High Altitude Disease: Consequences of Genetic and Environmental Interactions

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Abstract

High altitude disease (HAD) is a pathological effect of high altitude on humans, caused by acute exposure to low partial pressure of oxygen at high altitude. Millions of people who live at, or ascend to, altitudes above 2,500 m, are at risk for acute mountain sickness (AMS) and chronic mountain sickness (CMS). Among these populations, there are distinct patterns of adaptation to the environment, lowlanders are more susceptible to HAD than highlanders when they are exposured to hypoxia, Andean highlanders have higher incidence rate of HAD than Tibetans and Sherpas. People originating from the same sea level have different response to hypoxia. These situations showed that the incidence of HAD has a certain genetic background. With the development of modern research techniques, there is more and more evidences that indicates that the pathogenesis of HAD is associated with some mutant form of hypoxia-related genes, number and function of the genes encoding protein, such as HIF1A,EPO,EDN1,NOS3, and also associated with human leukocyte antigen (HLA)(define it) system, oxygen-carrying proteins and other genes. Although numerous molecular genetic linkage and association studies have been conducted to explain the pathology of high altitude disease, none are complete. With the availability of large scale DNA data sets, we will be able to identify more potential hypoxia related genes, and elucidate the genetics of high altitude diseases.

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High altitude disease (HAD), which can include dyspnea, nausea, headache, and disorientation, typically occurs at altitudes of over 2,500 m, and is primarily caused by lack of oxygen in the body (hypobaric hypoxia). Acute mountain sickness (AMS) and chronic mountain sickness (CMS) are the two main types of high altitude sickness.

AMS is a form of hypoxia that occurs acutely in humans and animals at high altitudes, and includes high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE). CMS develops over an extended time living at high altitudes and is characterized by polycythemia (increased hemoglobin and hematocrit), pulmonary hypertension and hypoxemia.

Millions of people who live at, or ascend to, altitudes above 2,500 m, are at risk for AMS and CMS. Among these populations, there are distinct patterns of adaptation to the environment, which can be attributed to their ethnic or individual susceptibility. For example, hemoglobin concentration is lower in Himalayan than in Andean highlanders,[1,2] and greater physical capacities have been reported in Tibetan than in ethnic Han Chinese who participated in high-altitude expeditions.[3] Similarly, lower CMS incidence rates were reported in Tibetan than in Han populations,[4] and higher hypoxic ventilatory response (HVR) rates are seen in Tibetans compared with Andeans.[5,6] These marked ethnic differences in response to hypoxia could be attributed to genetic differences, however, no specific genes have yet been identified to be responsible for adaptation to the high altitude environment.
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Genes and Environment

In nature, phenotypes derive from the interaction between genes and the environment. For protection, the colors of many animals change according to their environment. These gene-environment interactions have been demonstrated in experiments by placing animals in different environmental conditions. One such study stratified animals by genetic background, and showed variability of the serotonin (5-HT) transporter (5-HTT) gene regulatory region in monkey's cerebrospinal fluid.[7] In human, a similar modified gene-linked polymorphic region (5-HTTLPR), which causes allelic variation in 5-HTT expression, is associated with decreased 5-HT function and 5-HT-mediated psychopathology.[8]
Genetic factors interact dynamically with both pre- and post-natal environmental influences to shape development. Neonates and preschool children with histories of prenatal exposure to tobacco have displayed disproportionately poorer performances compared to controls, which was found to be correlated to a variation in the TaqIA polymorphism and dopamine receptor 2 expression.[9]

This type of phenomenon is readily apparent in chronic pulmonary diseases. For example, asthma is often associated with increased levels of $CD4^+$ lymphocytes,[10] and chronic obstructive pulmonary disease frequently involves $CD8^+$ lymphocytes.[11,12] An increasing number of studies are focused on pathogenesis analysis for complex diseases (including HAD), while there is a scarcity of studies linking pathologic physiology measurements with the disease.

