The Genetics of Altitude Tolerance

The Evidence for Inherited Susceptibility to Acute Mountain Sickness

Martin J. MacInnis, BSc, Pei Wang, MSc, Michael S. Koehle, MD, PhD, and Jim L. Rupert, PhD

Objective: Acute mountain sickness (AMS) has become a significant environmental health issue as improvements in transportation, "environmental tourism," and resource development lure more people to the highlands. Whether there is a genetic contribution to AMS susceptibility is a central question in high-altitude medicine. This article provides a systematic review of the evidence supporting such an innate predisposition.

Methods: Scientific literature databases were screened using the terms "acute mountain sickness/AMS" and "altitude illness" combined with the terms "DNA," "gene," "genetic," or "polymorphism."

Results: Sixteen genes from a variety of pathways have been tested for association with AMS and variants in eight showed positive associations suggesting that AMS is an environmentally mediated polygenic disorder.

Conclusions: The data suggest that genotype contributes to capacity to rapidly and efficiently acclimatize to altitude; nevertheless, the mechanisms by which this occurs have yet to be elucidated.

Acute mountain sickness (AMS) is a medical condition that can result from acute exposure to high-altitude environments (generally exceeding 2500 m). Each year, millions of people travel to the mountains and high plains for a variety of reasons including professional (eg, mining, military, and astronomy), recreational (eg, skiing, hiking, and mountaineering), or spiritual (eg, festivals and retreats) reasons or because of sociopolitical displacement (eg, refugees); consequently, large numbers of people are at risk of developing the condition.[1] The prevalence of AMS can be high (up to 93%)[2]; yet, public awareness is often lacking (45% of respondents at an American high-altitude ski resort had no knowledge of AMS).[3] Statistics such as these imply that precautionary measures are probably not well known, not acted upon, or not effective. Furthermore, AMS typically occurs in remote environments where medical treatment is limited or unavailable, frequently expensive,[2] and potentially hazardous for health care professionals—for example, in some combat operations in the Afghanistan highlands, 12% of costly and often risky medical evacuations were for personnel suffering from AMS.[4]

Overall, the development of AMS can endanger the health of high-altitude sojourners and decrease the productivity of those working in high-altitude environments. This can have a significant economic impact, especially in regions dependent on high-altitude tourism (such as the Cusco—Inca Trail—Machu Picchu corridor in Peru) or on major high-altitude engineering projects, such as the Golmud-Lhasa railroad in Tibet, the construction of which involved 30,000 to 50,000 people working above 4000 m.[1] In situations where time for acclimatization is limited, the impact on health can be severe. During the 1962 Sino-Indian War, more Indian soldiers died from altitude illness than from enemy fire because rapid deployment did not allow for sufficient acclimatization to altitude.[5]

Acute mountain sickness is less severe but much more common than the other major acute altitude illnesses: The frequency of high-altitude pulmonary edema and high-altitude cerebral edema (HACE) tends to be between 1% and 6% near 4000 m.[2] A review of selected studies from major mountainous areas distributed around the world with variable altitudes, ascent rates, and latitudes showed the incidence of AMS to range from 9% to 84%.[6] Establishing the incidence of AMS is challenging because of the large number of potential environmental cofounders (ranging from ascent profiles to pharmaceutical intervention); nevertheless, the 68% incidence in a large cohort of Nepalese religious pilgrims ascending on foot to 4380 m without prophylactic medications[7] may represent a "typical" incidence rate at moderate altitude.

