells and tissues of the body. However, these functions of HIF-1 have their negative impact too. For example, as discussed above, AHVR leads to vascular remodeling and structural changes in the lungs that result into HPV and PH (109). The latter is the hallmark of a number of HA related disorders and becomes the major cause of morbidity at HA. It worsens the condition of HAPE in sojourners and HAPH in HA natives (17, 88). Similarly, increase in red blood cell mass is undesirable and results in CMS in HA natives (88). Hence, HIF-1 mediates physiological responses to sustain hypobaric hypoxia but is also responsible for various environmentally induced mal-adaptive changes (114). In order to counteract these environmentally induced mal-adaptive changes, the HA adapted population have acquired several changes in their genetic makeup (22, 23, 29, 30, 89, 91, 104, 110). HA natives of Tibet (3000-5000 m) have hematological profile similar to what would be expected at sea level and are particularly resistant
to developing CMS (21, 88). On the other hand Andeans (3000-5000 m) have better $O_2$ saturation in their body (23, 91). Likewise, Ethiopians, the indigenous population residing on East African plateau (1500-3500 m) have Hb concentration and $SaO_2$ levels similar to the levels found at sea level (22). Genetics studies done by various groups have demonstrated the natural selection of few alleles in these populations, which correlates well with the beneficial phenotypes in HA natives. For example, genes pertaining to HIF pathway have shown many such changes regulating the phenotypic differences, which are discussed in details under the section ‘HA driven genomic expression’.

**Imbalance in vascular tone modulators**

Vascular tone modulators can be adversely affected by the hypobaric hypoxia environment at HA. Nitric oxide (NO), produced by endothelial nitric oxide synthase (NOS3) is one such molecule, which is highly crucial at HA (4, 21, 33, 49, 55). One of the primary adaptive features in response to HA is the increased blood flow for the improvement of oxygenation in the body and NO, being a potent vasodilator, seems to help in achieving it through counteracting maladaptive changes like VSMC proliferation and HPV. NO controls these functions by stimulating soluble guanylate cyclase (Figure 1c) to increase production of intracellular cyclic guanosine monophosphate (cGMP) that relaxes vascular smooth muscles (21). As expected, studies on circulating NO levels both in HA natives and sojourners have proved its relevance. NO levels were decreased in AMS (33, 55), HAPE (4, 49) and HAPH (156). On the contrary, HA natives had elevated circulating NO levels pointing to improved oxygenation (49). In addition, those molecules that regulate NO diffusion and its signaling are equally pertinent at HA. A recent study has shown that hemoglobin α (Hb α) present in Fe$^{2+}$ state in ECs scavenges
NO, whereas in Fe$^{3+}$ state facilitates NO diffusion from endothelium to VSMCs (142). This study complements those studies where deoxygenated globins and xanthine oxidoreductase residing in erythrocytes function as nitrite reductase to reduce nitrite to NO (151). NO production is also regulated by several other molecules; two among them are asymmetric dimethyl arginine (ADMA) and apelin (39, 79). ADMA, a naturally occurring amino acid, is the most abundant endogenous inhibitor of NOS (Figure 3). It is produced as a result of hydrolysis of post-translationally modified proteins and is degraded by dimethylarginine dimethylamino hydrolase-1 (DDAH1) (11). The inhibition of DDAH1 increases ADMA to reduce the levels of NO, which might lead to vasoconstriction in PAs (118). Significantly higher levels of ADMA were found in patients with HAPE (6), primary PH (118) and PH (11).

Apelin, the other NO modifier, is synthesized as 77-amino acid preproprotein (Figure 1c); however, its active forms apelin-36, -19, -17, -16 and -13 are known to maintain cardiovascular homeostasis by acting as peripheral vasodilators (37, 64). In the endothelium, apelin binds to its apelin receptor and activates NOS3 through AKT signaling pathway inducing vasodilatory effect in the pulmonary vasculature (37). However, it is possible that damaged intima disables apelin to perform its physiological function of vasodilatation at HA (37). Apelin null mice when exposed to chronic hypoxia developed more severe PH as compared to the wild type mice (37). The apelin null mice showed significant downregulation of NOS3 and Kruppel-like factor 2 (KLF2); as a consequence, decreased production of NO that might lead to the development of PH (37). The same study had also demonstrated decreased NOS3 and KLF2 expression in apelin null human pulmonary artery endothelial cells; moreover, serum levels of apelin in patients with PH were found to be reduced compared to healthy controls. Heme oxygenase (HMOX) could be another effector of vasodilatation at HA (42). It catalyzes the rate limiting step in heme
degradation to generate CO and biliverdin. These by-products enhance the \(O_2\) carrying capacity of blood in hypoxic condition (77, 93). CO, on one hand, assists in enhancing NO diffusion by ligating with \(Fe^{2+}\) of heme, whereas biliverdin through biliverdin reductase forms bilirubin, which is a potent antioxidant (77, 142). The HA adapted animals have enhanced HMOX-CO system regulating the pulmonary vascular function at HA (139).