Certain mountainous areas with both transient migrants and indigenous populations may offer abundant resources to study HAD. People in these regions have different nationalities, with varying durations at high altitudes and different degrees of exposure to hypobaric hypoxia. The characteristics of high altitude environments, with varying degrees of hypobaric conditions, hypoxia, ultraviolet radiation, dryness, and cold, may play contributing roles in gene mutation.[13,14,15] There are different genetic traits associated with native populations who have adapted to high altitude climates and to sojourners or migrants who are more susceptible to hypoxia at high altitude.[2,16] Tibetans may be the earliest resident population of these high elevation regions, having arrived there 25,000 to 50,000 years ago. Andeans have resided in these regions for about 10,000 years, followed by the Han who have been there for only several decades. Native Tibetans appear to be at lower risk for CMS than native Andeans,[17] which suggests that genetic backgrounds may contribute to an individual's adaptive capacity to hypoxic environments. Many studies have shown that genes play an important role in the pathogenesis of HAD. A majority of research is focused on identifying genes related to hypoxia, however, only a few reports mention familial or population genetics.

Risk Factors for High Altitude Disease

Other risk factors appear to be related to high altitude disease, including ethnicity, altitude, climate, ascending speed, physical activity level, duration at high altitude, and nutritional status, etc. Of all these factors, ethnicity is perhaps the most significant. Other contributing factors include sex, age, physical condition, and genetic susceptibility.

Sex

Reports have shown higher incidences of AMS and CMS in transient populations, as compared to resident populations at high altitude. Interestingly, males and females differ in their responses to hypoxia. CMS occurs more frequently in men. At 4,300 meters, CMS prevalence in Han females was only 1.76% as compared to 7.77% in Han males. The prevalence of CMS in Tibetan women and men is 0.56% and 1.78%, respectively.[18] The higher prevalence of HAD in males is also seen in South America; for example, the incidence of CMS is 11% in females and 28% males at a hospital in La Paz (3510 m) , and 26% in females and 54% in males at a hospital in El Alto (4,100m).[19] For AMS, the incidence rate appears to be similar for men and women;[20,21,22] however, there is at least one report of a higher incidence of AMS in women.[23,24] It is not entirely clear why this gender difference exists; however it may be because female hormones exert a positive effect on ventilation, oxygen utilization, and oxygen metabolism.[17,25]

Age

Age appears to be a major contributing factor for CMS, as research in Peru has shown that age-dependent polycythemia was based on an age-dependent loss of ventilation and arterial hypoxemia.[26] Research conducted in Tibet, however, did not show a relationship to age among native Tibetans.[27] The divergence of these findings indicates that the impact of age as a risk factor is not yet clear. There are no reports on age factor for AMS.

Ethnicity

It is clear that CMS is more common among migrant Han populations than native Tibetans. Important genetic differences have been found between these ethnic groups.[17,28] In a study involving several ethnicities, Tibetans had the lowest prevalence of CMS, and Han had the highest;[18] Tibetans also had a lower prevalence (0.91%) than Peruvian Quechuas (15.6%) at 4300 m.[29] In addition, native residents living at high altitude have higher ventilation and lower end-tidal PCO2 compared to other populations at the same altitude,[5] which may explain why Tibetans have a better tolerance to acute hypoxia than the Han population. These studies suggest that there is a strong correlation between ethnicity and CMS, which argues the case for conducting further studies on the relationship of genetic characteristics and CMS or AMS.

Individual difference and family inheritance

Individual difference appears to play a role in vulnerability to hypoxia, as some individuals appear remarkably resistant while others are notably susceptible. AMS in 847 mountaineers was investigated and, in susceptible individuals with rapid ascent and no pre-exposure its prevalence was 58%, it was 29% with pre-exposure only, 33% with slow ascent only, and 7% with both pre-exposure and slow

ascent.[30] Among the 235 affected subjects, the frequency of AMS in individuals who were naïve to high altitudes was two to three times higher than in individuals who had attained similar altitudes in the past without difficulties.[16]

Bartsch[31] considers individuals with a previous history of AMS to be more likely to suffer from hypoxic injury, suggesting an innate predisposition to high altitude disease. Subjects who are susceptible to HAPE have lower pulmonary ventilation response to hypoxia.[32] An MRI study has shown that regional pulmonary flow in HAPE-susceptible subjects becomes more heterogeneous when they are exposed to normobaric hypoxia.[33]

Another study shows that obesity exacerbates hypoxia-induced mean pulmonary artery pressure, right ventricular hypertrophy and pulmonary vascular remodeling.[34] A study on obese rats showed that they have lower HVR, which is attributed to the blunted ventilator response in obesity.[35] However, Ge et al.[36] found that HVR in mildly obese men was higher than in lean men, showing that respiratory chemosensitivity was not blunted; the authors suggested that the susceptibility of obese individuals to AMS may be at the genetic gene level. The obesity hormone, leptin, is over expressed in individuals in hypoxic environments and is associated with higher levels of HIF-1α.[37] Leptin may play an important role in obese individuals with AMS and CMS.

