Advances in Genomics and Genetics

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REVIEW

Oxidative stress at high altitude: genotype–phenotype correlations

This article was published in the following Dove Press journal:
Advances in Genomics and Genetics
7 May 2014
Number of times this article has been viewed

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Abstract: It has been well-documented that the hypobaric hypoxic environment at high altitude (HA) causes stress to both the permanent residents of HA and the sojourners. This oxidative stress primarily disturbs the oxygen-sensing and vascular homeostasis pathways, thereby upsetting normal human physiology, especially in sojourners. These environmental challenges have caused dynamic evolutionary changes within natives of HA, allowing them to develop adaptive plasticity. This review focuses on the genomic and biochemical features of the molecules involved in the oxygen-sensing and vascular homeostasis pathways with respect to HA pulmonary edema (HAPE) and adaptation. We review the role of genetic markers such as HIF-prolyl hydroxylase 2, endothelial PAS domain-containing protein 1, endothelial nitric oxide synthase, endothelin 1, cytochrome b-245 alpha polypeptide, and glutathione S-transferase pi 1, as well as three circulatory biomarkers (nitric oxide, endothelin 1, and 8-iso-prostaglandin F2α), by highlighting approaches such as candidate gene and genome-wide, adopted in deciphering the pathways. A disagreement between the two approaches has also been highlighted. In addition, we discuss that an overrepresentation of wild-type alleles in HA natives and mutant alleles of same polymorphisms in HAPE patients implies that the allelic variants at the same locus are involved in adaptation and HAPE, respectively. Moreover, healthy sojourners present a number of genomic features similar to HA natives, further strengthening the concept of genetic predisposition. A trend in correlation between protective and risk alleles and altered levels of circulatory markers clearly documents the phenomenon of genotype–phenotype correlations. We conclude that the genetic and biochemical markers discussed here both could prove to be potential targets for future diagnostic and therapeutic interventions.

Keywords: high altitude, oxidative stress, pulmonary edema, sojourners, natives

Introduction

Physiology at high altitude

The field of mountain medicine recognizes the high altitude (HA) region as comprising terrestrial elevations between 1,500 and 3,500 m.[1] HA is characterized by extreme environmental conditions such as low atmospheric pressure, low partial pressure of oxygen, low temperature,[1,2] and high ultraviolet radiation.[3] The higher the altitude, the more likely the adverse effects. An altitude above 1,500 m affects the homeostasis of human physiology, and 2,500 m makes humans much more susceptible to disorders. Altitude above 5,500 m cannot be tolerated by the human body for a long period of time.[1] The human body can adapt, as in HA natives, and acclimatize, as in sojourners (lowlanders), to the stressful environment of HA by having a higher breathing rate[2,4,5] and higher heart rate[1,2,4,5] and by making alterations in blood circulatory physiology.[6,]

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[7] In this review, we discuss how permanent and temporary dwellings have shaped human physiology, differentially leading to a number of genetic and circulatory differences in HA natives and sojourners, respectively. It is these differences that make the two populations considerably distinct in their ability to adjust to the environmental stress at HA.

Hemoglobin in HA acclimatization and adaptation

Alterations in blood circulatory physiology are a key phenomenon observed in both the sojourners visiting HA and long-term residents of the place (ie, HA natives). Hemoglobin is one of the important factors involved in the discussion of HA acclimatization and adaptation. It has been noticed that modifications in hemoglobin functions play a key role in mediating an adaptive response to HA hypoxia.[8,9] Hemoglobin is a specialized tetrameric protein found in the blood that is needed to transport oxygen molecules within the human body. The percentage saturation of hemoglobin with oxygen falls at HA, however, by increasing the concentration of hemoglobin, thus increasing the amount of oxygen that can be carried in the blood. Our body has devised a mechanism to overcome the effects of harsh environmental conditions at HA, but there is a negative aspect to this phenomenon as well, as too much hemoglobin makes blood viscous and difficult for the heart to pump effectively. This is a phenomenon commonly observed in a disease known as chronic mountain sickness. Population differences have been seen in hemoglobin levels, with lower hemoglobin levels found in Tibetans than in Aymaras and sojourners. This interpopulation difference has been accounted for by genetic factors for a very high proportion of the phenotypic variance found in hemoglobin concentration. The presence of significant genetic variance clearly indicates that natural selection leading to genetic adaptation, with respect to hemoglobin concentration, is going on in Tibetan and Aymara HA populations.[10] Separate studies have reported haplotypes and variants of genes, such as *endothelial PAS domain-containing protein 1* (*EPAS1*), *HIF-prolyl hydroxylase 2* (*EGLN1*), and *peroxisome proliferator-activated receptor alpha* (*PPARA*) associating with hemoglobin concentrations. rs10803083 of *EPAS1* is one of the most significant single nucleotide polymorphisms found at a significance level of a genome-wide study (GWAS) in the Aymara population.[11,12]

Hypobaric hypoxia and HA disorders

The inverse curvilinear correlation between barometric pressure, partial pressure of oxygen, and HA results in hypobaric hypoxia, which is the depletion of the oxygen supply to tissues, resulting from low atmospheric pressure. Hypobaric hypoxia has a number of deleterious effects on human physiology, and physical exertion in particular, in the unacclimatized sojourners, which creates respiratory and cerebral problems, eventually resulting in various mountain disorders.[1,13] Among the various disorders, acute mountain sickness, HA pulmonary edema (HAPE), and HA cerebral edema[1,2,13] afflict sojourners. In contrast, HA natives remain hale and hearty[5] under such harsh conditions. In this review, we concentrate mainly on HAPE in sojourners and adaptation in HA natives.

HAPE and adaptation

HAPE is a life-threatening form of noncardiogenic pulmonary edema that occurs in unacclimatized sojourners on exertion.[14–16] It is caused by acute exposure to low partial pressure of oxygen at HA. Speed of ascent and altitude attained are the key determinants in occurrence of HAPE,[1] as on rapid ascent, the body does not have sufficient time for the acclimatization process, such as deeper breathing, increased respiratory rate, higher urine production, and increased red blood cell production.