Among other vasoactive mediators, circulating levels of a potent, long acting vasoconstrictor ET-1 (endothelin-1) were reported lower in HA natives (6, 124) and higher in AMS (87) and HAPE (6, 38, 130). ET-1 promotes proliferation of VSMC (Figure 1c) and activates intracellular \(Ca^{2+}\), protein kinase C and early growth response genes like c-fos and c-jun (87, 130), which may possibly be contributing to vasoconstriction in HAPE. Hypobaric hypoxia also induces the expression of angiotensin-1 converting enzyme (ACE), another known vasoconstrictor, which catalyses angiotensin-I (AT-I) to angiotensin-II (AT-II) (35). The latter is also a known vasoconstrictor resulting in the proliferation of VSMCs and neo-intima in small PAs (1, 92).

Similarly, serotonin or 5-hydroxytryptamine (5-HT), a potent mitogen, is involved in proliferation of VSMCs and pulmonary fibroblasts (Figure 1c) and has been functionally demonstrated in PH (1). Importantly, upregulated circulatory levels of 5-HT were observed in HAPE suggesting its involvement in the pathophysiology (6). In a knockout study of 5-HT 1B receptor, the chronic hypoxic pulmonary hypertensive rats were observed to have less right ventricular hypertrophy and vasoconstriction indicating the mitogenic and vasoconstrictory effect of 5-HT (69). 5-HT also seems to plays a role in the activation of carotid bodies, which are required for AHVR, an important phenomenon towards acclimatization at HA. 5-HT is observed to evoke the sensitzation of carotid bodies via reactive oxygen species (ROS) by inducing the expression of NADPH oxidase (117).
Growth factors, another class of vasomodulators, are endogenous stimulators of cellular growth, proliferation and differentiation. They also act as chemoattractants for ECs and VSMCs, and as signaling molecules between cells. Erythropoietin (EPO), a hypoxia-inducible cytokine is required in the differentiation of erythroid progenitor cells and confers stimulatory effect on exercise performance of an individual (133). Since adequate oxygenation of the body is central to adaptive responses, the functions of EPO are well documented under hypobaric hypoxia (19, 32). EPO under low O₂ tension induces NO production in ECs, which might counter the hypertensive effect of increased red blood cells to maintain blood pressure homeostasis (24). Another study observed that a brief intermittent hypoxic (IH) stimulus could lead to effective stimulation of erythropoietin that could result into hematological adaptations (128). IH refers to the repeated episodes of hypoxia and reoxygenation, which appears to trigger a unique hypoxic response in comparison to continuous hypoxia (112, 113, 114). The study found an improvement in the arterial oxygen saturation in the subjects who were exposed to brief IH (128). This improvement in arterial oxygen saturation might be explained either by hyperventilation or by an enhancement in the alveolar-arterial oxygen conductance, both reflecting the processes of acclimatization. If V/Q mismatch existed in these subjects, then an improvement in mixed venous O₂ saturation would result in improved arterial O₂ saturation. Hence, in its other compensatory effects, elevated EPO production improves SaO₂ levels to counterbalance the maladaptive responses (128).

Vascular endothelial growth factor (VEGF), another extensively studied growth factor, is elevated upon HA exposure (149). VEGF acts as an endothelial mitogen and increases O₂ perfusion delivery through vascular remodeling by stimulating angiogenesis through angiopoietin (25). The transforming growth factor β (TGF-β) may also play an important role in
ECs and VSMCs by contributing to vascular remodeling through regulation of ET-1 activity (83). It has been extensively implicated in PH (46) and hence its importance can be anticipated at HA too. Adrenomedullin is another growth factor that has considerable influence on ECs and VSMCs (146). It is a systemic vasodilator which is increased to counter hypoxic vasoconstriction at HA (97, 98). Adrenomedullin activates adenylate cyclase to stimulate NO release (146). Its administration has been shown to improve pulmonary hemodynamics and exercise tolerance (97, 98). To conclude this section, numerous vascular tone molecules contribute to the development and the progression of pulmonary vascular remodeling under the extreme environment of HA. These endothelium dependent functional alterations help redefine the role of endothelial dysfunction. The responses are not limited to attenuation of endothelium dependent vasodilatation but also extend to the dysregulation in the release of vasoactive mediators, growth factor, matrix proteins and adhesion molecules in the ECs. The endothelium has emerged as a key regulator of vascular homeostasis and its dysfunction marks the overall dysregulation of the metabolic and transport functions of ECs.