A recent study shows that high altitude diseases may have a familial genetic trait. There is a family with three generations of members living in the Qinghai-Tibetan plateau that are affected with HAPE and which share the HIF2α haplotype. This may suggest that HAPE has a family genetic trait based upon a gene susceptibility to hypoxia.[38]

Hypoxia-related genes

High altitude diseases derive from interactions of the individual's genes with the (extreme, and sometimes hostile) environment. These interactions occur through direct and indirect mechanisms to regulate susceptibility to environmental stressors. Some of these genes work via their ability to sense oxygen, and include hypoxia inducible factor (HIF), erythropoietin (EPO), endothelin 1 (EDN1), and endothelial nitric oxide synthase (NOS3).

Hypoxia inducible factor (*HIF1A*)

Hypoxia inducible factor (HIF) mediates the effects of hypoxia on the cell. Six HIF genes have been identified in humans, with HIF-1 being the most important in regulating tissue oxygenation. It is a transcriptional activation factor that regulates the expression of a number of hypoxia related genes, including erythropoietin, heme oxygenase, nitric oxide synthetase, endothelin-1, and vascular endothelial growth factor.[39,40]

GT14, a novel dinucleotide-repeat polymorphism in the *HIF1A* gene has been shown to be present in higher frequencies in Sherpas than Japanese.[41] This may contribute to the greater tolerance for high altitudes exhibited by Sherpas. The frequency of G1790A polymorphisms in *HIF1A* is also higher in Sherpas than in Han Chinese,[42] and expression of *HIF1A* is markedly higher in natives of high altitudes than of low altitudes.[43]

Expression of *HIF1A* increases when oxygen concentrations are low, and decreases when they are high.[44,45] These studies indicate that HIF-1is associated with a hypoxia related adaptation by natives, who exhibit a physiological compensation during hypoxia stimulations. Overexpression of *HIF1A*, however, may have negative consequences, because it increases blood viscosity by excessively raising red cell mass through enhanced EPO activity, which may result in hypoxemia and CMS.[43,46] Engebretsen et al.[47] showed that there was an elevated expression of HIF-1 in rats with induced high altitude pulmonary edema. It is clear that HIF-1 has a hypoxia sensing role and that its measurement is a marker for identifying hypoxic injury in high altitude mountain diseases but it is not certain if it functions as a 'master gene' in this capacity.

Erythropoietin (*EPO*)

Erythropoietin (EPO) regulates red blood cell production. It is a hypoxia-dependent gene and helps the body adapt to hypoxic environments by increasing hemoglobin levels in order to increase oxygen capacity. It is also the means by which acute adaptations to high altitudes are achieved.[48] However, excessive EPO levels can produce increased red blood cell mass and viscosity, and may result in high altitude polycythemia (HAPC).[49] Pei et al.,[50] showed that patients living in the Qinghai Tibetan plateau with HAPC have higher levels of serum EPO. Exposure to high altitudes is associated with increased EPO secretion; EPO is markedly enhanced in patients with HAPE[51,52] and in animals with HAPC.[53] Other studies, however, report that EPO levels in patients with HAPC were not higher than healthy controls.[3,54,55] These contradictory findings suggest that higher expression of *EPO* is not associated with excessive red blood cell counts. In a study of 23 athletes, Gore et al.[56] reported that erythropoiesis did not accelerate despite the fact that there were increases in serum EPO when the athletes were exposed to intermittent hypoxia.

