Gene Expression in Chronic High Altitude Diseases

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Abstract

León-Velarde, Fabiola, and Olga Mejía. Gene expression in chronic high altitude diseases. *High Alt. Med. Biol.* 9:130–139, 2008.—Chronic mountain sickness (CMS) and high altitude pulmonary hypertension (HAPH) have been well described in different mountainous regions of the world as chronic high altitude (HA) diseases. This review briefly summarizes the available data from some genes known to be regulated by hypoxia-inducible factor 1 (HIF-1) and/or by hypoxia that have been studied in populations from these regions suffering from CMS and/or HAPH. Excessive erythrocytosis, caused by a lower oxygen saturation and hypoxic ventilatory response and/or ventilatory inefficiency, is the outstanding sign of CMS, and right ventricular enlargement, pulmonary hypertension, and remodeling of pulmonary arterioles are hallmarks of HAPH. Familial character and heritability studies have suggested that genetic factors could make a contribution to the pathogenesis of CMS and HAPH. Even though some alleles are more prevalent (G allele of eNOS polymorphism Glu298Asp in Sherpas and ACE I allele in HAPH Kyrgyz) or less prevalent (ACE D allele in HA Andeans) in the different high altitude populations, published data to date are insufficient for a rigorous test of any hypothesis regarding the implications of these gene polymorphisms in CMS or HAPH.

Key words: eNOS gene; ACE gene; HIF-1; hypoxia; polymorphisms; chronic mountain sickness; high altitude pulmonary hypertension

Introduction

HIGH ALTITUDE LONGTIME residents are at risk of developing chronic mountain sickness (CMS, Monge's disease), a clinical syndrome that occurs in some natives or lifelong residents above 2500 m. It is characterized by excessive erythrocytosis (EE) [females, hemoglobin (Hb): $Hb \geq 19$ g/dL; males: $Hb \geq 21$ g/dL], severe hypoxemia, and in some cases moderate or severe pulmonary hypertension, which may evolve to cor pulmonale, leading to congestive heart failure. The clinical picture of CMS gradually disappears after descending to low altitude and reappears after returning to high altitude. High altitude pulmonary hypertension (HAPH) is a clinical condition, which occurs in children and adults resident above 2500 m. It is characterized by a mean pulmonary artery pressure of >30 mmHg or a systolic pulmonary artery pressure of >50 mmHg measured at the altitude of residence, right ventricular hypertrophy, heart failure, moderate hypoxemia, and the absence of excessive erythrocytosis (León-Velarde et al., 2005). Even though CMS and HAPH represent separate manifestations of chronic hy-

poxia, that is, stimulation of erythropoiesis and stimulation of pulmonary hypertension, respectively, in many patients with severe CMS, both manifestations are present simultaneously (Peñaloza and Sime, 1971; Peñaloza et al., 1971). Thus, patients with moderate CMS may have little or no elevation of pulmonary artery pressure or resistance beyond the normal increase at high altitude (Antezana et al., 1998; Vargas et al., 2003); alternatively, particularly in children and young adults, life-threatening HAPH may occur with little or no increase in Hb (Lin and Wu, 1974; Sui et al., 1988; Anand and Wu, 2004).

Alveolar hypoventilation and or ventilation/perfusion mismatch (Hurtado, 1942; Monge-M and Monge-C, 1966; Cruz et al., 1969; Ergueta et al., 1971; Ge, 1989; Vargas and Villena, 1992) play a central role in the physiopathology of CMS; this causes an overstimulation of erythropoiesis, leading to increased red cell mass and blood viscosity (Winslow and Monge, 1987). Hypoventilation and/or respiratory disorders could worsen with age, with obesity and chronic obstructive pulmonary disorders, with menopause, and during sleep in high altitude residents, further aggravating exces-

sive erythropoiesis (Sime et al., 1975; León-Velarde et al., 1993, 1994, 2001; Sun et al., 1996; Bernardi et al., 2003; Spicuzza et al., 2004).

Vasoconstriction is the cause of HAPH. A unique feature of pulmonary, as opposed to systemic, circulation is vasoconstriction observed at decreased levels of alveolar oxygen. When hypoxia is prolonged, increased muscularization occurs in the small pulmonary arteries of rats and humans, leading to increased pulmonary vascular resistance and sustained elevation of pulmonary arterial pressure (Arias Stella and Saldaña, 1963; Meyrick and Reid, 1980). The resulting pulmonary hypertension leads to right ventricular hypertrophy and in some individuals to "excessive" HAPH (Peñaloza and Sime et al., 1971).