In addition, at HA, because of the low pressure, water vapor is lost from the lungs at an increased rate, leading to dehydration, which also contributes to the symptoms of HAPE.[17] Headache is the first symptom commonly experienced on ascent to HA and is often a result of dehydration. Exaggerated hypoxic pulmonary vasoconstriction is a hallmark of HAPE, characterized by abnormal build-up of fluid in the lungs, preventing air spaces from opening up and filling with fresh air after each breath.[14–16,18] This abnormal fluid build-up leads to dyspnea, cough, congestion, tachypnea, tachycardia, central cyanosis, and wheezing while breathing. These symptoms constitute the diagnosis of HAPE.[14–16,18,19] Chest radiographic infiltrates consistent with pulmonary edema confirm the disorder.[14,16,18] The symptoms and signs disappear within 3 days of the start of treatment with supplemental oxygen and bed rest. The incidence rate of HAPE is variable but is more pronounced in unacclimatized sojourners than in HA natives.[16,18] It seems that over the generations, HA natives have undergone positive natural selection for the beneficial traits (ie, an adaptive evolution has been undertaken). The adaptive plasticity inherited in subsequent generations has resulted in a large population of HA natives well-adapted to the strenuous HA settings.[20–23] Visible adaptive changes observed in HA natives are higher resting ventilation,[5] higher exhaled NO,[24,25] blunted hypoxic

vasoconstrictor response,[5] and thin-walled pulmonary vasculature.[26]

While restricting ourselves to hypoxia-induced oxidative stress, we do recognize that adaptation in HA natives and HAPE in sojourners can be nongenetic, consisting of physiological, biochemical, or behavioral adjustments in response to environmental changes. Hence, it becomes difficult to differentiate the contribution of each marker to a particular phenotypic trait, which has convoluted the inference of genetic adaptation and HAPE. In this review, however, we have tried to look on and discuss a few of the important genes related to vascular homeostasis and oxygen-sensing pathways, respectively, as well as their associated circulatory biomarkers in HAPE and adaptation.

Hypobaric hypoxia-induced oxidative stress

One of the most detrimental effects of hypobaric hypoxia is oxidative stress,[27] which is the consequence of increased reactive oxygen species (ROS),[28] reactive oxygen and nitrogen species (RONS),[29] decreased antioxidants,[30] and free radical-mediated reduction in pulmonary NO bioavailability.[31,32] The ensuing oxidative stress primarily contributes to endothelial damage and vascular wall remodeling, which are potentially involved in the development of HA-related disorders in sojourners.[1,13]

Thus, we can say that harsh climatic conditions at HA initiate a series of events in which there is an increased production of reactive intermediates,[29,30,32,33] vasoconstrictors such as endothelin 1 (ET-1),[32,34–36] 5-hydroxytryptamine (5-HT), 8-iso-prostaglandin F2α (8-iso-PGF2α),[34] and various other proinflammatory cytokines.[29,35,37–39] In contrast, there is a decrease in the production of vasodilators such as NO,[32,34,40,41] prostacyclins,[38] antioxidants,[29,30,38] and anti-inflammatory cytokines.[37,38] This alteration in the production of these moieties is a fail-safe mechanism of the body to divert resources toward the energy-starved parts and to restore vascular homeostasis. However, this increase and decrease in the levels leads to an imbalance between the body's ability to sustain the damage and detoxify the ROS/RONS and their continued manifestation in higher and higher amounts. An increase in the levels of vasoconstrictors and proinflammatory cytokines, in the absence of counteracting partners, leads to higher production of ROS/RONS and, hence, both to more damage to the vascular homeostasis and to vascular dysfunction.[32,42] We hypothesize that this imbalance is a key mechanism implicated in different HA-related disorders. Thus, in effect, oxidative stress at HA is a vicious cycle that is reinforcing itself through a feedback loop, aggravating the damage in an effort to resolve the problem and giving rise to the initial set of conditions. Figure 1 explains HA-associated oxidative stress. Of note, there is a difference in levels of antioxidants expressed in sojourners and HA natives, which provides insight into the differential adaptation pattern and tolerance to HA by HA natives.[28,43]

Gene–environment interaction

There is an interplay between gene and environment (ie, genes and the environment [G×E] interact to produce a phenotypic outcome, which is governed by both entities).[44–47] One way to assess G×E interactions[48] is to perform candidate gene studies. In such studies, genotyping is done in a case-control format, which tells about the risk or minor genotype (found more in cases) or protective or major genotype (found more in controls) of a gene. SPSS[49,50] (IBM Corporation, Armonk, NY, USA) and MDR (Vanderbilt University Medical School, Nashville, TN, USA)[51,52] are two well-known software programs that can be used to study such interactions. The input file format depends on the software being used. Accordingly, this tells how the environment interacts with genes or vice versa. Statistically significant interactions are indicated by $P<0.05$ or any other cut-off set by the user. Interaction can be synergistic or redundant, depending on the information gained or lost through the interaction, respectively.[51]

A common way of analyzing G×E interactions by multinomial logistic regression analysis follows:

- Problem: Understanding the effect of the mutant genotype of rs2238625 (T/C) on HAPE patients (cases).
- Solution: For this, a single nucleotide variant, rs2238625 (T/C), of *GNA11* (*guanine nucleotide binding protein [G protein], alpha 11 [Gq class]*) will be genotyped in HAPE patients (cases) and related controls. For regression analysis, an input file needs to be prepared.
  - Coding for an input file is such that:
    - cases are coded as 0 and controls as 1 (reference),
    - males are coded as 1 and females as 0, and
    - a major genotype is coded as 1 and a minor genotype as 0.
  - After genotyping, an initial counting of the common homozygotes, heterozygotes, and rare homozygotes is prepared, as shown in Table 1.
  - Here, TT emerged as a major genotype (a genotype found majorly in both cases and controls) and CC as minor or concern genotype. While performing an analysis between major genotype TT and a heterozygote (ie, CT), we code TT as 1 and CT 0