**K⁺ and Ca²⁺-gated channelopathy**

Hypoxia results in impairment of voltage-gated (Kv) channel α subunit causing significant membrane potential depolarization (126), due to increased intracellular Ca²⁺ (Figure 1c). This reverse reaction results in an array of pathophysiological responses underlying vasoconstriction, PASMC contraction, medial hypertrophy and hyperplasia. For example, uptake of cytosolic Ca²⁺ not only activates HPV (111, 126) but also sensitizes carotid bodies (17), activates immediate early genes and proliferative genes that result in unchecked cell proliferation (111, 125). In addition to the impairment of voltage gated channels, the uptake of intracellular Ca²⁺ is also
regulated by some other nonselective cation channels like transient receptor potassium channels (TRPCs). One of the seven members of TRPCs, TRPC6 is demonstrated to be essential for acute HPV and is believed to be activated by diacylglycerol (53). Chronic hypoxia upregulates the store-operated Ca\(^{2+}\) channel expression of TRPC1 and TRPC6 (114). The exact mechanism of channelopathy under hypobaric hypoxia is unclear but involvement of various transcription factors is anticipated; for instance, HIF induces its target genes like ET-1, VEGF, PDGF and EPO, which are involved in pulmonary vasoconstriction and vascular remodeling. Also, HIF1α appears to regulate the expression of TRPC1 and TRPC6 (114). Therefore, one can hypothesize the involvement of these genes in the induction of channelopathy, which consequently leads to pathophysiological developments in hypoxic lungs (134).

**Oxidative overload**

Production of enhanced ROS and reduced antioxidants result in oxidative damage at HA (Figure 4). Studies on HA disorders such as HAPE have reported oxidative stress, which might primarily contribute to endothelial damage and vascular wall remodelling (6, 13, 45, 85). The oxidative overload impairs several functions; it disrupts vascular homeostasis, increases intracellular Ca\(^{2+}\), damages proteins, lipids and DNA (136). Superoxide anions, produced under the stress, decrease the NO-mediated vasodilatation by generating peroxynitrite (ONOO\(^-\)) (103). The latter is a highly reactive nitrogen species that further induces stress in the body (102). It readily crosses biological membranes to modify proteins with heme prosthetic group. It inactivates enzymes by oxidizing the thiol groups in an amino acid to alter the structure. Importantly, peroxynitrite also triggers lipid peroxidation in membranes, which degenerate membrane lipid causing membrane permeability and fluidity. Exaggerated lipid peroxidation in the form of 8-iso prostaglandin F2α
has been reported in HAPE (6, 85). Alveolar leakage in HAPE could possibly be one of the pathological consequences of increased oxidative stress. Furthermore, peroxynitrite yields nitrosative stress by producing nitrotyrosine and it also reacts with CO₂ to form carbonate radicals, which are likely to be more toxic than hydroxyl radicals. Of relevance, the circulating levels of one of the lipid peroxidation products, 8-iso-prostaglandin F₂α were found elevated in HAPE, indicating the role of oxidative stress in the progression of this disorder (6, 85). Apart from causing vascular damages, ROS also function as important intracellular and intercellular secondary messengers modulating several downstream signaling molecules involved in VSMC growth and migration (73, 147). It is also strongly implicated in sensitization of carotid bodies through 5-HT by inducing the expression of NADPH oxidase that increases the production of ROS (117).

**HA driven genomic expression**

Hypoxic physiological stresses occur in all subjects at HA but only few susceptible individuals suffer from HA disorders. This could be partly attributed to genetic variation that alters the biomolecules involved in responses to hypobaric hypoxia. An approach is needed for identifying the polymorphisms of modifying gene(s), which explain the hyper- and hypo-responsiveness among individuals and help in deciphering the mechanisms. The genetic approaches like, candidate gene, sequencing and genome-wide association have been successful in delineating the convergent evolution of the HA populations living in a similar stressful environment (2, 20, 29, 65, 86, 110, 138, 150, 155, 158).

The role of a single gene in major monogenic disorders is well established; however, the same does not apply to complex diseases. Similar to several other diseases, the significance of genetic
polymorphisms in the physiological pathways in HAPE has yet to be resolved; although, several candidate genes have been reported to associate with HAPE. For example, a number of polymorphisms of NOS3 and ET-1 genes associate with HAPE (3, 4, 34, 124). Polymorphisms in these genes are known to affect the respective circulating levels of NO and ET-1 that shape the physiological set up, adaptive or maladaptive, in an individual (55, 77). However, in spite of the polymorphisms associating with their respective levels, their clinical and biological relevance has yet to be established. This can be attributed to several factors. Importantly, the influence of any individual polymorphism on the levels, in general, is modest, and expectedly several polymorphisms of one or several genes, in fact, are involved to bring in the overall influence. Furthermore, the expression of the gene is influenced by genetic factors within a gene itself and also by environmental factors surrounding it. Hence, it has remained a challenge to differentiate the direct effect of any individual polymorphism on a given phenotype to establish the physiological tone and the role in the therapy regimen. Further, the individual pharmacological responses of each patient may be different based on genomic profile. Identification of individual’s response profile will minimize the adverse effect of a given drug and will increase the efficacy of therapy. Nevertheless, the research on HAPE especially, up till now, has been encouraging and will soon bear fruits. Keeping this in mind, the following text covers few of the pertinent genes that have been screened for their polymorphisms in HAPE and even in HAPH.