In the Andean countries of South America, some 35 million people live above 2500 m. Three of the capital cities in the Andes (La Paz, Bolivia; Bogotá, Colombia; Quito, Ecuador) are at high altitude. In Asia, ~80 million people live above this altitude. For countries such as China and India, this number includes ~2% 3% of the population respectively; for Afghanistan and Kyrgyzstan, 10%; and for Nepal and Bhutan, between 35% to 45% of ~22 and 2 million people, respectively. In Kyrgyzstan, the high mountains of the Tien Shan and Pamir ranges account for 90% of its territory, and more than 200,000 people live above 3000 m. In China alone, there are four high plateaus (Qinghai-Tibet, Inner Mongolia, Yun-Gui, and the Yellow Land Plateau), with a total population of nearly 40 million people. North America, Mexico and the western United States have relatively smaller (~15 million people), but increasing, high altitude populations (Niermeyer et al., 2001). It is estimated that between 5% and 10% of high altitude inhabitants may develop CMS or HAPH (León-Velarde et al., 2005). Large interindividual differences exist in the magnitude of the ventilatory response to hypoxia, the severity of erythremia, and the pulmonary pressure response to hypoxia, with some subjects demonstrating low ventilatory levels and/or exaggerated increases in pulmonary arterial pressure at high altitude. These features could imply a genetic susceptibility to CMS and HAPH; further, ethnic differences, familial clustering, and animal studies, which seem to show some genetic association with EE and the pulmonary pressure response to hypoxia, are suggestive of a genetic involvement in Monge's disease and in HAPH. In addition, quantitative genetic analysis has revealed, that the proportion of phenotypic variance in hemoglobin concentration attributable to genetic factors (i.e., the heritability of hemoglobin concentration) was 86% in the Tibetans and 87% in the Andeans (Beall et al., 1998). Although, these results indicate that genetic factors account for a large proportion of phenotypic variance in hemoglobin concentration in these populations, they do not identify the specific genetic factor underlying intrapopulation or interpopulation variation in response to altitude.

In general, very few genetic studies have been conducted on highland native populations. This paper reviews only those that attempt to provide a genetic explanation and understanding of CMS and HAPH. Even though much more research has been pursued in connection to the genetic basis of acute high altitude responses, this paper only deals with research that appears to be relevant for the purpose of this review.

Candidate genes in CMS and HAPH—which genes?

If there is a genetic involvement in CMS and HAPH, the candidate genes could be key components within the respiratory and cardiovascular system sensitive to hypoxia, that is, genes known to be regulated by HIF and/or by hypoxia. An intermediate biochemical phenotype that reflects the HIF-related activation of transcription by hypoxia can be linked downward to common genetic variation within the HIF signaling pathway and upward to variation in integrative respiratory and cardiovascular responses to hypoxia.

Hypoxia-inducible factor 1 (HIF-1)

Hypoxia-inducible factor 1 was originally identified as a transcription factor in a hepatoma cell line that produced erythropoietin, but subsequently was shown to be present in many more cell types. It is a heterodimer consisting of HIF-1 α and HIF-1 β subunits. It is now recognized that several hundred genes are either directly or indirectly regulated by HIF. The discovery of HIF has been a great step forward in our comprehension of hypoxia response. HIF-1 is a transcription factor that is a key regulator of oxygen homeostasis, and, in addition to erythropoietin, HIF-1 responsive genes include regulators of functions such as cellular energy metabolism, iron metabolism, catecholamine metabolism, vasomotor control, and angiogenesis, suggesting an important role in the coordination of oxygen supply and cellular metabolism (reviewed in Semenza, 2004).

The primary mode of HIF regulation is through proteasomal degradation, which requires the presence of the von Hippel-Lindau (VHL) tumor suppressor protein to act as the recognition component of a ubiquitin E3 ligase complex. The interaction between HIF and VHL is brought about by the hydroxylation of two proline residues (in both HIF-1 α and HIF-2 α). The transactivation domain function of HIF-1 α has also been shown to be regulated by asparaginyl hydroxylation in an O₂-dependent manner (Semenza, 2004). These catalytic reactions will be significantly affected by variation in substrate, in enzyme concentration, and any structural variations that affect the reaction rates. Genetic variation surrounding these key O₂-sensing steps therefore has the potential to influence greatly the overall function of the HIF system. In mice partially deficient for HIF-1, exposure to chronic hypoxia induces less pulmonary hypertension, and they have pulmonary vascular myocytes that depolarize less with hypoxia and have impaired carotid body and ventilatory acclimatization responses to hypoxia compared with control animals (Yu et al., 1999; Shimoda et al., 2001; Kline et al., 2002).