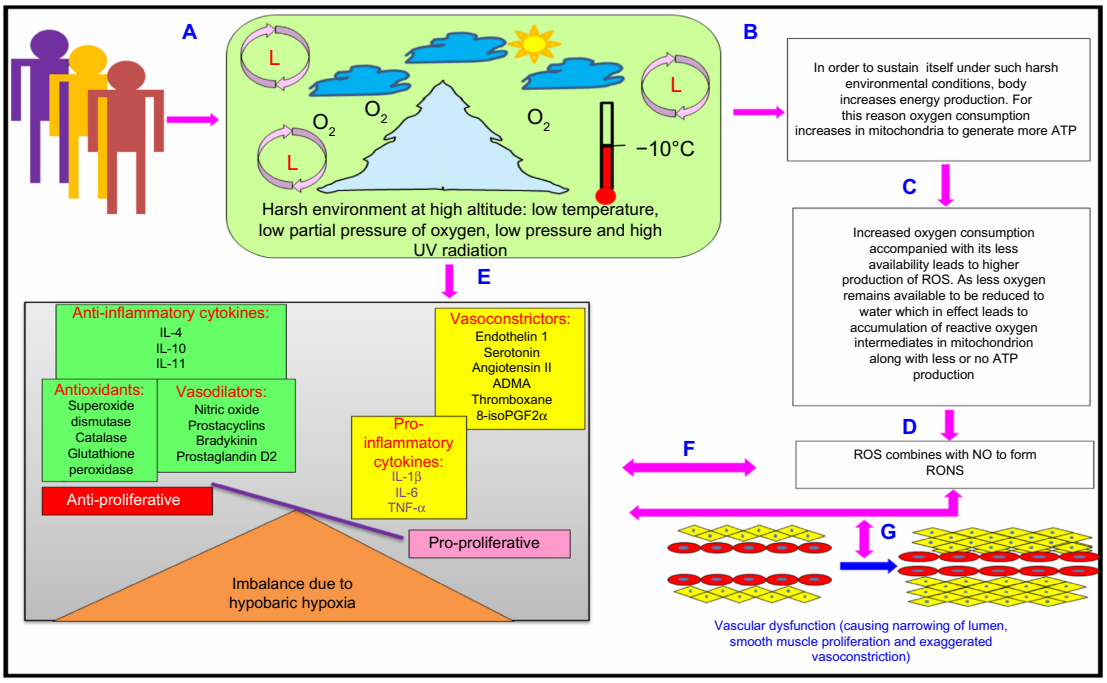

Figure 1 High-altitude-associated oxidative stress.

Notes: The human body, on ascent to high altitude, to acclimatize and to maintain homeostasis, needs energy to sustain itself under the harsh environmental conditions. More oxygen also is required, as oxygen is consumed for energy generation (ie, ATP production through oxidative phosphorylation in mitochondrion), but the same is not available. So the human body (**A**) increases oxygen consumption to generate energy (**B**), but because of low oxygen availability, reactive oxygen species (ROS) accumulates in mitochondrion as less oxygen is available to be reduced to water (**C**). ROS combines with nitric oxide (NO) in vasculature to generate reactive oxygen and nitrogen species (RONS) (**D**). High-altitude exposure also leads to imbalance in the levels of vasoactive modulators (**E**). This imbalance causes more generation of ROS/RONS. In turn, ROS/RONS cause more imbalance in vasoactive modulators (**F**). Together, both lead to vascular dysfunction (**G**), which aggravates imbalance in vasoactive modulators and ROS/RONS. Thus, this vicious cycle of oxidative stress goes on until the subject receives medical help.

Abbreviations: UV, ultraviolet; ATP, adenosine triphosphate; IL, interleukin; TNF, tumor necrosis factor; ADMA, asymmetric dimethylarginine.

(model 1), and we delete the CC genotype, as between TT and CC (TT =1, CC =0, CT=deleted; model 2).

- Input file formats are provided, as in Tables 2 and 3.

- Conclusion: A significant *P*-value suggests that there is an effect of mutant genotype (ie, CC) on cases. Since our cases are HAPE patients, it implies that the mutant genotype is causing a predisposition to HAPE in sojourners and there is a possible interaction between hypobaric, hypoxic environments at HA and *GNA11*.

A snapshot of the SPSS software for performing the analysis is provided in Figure 2. Clinical characteristics influencing the results, such as age and sex, are entered in the covariate section of the multinomial regression analysis to nullify their effect.

Table 1 Initial counting of common homozygotes, heterozygotes, and rare homozygotes

Gene (rsID) (*GNA11*) and genotype/allele	Cases	Controls
rs2238625		
TT	86 (40%)	107 (51%)
TC	99 (46%)	90 (43%)
CC	30 (14%)	11 (5%)

Abbreviation: *GNA11, guanine nucleotide binding protein [G protein], alpha 11 [Gq class].*

After performing the analysis, it is determined that what kind of G×E interaction is taking place may result in favorable as well as detrimental effects. In highland natives, this interaction has caused visible favorable changes, making them commodious enough to dwell in the HA settings, whereas the same interaction has remained neutral for a large number of sojourners but risky for a few. Differences in the sojourners' response to the HA environment clearly demarcate the genetic predisposition, which has been well-documented in the HA natives[11,46,53–55] and in one of the most common HA disorders (ie, HAPE).[46,53,55] We attribute this genetic predisposition to evolutionary processes, suggesting that adaptation in HA natives stemmed from ancient ancestors favoring a "thrifty" genotype, which enabled survival and settlement under the harsh climatic conditions of HA. In contrast, lowlanders, who had never experienced such harsh environmental conditions, did not have the genotype. As a consequence, when unacclimatized sojourners (lowlanders) ascended to HA, they suffered with HA-related disorders such as HAPE. Thus, the thrifty genotype became protective in nature at

Table 2 Input file formats

rs2238625	Sample	Age	Sex	SBP	DBP	SaO_2	Body mass index, kg/m²	MAP
Main file								
T/T	Case	45	Male	150	90	62	25.65437	110
C/T	Case	29	Male	147	80	68	32.04615	102.3333
C/C	Case	24	Male	150	100	82	37.46098	116.6667
C/T	Case	32	Male	121.92	83.46	69.6	25.25	96.3
C/C	Case	32	Male	121.92	83.46	69.6	25.25	96.3
T/T	Case	32	Male	121.92	83.46	69.6	25.25	96.3
T/T	Control	26.6	Male	110	70	93	16.51473	83.33333
C/T	Control	26.6	Male	93	77	87	19.31295	82.33333
C/T	Control	21	Male	130	80	81	18.42024	96.66667
C/T	Control	22	Male	100	70	76	20.02884	80
T/T	Control	24	Male	101	74	93	17.90886	83
T/T	Control	21	Male	120	80	85	21.09375	93.33333
Model 1								
1	0	45	1	150	90	62	25.65437	110
0	0	29	1	147	80	68	32.04615	102.3333
0	0	32	1	121.92	83.46	69.6	25.25	96.3
1	0	32	1	121.92	83.46	69.6	25.25	96.3
1	1	26.6	1	110	70	93	16.51473	83.33333
0	1	26.6	1	93	77	87	19.31295	82.33333
0	1	21	1	130	80	81	18.42024	96.66667
0	1	22	1	100	70	76	20.02884	80
Model 2								
1	0	45	1	150	90	62	25.65437	110
0	0	24	1	150	100	82	37.46098	116.6667
0	0	32	1	121.92	83.46	69.6	25.25	96.3
1	0	32	1	121.92	83.46	69.6	25.25	96.3
1	1	26.6	1	110	70	93	16.51473	83.33333
1	1	24	1	101	74	93	17.90886	83
1	1	21	1	120	80	85	21.09375	93.33333
1	1	22	1	120	92	93	18.73049	101.3333

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; SaO_2, arterial blood oxygen saturation; MAP, mean arterial pressure.

