Gene Polymorphisms and High-Altitude Pulmonary Edema Susceptibility: A 2011 Update

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Abstract
High-altitude pulmonary edema (HAPE) is a severe disease caused by high-altitude hypoxia. Since some individuals are more susceptible to high altitude than others, the incidence is variable and cannot be predicted. Furthermore, multiple genes can contribute to the occurrence of HAPE, making it even more difficult to predict. The genes associated with HAPE include those in the renin-angiotensin-aldosterone system pathway, the nitric oxide pathway and the hypoxia-inducible factor pathway. Other genes associated with HAPE include tyrosine hydroxylase (TH), vascular endothelial growth factor (VEGF), pulmonary surfactant proteins and \(\beta_2\)-adrenergic receptor. The association of the polymorphisms of these genes with HAPE susceptibility has previously been investigated. Among the genes evaluated, polymorphisms of NOS3, ACE, CYP11B2, Hsp70 and endothelin-1 and pulmonary surfactant proteins A1 and A2 were shown to be associated with HAPE incidence, while associations between TH, VEGF and HAPE remain to be fully elucidated. Novel technological approaches, including genome-wide association studies and next-generation sequencing, are currently being used to identify new HAPE susceptibility genes. The goal of this review article is to summarize the current literature and to define the outstanding areas of research that need to be explored to advance our ability to predict when HAPE will occur.

Introduction
High-altitude pulmonary edema (HAPE) is an acute idiopathic disease that occurs as a result of a rapid exposure to high altitude. It develops quickly and can have a dramatic negative impact. The HAPE morbidity rate following exposure to altitudes higher than 3,700 m above sea level ranges from 0.4 to 2\% \([1, 2]\). HAPE occurs most frequently in people who are ascending to high altitudes for the first time. It can also occur, however, in those who are returning to a high altitude after spending a period of time at sea level \([3]\). Animal studies and epidemiology investigations have demonstrated that HAPE has race-specific and family-susceptible tendencies, as well as individual susceptibility tendencies \([4]\). For example, the incidence in native Tibetans is much lower than the incidence seen in the immigrant Han population. There have been only 21 cases of HAPE among Tibetans out of the...
3,184 high-altitude disease cases seen in the past 50 years at the Tibet Military General Hospital [5]. These results demonstrate that its incidence in the Tibetan population is low, and possibly related to differences in their genetic background. For patients diagnosed with HAPE who return to areas of high altitude, there is a high risk of recurrence. HAPE frequently occurs in the same family, consistent with the genetic influence; for example, three generations from one family had six family members who suffered from it [6]. This indicates that its occurrence and susceptibility are prompted by both environmental and genetic factors.

Genetic contributions to its pathogenesis of HAPE have been frequently evaluated. Currently, the possible mechanisms that have been identified to explain HAPE include the following: (1) hypoxic pulmonary vasoconstriction, hypoxic pulmonary hypertension and dynamic changes in lung water movement; (2) damage to the lung vascular endothelial cells mediated by inflammation, increasing pulmonary vascular permeability, and (3) reduced amiloride-sensitive sodium channel and water channel protein activities, decreasing lung water clearance [7, 8]. Genes that may contribute to the occurrence of HAPE include: renin-angiotensin-aldosterone system (RAAS) pathway genes, nitric oxide (NO) pathway genes, hypoxia-inducible factor (HIF) pathway genes, tyrosine hydroxylase (TH), vascular endothelial growth factor (VEGF), lung surfactant protein A, Hsp70, endothelin-1 (ET-1) and β2-adrenergic receptor (fig. 1) [1, 9]. Gene polymorphisms in humans include tandem repeat series, deletion and insertion polymorphisms and single nucleotide polymorphisms (SNPs). SNPs are the most common form of polymorphism in the human genome and are characterized by single nucleotide substitution. The frequency of the polymorphism in the population is often greater than 1% [10]. Gene polymorphisms have become an important component of disease genomics, functional genomics, pharmacogenomics and environmental genomics studies [3].

We will summarize the current literature on the role of the genes associated with HAPE susceptibility and discuss key research gaps in order to better understand the genomic contribution to HAPE susceptibility.