In recent past, selection of regions of important candidates of O₂-sensing pathway has been shown in adaptation in different HA populations. Interestingly, HIF2α or EPAS1 has shown positive selection in Tibetans (20, 73). The selected loci associated with low hemoglobin (Hb) concentration. Another candidate of this pathway EGLN1, however, has shown positive selection in Andeans and Tibetans (2, 86, 110, 138, 150, 156). Similar to EPAS1, the EGLN1 loci
associated with low Hb concentration in Tibetans, a sign of HA adaptation (138). Increased red blood cell mass is a maladaptive response (23) and Tibetans, as evidenced through their hematological profile, have overcome this response through the process of natural selection. Our laboratory has also shown selection of rs1538664, rs479200, rs2486729, rs2790879, rs480902, rs2486736 and rs973252 in adaptation and HAPE (2, 86). The risk variants rs1538664A, rs479200T, rs2486729A, rs2790879G, rs480902C, rs2486736A and rs973252G that were over-represented in HAPE, correlated with decreased SaO2 level and increased EGLN1 expression (86). On the contrary the beneficial alleles rs1538664G, rs479200C, rs2486729G, rs2790879T, rs480902T, rs2486736G and rs973252A were associated with higher SaO2 level in the two control groups namely, HA Ladakhi population and HAPE-free controls. Likewise, the risk alleles associated haplotypes A-T-A-G-C-A-G, G-T-A-G-C-A-G and G-T-G-T-C-G-A predicted greater risk for HAPE (86). It is obvious from these studies that HIF signaling pathway is the core pathway involved in O2 homeostasis at HA; nevertheless, the complete genetic picture has yet to be deciphered. In addition, there are nearly 300 downstream target genes of HIF signaling pathway that are regulated by HIF under such environment and their functions play an equally important role in deciding the physiology and pathophysiology at HA (3, 4, 38, 124). For example, the downstream target genes of HIF, such as NOS3 and ET-1, have shown significance under hypobaric hypoxia as few of the variants of these genes have emerged as markers at HA (3, 4, 38, 124). Selection of 894T and 4a variants of NOS3 G894T (rs1799983) polymorphism and 4b/4a repeat (27 base pair variable number tandem repeat in intron 4) in HAPE and 894G and 4b variants in HA population has been observed (3, 4). Presence of 894T variant encodes for aspartic acid instead of glutamic acid, which renders the enzyme inactive thereby depleting NO level. Likewise, 4a variant also termed as short intronic repeats RNA seems responsible for
decreasing NOS3 expression through miRNA-mediated inhibition (160). ET-1, encoding for a potent vasoconstrictor endothelin-1, too has shown the evidence of positive selection in Tibetans and Andeans (38, 89, 124). The prevalence and correlation of ET-1 longer-repeats of (CT)n–(CA)n repeat in 5’ UTR microsatellite and G allele of 2288G/T (rs2070699) intronic polymorphism with lower ET-1 levels in the natives pointed to their functional role at HA (57).

Several other candidates of hypoxia-signaling pathway like HIF-prolyl hydroxylase 3 (89); protein kinase, AMP activated, alpha 1 catalytic subunit (29, 89); phosphoinosite-3-kinase, catalytic alpha polypeptide (29) have shown signals of selection in Andeans. Similarly, Tibetans have shown signals for selection of other signaling molecules like Fetal hemoglobin (HBB/HBG) (158), PPARα (138), Heme oxygenase 2 (138), calcium/calmodulin-dependent protein kinase II delta (138), angiopoietin-like 4 (155) and many more. Table 1 provides the details of positively selected genes and their pathways in various HA populations that point to natural selection towards adaptation to the extreme environment of HA.

Oxidative overload is a common phenomenon at HA. Cytochrome b-245 α polypeptide (CYBA) encoding for p22phox subunit of NADPH oxidase of oxidative stress pathway was reported to show significant association with ROS in HAPE (85). The CYBA risk alleles G and C of −930A/G (rs9932581) and 4325C/T (rs4673), respectively, associated with excess ROS and thereby with HAPE (85). The Interaction between the two alleles also revealed over-representation of most of the risk-alleles-associated haplotypes in HAPE and protective-alleles-associated haplotypes in healthy highlanders. Another pathway, renin-angiotensin-aldosterone system (RAAS) that maintains electrolyte homeostasis, cardiovascular remodeling and vascular tone also has been documented in HA physiology (84). Increased renin activity, AT-II and aldosterone levels were reported in HAPE indicating the imbalances of RAAS (120). Perhaps, all
the members of this pathway are the candidates for investigation at HA. The association of insertion (I) allele of ACE insertion/deletion (I/D) polymorphism in relation to HA adaptation was reported (120); the same allele associated with lower plasma ACE level (145) and elevated SaO2 level (30, 154). Insertion allele, one of the rare genetic markers, has been associated with athletic excellence and endurance and thus with healthier cardiovascular system (54). A recent meta-analysis associated the ACE D allele carriers with greater risk of developing HAPE (121); the allele accounted for higher ACE level and its activity and consequently, with right ventricular hypertrophy. Contrary to all these findings, the II genotype or the I allele associated with HAPH in Kyrgyz highland population (5).