HA.[56] This may well be the case for the genes associated with the major physiological pathways that play crucial roles under the hypobaric hypoxic environment of HA, such as vascular homeostasis,[34,46] oxygen sensing,[57,58] and inflammatory pathways.[40,42,59,60] The genes that majorly associate with these pathways can easily be recognized as *ET-1*,[35,36,61] *angiotensin-converting enzyme* (*ACE*),[62–65] *endothelial nitric oxide synthase* (*NOS3*),[60,66,67] *cytochrome b-245 alpha polypeptide* (*CYBA*),[68–70] *glutathione S-transferase pi 1* (*GSTP1*),[71–74] *5-HT and the related 5-hydroxytryptamine transporter* (*5-HTT*),[75] *bone morphogenetic receptor type isoforms* (*BMPRI* and *BMPRII*),[76–79] and *rho-associated coiled coil kinase isoforms* (*ROCKI* and *ROCKII*).[75,80–83]

The significance of these genes in the pulmonary vasculature has been well-established, which renders the lead for the ongoing studies. These genes can easily be identified as potential candidates in determining the phenomenon of adaptation and HAPE at HA. The selection of genetic variants from such

Table 3 A typical result of a multinomial logistic regression analysis

Sample[a]	Parameter estimates						95% confidence interval for Exp(*B*)	
	B	Standard error	Wald	df	Sig	Exp(*B*)	Lower bound	Upper bound
0								
Intercept	−1.940	0.757	6.576	1	0.010	–	–	–
Age	0.098	0.016	39.815	1	0.000	1.103	1.070	1.137
Sex	−1.169	0.631	3.430	1	1.064	0.311	0.090	1.070
[rs2238625 = 0]	0.290	0.220	1.1741	1	0.187	1.337	0.868	2.058
[rs2238625 = 0]	0[b]	–	–	0	–	–	–	–

Notes: [a]The reference category is 1; [b]this parameter is set to zero because it is redundant.
Abbreviations: df, degrees of freedom; Sig, significance.

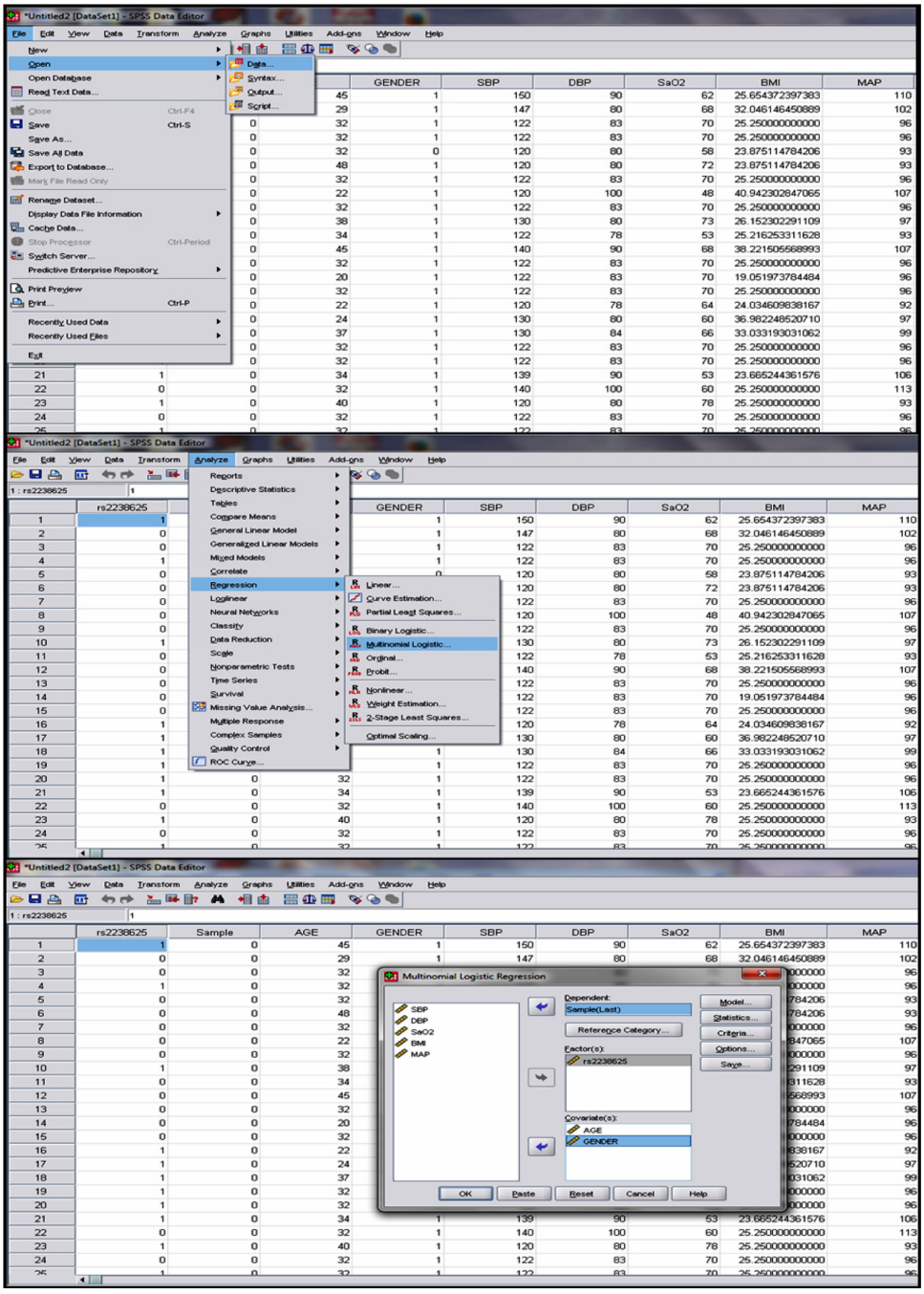

Figure 2 Snapshot of SPSS software (IBM Corporation, Armonk, NY, USA).

genes that are facilitating adaptation at HA must be decisive to offset the detrimental consequences of hypoxia. Accordingly, the alleles favored at HA should be overrepresented in natives. Inversely, underrepresentation of the same genetic variants may lead to HA disorders such as HAPE. This can be established by association studies with three study groups: patients, related controls from lowland, and healthy highland natives. In view of these facts, we suggest that G×E toward acquiring the beneficial traits in HA natives is immense at the same time it is rendering sojourners susceptible to HAPE.