Modifications within the HIF pathway may give rise to the very marked variations in the responses of normal humans to hypoxia. Thus, interindividual variation in HIF-regulated gene expression at the cellular level may relate to variations in responses at the integrative level. Appenzeller et al. (2006) have found correlations between the high levels of expression of several genes, including HIF-1, symptoms at high altitude, and CMS. They also underscore the changes in gene expression levels in CMS subjects after arrival to sea level, where their values never reached sea-level values. This may indicate the existence of alterations in regulators of HIF-1 α , keeping higher levels in the absence of hypoxia stimulus. With another approximation, using genetic markers located

in close proximity to the HIF-1 α gene and to genes that regulate its stabilization and degradation, such as von Hippel-Lindau (VHL), "prolyl hydroxylase domain containing" 1, 2, 3 (PHD1, PHD2, PHD3), and phosphatase and tensin homologue deleted on chromosome 10 (PTEN), Mejía et al. (2005) addressed the association with severe polycythemia and CMS in CMS subjects and high-altitude controls, and found no evidence of association. But this does not exclude variations in these genes contributing to polycythemia and possibly CMS. Indeed, mutations in the PHD2 gene have been reported to be associated with familial erythrocytosis (Percy et al., 2006) and erythrocytosis with unidentified etiology (Al-Sheikh et al., 2008). Thus, taking into account the still reduced data that links HIF1 with polycythemia and CMS, its role in this disease remains to be defined.

Erythropoiesis genes

Erythropoietin (EPO) is a hormone that has a very important erythroid function, but also nonerythroid purposes; it protects the brain from ischemia and the spinal cord and retina from injury in humans and mice, among others functions (Höpfl et al., 2003). HIF-1 α is the key regulator of EPO production. Oxygen deficiency stimulates both HIF and EPO production, which, in turn, increases red cell production, the outstanding sign of CMS. In CMS, EPO is variably expressed, but in patients with very high hemoglobin concentrations, EPO is usually highly expressed (León-Velarde et al., 1991).

As part of the genetic association analysis performed by Mejía et al. (2005), erythropoietin (EPO) and erythropoietin-receptor (EPOR) genes were also included as functional candidate genes. Further, Mejía et al. (unpublished data) sequenced the coding and downstream regulatory regions of the EPO gene from cell lines derived from the high altitude natives studied and found no variations in the coding sequences, intron-exon boundaries, or any regulatory domain. The association between the polymorphisms linked to EPO and EPOR and erythremia failed to show evidence of a major monogenic contribution of the loci tested to excessive erythrocytosis in CMS. This seems to indicate that the excessive erythropoiesis might be caused by factors other than the changes in the EPO gene. However, we cannot exclude the possibility that variations in the studied genes contribute to the risk of erythremia and CMS. The role of the high EPO levels in the genesis of CMS, if there is one, is still waiting to be elucidated.

Endothelial nitric oxide synthase (eNOS)

The synthesis of nitric oxide (NO) is catalyzed by endothelial nitrate synthase (eNOS), among other oxide synthases. eNOS catalyzes the oxidation of the amino acid L-Arginine to give rise to citrulline and NO. NO activates a soluble guanylate cyclase and elicits smooth muscle relaxation by protein kinase activation. Thus, eNOS is a key catalytic enzyme responsible for providing NO release in the vascular bed, in general, and of basal pulmonary NO release, in particular, thus playing an important role in vessel tone, blood pressure, and blood flow (Garg and Hassid, 1989; Sarker et al., 1996).

Hypoxia regulates the activity and endothelial cell expression of genes, including eNOS (Coulet et al., 2003), and the association of polymorphisms in this gene with hypoxia is ex-

pected to alter vascular mechanical forces and vascular remodeling. Based on these data, it has been put forward that eNOS gene mutations might be involved in the pathogenesis of HAPH. Thus, in several high altitude populations, the G894T [nucleotide substitution in exon 7, which causes amino acid substitution of glutamic acid (Glu) by aspartic acid (Asp) at the 298th position] and 4B/4A (27-bp variable number of tandem repeats (VNTR) within intron 4] polymorphisms have been examined. Overall, the T allele has been found to be associated with vascular disorders and pulmonary hypertension that are mediated by NO (Hampl and Herget, 2002), without modifying the NO-mediated vascular responses (Sofowora et al., 2001). This polymorphism might indeed have physiological significance in the presence of endothelial dysfunction. The 27-bp VNTR is perhaps less likely to be functional; in fact, conflicting associations between the intron 4 polymorphism and nitric oxide pathway activity have been described. Among the populations who live exposed to chronic hypoxia, blood flow is likely to be a major determinant of oxygen delivery; accordingly, recent research among Andean highlanders demonstrates that an increased NO synthesis is an essential attribute for oxygen delivery at high altitude by allowing better pulmonary perfusion (Beall et al., 2001).