Acute mountain sickness symptoms are nonspecific: headache, nausea, vomiting, anorexia, lethargy, dizziness, and insomnia.[8] Symptoms generally appear during the first night at altitude (6–12 hours after arrival),[9] and the severity of AMS can range from mild to extreme but is generally moderate.[6] Both faster ascent rates and increased altitudes are associated with a greater incidence and severity of AMS (for example, see[10]). Acute mountain sickness history is a predictor of subsequent affliction,[11] although recent exposure to altitude also decreases the risk of AMS, potentially through some degree of residual acclimatization. Other risk factors have been investigated, but their association with AMS is either less significant than those discussed earlier, equivocal, or undetectable. Finally, although fitness is not an independent risk factor, exertion at altitude does seem to increase one's risk of developing AMS,[12] and individuals who are more fit might be more likely to exert themselves at altitude or while ascending.

ASSESSMENT AND TREATMENT

The most common tool used to diagnose and measure the severity of the symptoms of AMS is the Lake Louise scoring system (LLS).[13] A positive LLS diagnosis requires a recent increase in altitude, a headache, and at least one additional symptom from a list of four: gastrointestinal symptoms, fatigue/weakness, dizziness/lightheadedness, and difficulty sleeping. Each symptom (including headache) is subjectively rated from 0 to 3, with 3 being the most severe rating, and an overall cumulative score greater than 3 is usually considered diagnostic.

Acute mountain sickness can be acutely incapacitating, but in most cases, proper and timely diagnosis followed by simple interventions rapidly alleviates symptoms.[8] The initial treatment is rest with no further ascent until the condition resolves. If there is no improvement, descent is advised. If symptoms worsen, descent becomes imperative because AMS can advance to HACE, a potentially fatal condition.[6] Often descending 500 to 1000 m is sufficient to alleviate AMS,[8] (and "simulated" descent using supplemental oxygen or hyperbaric therapy can be used if physical descent is not an option).[2] In addition to ascent and descent strategies, various pharmaceuticals such as acetazolamide and dexamethasone have been assessed for their ability to prevent or reduce AMS symptoms and severity.[8,14] Studies of antioxidant supplements and *Gingko biloba* have had mixed results with respect to the prevention and treatment of AMS,[15,16] and although there is anecdotal support for chewing or

From the School of Human Kinetics (Mr MacInnis, Ms Wang, and Drs Koehle and Rupert) and Allan McGavin Sport Medicine Centre, Department of Family Practice (Dr Koehle), University of British Columbia, Canada.

Address correspondence to: Jim Rupert, PhD, School of Human Kinetics, University of British Columbia, 6081 University Boulevard, Vancouver, BC V6T 1Z1, Canada; E-mail: rupertj@interchange.ubc.ca.

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brewing coca leaves to keep *soroche* at bay, this traditional Andean method has not been systematically investigated to determine either its efficacy or its safety.

PATHOPHYSIOLOGY AND ACCLIMATIZATION

Although the pathophysiology of AMS is not entirely understood, the condition is believed to be a manifestation of neurological complications linked to hypoxia. As altitude increases, barometric pressure declines resulting in a decrease in the partial pressure of inspired oxygen. There are a number of acclimatization responses that will attempt to minimize the impact of this on blood oxygen levels, but if they are inadequate or too slow, systemic hypoxemia will occur. This will reduce the amount of oxygen delivered to cells. In the highly oxygen-dependent brain, this results in cerebral swelling (both extracellular and intracellular) and increased oxidative stress in the brain.[17,18]

Acute mountain sickness was hypothesized to be a mild form of HACE,[19] but recently, this hypothesis has lost some support because studies have failed to associate cerebral edematous brain swelling or increased intracranial pressure with AMS.[18] During hypoxia, the decrease in cerebrospinal fluid volume and the increase in cerebral blood flow were equivalent in individuals with and without a history of AMS.[20] Thus, the hypothesis that AMS is the result of inadequate cerebrospinal compliance (ie, a "tight-fitting" brain)[21] is not fully supported. A revised version of this hypothesis proposes that although brain swelling does occur in AMS patients, it is not exclusive to AMS patients, and it is not responsible for the symptoms of AMS; rather, it was proposed that increased free radical generation directly activates the trigeminovascular system and indirectly triggers intracellular swelling.[18] Hypoxia-mediated intracellular swelling increases nitric oxide (NO) production, which also activates the trigeminovascular system.[18] Activation of the trigeminovascular system may explain the headache that is strongly associated with AMS.[17,22] In addition to the absolute hypoxia of altitude, the decreased ambient pressure (hypobaria) may also have a role in the pathophysiology of AMS. In hypobaric chambers, a lower barometric pressure results in a greater mean LLS than a higher barometric pressure for an equivalent partial pressure of inspired oxygen.[23] The mechanism underlying the contribution of decreased barometric pressure to AMS is unknown.[24]