Candidate gene approach

A candidate gene approach is a hypothesis-driven approach that directly tests the effects of the genetic variants of a potentially contributing gene in an association study. These studies include either members of an affected family or

unrelated cases and controls. These studies can be performed relatively quickly and inexpensively and may also allow identification of genes with small effects.[66] The candidate gene approach has helped in identifying many candidates,[6] but here we highlight only *ET-1, NOS3, CYBA*, and *GSTP1*, which are majorly implicated in vascular dysfunction resulting from hypoxia-induced oxidative stress at HA.

Criteria for marker inclusion

The reasons for studying these genetic and associated circulatory markers are as follows. First, *ET-1*,[36,84] *NOS3*,[85–87] *CYBA*,[88] and *GSTP1*[74,88] are by far the four most well-characterized genes that differentially stimulate the physiological processes to bring in cellular changes at HA. Second, functionally counteracting partners have been chosen to study (eg, *ET-1* counters *NOS3* and *GSTP1* counters *CYBA*) to understand in entirety the intriguing phenomenon of oxidative stress at HA[89] (gene ID: 2950, gene ID: 1535). Third, these genes perform functions that are multifactorial in nature (gene ID: 4846, gene ID: 1906, gene ID: 2950, gene ID: 1535). Finally, the number of genes involved in physiological regulation, apart from these genes, is enormous and includes many genes with debatable functions. Circulatory biomarkers such as ET-1,[90] NO,[91] and 8-iso-PGF2α[88,92] have been chosen because of their association with *ET-1*,[90] *NOS3*,[91] *CYBA*,[88,92] and *GSTP1*,[88] respectively, as well as because of their established multifactorial functions,[93] such as vasoconstrictory and vasodilatory functions in oxidative stress at HA.

ET-1

ET-1 is a 21 amino acid long peptide hormone, the production of which is significantly regulated by hypoxia. It is a potent, long-lasting vasoconstrictor[94–96] with mitogenic properties[94,97–99] promoting smooth muscle cell proliferation,[92,100] regulating vascular tone,[94,95] and manifesting its effect through endothelin receptor type A.[94,98] ET-1 has been studied at both the genetic[101–104] and biochemical level.[36,61,84,101,105] Three *ET-1* polymorphisms (viz, CT_n–CA_n repeat, Lys198Asn, and G2288T) were studied in HA natives and HAPE patients, and the variants of these polymorphisms were found to be associated with adaptation or disease. The CT_n-CA_n repeats are the dinucleotide repeat polymorphism in *ET-1*. The (CT)n-(CA)n repeats were segregated and recognized as shorter (13–30 base pairs) and longer (31–45 base pairs).[106,107] Among the variants, an overrepresentation of CT_n-CA_n shorter repeats, Asn198, and T alleles was reported in HAPE patients compared with in ethnically matched related healthy sojourners (HAPE-resistant, controls [HAPE-r]). Moreover, the HAPE patients had significantly higher ET-1 levels, which correlated positively with risk variants. In contrast, an overrepresentation of CT_n-CA_n longer repeats, G allele, and the combination of wild-type genotypes (ie, longer-repeats/GG, longer-repeats/Lys198Lys) were reported in both HA natives and healthy sojourners (HAPE-r). Consequent to this, plasma ET-1 levels were significantly lower in HA natives. The wild-type genotypes (ie, longer-repeats, GG, and Lys198Lys genotypes) associated with significantly lower ET-1 levels in the highlanders (HLs), as expected.[106,107] It is obvious from these findings that elevated ET-1 levels cause exaggerated vasoconstriction and increased endothelial permeability (Figure 3). Moreover, the correlation of these variants (CTn-CAn shorter repeats, Asn198, and T alleles with elevated ET-1 levels in HAPE patients) accounts for the same function, and vice versa in HA natives and HAPE-r (Table 4).

NOS3

NOS3 is an enzyme that generates NO, a potent vasodilator with multiple other functions in blood vessels, by converting L-arginine to L-citrulline, using nicotinamide adenine dinucleotide phosphate as an energy source.[91,108] NO is involved in regulating vascular tone by inhibiting smooth muscle contraction,[91,108–110] monocyte adhesion,[108] and platelet aggregation.[91,108,110] The gaseous molecule also counteracts many environmentally induced maladaptive changes such as smooth muscle cell proliferation[91,108–110] and hypoxic pulmonary vasoconstriction.[108,111] NOS3 gets activated by higher concentrations of divalent calcium ions,[109,111] leading to vasodilation via NO and thereby countering exaggerated vasoconstriction, a hallmark of HAPE.[18,112] It has been the most investigated candidate gene because of its relevance in the maintenance of vascular homeostasis.[91,111]

A number of polymorphisms are reported, but the favored polymorphisms in the gene were Glu298Asp (G894T)[86,87,113,114] and 4b4a[86,87,114] (a 27 bp variable number of tandem repeats). Glu298Asp has also been reported to have a functional effect on NOS3 protein by causing differential susceptibility of NOS3 protein to cleavage on allele change from G894T.[115] It was reported that Asp298 and 4a alleles of Glu298Asp and 4b4a polymorphisms, respectively, were overrepresented in HAPE patients compared with in the healthy sojourners (HAPE-r). The interaction between these two polymorphisms was significant with an overrepresentation of the haplotype Glu2984b in the controls and Asp2984a in HAPE patients. In addition, it was also observed that NO levels were significantly reduced in HAPE patients; moreover,