Endothelin 1(*EDN1*)

Endothelins (ET) are proteins that cause vasoconstriction and raise blood pressure. Endothelin consists of three isoforms (ET-1, ET-2 and ET-3) with varying regions of expression and which bind to two key receptor types, ET_A and ET_B. ET-1 has greater vasoconstriction properties, and is a major factor in the development of hypoxia pulmonary hypertension.[57,58,59] This occurs because the proximal promoter of the ET-1 gene contains an HIF-1 binding site.[60] ET-1 receptor antagonists have been shown to prevent hypoxia pulmonary hypertension,[61,62] to reduce hypoxia induced mortality,[63] and to inhibit hypoxia induced apoptosis.[64] *EDN1*expression is different in natives (and animals) at high altitudes as compared to lower altitudes. The levels of ET-1 mRNA in lung tissue are higher in rats[65] and sheep[66] during hypoxic episodes. A genetic analysis,

involving 426 highlanders and 236 lowlanders, showed that overexpression of the longer repeats, -3A/-3A, GG and Lys198Lys genotypes on *EDN1*, were associated with significantly lower ET-1 levels in the Highlanders than in the lowlanders.[67] Another study showed that *END1* variants and ACE have roles in HAPE susceptibility.[68]

Endothelial nitric oxide synthase (*NOS3*)

Endothelial nitric oxide synthase is responsible for making nitric oxide (NO) in vascular tissues. NO is a gas with several physiological functions, including maintaining pulmonary vascular tone and altitude adaptation. It was reported that the level of NO was reduced in HAPE patients.[69,70] Tibetans and Aymara have higher levels of NO in their exhalation.[71,72] Ahsan et al. showed that Ladakhi have higher levels of circulating NO.[73] These studies support the suggestion that higher levels of NO and *NOS3* have adaptive roles at high altitude. The reports showed G894T and 27 base-pair 4b/4a variants in *NOS3*, and overexpression of Glu and 4b alleles in Ladakhi.[73,74] and Sherpers,[75] which supports the notion that higher levels of NO and *NOS3* have an adaptive role at high altitude.

NO is made from arginine by NOS, and polymorphisms in the *NOS3* gene are associated with HAPE susceptibility in Japanese people.[76] The variant alleles of *NOS3* contribute to reduced NO in HAPE individuals[77,78] mice with *NOS3* deficiency also more readily develop HAPE. Another study found that pulmonary vascular resistance was greater in *NOS3* deficient mice than in wild-type mice. Increased pulmonary vascular resistance also occurred in wild-type mice where three NOS isoforms were inhibited by L-NAME.[79] Hence, we suggest that susceptibility to hypoxia has a genetic basis.

Other genes

Many other hypoxia related genes participate in the development of high altitude disease, including vascular endothelial growth factor gene, angiotensin converting enzyme-I/D, β2-adrenergic receptor, tyrosine hydroxylase, etc., to form a complex signal pathway network. Through this network, hypoxia related genes contribute to the development of AMS and CMS. Overall, the role of hypoxia-related genes in high altitude disease pathogenesis remains a 'work in progress.'

HLA system

The classic view of one gene encoding one protein has recently changed.[80] It now accepted that two or more genetic loci may determine a single protein function, or that one gene may be associated with multiple protein products and functions. The human leukocyte antigen (HLA) system exemplifies this new view as it includes multiple genes that code for an array of protein products.

A wealth of data shows that HLA is associated with multiple immunologic diseases.[81,82] Genetic association studies have shown that certain HLA alleles are associated with susceptibility to some non-immunological diseases. This is especially true for associations between HAPE and HLA-DR6 or DQ4,[83] and of pulmonary hypertension with HLA-DR6.[84]

Pulmonary artery cells from animals with hypoxia induced pulmonary hypertension express leukocyte/monocyte markers.[85] The expression levels of mbHLA-G1 and sHLA-G1 at 2% oxygen are twice as those at 20% oxygen.[86] Mouillot et al.[87] demonstrated that modulation of HLA-G gene expression is dependent on HIF-1 stabilization and thus might be relevant for the control of HLA-G gene expression in hypoxic tumors. The authors found that there is markedly different HLA-II allele frequency distributions between Tibetans with CMS compared to healthy Tibetans who live at the same altitudes.[88] Overall, this research suggests that the presence or absence of certain HLA alleles affects individual's susceptibility to hypoxia, but it does not explain the entire phenomenon.