In Asia, the Sherpa adaptation to high altitude has been hypothesized as being due to a genetic basis, because they have had a considerable period of time for gene selection against different life-threatening high altitude diseases, for example, against HAPH. In this sense, Ahsan et al. (2005) investigated the role of the eNOS gene in high altitude adaptation among Ladakh natives (over 3600 m India). They reported that wild-type alleles G and 4b were significantly overrepresented in these natives, and the combination of wild-type genotypes GG and BB, which contributed to the higher NO levels, were associated with high altitude adaptation. More recently, Droma et al. (2006) have postulated that the wild-type alleles of these polymorphisms may be a benefit for Sherpa adaptation to high altitude, based on the finding that the frequencies of these alleles were also significantly higher in Sherpas at 3440 m ($n = 105$; G: 85.7%; B: 96.7%) than in non-Sherpa Nepalis (G: 77.9%; B: 90.5%). In addition, the combination of GG and BB was significantly greater in Sherpas (66.7%) than in non-Sherpas (47.7%) (Table 1). They also measured serum concentrations of the metabolites of NO (nitrite and nitrate) and found that they were significantly lower in Sherpas ($53.2 \pm 4.6 \mu\text{mol/L}$) than

TABLE 1. GENOTYPE AND ALLELE FREQUENCIES OF THE eNOS POLYMORPHISM IN LOWLANDS (LL, SEA LEVEL), HIGHLANDS (HL, ANDEANS), AND CHRONIC MOUNTAIN SICKNESS (CMS) SUBJECTS, CERRO DE PASCO, PERÚ (4300 M)

Genotypes	LL (n = 57) n (%)	HL (n = 48) n (%)	CMS (n = 42) n (%)
GG	41 (72)	38 (79)	32 (76)
GT	15 (26)	10 (21)	9 (22)
TT	1 (2)	0 (0)	1 (2)
G	97 (85)	86 (90)	73 (87)
T	17 (15)	10 (10)	11 (13)

From Mejía et al., 2000.

in non-Sherpas ($107.3 \pm 9.0 \mu\text{mol/L}$). However, the concentration values had very wide ranges and were not associated with the eNOS polymorphisms.

Exaggerated constriction of vessels in the lungs appears to contribute to both HAPH and high altitude pulmonary edema (HAPE) (Maggiorini and León Velarde, 2003). In line with this proposition, well-adapted high altitude populations exhaled significantly higher concentrations of NO compared to lowland populations (Beall et al., 2001), but HAPE patients had lessened NO in their lungs (Busch et al., 2001). Despite the similarities of the acute (HAPE) and chronic (HAPH) diseases, Droma et al. (2002), in Japan, found that rare alleles of G894T and 4B/4A polymorphisms in the eNOS gene occurred significantly more often in subjects who suffered HAPE than in a group of mountain climbers who had never experienced the condition. In contrast with the notion that these polymorphisms may be a benefit for the Sherpa adaptation to high altitude, the HAPE group was healthy and athletic at low altitudes, but suffered HAPE at least once while mountain climbing. The control group, on average, had made more than 100 climbs at elevations exceeding 2800 m above sea level without experiencing HAPE. Only 9.8% of the control group carried the G894T polymorphism, compared with 25.6% of the HAPE patients. Regarding the 4B/4A polymorphism, Droma et al. found that the variations occurred in 6.9% of the control group and in 23.2% of the HAPE patients; and 26.8% of the HAPE group, but none of the control group, carried the two eNOS polymorphisms. The difference between both studies by Droma et al. (2002 and 2006) might be explained based on the proportion of each polymorphism found in each population. Actually, the use of pulmonary pressure as a continuous outcome together with a gene-based approach using SNPs could be used as a more informative approach for assessing the effect of the NOS3 variation on HAPH or HAPE.