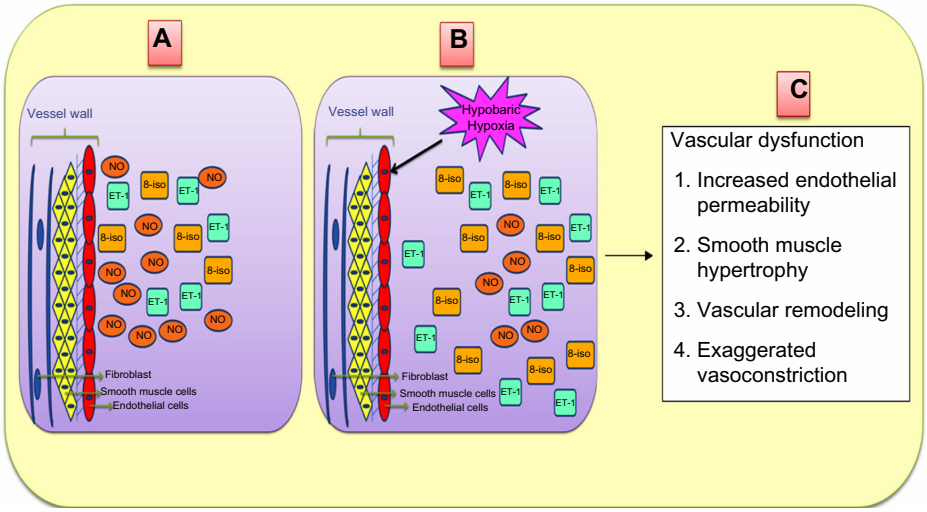

Figure 3 Significance of biomolecules in vascular homeostasis.
Notes: (**A**) Levels of biomarkers; namely, nitric oxide (NO), endothelin 1 (ET-1), and 8-isoprostaglandin F2α (8-iso-PGF2α or 8-iso) under normal conditions. NO is a vasodilator, whereas ET-1 and 8-iso-PGF2α are vasoconstrictors. (**B**) Under the stress of hypobaric hypoxia, the levels of NO decrease, whereas levels of ET-1 and 8-iso-PGF2α increase. This increase in the concentration of vasoconstrictors and decrease in the concentration of vasodilators lead to exaggerated vasoconstriction and an increase in pulmonary vascular resistance, leading to high-altitude pulmonary edema. (**C**) This alteration in the levels of various biomarkers results in oxidative stress, which in effect leads to vascular dysfunction through smooth muscle cell proliferation, vasoconstriction, and vascular remodeling.

the HAPE patients who overrepresented the minor alleles had significantly lower levels of NO. Conversely, Glu298 and 4b alleles of Glu298Asp and 4b4a polymorphisms, respectively, were overrepresented in HA natives and healthy sojourners. The interaction between these two polymorphisms was significant, with an overrepresentation of the haplotype Glu2984b in the HA natives. A correlation analysis of these variants revealed that the subjects with wild-type alleles or their combinations 894GG+4bb 894G (298Glu) and 4b were associated with higher levels of circulating NO, whereas those with the minor alleles were associated with lower levels of NO.[85,116,117] As elevated NO levels cause increased vasodilatation (Figure 3), it can be said that respective correlated variants (ie, Glu298and 4b in HA natives and HAPE-r; Table 4) also account for the same function, and vice versa in HAPE patients.

CYBA

CYBA encodes for CYBA, also known as p22phox subunit. This protein forms a critical component of the nicotinamide adenine dinucleotide phosphate oxidase system, which is involved in the production of superoxide anion, a reactive free radical.[118] These superoxides are involved in causing oxidative stress, one of the debilitating effects of HA exposure, leading to HAPE.[29] The gene has functional variants that have been associated with various oxidative stress-related diseases.[69,70,118–121] The polymorphisms in focus are –930A/G and H72Y (C242T).

It has been found that elevated free radical formation (a hallmark of oxidative stress), along with antioxidant deficiency, can be reliably assessed by estimating the levels of 8-iso-PGF2α. 8-iso-PGF2α, a potent vasoconstrictor, is a prostaglandin-F2-like compound belonging to the F2 isoprostane class that is produced in vivo by the free radical-catalyzed peroxidation of arachidonic acid. It is a reliable biomarker of oxidative stress, and its concentration is increased in the bronchoalveolar lavage fluid and urine of patients with interstitial lung diseases and chronic obstructive pulmonary disease.[122–124] 8-iso-PGF2α levels were invariably elevated in HAPE patients; moreover, it has also been found to be associated with the polymorphisms of *CYBA* mentioned earlier. The genotype distributions of –930A/G and C242T differed significantly in HAPE patients. The alleles, G of –930A/G and C of C242T, were overrepresented in HAPE patients, as was the haplotype G-C. Furthermore, 8-iso-PGF2α has been found to be significantly increased in HAPE patients, and risk alleles G and C of –930A/G and C242T associated significantly with higher levels of 8-iso-PGF2α in HAPE patients. In contrast, allele A of –930A/G and T of C242T, respectively, and their respective haplotypes A-T were over-represented in HA natives and healthy sojourners. Furthermore, 8-iso-PGF2α has been found decreased significantly in HA natives and sojourners. Protective alleles A and T of –930A/G and C242T associated with significantly lower levels of 8-iso-PGF2α in HA natives and healthy sojourners.[88] As apparent from the earlier text, elevated 8-iso-PGF2α levels

Table 4 Single nucleotide polymorphisms (SNPs) and biomarkers under hypobaric hypoxia

Biomarker	Gene	SNPs	Variant (Bio-levels)		Functions	Reference
			HA natives	Lowlanders		
Endothelin-1, pg/mL	*ET-1*	(CT)n–(CA)n repeat	**Longer repeat*** (1.4±0.8) Shorter repeat (2.2±0.8) $P<0.0001$	**Longer repeat** (3.3±0.7) Shorter repeat* (3.7±0.9) $P<0.001$	(CT)n–(CA)n longer repeats, GG and Lys198Lys genotypes of G2288T and Lys198Asn, respectively, correlated with lower ET-1 levels, signifying the protective nature of these repeats and alleles to HAPE.	100
		G2288T	**GG*** (1.5±0.9) GT/TT (2.1±0.9) $P<0.0001$	**GG** (3.4±1.0) GT/TT* (4.0±0.9) $P<0.001$		
		Lys198Asn	**Lys198Lys*** (1.6±0.8) Lys198Asn/Lys198Asn (2.0±0.9) $P<0.0001$	**Lys198Lys** (3.5±0.9) Lys198Asn/Lys198Asn* (3.7±0.7) $P>0.05$		
Nitric oxide, μM	*NOS3*	G894T	**GG*** (96.56±34.89) GT (105.46±36.52) TT (–) $P>0.05$	**GG** (92.51±30.19) GT (87.68±28.87) TT* (58.62±12.72) $P<0.05$	894T and 4a variants, associated with decreased *NOS3* activity, thus lower NO levels and were found as risk alleles, clearly establishing their association with HAPE. Moreover, it was noted that presence of T allele renders the enzyme susceptible to intracellular proteases which may lead to impaired endothelial-dependent vasodilatation, a hallmark of HAPE.	12, 109, 133
		4B/4A	**BB*** (98.00±36.15) AB (104.82±28.16) AA (–) $P>0.05$	**BB** (88.49±27.74) AB (94.90±34.23) AA* (83.5±23.8) $P>0.05$		
8-isoprostaglandin F2α, pg/mL	*CYBA*	–930**A**/G	G (β=0.565, $P<0.0001$)	G* (β=0.254, $P<0.05$)	–930G increases transcriptional activity of *CYBA* promoter and change of His72 to Tyr72 results in loss of oxidative function and superoxide production.	81, 111, 113, 134
		H72Y(C/**T**)	C (β=0.367, $P<0.05$)	C* (β=0.237, $P<0.05$)		
	GSTP1	**Ile**105Val	G (β=0.750, $P<0.0001$)	G* (β=0.301, $P<0.01$)	Valine at either of the two loci ie, 105 or 114 results in decreased substrate-binding affinities and reduced catalytic efficiencies, ultimately resulting into differential enzyme activity.	
		Ala114Val	T (β=0.212, $P>0.05$)	T* (β=0.046, $P>0.05$)		