Angiotensinogen (AGT) is a precursor molecule of AT-I, hence AGT has been studied for its variants and among the most studied SNPs are –6G/A, T174M (rs4762) and M235T (rs699); however, only M allele of M235T was found to associate with HAPE (62, 140). AGTR1 located on chromosome 3 was also associated with HAPE susceptibility through 1517G/T (rs5189) polymorphism present in 3’ UTR (140). Aldosterone synthase (CYP11B2) is another important member of RAAS, encoding for aldosterone, whose major role is salt and water retention. Hyperaldosteronism results in cardiac failure, inhibits NO synthesis, increases collagen deposition and myocardial stiffness, which might lead to HA disorders (62, 63). The functional polymorphisms in CYP11B2 were suggested to cause alterations in regulatory controls of this gene such as the Intron 2 conversion (Iw) polymorphism in which most of the intron 2 of CYP11B2 is replaced by that of CYP11B1 (153). Likewise, –344T/C (rs1799998) polymorphism of the same gene was reported to affect transcriptional activity because of its location in the putative binding site of transcriptional regulatory factor SF-1 (106, 123). The wild-type alleles Iw and −344T, reported to be overrepresented in HA natives, correlated with lower aldosterone
levels, whereas the minor or risk alleles Ic and −344C, overrepresented in HAPE, correlated with increased aldosterone levels (122, 123). *Adrenergic beta-2 receptor* (*ADRB2*), encoding for G protein-coupled receptor, is an important member of RAAS as well as sympathetic nervous system. It contributes to vasodilatory effect through NO mediated action and plays crucial role in lung fluid clearance (48, 141); however, nothing concrete has emerged from its genetics study so far (141). To conclude, Tibetans have genetics adaptations that enable their successful survival in an extreme environment of HA; whereas, the sojourners differ from these natives. For example, sojourners with beneficial alleles perform physical activities without showing signs of discomfort; on the contrary, sojourners having risk alleles may experience various levels of discomfort upon physical exertion and develop mountain illnesses. There is strong evidence that genes of O2-sensing pathway like *EPAS1* and *EGLN1* bear strong signals of natural selection that correlate with relevant clinical and bimolecular levels at HA. Risk variants of genes like *NOS3*, *ET-1*, *CYBA*, *ACE* and others of vascular homeostasis pathways have contributed in the pathophysiology of HA mal-adaptation. However, in spite of extensive association studies, we are still not able to fully elucidate the complex pathophysiological mechanisms behind these HA illnesses. Hence, a lucid understanding of the contribution of various genetic variants associating with HA adaptations and mal-adaptations by the exploring their functionality is required. In addition, these investigations can also be translated for practical applications by devising new therapeutic targets to enhance the health management at HA.

**Small RNAs potential role**

Small RNAs or microRNAs (miRNAs) are endogenous small non-coding gene regulatory molecules that downregulate gene expression either by post-transcriptional degradation of
mRNA of a gene or by translational repression (15). The interactions between miRNAs and cellular hypoxia have been documented (58, 72), however their role in hypobaric hypoxia has yet to be explored. A study on signature miRNAs of hypoxia has reported several hypoxia-regulated microRNAs (58, 72). These miRNAs not only provide a link between tumor-specific stress factor and gene expression but also correlate well with the expression of hypoxia-inducible genes (72). In addition, miRNAs such as miR-210, -26 and -181 regulate HIF to modify endothelial cell response and activate the genes involved in pathways such as cell proliferation, apoptosis, angiogenesis, DNA repair, chromatin remodeling, metabolism, migration and cell death regulators (58, 143). These biological functions that are affected by miRNAs are also implicated in HAPE. Furthermore, PH being a hallmark of this disease, the miRNAs studied in hypoxic PH may also be implicated in these disorders. The role of specific miRNAs such as miR-21, -22, -30, -322, -328 and -451 in the etiology of hypoxic PH has been observed (44, 105). miR-21 was found to be upregulated in patients with PH, it exerts its effect by downregulating BMP receptor type 2 signaling, which increases pulmonary arterial hypertension (PAH) (105). miR-23b and 26a were shown to inhibit TGFβ signaling that might further contributed to aggravation of PAH (44, 159). On the contrary, miR-328 was found to be significantly downregulated in hypoxic PH and was shown as a protecting factor against PA vasoconstriction and remodeling (59). It mediates its protective role by posttranscriptional repression of L-type calcium channel-α1C and insulin growth factor 1 receptor, thereby inhibiting the VSMCs proliferation (59). The investigation of these miRNAs under hypobaric hypoxia or directly in HA diseases like HAPE will not only help explain their roles, but also help find novel pharmacologic approaches. For example, miRNAs that play a crucial role in the development of pathophysiology can be targeted
with AMOs (antimicroRNA oligonucleotides) and LNAs (locked nucleic acids) to provide a new line of therapeutics for HA disorders.

