Lungs at high-altitude: genomic insights into hypoxic responses

Aastha Mishra,1,2 Ghulam Mohammad,3 Tsering Norboo,4 John H. Newman,5 and M. A. Qadar Pasha1

1Department of Genomics and Molecular Medicine, Genomics and Molecular Medicine, CSIR-Institute of Genomics and Integrative Biology, Delhi, India; 2Department of Biotechnology, University of Pune, Pune, India; 3Department of Medicine, SNM Hospital, Leh, Ladakh, J&K, India; 4Ladakh Institute of Prevention, Leh, Ladakh, J&K, India; and 5Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

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Address for reprint requests and other correspondence: M. A. Qadar Pasha, CSIR-Institute of Genomics and Integrative Biology, Mall Rd., Delhi 110 007, India (e-mail: qpasha@igib.res.in).

Mishra A, Mohammad G, Norboo T, Newman JH, Pasha MAQ. Lungs at high-altitude: genomic insights into hypoxic responses. J Appl Physiol 119: 1–15, 2015. First published April 24, 2015; doi:10.1152/japplphysiol.00513.2014.—Hypobaric hypoxia at high altitude (HA) results in reduced blood arterial oxygen saturation, perfusion of organs with hypoxic 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 nonhomeostatic responses in gene regulation that 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 maladaptations and will lead to new therapeutics for HA disorders related to hypoxic lungs.

high altitude; acute hypoxic ventilatory response; asymmetric dimethyl arginine; hypobaric hypoxia; hypoxia-inducible factor; high-altitude pulmonary edema; acute mountain sickness; vascular remodeling; hypoxic pulmonary vasoconstriction response

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 PO2, and decreased blood arterial oxygen saturation (SaO2) (57, 76, 135, 137). Despite the hypoxemic 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 artery pressure (mPAP) compared with 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 a clinical priority; however, as the focus of this review is the lung-associated HA disorders

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such as HAPE, 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 noncardiogenic, noninflammatory edema in HAPE results from 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 in depolarization, which induces calcium-dependent release of neurotransmitters to initiate acute hypoxic ventilatory responses (AHVR) (114). AHVR presages the sequence of physiological changes including hypcopnea 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 (101). Hypoxia exposure also induces an increase in the proliferation of VSMCs and adventitial fibroblasts as shown in Fig. 1A (52, 139a). 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 O₂-sensing molecules are important at HA as they maintain cellular and systemic O₂ 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 O₂-sensing mechanism of the body (135). A schematic presentation, in Fig. 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 COOH-terminal O2-dependent degradation (ODD) domain is responsible for the protein stability and the COOH-terminal transactivation domain (CTAD) mediates its transcriptional response (28).

EGLN1, a member of the ubiquitous Fe(II) and 2-oxoglutarate-dependent oxygenase superfamily, under normoxic conditions, catalyzes hydroxylation of proline residues at positions 402 and 564 of the ODD domain of HIF-1α so that HIF-1α gets recognized by von Hippel-Lindau ubiquitination complex to stimulate polyubiquitination and proteasomal degradation (43). Concurrently, HIF1AN hydroxylates the 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 the HIF-signaling pathway (75). However, under hypoxia, the EGLN1 and HIF1AN activities are inhibited, which paves the way for normal functioning of HIF-1α so that HIF-1α gets recognized by von Hippel-Lindau ubiquitination complex to stimulate polyubiquitination and proteasomal degradation (43).

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 in 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 maladaptive changes (114). To counteract these environmentally induced maladaptive changes, the HA adapted population has acquired several changes in their genetic makeup (22, 23, 29, 30, 89, 91, 104, 110). HA natives of Tibet (3,000-5,000 m) have a 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 (3,000–5,000 m) have better O2 saturation in their body (23, 91). Likewise, Ethiopians, the indigenous population residing on the East African plateau (1,500–3,500 m), have Hb concentration and SaO2 levels similar to the levels found at sea level (22). Genetics studies done by various groups have demonstrated the natural selection of a 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 below (see 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 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 (Fig. 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 have 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 the Fe$^{2+}$ state in ECs scavenges NO, whereas in the Fe$^{3+}$ state it 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 (Fig. 3). It is produced as a result of hydrolysis of posttranslationally 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 a 77-amino acid preproprotein (Fig. 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 the AKT signaling pathway, inducing a vasodilatory effect in the pulmonary vasculature (37). However, it is possible that damaged intima disables apelin from performing its physiological function of vasodilatation at HA (37). Apelin null mice when exposed to chronic hypoxia developed more severe PH compared with the wild-type mice (37). The apelin null mice showed significant downregulation of NOS3 and Kruppel-like factor 2 (KLF2); and, 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 with 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 O2 carrying capacity of blood in hypoxic condition (77, 93). CO, on the one hand, assists in enhancing NO diffusion by ligating with Fe2+ of heme, whereas biliverdin through biliverdin reductase forms bilirubin, which is a potent antioxidant (77, 142). The HA-adapted animals have and enhanced HMOX-CO system regulating the pulmonary vascular function at HA (139).