Notes: Significantly associated protective variants (bold) in high-altitude natives and lowlanders correlated with desirable levels of biomarkers, and vice versa for risk variants. Risk and protective variants were overrepresented (*) in lowlanders and high-altitude natives, respectively, indicating genetic selection and predisposition of sojourners to disease and adaptive evolution in high-altitude natives.

Abbreviations: *ET-1*, endothelin 1; HAPE, high-altitude pulmonary edema; *NOS3*, endothelial nitric oxide synthase; *CYBA*, cytochrome b-245 alpha polypeptide; *GSTP1*, glutathione S-transferase pi 1.

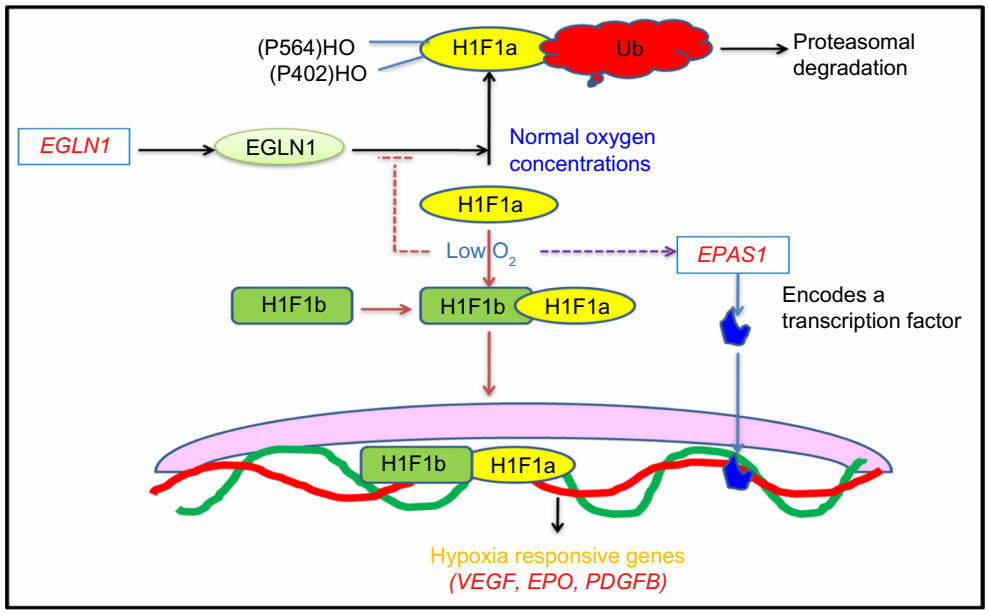

Figure 4 Oxygen-sensing pathway.

Notes: Hypoxia-inducible factor 1α subunit (HIF1α) is a constitutively active, ubiquitously found protein crucial to the cellular responses under hypoxic conditions. Under normal conditions (ie, normobaric normoxia), Egl nine homolog 1 (EGLN1) phosphorylates HIF1α at proline residues to facilitate ubiquitin-mediated proteasomal degradation. However, under hypoxic conditions, *EGLN1* is inhibited, whereas another gene (*EPAS1*) gets activated. *EGLN1* inhibition stabilizes HIF1α, which then joins with its counterpart HIF1β to form a dimeric complex. This complex translocates to the nucleus and binds to the DNA. In contrast, *EPAS1* encodes a basic-helix-loop-helix domain bearing a transcription factor that binds to DNA. These bindings activate hypoxia-responsive genes such as *erythropoietin* (*EPO*), *vascular endothelial growth factor* (*VEGF*), and *platelet-derived growth factor B* (*PDGFB*). Activation of these genes leads to the alteration in vascular homeostasis.

cause exaggerated vasoconstriction and increased oxidative stress (Figure 3). For this reason, it can be said that respective correlated variants (ie, G of –930A/G and C of C242T in HAPE patients; Table 4) also account for the same function, and vice versa in HA natives and HAPE-r.

GSTP1

GSTP1 encodes the enzyme glutathione S-transferase pi 1, which plays an important role in the detoxification of reactive intermediates by scavenging ROS with reduced glutathione, thereby maintaining homeostasis and preventing tissue damage[125] (gene ID: 2950). *GSTP1* is a polymorphic gene, and its exonic variants, I105V (A315G) and A114V (C342T), have been reported to be capable of altering catalytic activity.[126,127] Moreover, these polymorphisms are associated with diseases involving hypoxia,[73,74,128,129] and 8-iso-PGF2α, a reliable indicator of oxidative stress,[122–124] has been found to be associated with the two polymorphisms of *GSTP1* mentioned earlier. In the case of HAPE, the G allele of I105V (A/G) and T allele of A114V (C/T) were prevalent in HAPE patients and associated with increased 8-iso-PGF2α levels. Haplotype G-T, formed of risk alleles of I105V (A/G) and the T allele of A114V (C/T), were overrepresented in HAPE patients. Contrary to these findings, the A allele of I105V (A/G) and C allele of A114V (C/T) were prevalent in HA natives and healthy sojourners and were associated with decreased 8-iso-PGF2α levels. Haplotypes A-C, formed of protective alleles of I105V (A/G) and A114V (C/T), were overrepresented in HA natives and healthy sojourners (lowlanders).[88] In view of the fact that elevated 8-iso-PGF2α levels cause exaggerated vasoconstriction and increased oxidative stress (Figure 3), it can be said that respective correlated variants (ie, G allele of 105V [A/G] and T allele of A114V [C/T] in HAPE patients; Table 4) also account for the same function, and vice versa in HA natives and HAPE-r.