**RAAS Pathway Gene**

Many studies have suggested that there is a close relationship between the occurrence of HAPE and the RAAS (fig. 2), even though one study found that angiotensin-converting enzyme activity was unchanged in subjects moving from 700 to 3,800 m [11]. Renin is a protease synthesized by the renal-afferent-artery proximal cells that is then stored in the kidney until its release into the blood. Renin has a direct effect on the conversion of angiotensinogen to angiotensin I (Ang I). Ang I has no biological activity and requires angiotensin-converting enzyme (ACE) for conversion to Ang II. Angiotensinogen is secreted by the liver, and ACE is produced by many organs including the lung, kidneys and heart [12]. Ang II has a high biological activity that includes a strong vasoconstrictor effect. The vasoconstrictor effect of Ang II is 10–40 times stronger than that of adrenaline. Ang II can stimulate aldosterone which is regulated by aldosterone synthetic enzyme (CYP11B2) to stimulate water and sodium retention.

Hypoxemia caused by the rapid ascent to high altitude can activate RAAS, increase the activity of renin, Ang II
and aldosterone in the plasma [13, 14]. Ang II can affect the tension of the systemic arteries, and pulmonary vasoconstriction can increase pulmonary artery pressure. A high altitude may directly damage the pulmonary vascular smooth-muscle-cell membrane, which serves to close the membrane K⁺ channels and leads to cell depolarization to stimulate extracellular Ca²⁺ influx. Pulmonary vascular endothelial cells produce many active substances that induce pulmonary vasoconstriction and lead to pulmonary hypertension [14, 15]. High blood pressure and hypoxic conditions induce the renal artery to constrict, increase the aldosterone concentration and cause sodium and water retention. The net result of this response is an increase in the pulmonary vascular hydrostatic pressure to a level significantly greater than that of the fluid in the lung tissue. HAPE forms when fluid leakage exceeds nonlymphatic transport capacity.

There have been many reports of polymorphisms linked to HAPE in recent years, but the results do not necessarily translate across different populations. For example, Dehnert et al. [16] studied 20 controls and 19 cases of HAPE patients and found that the ACE insertion or deletion polymorphism had no association with susceptibility to HAPE among the mountaineer population. However, another study examined 163 controls and 160 cases of HAPE patients and found that the allele frequencies were significantly different in the Indian population (p < 0.05) [17]. Although genotypes of ACE associated with HAPE susceptibility have been reported, the results have not been consistent. For example, Charu et al. [18] found the ID/DD genotypes of ACE I/D were more frequent in HAPE patients than controls (p = 0.03), with the D allele being the most frequent in HAPE patients. Aldashev et al. [19] also compared the ACE I/D genotypes and revealed a 3-fold higher frequency of the I/I genotype in highlanders with high-altitude pulmonary hypertension. Furthermore, the mean pulmonary artery pressure was higher in highlanders with the I/I genotype compared with the I/D genotype or the D/D genotype. Although RAAS has been shown to be involved in HAPE occurrence, some reports have associated this with a particular ACE insertion or deletion polymorphism. Other reports have claimed that the same ACE insertion or deletion polymorphism was not associated with HAPE [16, 20]. Qi et al. [21] performed an exhaustive literature search and a meta-analysis to study the association of the ACE insertion and deletion polymorphism with HAPE. A total of 5 studies, with a sample size of 662 controls and 305 cases, were examined by meta-analysis. The summary odds ratio indicated that there were no significant differences in the risk of developing HAPE between carriers of the two alleles. However, genotype association under the dominant mode showed that the deletion allele significantly conferred a 1.55-fold increase in HAPE risk compared with insertion genotype carriers. From these results, the authors concluded that ACE deletion allele carriers were at a significantly increased risk of developing HAPE.