Among other vasoactive mediators, circulating levels of a potent, long-acting vasoconstrictor, endothelin-1 (ET-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 (Fig. 1C) and activates intracellular Ca2+, 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 catalyzes angiotensin-I (AT-I) to angiotensin-II (AT-II) (35). The latter is also a known vasoconstrictor resulting in the proliferation of VSMCs and neointima in small PAs (1, 92). Similarly, serotonin or 5-hydroxytryptamine (5-HT), a potent mitogen, is involved in proliferation of VSMCs and pulmonary fibroblasts (Fig. 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-HT1B receptor, the chronic hypoxic pulmonary hypertensive rats were observed to have less right ventricular hypertrophy and vasoconstriction indicating the mitogenic and vasoconstrictive effect of 5-HT (69). 5-HT also seems to play a role in the activation of carotid bodies, which are required for AHVR, an important phenomenon toward acclimatization at HA. 5-HT is observed to evoke the sensitization 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 a 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 appear to trigger a unique hypoxic response compared with 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 ventilation/perfusion 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⁺ (Fig. 1C). This reverse reaction results in an array of pathophysiological responses underlying vasoconstriction, PASM 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 Ca2⁺ 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 vasconstriction 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 (Fig. 4). Studies on HA disorders such as HAPE have reported oxidative stress, which might primarily contribute to endothelial damage and vascular wall remodeling (6, 13, 45, 85). The oxidative overload impairs several functions; it disrupts vascular homeostasis, increases intracellular Ca2⁺, and 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

Fig. 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, lipoxygenase, and uncoupled NOS3 produce a limited amount of ROS at all times 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 superoxide anion (O²⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH·), and lipid radicals (LOO·), and reduced production of antioxidants result in vascular dysfunction and remodeling through oxidative damage. GSSG, glutathione disulfide; ONOO⁻, nitrogen radicals; SOD, superoxide dismutase.
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 a 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 hyporesponsiveness among individuals and help in deciphering the mechanisms. The genetic approaches, such as candidate gene, sequencing, and genomewide 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, 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, despite 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 an 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, until now, has been encouraging and will soon bear fruits. Keeping this in mind, the following text covers a few of the pertinent genes that have been screened for their polymorphisms in HAPE and even in HAPH.

In the 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 overrepresented 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 the 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 the 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 asparatic 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 vasconstrictor endothelin-1, also 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 the 5’t untranslated region (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); and 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 2 provides the details of positively selected genes and their pathways in various HA populations that point to natural selection toward 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 the 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 overrepresentation of most of the risk alleles-associated haplotypes in HAPE and protective alleles-associated haplotypes in healthy highlanders. Another path-
way, the 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

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>
<th>Group (Ref. No.)</th>
<th>Approach</th>
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<tr>
<td>Tibetans</td>
<td>Endothelial PAS domain-containing protein 1 (EPAS1)</td>
<td>HIF signaling</td>
<td>Peng et al. (110)</td>
<td>GWAS</td>
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<td>Simonson et al. (138)</td>
<td>GWAS</td>
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<td>HIF signaling</td>
<td>Simonson et al. (138)</td>
<td>GWAS</td>
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<td>Calcium/calmodulin-dependent protein kinase II delta (CAMK2D)</td>
<td>Calcium/calmodulin-dependent signaling</td>
<td>Simonson et al. (138)</td>
<td>GWAS</td>
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<td>Heme oxygenase 2 (HMOX2)</td>
<td>Heme degradation</td>
<td>Simonson et al. (138)</td>
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<td>Simonson et al. (138)</td>
<td>GWAS</td>
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<td>GWAS</td>
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<td>Oxygen transport</td>
<td>Yi et al. (158)</td>
<td>GWAS</td>
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<td>GWAS</td>
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<td>Yi et al. (158)</td>
<td>GWAS</td>
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<td>GWAS</td>
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<td>Bigham et al. (29)</td>
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GWAS, genomewide association studies.
to HA adaptation was reported (120); the same allele associated with lower plasma ACE level (145) and elevated SATo level (30, 154). Insertion allele, one of the rare genetic markers, has been associated with athletic excellence and endurance and thus with a 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 the 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 the 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 the 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, and 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 the sympathetic nervous system. It contributes to the vasodilatory effect through NO-mediated action and plays a 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 the 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 maladaptation. However, despite 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 maladaptations by 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 noncoding gene regulatory molecules that downregulate gene expression either by posttranscriptional 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 contribute 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 pharmacological approaches. For example, miRNAs that play a crucial role in the development of pathophysiologic 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 these regulations at HA might help in deciphering the complex interactions between the genome and hypobaric hypoxia environment (27). Several of the posttranslational 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 de-
cipher the impact of hypobaric hypoxia on various biological pathways at HA. Not many studies on HA have targeted these regulations in the context of long-term or short-term hypoxia exposure; however, preliminary results are alluring and demand attention (7, 131). Nanduri et al. (100) 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 apneas, 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 genomewide signals of epigenetic differences coming from the CpG sites near these genes indicated the importance of such epigenetic regulations (7). All of these investigations including our ongoing research on the role of DNA methylation in mediating the effect of hypobaric hypoxia, show much promise, and their further investigation, including some other epigenetic regulations like glycosylation, acetylation, and histone modification, will surely assist in understanding the responses of an organism toward 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 chemo receptors 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.

![Fig. 5. Therapeutic targets at HA. The treatment at HA, in general, intends to elevate vasodilatation in the body either by increasing cGMP/cAMP concentration or by decreasing PKC levels, which alleviate vasoconstrictory effects. AT-1R, angiotensin-II type I receptor; cG(A)MP, cyclic guanylate (adenylate) monophosphate; PDE5, phosphodiesterase type 5; MLCK, myosin light chain kinase; VIP, vasoactive intestinal peptide. See Fig. 1 for other abbreviations.](image)
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). Silde-nafil, 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 (Fig. 5), is also effective in both prevention and treatment of HAPE (16). Among the other medications, the endothelin antagonist, bosantan, has been established to alleviate HPV (40). Because platelet aggregation leading to thrombotic lesions is also implicated in HA disorders (Fig. 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). Fusadil, a rho kinase inhibitor (94); KT5926, myosin light chain kinase inhibitor (99); SP600125, c-Jun NH2-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 that 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 useful.

**CONCLUSIONS**

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 cross-talk among multiple interacting pathways and responses needs 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-fullly understood, may be the basis of therapeutic targets in the 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.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

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