Acclimatization to hypoxia occurs through a suite of acute and chronic molecular, cellular, tissue, and systemic responses that increase oxygen supply and decrease oxygen demand in an attempt to reach homeostasis.[25] These responses include increased ventilation and cardiac output, bicarbonate diuresis, hemoconcentration, increased anaerobic metabolism, and over time, angiogenesis, erythropoiesis, and additional hemoglobin production.[26] Individual variation exists in the speed and extent of these responses but the degree to which the variation is acquired or innate is uncertain. Because genetics has a prominent role in determining an individual's physiology (both intrinsic and adaptive), genetic studies will improve our understanding of the molecular mechanisms that underlie the physiological manifestations of AMS.

EVIDENCE FOR THE GENETIC ETIOLOGY OF AMS

Methods

We identified appropriate articles to review through searches of PubMed and other online databases using the terms "acute mountain sickness," "AMS," and "altitude illness" in combination with the terms "gene," "genetic," "DNA," and "polymorphism." We identified additional articles from the bibliographies of selected articles. The majority of articles were published in English, but several studies published in Chinese were translated and included. Any article available before the middle of June 2010 was included in this review.

Human Genetic Variation

The approximately 3 billion–base pair human genome is common to all humans, differing only slightly between individuals (averaging 1 difference per 1000 base pairs for two unrelated individuals).[27] These disparities contribute much to the innate differences between individuals. Genetic variations in populations result from individual mutations that spread through breeding (above a frequency of 1% these mutations are termed "polymorphisms"). The number of genetic variants ("alleles") at a polymorphic site depends on the type of polymorphism; nevertheless, for most polymorphisms, an individual can carry a maximum of two alleles (excluding genes located on the X and Y chromosomes, for which males carry only a single gene) either homozygously (two copies of one allele) or heterozygously (one copy of two different alleles). The classes of DNA polymorphisms are listed in Table 1.

The most common and most frequently studied polymorphisms are single nucleotide polymorphisms (SNPs), which result from a DNA base substitution (eg, an A for a T). There are an estimated 10 million SNPs in the global human population.[28] Other types of polymorphisms include insertions/deletions and repeats, both of which affect not only the code but the length of the affected strand. In an insertion/deletion the variation occurs because a segment of DNA is either present or absent. A repeat is a type of polymorphism in which single bases or segments of DNA are present in variable copy numbers arranged in tandem.

TABLE 1. Types of Common Genetic Polymorphisms

Polymorphism	Description	Number of Variants; Variant Type
Single nucleotide polymorphism	A change at one base pair.	
Substitution	One base is substituted for a different base.	Usually two variants; one of two alternate bases present at each allele.
Insertion/deletion	One base pair is inserted or deleted.	Usually two variants; one base is present or absent at each allele
Variable number of tandem repeats	Multiple tandem repeats of identical DNA sequences.	
Microsatellites	Very short repeats (~1–10 base pairs)*	Multiple variants†; number of repeats (ie, length of variant) at each allele.
Minisatellites	Longer repeats (~10–100 base pairs) *	Multiple variants; number of repeats (ie, length of variant) at each allele.
Insertion/deletion	The insertion or deletion of more than one base pair.	Usually two variants; presence or absence of multiple base pairs at each allele.

*Definitions by length vary.