In high altitude Amerindian natives living at 4300 m, Mejía et al. (2000) were not able to show that normal high altitudes natives or CMS subjects have a different prevalence of the G894T and 4B/4A polymorphisms, as happens in Sherpas (Tables 1 and 2) (Droma et al., 2006). However, it should be mentioned, that the pathological profile of chronic high altitude diseases is very different between Asian and Andean populations (Groves et al., 1993; León-Velarde et al., 2005). In addition, in the evolutionary time scale at high altitude,

the altitude of residence between Sherpas and Andeans is rather different.

Angiotensin-converting enzyme (ACE)

ACE is a component of the renin-angiotensin system (RAS), which is involved in the regulation of blood pressure and blood flow and volume. ACE activates the potent vasoconstrictor angiotensin II, which regulates pressure, as well as water and salt retention, by stimulation of aldosterone release. But ACE also degrades bradykinin by activation of the BK₂ receptor, which stimulates the release of nitric oxide and prostaglandins and may counteract Ang II-mediated effects (Zisman, 1998). Because of these important effects, some studies, such as that of Morrell et al. (1995) in rats, have implicated ACE in the etiology of hypoxia-induced pulmonary hypertension. However, it should be taken into account that different effects of ACE inhibitors on the affinity of the enzyme have raised the question that tissue-bound ACE might have a different effect than that of circulating ACE. In fact, it has been reported that ACE inhibitors with more hydrophobic moieties have a greater affinity for the C-terminal than for the N-terminal active site of the enzyme. This differential affinity could be further modulated by organ type. Although the mechanism for these organ-specific effects is not known, it has been proposed that the two active sites of ACE may interact differently with particular ACE inhibitors, and the specific organ in which ACE is tissue bound may also affect the inhibitor affinity for the active sites of the enzyme (Zisman, 1998).

The gene for human ACE is located on chromosome 17, and a frequently studied polymorphism is the 287 bp Alu insertion (I)/deletion (ID) polymorphism located in intron 16 (Rigat et al., 1990). The I allele has been associated with lower ACE protein function compared to the D (Danser et al., 1995; Costerousse et al., 1997). Serum ACE levels and the RAS system are modified by hypoxia (Milledge and Catley, 1984), and these effects in turn may mediate the cardiopulmonary responses to high altitude.

Montgomery et al. (1998) reported an overrepresentation of the I allele in elite mountaineers who ascended beyond 8000 m without supplemental oxygen, suggesting an I-allele performance benefit for mountaineers ascending to extreme altitude. In another study of recreational climbers ($n = 248$) who climb to 4800 m, the I allele again predicted success in reaching the summit (Tsianos et al., 2005), and Alvarez et al. (2000) have also shown that the I allele occurred at a significantly higher frequency in elite athletes ($n = 120$) compared to controls. To explain this apparent performance benefit, there is evidence that individuals of I/I genotype maintain higher arterial oxygen saturation (SaO_2) at rest and during exercise at high altitude. A study shows that subjects of European ancestry with the I/I genotype who ascended to above 5000 m in 12 days were able to maintain higher SaO_2 , especially as altitude increased (Woods et al., 2002). In addition, Patel et al. (2003) have shown that the hypoxia-induced rise in minute ventilation was significantly greater among those of II genotype (4.1% vs. 2.2% for II versus DD) in subjects ($n = 60$; 14 II, 30 ID, 16 DD) who exercised for 6 min at 50% of the workload at the ventilatory threshold, while breathing 12.5% oxygen. Bigham et al. (2008) have also shown an association of ACE I/I genotype with higher resting and submaximal exercise SaO_2 in two groups of Peru-

TABLE 2. GENOTYPE AND ALLELE FREQUENCIES OF THE eNOS POLYMORPHISM IN NON-SHERPAS (LL, *SHERPA NEPALIS* RESIDING IN KATHMANDU, 1330 M) AND IN HIGHLANDS SUBJECTS (HL, *SHERPAS*, *NAMCHE BAZAAR*, 3440 M)

Genotypes	LL (n = 111) n (%)	HL (n = 105) n (%)
GG	68 (61)	76 (72)
GT	37 (33)	28 (27)
TT	6 (5)	1 (1)
G	173 (78)	180 (86)*
T	49 (22)	30 (14)

* $p < 0.05$ when compared with LL.
From Droma et al., 2006.

vians (one born at sea level, with high altitude ancestors, and one born at high altitude) who were tested at 4300 m. In both study groups, individuals of I/I genotype maintained an ~ 2.3 percentage point higher SaO_2 , explaining about 4% of the total variance in resting and exercise values. These authors suggest that central cardiopulmonary effects of ACE could underlie the association of the I allele with ventilation and SaO_2 . Indeed, angiotensin II modulates hypoxia-induced pulmonary vasoconstriction (Kiely et al., 1995), and differences in circulating ACE could affect ventilation-perfusion (V/Q) relationships within the lung.