Oxygen-carrying proteins

The oxygen-carrying protein family includes hemoglobin, cytoglobin, neuroglobin, and myoglobin. Although they are all involved in the transfer of oxygen, hemoglobin has by far been the most studied .

Hemoglobin

Elevated hemoglobin levels are a notable characteristic of CMS. The excessively high concentrations of RBCs slow their own transit and may result in cell and organ injury, and the development of CMS. This process may be associated with hypoxia related genes, such as EPO.[48,49,50,52] In our own work on this topic, the coding region of the alpha-globin gene of the Tibetan antelope was obtained, and compared with the sheep alpha-chain modifications of α117 Glu/Asp and α132 Asn/Ser in important regions.[89] These gene mutations provide essential information for elucidating the possible roles of hemoglobin in the Tibetan antelope's ability to adapt to extremely high altitudes.

Cytoglobin

Cytoglobin is the protein product of CYGB, and is located in the brain, and is thought to be involved in protection against hypoxia.[90] Singh et al.[91] showed that cytoglobin is also a hypoxia-induced hemoprotein in hypertrophic myocardium and that cytoglobin transcript levels are high in the adult heart. Transcriptional analysis of the CYGB 5′ flanking region found conserved binding sites for the transcription factors HIF-1, AP-1, and NFAT. They also showed that calcineurin activity affects cytoglobin transcription and enhances NFAT and AP-1 binding to the putative cytoglobin promoter. Under hypoxic conditions, inhibition of calcineurin, NFAT, and/or AP-1 activities decreases endogenous cytoglobin levels. In general, CYGB affects molecular structure to enhance oxygen affinity in hypoxic environments.[92]

Neuroglobin

Neuroglobin is an intracellular hemoprotein expressed in the central and peripheral nervous systems, and has a higher affinity than hemoglobin for oxygen.[93] Neuroglobin DNA

sequences are highly conserved across humans, rats, and Tibetan chickens.[92,94,95] Another report showed that the Lys-2224(4)-Asn mutation in exon 4 of the neuroglobin gene exists only in the Tibetan chicken and not in lowland chickens, suggesting that this mutation may be associated with hypoxia adaptation.[95]

Myoglobin

The Tibetan antelopes live at altitudes of 4000m-5000m, where it can run continuously for four hours at speeds of 75 kilometers/hour, despite the hypoxic environment. They have strong muscles and an unique ability to regulate oxygen in muscles. Numerous studies have reported that myoglobin plays an important role in muscle activity,[96,97] and can be increased by hypoxic stimulation.[98,99] Research has revealed that hypoxia in the presence or absence of exercise-induced stimuli reprograms calcium signaling and modulates myoglobin gene expression.[99] Research studies have succeeded in cloning the coding region of the myglobin gene in the high-altitude Plateau Pika,[100] a small hamster-like animal endemic to China, and the Tibetan antelope.[101] Two mutations in the myoglobin gene of the Tibetan antelope were observed at 21(GGT→GAT) and 78 (GAA→AAG), which results in translational changes: Gly→Asp and Glu→Lys. Two myoglobin gene polymorphisms in Pika were identified by comparing it with the amino acid sequence of previously reported sequences of American Pika.[102]

Human genome studies

Although numerous molecular genetic linkage and association studies have been conducted to explain the pathology of high altitude disease, none are complete. With the availability of large scale DNA data sets, we will be able to identify more potential hypoxia related genes, and elucidate the genetics of high altitude diseases. Gigante et al.,[103] analyzed the risk of coronary heart disease (CHD) using HapMap and found that coagulation factor II receptor genetic variants are associated with CHD. Such HapMap studies enhance our ability to study gene variations in response to environmental stimuli such as hypoxia.

Conclusion

Human genetic variations contribute substantially to differences in response to environmental changes. Variability in a number of genes may play roles in human genetic adaptation to hypoxia and the development of high altitude diseases. Because genetic mechanisms of high altitude diseases comprise a complex genetic background, we need to focus our efforts on multiple possibilities. Currently, our research group is studying susceptibility genes for chronic hypoxia in Tibetans with and without CMS using HapMap. In addition, we are studying the familial epidemiology of CMS in the Qinghai-Tibetan plateau. We are also conducting research to identify familial CMS, especially in twins, which may determine which genes are responsible for CMS.

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