Hotta et al. [22] used PCR-RFLP to analyze 55 controls and 49 patients with HAPE. They evaluated the A1166C and G1517T SNPs of Ang type I receptor (AT1R) and found that the frequencies of the AT1R alleles 1517 G or 1517T were significantly different in two groups of patients with HAPE. They concluded that the AT1R polymorphism was related to HAPE susceptibility by increasing pulmonary vascular resistance to increase pulmonary artery pressure, causing HAPE. Qi et al. [23] analyzed 10 SNP loci of five RAAS-related genes from 144 healthy controls (who were working on the Qinghai-Tibet railway construction project) and 140 HAPE patients. They found that the C344T polymorphism of the CYP11B2 gene and the A240T and A2350G polymorphisms of the ACE gene were closely associated with susceptibility to HAPE. The 240A and 2350G polymorphisms of the ACE gene and the 344T polymorphism of the CYP11B2 gene were not associated with susceptibility to HAPE. Travelers visiting high-altitude climates (>2,500 m) are more susceptible to high-altitude disease than subjects born at high altitude who are well adapted to the extreme hypoxic environment. For native populations, the chance of developing high-altitude disease is diminished. Rajput et al. [24] studied the –344T/C, intron-2 conversion (Iw/Ic), K173R and A5160C polymorphisms of the role of CYP11B2 in the hypoxia adaptation. They found that the high frequency wild-type –344T allele was positively associated with adaptation to high altitude. Stobdan et al. [17] screened 163 controls and 160 HAPE patients from Indian descent for eight polymorphisms of four RAAS genes (ACE, Ang, AngR1 and AngR2) in a case-control study. Significant difference in genotype and allele frequencies of the ACE I/D and AGT M235T polymorphisms were observed between the two groups. All of these suggested that polymorphisms of the RAAS pathway gene relate to the susceptibility to HAPE.

**NO Pathway**

NO is an important vasodilator synthesized in the pulmonary vascular endothelial cell. Its levels are regulated by endothelial cell-derived NO synthase 3 (NOS3). It is
resistant to oxidative damage and can serve as both a second messenger and a neurotransmitter to mediate various physiological functions. While it does not have a significant effect on pulmonary circulation, it can decrease edema associated with HAPE by improving pulmonary ventilation and blood oxygen saturation, which serves to transport fluid out of the edematous region [25–27].

For the NO pathway, many groups have examined the NOS3 polymorphism and HAPE and have observed positive associations. For example, Droma et al. [28] examined 51 healthy controls and 41 cases of HAPE patients and found that the frequency of the Glu298Asp variant of the 7th exon and the 27-bp variable number of tandem repeat (VNTR) of the 4th intron from gene NOS3 in the HAPE cases were higher than those in the control group. In another study, 72 healthy controls and 60 HAPE patients were analyzed for G894T, 27-bp repeat sequence, –A 922G and –T 786C polymorphisms of the NOS3 gene. They found that NO levels in healthy control group plasma were significantly higher than in the HAPE group. The frequency of the 27-bp repeat sequence in the two groups was significantly different by genotyping, and the genotype of the 894T, –922G and –786C polymorphisms had higher frequencies in the HAPE group [29]. In China, Yu-jing et al. [26] examined whether the polymorphisms of the NOS3 gene were associated with susceptibility to HAPE in Chinese railway construction workers. A case-control study was conducted including 160 healthy controls and 149 HAPE patients. Three polymorphisms of the NOS3 gene, T-786C in promoter, 894G/T in exon 7 and 27-bp VNTR in intron 4 were genotyped. They found the frequencies of the 894T allele and heterozygous G/T of the 894G/T variant were significantly higher in HAPE patients than in the control group. Haplotype analysis revealed that the frequencies of two haplotypes (T-T-5 repeats of 27 bp VNTR; C-G-4 repeats of 27-bp VNTR) were also significantly higher in the HAPE patients [26]. This finding suggests that HAPE could be caused by a reduction in NO levels. The genotypes of 894T, –922G, and –786C from NOS3 may be closely related with the formation of NO and are predictors of HAPE.

While the above-mentioned studies demonstrated a positive association between NOS3 and HAPE, other studies have failed to demonstrate any such association. For example, Weiss et al. [30] found no significant differences in the G894T, T786C and catecholamine (CA) repeat polymorphisms in the NOS3 gene when 51 HAPE patients were compared to 52 control individuals. This may have been due to low sample sizes or to potential variability in the populations examined.

Tyrosine Hydroxylase

Since TH is the rate-limiting enzyme of CA synthesis, and increasing CAs can cause pulmonary vasoconstriction and pulmonary hypertension, TH has been examined as a potential mediator of HAPE. Hanaoka et al. [31] analyzed 43 patients and 51 controls for the distribution of small satellites of the TH gene intron 1 and the Met81Val variant of the TH gene intron 2. They found that there was no association of the variants with susceptibility to HAPE. Whether TH has a role in HAPE, therefore, remains to be determined.