There is also evidence that native groups such as the Quechua have an increased I-allele frequency (0.72). However, the Quechua I-allele frequency does not appear to be increased relative to all other Native American populations. In fact, many low altitude Native Americans exhibit I-allele frequencies greater than that observed in Quechua (Rupert et al., 1999). Also, in the highland native Ladakhis from Northern India, the I-allele frequency has been documented at 0.67 (Qadar Pasha et al., 2001). These frequencies are slightly higher than the mean European, African, or Asian I-allele frequencies in the literature. In the Kyrgyz population, Aldashev et al. (2002) found that I-allele frequencies differed between highlanders and lowlanders (I allele being more frequent in lowlanders, but, on average were similar to that found in other Asian populations. This difference might be explained by the fact that their lowland control group was recruited from patients attending hospitals and thus may not have been representative of the Bishkek male population. Aldashev et al. have also shown that the variance in plasma ACE activity that could be attributed to the ACE I/D polymorphism was 16.3%, compared with 28% in a European population (Tiret et al., 1992). The explanation given for this difference is that plasma ACE activity in the Kyrgyz population could be under greater control from other polymorphisms in the ACE gene or other regulatory genes.

With regard to HAPH, the Kyrgyz revealed threefold higher frequency of I/I genotype at high altitude in subjects

FIG. 1. Distribution of pulmonary arterial pressures of male highlanders according to ACE genotype. The mean pulmonary arterial pressure (MPAP) was higher in the I/I genotype group than in the I/D or D/D genotype group. $*p < 0.05$. From Aldashev et al., 2002.

FIG. 2. Distribution of hemoglobin concentrations of healthy male highlanders according to ACE genotype. The mean hemoglobin concentrations (Hb) was higher in the D/D genotype group than in the I/I genotype group. $*p < 0.05$.

with this condition (Morrell et al., 1999; Aldashev et al., 2002) (Fig. 1). As mentioned, the highland Kyrgyz had lower I-allele frequency (0.56, $n = 87$) compared to a Bishkek lowland control group. However, the Aldashev et al. study has determined that HAPH, as defined by the prevalence of ECG signs of right ventricular hypertrophy in the absence of chronic airflow obstruction, is much more common among the highland population of Kyrgyzstan than in other high altitude populations, likely because the Kyrgyz migrated to the Tien-Shan and Pamir Mountains from lower altitudes relatively recently, in about the 9th and 10th centuries AD, thus with a shorter period of time for selection against HAPH.

In contrast to the Kyrgyz, the Amerindian population at high altitude show more prevalence of EE than of HAPH. When evaluated for the ACE polymorphism, hemoglobin was higher in healthy highlanders with the D/D genotype (19.2 ± 1.1 g/dL) compared with the natives with I/I genotype (18.0 ± 1.3 g/dL; $p < 0.05$) (Mejía et al., 2000) (Fig. 2). If I/I genotype is associated with a good athletic performance and with good capacity to maintain a high SaO_2 as altitude increased, it can be speculated that the higher hemoglobin concentrations with the D/D genotype is due, in part, to their poor capacity to maintain a higher SaO_2 at their altitude of residence.

In relation to the differences between Andean and Kyrgyz populations (Tables 3 and 4), it could also be speculated that the ACE I/I genotype represents a genetic marker for HAPH susceptibility, independent of its effects on ACE activity in other systems. Aldashev et al. (2002) also hypothesized that subjects with the I/I genotype may have enhanced endurance performance, as described in elite athletes. An increased level of exercise would lead to an elevation of cardiac output and pulmonary artery pressure, exacerbating any tendency to HAPH. With regard to the higher hemoglobin in highlanders with the D/D genotype, as the I allele has been associated with lower ACE protein function compared to the D allele, a preponderate D/D genotype might indicate higher ACE protein function and higher levels of angiotensin II. This, in turn, would constrict the vascular smooth muscle cells and play a role in vessel tone, blood flow, and thus blood pressure, which

TABLE 3. GENOTYPE AND ALLELE FREQUENCIES OF THE ACE I/D POLYMORPHISM IN LOWLANDS (LL, SEA LEVEL), HIGHLANDS (HL) ANDEANS AND SUBJECTS WITH CHRONIC MOUNTAIN SICKNESS (CMS), CERRO DE PASCO, PERÚ 4300 M

Genotypes	LL (n = 57) n (%)	HL (n = 48) n (%)	CMS (n = 42) n (%)
II	20 (35)	27 (57)*	23 (54)*
ID	26 (46)	18 (38)	14 (33)
DD	11 (19)	3 (5)	5 (13)
I	66 (58)	73 (76)*	60 (71)*
D	48 (42)	23 (24)*	24 (29)*

From Mejía et al., 2000.