**Epigenetic aberrations**

Mechanisms independent of nucleotide sequence create different phenotypes in different cells (27). These mechanisms are influenced by the immediate environment that results in varied epigenetic regulations in the body. Understanding of these regulations at HA might help in deciphering the complex interactions between the genome and hypobaric hypoxia environment (27). Several of the post-translational modifications like glycosylation, acetylation, methylation, histone modification and DNA methylation of CpG islands of promoter of several genes work together, to regulate various physiological functions (27). Around 50% of total genes including the coding genes and small RNAs have CpG islands, whose transcription is influenced by methylation of DNA and acetylation of histone (148). Therefore, exploration of such epigenetic regulations will decipher the impact of hypobaric hypoxia on various biological pathways at HA. Not many studies on HA have targeted these regulations in context of long term or short term hypoxia exposure; however, preliminary results are alluring and demand attention (7, 131). Nanduri et al have examined the role of DNA methylation in mediating the long-term effect of neonatal IH on carotid body responses to hypoxia in the same rats upon adulthood (100). Carotid bodies are the specialized sensory organs that detect hypoxia and mediate ventilatory responses (116). Their study demonstrated that when adult rats that were exposed to IH as neonates exhibited augmented hypoxic sensitivity of the carotid body, respiratory abnormalities manifested by a greater number of spontaneous apnoeas, systemic hypertension and elevated plasma catecholamines (100). The global DNA methylation was increased in the carotid bodies.
Moreover, genes encoding antioxidant enzymes were downregulated and oxidative stress was increased in the carotid bodies in these adult rats. Further, the same group showed that the marked hypermethylation of the promoter region of the superoxide dismutase 2 gene contributed to reduced expression of antioxidant gene. Likewise, another group demonstrated that long term exposure to hypoxia globally caused reduced methylation and histone acetylation in fetal pulmonary arterial smooth muscle cells (157). Based on these observations, they hypothesized that this long term exposure to hypoxia resulted in epigenetic alterations, which in turn might lead to VSMC proliferation and vascular remodeling, the major hallmarks of HAPE (157). Further, hypomethylation has also been attributed to genetic instability due to ionizing radiation at HA (71, 107). Another recent comparative study on Ethiopian highlanders and lowlanders has reported variation of methylation between Amhara and Oromo populations (7). They reported significant methylation of four CpG sites around the genes involved in HIV-1 infection. Although none of the genes were hypoxia candidate genes; however, HIV/AIDS has been a major health problem in Ethiopia and genome wide signals of epigenetic differences coming from the CpG sites near to these genes indicated the importance of such epigenetic regulations (7). All of these investigations including our ongoing research on the role of DNA methylations in mediating the effect of hypobaric hypoxia show lot of promise and its further investigation including some other epigenetic regulations like glycolysation, acetylation and histone modification will surely assist in understanding the responses of an organism towards the hypobaric hypoxic environment.
Therapeutic intervention

Descent to lower altitude is the most effective treatment for HA disorders (61). The Gamow bag, has simplified field treatment (67). The patient is placed inside the sealed, pressurized bag, which simulates a descent to lower altitude by raising barometric pressure (51). Figure 5 demonstrates several of the important therapeutic molecules along with the pathways regulated by these drugs. Several therapies are available, but acetazolamide (Diamox) and dexamethasone are the two best tried and tested drugs, and are often used as preventive medication during HA exposure and for the treatment of AMS and HACE (60, 80, 127). Acetazolamide, a potent carbonic anhydrase inhibitor, works by inducing renal bicarbonate excretion that leads to metabolic acidosis. This causes peripheral chemoreceptors to increase ventilatory drive during exposure to low oxygen tensions (60). Dexamethasone does not play a role in acclimatization but buys time when it is problematic to descend. It blocks the arachidonic acid pathway thereby decreasing inflammatory mediators like prostaglandins in brain (60). In addition, dexamethasone effectively lowers PAP by increasing the expression of NOS3 (31) and is also known to block the action of VEGF (70). Further, it reduces vascular permeability by reducing the surface tension and microvascular transmural pressure by increasing surfactant phospholipid and protein secretion into the alveolar lining fluid.

In addition to acetazolamide and dexamethasone, oxygen therapy effectively treats AMS and HAPE (108). The vasodilatory function of NO is mediated by cGMP, but phosphodiesterase type 5 (PDE5) readily degrades the latter (68). Sildenafil, a PDE5 inhibitor provides an acute pulmonary vasodilatory effect and improves gas exchange, which might help in prevention and treatment of HAPE (81). Nifedipine, a calcium channel blocker (Figure 5) is also effective in both prevention and treatment of HAPE (16). Among the other medications, the endothelin
antagonist, bosentan, has been established to alleviate HPV (40). Because platelet aggregation leading to thrombotic lesions is also implicated in HA disorders (Figure 5), antiplatelet therapy with aspirin and ibuprofen could also be used in the treatment (34, 56).

Other newer therapies have not been adequately tested in HA disorders but may have promise. Although protein kinases have yet to be associated with HA disorders, their involvement in PH has been shown (94). Fasudil, a rho kinase inhibitor (94); KT5926, myosin light chain kinase inhibitor (99); SP600125, c-Jun N-terminal kinase inhibitor (26); AG1879, SRC-family tyrosine kinases inhibitor (119) and gefitinib, an epidermal growth factor receptor inhibitor (106) are examples of some of the inhibitors, which may find relevance.