Vascular Endothelial Growth Factor

VEGF can increase the capillary permeability and stimulate vascular endothelial cell proliferation to promote angiogenesis. It may, therefore, play a special role in the pathogenesis of many diseases including HAPE. VEGF was increased in the plasma of HAPE patients before treatment with dexamethasone and aminophylline, and restored to control levels after treatment [25]. However, SNP allele frequencies of VEGF C2578A, G1154A, T460C, G405C and 5’-C936T showed no differences between patients susceptible to HAPE and the controls [32]. Whether VEGF genetic variation plays a role has still to be elucidated.

Pulmonary Surfactant Proteins

Oxidative damage stimulated by hypoxia can cause pulmonary endothelial damage and increase pulmonary vascular permeability, both of which can cause HAPE. There are two types of pulmonary surfactant proteins: SP-A1 and SP-A2, which are both distributed in the alveolar surface at high concentrations and have strong negative effects on resistance to oxidative damage. In addition, surfactant proteins are closely linked to the incidences of other pulmonary diseases such as chronic obstructive pulmonary disease and respiratory distress syndrome. The evaluation of 15 healthy controls and 12 cases of HAPE patients showed that the alleles of SP-A1 1101 T, 3192C, 3234C and SP-A2 3265C were associated with susceptibility to HAPE [33].
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**HIF Pathway Gene**

HIF-1α shares 48% amino acid sequence identity with the HIF-2α subunit, which is also known as the endothelial Per/ARNT/Sim (PAS) domain protein-1 (EPAS-1) [34]. From 2010 to 2011, four independent groups showed that in Tibetan populations the gene encoding HIF-2α contains key gene polymorphisms, which may explain the ability of these people to adapt to living at high altitudes [35–39]. Hypoxia-inducible transcription factors were first identified by the description of HIF-1 [40], which was subsequently found to enhance the transcription of multiple genes that encode the proteins necessary for rescuing from hypoxic exposure. Included among these genes were erythropoietic-, angiogenic- and glycolytic-related genes. Although HIF-1 is highly similar to HIF-2α in the amino acid sequence and has the potential to bind and mediate many of the same genes, the biological actions of HIF-1 in response to hypoxia are distinct from those of HIF-2 [34, 41–45]. In recent years, several HIF-2α mediated processes have been found to be linked to the increase in vascular permeability seen in the human response to high-altitude exposure (fig. 3) [46–48]. In animal studies, it was found that HIF-2α heterozygous-null mice exhibit exaggerated carotid body sensitivity, breathing instability and hypertension in response to hypoxia [49]. Duan et al. [50] reported that hypoxia controls endothelial function directly via the upregulation of EPAS-1-regulated Tie-2 expression. Since EPAS-1 can bind directly to the VEGF promoter [51], both endothelial functions and VEGF changes may relate to the occurrence of HAPE. Lorenzo et al. [6] evaluated one family by a genome-wide association study using Affymetrix 5.0 chip and failed to find an association of HIF-2α haplotypes with HAPE susceptibility. While this study is intriguing, the samples size was very small and additional large sample, multicenter studies are needed to confirm these results.

Egl nine homolog 1 (EGLN1) hydroxylates constitutively expressed HIF at two proline residues to cause polyubiquitination by the von Hippel-Lindau E-3 ligase complex and subsequent degradation by the proteasome machinery [52]. Hypoxia can inactivate EGLN1, thereby activating HIF pathway genes and promoting the hypoxia-adaptive responses through upregulation of glycolytic enzymes, hemeoxygenase (at the cellular level), VEGF (at the local level) and erythropoietin (at the systemic level). Aggarwal et al. [53] studied the association of common variations (rs480902 and rs479200) in the EGLN1 gene with HAPE susceptibility. Interestingly, the TT genotype of rs479200 had a significantly higher frequency (0.44) in HAPE patients compared to in natives of high altitude (0.05). Higher expression of EGLN1 correlated inversely to HIF activity. The TT genotype of rs479200, which was correlated with a higher expression of EGLN1, associated with HAPE patients.

**β2-Adrenergic Receptor**

The roles of β2-adrenergic receptor in pulmonary oxygenation during altitude acclimatization, physical performance and lung fluid clearance have been ascertained. In a case-controlled study, 143 unrelated controls and 110 unrelated HAPE patients were selected. The eight SNPs including three tag-SNPs were genotyped from promoter and exonic regions of the β2-adrenergic receptor. The haplotypes from 46A/G and 79C/G SNP were associated with HAPE. Its haplotype 46G-79C-523C was also significantly overrepresented in the controls [54].