* $p < 0.05$ when compared HL and CMS with LL.

is increased in CMS, in contrast with normal high altitude populations, who have a tendency to present lower systemic blood pressure levels (Banchero et al., 1966; Marticorena et al., 1969). Unfortunately, the level of ACE in plasma has not been measured along with the frequency of ACE D/D genotype; thus this genetic marker could also be independent of its effects on ACE activity.

As Rupert et al. (1999) pointed out, from the accessible data it is difficult to verify natural selection as an explanation for the relatively low D-allele frequency in high altitude Quechua. As well, natural selection cannot be adopted as an explanation for the relatively high I-allele frequency in high altitude Kyrgyz. Unfortunately, the ACE I/D polymorphism is still poorly understood, and it is likely that the adaptive process involves the selection of alleles at multiple loci with beneficial effects on the phenotype, which can also include a gene-gene interaction related to the different ethnic altitude of origin.

Vascular endothelial growth factor (VEGF)

VEGF is an important signaling protein involved in both vasculogenesis and angiogenesis. VEGF activity has been mostly studied in cells of the vascular endothelium, although it does have effects on a number of other cell types (e.g., stimulation monocyte/macrophage migration, neurons, cancer cells, kidney epithelial cells). In vitro, VEGF has been shown to stimulate endothelial cell mitogenesis and cell migration. VEGF is also a vasodilator, and increases microvascular permeability; it was originally referred to as vascular permeability factor (Gibbons and Dzau, 1994; Dvorak et al., 1995).

The degree of induction by hypoxia of VEGF in peripheral blood monocytes correlates both with the degree of formation of collateral blood vessels in coronary heart disease (Schultz et al., 1999). In relation to HIF, which regulates VEGF production, a single nucleotide (C to T) polymorphism (SNP) in exon 12 of HIF-1 α that changes residue 582 of HIF-1 α from proline to serine has been shown to be associated with significantly less collateral vessel formation in patients with ischemic heart disease (Resar et al., 2005). Even though the VEGF dimeric protein could be composed of 121, 165, 189, or 206 amino acid subunits, the main VEGF isoform expressed by many cell types is VEGF165. Appenzeller et al. (2006) found that, when measuring VEGF121 and VEGF165 subunits, the latter have significantly higher expression in CMS patients in CP ($p = 0.006$) compared with controls. VEGF 165 was negatively correlated with arterial saturation

($R = 0.79$; $p < 0.001$) in CMS patients. These findings could be related to its angiogenic effects, or to VEGF's role in neuroprotection. VEGF is a modifier of motor neuron degeneration and is involved in neuronal death in spinal cord and cerebral ischemia. There is evidence of its role in ischemic neuropathy, nerve regeneration, Alzheimer's disease, and multiple sclerosis (Storkbaum et al., 2004). Thus, increased VEGF expression, as found in CMS, might also be related to neuron survival and/or neurodegeneration protection in chronic hypoxia (Appenzeller et al., 2006).

Other Candidate Genes That Have Been Related to Hypoxia Tolerance

Phosphoglycerate kinase (PGK)

Another hallmark of cellular responses to hypoxia is the upregulation of oxygen-independent metabolic pathways to supply the additional energy necessary for cell survival under diminished oxygen availability. Thus, in hypoxia, enzymes involved in the glycolytic pathway and glucose transporters are upregulated by HIF-1, including PGK 1 (Seagroves et al., 2001).

PGK is involved in the transformation of 1,3-disphosphoglycerate into 3 phosphoglycerate and ATP production, an important step in glycolysis. The gene coding for PGK, which is expressed in every tissue except testes, is located on the X chromosome. Gene structure, cDNA, and protein sequences have been identified, and a few mutations have been described. Patients present with hemolytic anemia, which may be associated with various neurological disorders. Some individuals are affected by muscular disorders alone or these combined with hemolytic anemia (Beutler, 2007).