Efficacy of treatment recommended at HA: Acetazolamide is the preferred drug of choice as prophylaxis against all forms of AMS. The increased alveolar ventilation increases the amount of oxygen in the blood and helps in the treatment of HAPE. The diuretic effect of acetazolamide contributes to management of the edematous state. In patients who cannot take acetazolamide, dexamethasone can be substituted but has not been validated for HAPE and must be taken for the duration of exposure. In rescue from AMS, dexamethasone is a drug of choice for cerebral illness. In patients that have stable systemic blood pressure, nifedipine is appropriate for HAPE, and in those with marginal blood pressure, either sildenafil or tadalafil. In all cases of mountain sickness, added O₂ is immediately efficacious. Descent is effective when possible and hyperbaric treatment works well when available (78).
Conclusion

Understanding the response of lungs to hypobaric hypoxia holds promise to elucidate complex mechanisms in numerous diseases where hypoxia is a cause as well as a consequence. To achieve this, the crosstalk among multiple interacting pathways and responses need to be defined. Some pathways such as oxygen-sensing, RAAS and oxidative stress have been strongly implicated in hypobaric hypoxia. However, association is not enough to elucidate the causal mechanisms operative in hypoxic lungs. The mechanisms of vascular tone modulators such as apelin, ADMA, and 5-HT once more fully understood may be the basis of therapeutic targets in near future. Current knowledge of genetic structure of populations at HA shows evidence of natural selection of candidate genes, and helps in understanding the processes of natural selection and human evolution. Likewise, association of genetic markers with various HA diseases indicate their role in the pathophysiology of these diseases. However, comprehensive investigations are still needed to decipher HA biology and to bring out the markers of global efficacy.

Contributors

Aastha Mishra researched and wrote the manuscript. Qadar Pasha and John H. Newman conceptualized, researched, wrote and edited the manuscript. Ghulam Mohammad and Tsering Norboo provided needful inputs and contributed to clinical and therapeutic section of the review. All the authors were responsible for the decision to submit the manuscript for publication.

Conflicts of interest

No conflicts of interest, financial or otherwise, are declared by the authors.
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Legends to the Figures and Tables

Figure 1: Schematic representation of the pathophysiological changes occurring in PAs of lungs at HA.

(a) Distal PAs of the lungs, (b) cross-sectional view of an affected PA with inner lining of dysfunctional ECs followed by VSMCs proliferation. (c) The pathophysiological mechanisms occurring in ECs and VSMCs, which are involved in the development of HA illnesses.

ACE, angiotensin-1 converting enzyme; AGTR1, angiotensin-II type I receptor; AT-I, angiotensin-I; AT-II, angiotensin II; ADMA, asymmetric dimethyl arginine; Ap, apelin; BMP, bone morphogenetic protein; BMPRII, bone morphogenetic protein receptor type II; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; Ecs, endothelial cells; NOS3, endothelial nitric oxide synthase; ET-1, endothelin-1; ET-R, endothelin receptor; 5-HT, serotonin; 5-HTR, serotonin receptor; 5-HTT, serotonin transporter; IP3, inositol trisphosphate; MAPKs, mitogen activated protein kinases; NO, nitric oxide; P, phosphorylation; PGI2R, prostacylin receptor; PIP2, phosphatidylinositol 4, 5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; ROS, reactive oxygen species; TF, transcription factor; VSMCs, vascular smooth muscle cells.

Figure 2: Regulation of adaptive homeostatic responses under hypobaric hypoxia.

In the normoxic condition, the protein levels of HIF-1α as well its transactivational activity are regulated by EGLN1 and HIF1AN, respectively. However, under hypobaric hypoxia, EGLN1 and HIF1AN are inhibited to allow proper functioning of HIF-1α for maintenance of O2 and cellular homeostasis.
ARNT, aryl hydrocarbon nuclear receptor translocator; Asn, asparagines; EGLN1, HIF-prolyl hydroxylase 2; HIF-1α, hypoxia inducible factor-1α; HRE, hypoxia response element; O₂, oxygen; OH, hydroxyl group; ub, ubiquitin proteins; VHL, von Hippel-Lindau tumour suppressor protein; p300/CBP, E1A binding protein p300-CREB-binding protein; Pro, proline.

Figure 3: Metabolism and physiology of asymmetric dimethyl arginine (ADMA).
ADMA is a naturally occurring amino acid produced during protein arginine methylation catalyzed by the family of intracellular enzyme PRMT and is degraded by a key catabolic enzyme DDAH-1. DDAH-1, dimethylarginine dimethyl amino hydrolase-1; NOS, nitric oxide synthase; PRMT, protein arginine methyltransferase enzyme.

Figure 4: Oxidative status at HA.
The increased production of ROS and decreased production of antioxidants results into oxidative overload at HA, which is one of the crucial factors affecting the development of HA pathophysiology. Mitochondrial respiratory chain, NADPH oxidase, xanthine oxidase, cyclooxygenase, lipooxygenase and uncoupled NOS3 produces a limited amount of ROS at all the time in the body which is scavenged out by antioxidants like SOD, catalase and glutathione peroxidase. **CYBA** encoding p22phox subunit of NADPH oxidase has been found to be significantly associated with HA. Under the stressful conditions, enhanced production of ROS such as O₂⁻, H₂O₂, hydroxyl radical (OH˙), LOO˙ and reduced production of antioxidants result in vascular dysfunction and remodeling through oxidative damage. GSSG, glutathione disulfide; H₂O₂, hydrogen peroxide; LOO˙, lipid radicals; ONOO˙, nitrogen radicals; O₂⁻ superoxide anion; SOD, superoxide dismutase.
Figure 5: Therapeutic targets at HA.