**Endothelin-1**

ET-1, both in terms of the presence of gene variants and levels, was found to associate with hypoxia adaptation for high-altitude natives [55]. Charu et al. [18] explored the ET-1 5′-untranslated region microsatellite (CT)n-(CA)n, −3A/−4A (rs10478694), G2288T (rs2070699) and Lys198Asn (rs5370) polymorphisms in 64 HAPE patients and compared the prevalence with 53 control subjects. They reported that the GT+TT genotypes of ET-1 G2288T polymorphisms were overrepresented in HAPE.

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**Fig. 3.** HIF pathway genes related to the occurrence of HAPE.
patients ($p = 0.002$), with the T allele being more frequent. The ET-1 G2288T polymorphism emerged as a noteworthy variant showing a strong association with HAPE susceptibility.

**Hsp70 Family Genes**

The heat shock protein family members, especially HSP70, can protect cells and organs against many different types of damage. The association between polymorphisms of the HSP70 family genes and a risk of acute high-altitude illness was evaluated in 56 patients and in 100 matched controls by genotyping for the polymorphisms +190 G/C, +1267 A/G, 2437 G/C in the hsp70-1, hsp70-2, and hsp70-hom genes. There was a significantly higher frequency of hsp70-2 B/B and hsp70-hom A/A and B/B genotypes and a significantly lower frequency of the hsp70-hom A/B genotype in the patients than in the controls ($p < 0.05$ for all). These results suggested that individuals with hsp70-2 B/B and hsp70-hom A/B and B/B genotypes may be more susceptible to high-altitude illness, whereas those with the hsp70-hom A/B genotype may be tolerant to high-altitude illness [56]. Qi et al. [57] also studied the five common polymorphisms within HSPA1A (rs1043618 and rs1008438), HSPA1B (rs1061581 and rs539689) and HSPA1L (rs2227956) of the Hsp70 family, to explore their potential interactions with susceptibility to HAPE in the Chinese population (148 HAPE patients and 483 matched controls). Significant differences were observed in the genotype and allele distributions of rs1008438 and in the rs1061581 allele distribution between HAPE patients and controls. Haplotypes Hap4 (G-C-A, in order of rs1061581, rs1043618 and rs1008438) and Hap5 (G-G-A) had an 86% reduced susceptibility risk for HAPE, and Hap7 (A-C-C) had a 2.43-fold increase in risk for HAPE. This study demonstrated strong interactions between rs1061581, rs1043618 and rs1008438 polymorphisms within the Hsp70 family and susceptibility to HAPE in Chinese. Moreover, polymorphism rs1008438 might cause the development of HAPE via a change in HSPA1A promoter activity.

**Future Directions and Conclusions**

HAPE encompasses a broad class of medical conditions resulting from acute exposure to a hypoxic environment. Our understanding of the underlying pathophysiology of HAPE has been unclear but is expanding. This review summarized the recent scientific findings pertaining to the genetic etiologies of HAPE. Currently, there is evidence for at least a partial genetic basis for HAPE. Differences in susceptibility among different individuals may be due to ethnic variations, which complicate data interpretation. Familial clustering of cases and some positive associations of nuclear genetic variants all support a role for genetics in HAPE susceptibility. The majority of genetic investigations into HAPE susceptibility have focused on candidate-gene association studies. Of the genes evaluated, NOS3, ACE, CYP11B2, Hsp70, ET-1 and pulmonary surfactant proteins A1 and A2 have been shown to have polymorphisms that associate with HAPE incidence, while associations between TH, VEGF and HAPE have still to be fully elucidated.

While much work remains to be done, an initial list of susceptible genes is emerging. Care should be taken when evaluating these studies, however, because phenotypic and cohort definitions may limit the ability to merge studies [58]. It is not likely that a single genetic polymorphism will be identified as the sole determinant of HAPE; instead, the pathogenesis of HAPE is likely polygenic, with multiple genes and the interactions among these genes contributing to the complete HAPE phenotype. More sophisticated investigations are needed to study the genetics and epigenetics of HAPE, including genome-wide association studies and next-generation sequencing [59]. Due to the rare occurrence of HAPE, large sample sizes and a variety of ethnic populations are needed to fully test for associations between polymorphisms and HAPE susceptibility [60]. An increased understanding of the role that genetics plays in the development and severity of altitude illnesses will enhance our ability to prevent, diagnose and treat HAPE [61].

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