In CMS, the PGK 1 gene has been found to be expressed at higher levels than in the white cells of HA normal natives at 4300 m (Appenzeller et al., 2006). These proteins are also upregulated by oxidative stressors at sea level and may be associated with the release of reactive oxygen species (ROS), which are implicated in aging. However, it could be also hypothesized that an upregulation of oxygen-independent metabolic pathways, like the glycolytic pathway and glucose transporters, might be necessary to supply the additional energy necessary for cell survival under severe hypoxemia, for example, in subjects with CMS (Appenzeller et al., 2006).

TABLE 4. GENOTYPE AND ALLELE FREQUENCIES OF THE ACE I/D POLYMORPHISM IN LOWLANDS (LL) AND HIGHLANDS (HL) KYRGYZ AND IN HIGHLANDS SUBJECTS WITH HIGH ALTITUDE PULMONARY HYPERTENSION (HAPH), NARYN AREA, 2800 TO 3100 M ABOVE SEA LEVEL, OR PAMIR REGION, 3200 TO 3600 M ABOVE SEA LEVEL

Genotypes and alleles	LL (n = 212) n (%)	HL (n = 30) n (%)	HAPH (n = 48) n (%)
II	90 (43)	4 (13)	21 (44)*
ID	96 (45)	15 (50)	22 (46)
DD	26 (12)	11 (37)	5 (10)
I	276 (65)	23 (38)	64 (67) [†]
D	148 (35)	37 (62)	32 (33)

From Aldashev et al., 2002.

* $p < 0.003$; [†] $p < 0.0005$ when compared HL subjects.

Heme oxygenase-1 (HMOX-1), heat shock protein 70 (hsp70), ataxia telangiectasia mutated (ATM)

Stressed mammalian cells upregulate heme oxygenase, which catabolizes heme to biliverdin, carbon monoxide, and free iron. Heme oxygenase occurs as two isozymes, an inducible heme oxygenase-1 (HMOX-1) on chromosome 22 and a constitutive heme oxygenase-2 on chromosome 16 (Bainbridge and Smith, 2005). Expression of heat shock protein 70 (hsp70) is stimulated during ischemia and stress and also participates in innate immunity (Li et al., 2002; Schmitt et al., 2006). Ataxia telangiectasia mutated (ATM), a phosphatidylinositol-3-OH-kinase-like serine/threonine kinase, and the related ATR (ATM and Rad3 related) kinase regulate cellular responses to DNA damage by controlling cell cycle arrest and DNA repair pathways when recruited and activated by DNA double-strand breaks. It is a well-characterized tumor suppressor that plays a key role in the maintenance of genomic stability, with functions in cell cycle arrest and apoptosis (Smith et al., 1999). Knockout mice lacking the ATM protein exhibit impaired detection and repair of DNA damage and increased embryopathies (Bhuller et al., 2006).

When RNA is extracted from venous blood white cells of CMS patients and evaluated for the gene expression of HMOX-1, hsp70 and ATM, significantly higher levels have been found in CMS when compared with Andean controls at 4300 m (Appenzeller et al., 2006). Hence, these results may be associated with increased apoptosis or, alternatively, with the development of, as yet to be determined, mechanisms for cellular protection that mitigate the effects of increased cellular death rates and aging.

However, in the Appenzeller (2006) study it would have been worthwhile to consider the inclusion of alternative covariates in the analysis (e.g., age) and evaluate if, after adjusting for multiple comparisons, *p*-values could be still considered significant. Unfortunately, the number of subjects was too small to allow these kinds of comparisons.

Conclusion

With no doubt, genetic studies will have important implications for the etiology of CMS and HAPH. However, concerning the exploration of the polymorphisms that are related to the different hypoxic responses, published data to date are insufficient for a rigorous test of any hypothesis, probably because the genetic effects are small (polygenic). In addition, gene-gene or gene-environment interaction should be taken into account in the search for a genetic cause of these high altitude diseases. In future studies we also recommended the use of SNP databases and genome-wide haplotype mapping to increase the power to detect the association between a potential susceptibility loci and CMS or HAPH or, alternatively, with the main characteristics of these diseases, that is, hypoventilation and/or EE and pulmonary hypertension.

Also, it is worthwhile to note that the variability of the results obtained in the genes studied so far seems to indicate that the genetic background of the populations is so vast that the findings could represent instead genetic markers for other susceptibilities, independent of the effects on the downward effectors in the suspected systems affected by CMS and/or HAPH. Different and combined approaches

may help to identify the genetic basis responsible for the intolerance to hypoxia in different high altitude populations and should finally help to identify individuals at increased risk of CMS and HAPH.

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