The treatment at HA, in general, intends to elevate vasodilation in the body either by increasing cGMP/cAMP concentration or by decreasing PKC levels, which alleviate vasoconstrictory effects.

ADMA, asymmetric dimethyl arginine; AT-II, angiotensin-II; AT-1R, angiotensin-II type I receptor; PKA, protein kinase A; cG(A)MP, cyclic guanylate (adenylate) monophosphate; DAG, diacylglycerol; PDE5, phosphodiesterase type 5; DDAH, dimethylarginine dimethyl amino hydrolase-1; ET-1, endothelin-1; ET-R, endothelin receptor; 5-HT, serotonin; 5-HTT, serotonin transporter; 5-HTR, serotonin receptor; IP3, inositol triphosphate; MLCK, myosin light chain kinase; PLC, phospholipase C; PKC, protein kinase C; PIP2, phosphatidyl inositol bisphosphate; NO, nitric oxide; VIP, vasoactive intestinal peptide.

Table 1: Diagnostic classification of various HA disorders.

Table 2: The positively selected genes and their pathways in different HA populations like Tibetans, Andeans, Daghestanis, Eurasians and Ethiopians through candidate approach and GWAS.
Figure 1
Figure 2
Figure 3

Therapeutic step: farnesoid X receptor agonist GW 4064 increases the level of DDAH and decreases ADMA.
Figure 4
Figure 5
Table 1: Diagnostic classification of patients with high-altitude disorders

<table>
<thead>
<tr>
<th>Acute mountain sickness</th>
<th>High-altitude cerebral edema</th>
<th>High-altitude pulmonary edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best assessed by Lake Louise scoring</td>
<td>Mental impairments like hallucination, disorientation and confusion.</td>
<td>Dyspnea both at rest and during exercise.</td>
</tr>
<tr>
<td>system.</td>
<td>Headache followed by ataxia.</td>
<td>Severe pulmonary rales, tachypnea and cyanosis.</td>
</tr>
<tr>
<td>Shortness of breath, tachypnea.</td>
<td>Brain MRI show variable degrees of edema in subcortical white matter and the splenium of the corpus callosum</td>
<td>Moist cough, extreme weakness, profuse perspiration and fever.</td>
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<tr>
<td>Pulmonary rales and ataxia</td>
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<td>Low blood SaO₂ level.</td>
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<td>Headache, insomnia, laziness, lassitude,</td>
<td></td>
<td>Chest X-ray and echocardiography show patchy</td>
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<td>anorexia, vomiting and fatigue.</td>
<td></td>
<td>edema of lungs.</td>
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<tr>
<th>Sub-acute mountain sickness</th>
<th>Chronic mountain sickness</th>
<th>High-altitude pulmonary hypertension</th>
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</thead>
<tbody>
<tr>
<td>Described predominantly in Han-Chinese</td>
<td>Visible polycythemia and hypoxaemia with dark red cheeks and lips.</td>
<td>Pulmonary arterial hypertension and dyspnoea</td>
</tr>
<tr>
<td>infants and Indian soldiers; it is of two</td>
<td>Neuropsychological symptoms like memory loss, mental confusion, sleep disturbance, headache, anorexia and fatigue.</td>
<td>followed by fatigue, weakness and anginal chest</td>
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<tr>
<td>kinds: Infantile SMS and adult SMS.</td>
<td></td>
<td>pain.</td>
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<td>Severe hypoxic pulmonary hypertension</td>
<td></td>
<td>High pulmonary vascular resistance with mPAP</td>
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<tr>
<td>with extreme medial hypertrophy.</td>
<td></td>
<td>and sPAP of &gt;25 and 50 mm Hg, respectively.</td>
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<tr>
<td>Exertional dyspnea, edema and pericardial</td>
<td></td>
<td>Right ventricular wall thickness of &gt;0.5 cm as</td>
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<tr>
<td>effusion.</td>
<td></td>
<td>determined by Doppler echocardiography.</td>
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</table>
Table 2: The positively selected genes and their pathways in different HA populations like Tibetans, Andeans, Daghestanis, Eurasians and Ethiopians through candidate approach and GWAS.

<table>
<thead>
<tr>
<th>Population</th>
<th>Genes</th>
<th>Pathway</th>
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<td>Peng et al. 2011</td>
<td>GWAS</td>
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<td>HIF signaling</td>
<td>J et al. 2012</td>
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<td>disrupted in schizophrenia 1 (DISC1)</td>
<td>Neuronal</td>
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<td>Ethiopians</td>
<td>Mitochondrial calcium uptake 1 (CAB1A1)</td>
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<td>Thyroid hormone receptor beta (THRB)</td>
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