In view of the facts mentioned earlier, it may be inferred that the overrepresentation of wild-type alleles (protective variants) in HA natives and mutant alleles (risk variants) of the same polymorphism in HAPE patients are involved in adaptation and HAPE. The trend in correlation of alleles with biolevels further documents the phenomenon of genotype–phenotype correlations, as it was seen that protective variants correlated with lower ET-1 and 8-iso-PGF2α levels and higher NO levels, and vice versa with risk variants. Elevated NO, decreased ET-1, and 8-iso-PGF2α levels in HA natives suggest their crucial role in HA adaptation and acclimatization, and vice versa for decreased NO level, and increased ET-1 and 8-iso-PGF2α in HAPE patients, compared with in the healthy sojourners, signifies their role in hypobaric

Table 5 A comparative view of candidate gene association studies and genome-wide association studies

Candidate gene association studies	Genome-wide association studies
Initial description	
Pathway- and disease-based	Only disease-based
Conducted on specified genes	Performed on whole genome
Priori knowledge present about the genes	No prior knowledge about the genes
+ points of candidate gene association studies	
Economical	Needs large amount of expenditure
To date, many markers have been found to be associated	Fewer markers deciphered to date
Easy statistical analysis, less statistical stringency	Complex statistical analysis, high statistical stringency
Analysis can be performed on normal desktop	Needs a workstation for analysis
Not very large sample size required	A large sample size required for validation of the identified variants
Can be performed easily in any laboratory	Needs specialized, costly equipment
	+ points of genome-wide association studies
Approach limited to the existing knowledge	No such limitation
Counted number of variants can be studied	A large number of variants are studied

hypoxia-induced oxidative stress at HA. Figure 3 describes the consequences of increased and decreased levels of a few of these molecules under hypobaric hypoxia-induced oxidative stress at HA.

GWAS

The candidate gene approach is restricted in a large part by an understanding of the underlying and associated biology of the disease being investigated, GWAS are being performed. These are noncandidate-driven, unbiased studies. GWAS are one of the most exhaustive techniques, employing high-throughput technology to scan the whole genome for a large variety of variants such as copy number variations and single nucleotide polymorphisms.[130–133] The application of genome-wide scans in the HA investigations has revealed two chromosomal regions associated with adaptive evolution in the two highland populations; that is, Andean and Tibetan. The identified genes are *EGLN1* and *EPAS1*, of an oxygen-sensing pathway, and are particularly important in adapting to the hypobaric hypoxic environment at HA.[21,53,134–138] In simultaneous investigations, it has been found that few variants in these genes compared with in the lowland population are associated with low hemoglobin concentration in blood, and thus with adaptation.[134,139–141] Figure 4 mentions the oxygen-sensing pathway involving concerned genes such as erythropoietin, vascular endothelial growth factor, and platelet-derived growth factor B. Activation of these genes leads to the alteration in vascular homeostasis, leading to oxidative stress at HA.

Candidate gene and genome-wide approach: the disagreement ensues

It is of consequence to add that a comparison between the candidate gene and genome-wide approaches has revealed astonishing differences. The candidate gene approach has identified several markers of significance; however, none could be validated through GWAS. Of note, we are not against GWAS, since this approach has identified novel genes and pathways in studies of other diseases and biologic phenotypes, but in the study of HA physiology and disease, the amount of knowledge gained has been negligible and conflicting. The basis for the discrepancy between candidate-gene and GWAS-identified genes is unknown. As adaptation and maladaptation of the complex diseases are the phenomena involving a number of genes, it raises a fundamental question and an argument that genome-wide approach is not yielding as many markers, despite the candidate gene approach representing the association of several loci. Can it be attributed to the method, the smaller sample size, or the statistical tools? We suggest that without compromising the stringency of the statistical measures, an appropriate approach needs to be found so that an enormous amount of capital being put up in such high-throughput experiments, such as GWAS, is more productive and that the information retrieved is helpful for the cause of human establishment. Table 5 represents a comparative view of candidate gene and genome-wide association studies, respectively.

Conclusion

To sum up, the last decade has experienced phenomenal progress in understanding the genetic association of variants with HA-related disorders and adaptation patterns at HA. Simultaneously, the molecular aspects and biochemical parameters of HA also received equal attention. Genetic and biochemical studies until now have highlighted the significance of few oxidative stress markers in relation to adaptation and disease; together, both could prove to be potential candidates for diagnostic and therapeutic intervention. The near absence of HA disorders such as HAPE in natives, the relative exaggerated susceptibility of few sojourners to HAPE, and the neutrality of a large number of sojourners clearly indicates the involvement of genetic variants in differentially regulating physiology, leading to disease in sojourners and adaptation in HA natives.

The overrepresentation of wild-type or protective alleles in HA natives and their association with desirable levels of circulatory biomarkers strengthens their involvement with HA adaptation. In contrast, overrepresentation of mutant or risk alleles and their association with disturbed levels of biomarkers in HAPE patients strengthens their involvement in hypoxia-induced oxidative stress, and thereby in HAPE. Moreover, healthy sojourners present a number of genomic features similar to HA natives, further strengthening the concept of genetic predisposition to HAPE. In this regard, we try to provide a succinct overview in Table 4, where a correlation between significantly associated variants of selected genes (ie, *ET-1*, *NOS3*, *CYBA*, and *GSTP1*) and associated biomarkers (NO, ET-1, and 8-iso-PGF2α) is provided for a quick understanding of HA adaptation and susceptibility to HA disorders. Genome-wide association studies have additionally facilitated deciphering newer pathways concerned with hypoxia-induced oxidative stress. Although human physiology is complex, and many genes are involved in vascular dysfunction resulting from hypoxia-induced oxidative stress, we could just provide a glimpse of a few established genes and biomarkers pertaining to HAPE, adaptation, and maladaptation. Studies would continue to identify, validate, and establish newer markers of global significance, and taken together, both genetic and biochemical markers discussed in the review could be potential targets for future diagnostic and therapeutic interventions for HA-related disorders.

Author contributions

Priyanka Pandey wrote the manuscript. MA Qadar Pasha conceptualized and edited the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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