†As humans are diploid for all chromosomes (except the X and the Y in males), individuals can only carry a maximum of two variants for any given polymorphism regardless of how may variants exist in the population.

The human genome contains an estimated 25,000 *genes*, which can be defined as segments of contiguous DNA sequence that encode a product (usually a protein) and the associated sequences that regulate the formation of that product (ie, control timing, location, and quantity). Whether or not a DNA variant affects the phenotype (the observable characteristics of an organism [eg, susceptibility to AMS]) depends on the location and the nature of the variant in the gene. Most genes consist of exons, which contain protein-coding sequence; introns, which contain noncoding sequence; and upstream (5′) and downstream (3′) flanking regions (Figure 1). For a gene to be expressed ("turned on"), its DNA sequence must be transcribed into messenger RNA (mRNA), which is then used as a template for protein synthesis (ie, the process of translation). Only part of the gene is transcribed into mRNA, and the final mRNA molecule is composed of exons, as introns are removed ("spliced out"). Similarly, in the final mRNA molecule, the protein-coding sequence is flanked by 5′ and 3′ untranslated regions that are not translated into the protein's amino acid sequence. Regulatory elements can be located in various regions of the gene and are essential in governing expression either by following a preordained pathway (such as during development) or in response to a plethora of internal or external stimuli. Variants in the protein-coding sequence itself may potentially affect the structure and function of the protein (Table 2), whereas variants within flanking regions, untranslated regions, or introns may affect gene

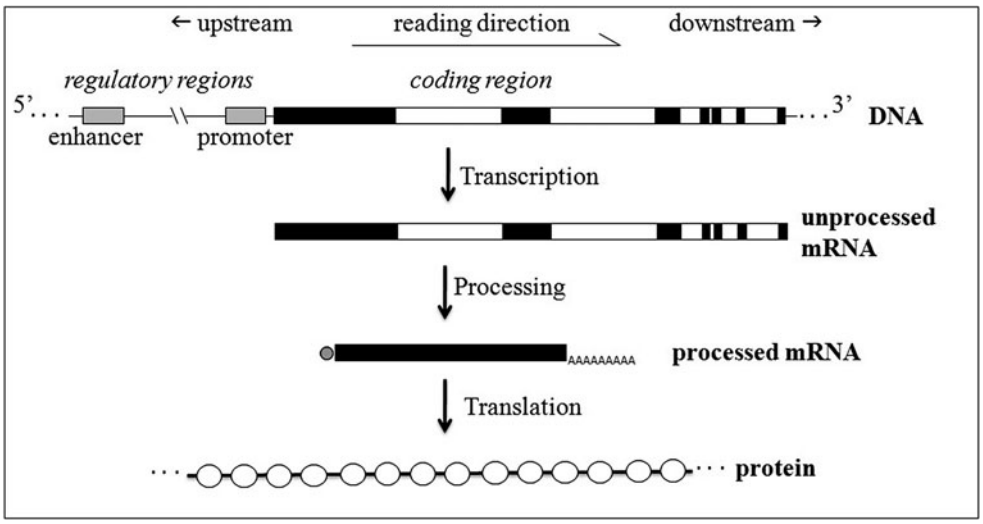

FIGURE 1. A cartoon of a typical protein-encoding mammalian gene. Exons (black) are separated by introns (white), which will be excised during processing and thus not be part of the mature RNA transcript. The gray boxes represent upstream regulatory regions where reading of the gene is initiated and controlled. The gene is transcribed as messenger RNA (mRNA), which is then processed (addition of a 3′ poly-A tail and 5′ cap and intron excision). The sequence of the mature mRNA is read by ribosomes and translated into the amino acid sequence that forms the structural core of the encoded protein. Upstream (5′) and downstream (3′) refer to the chemical orientation the DNA molecule and the information orientation of the gene. By convention, DNA is presented in a 5′ to 3′ orientation, which is the functional direction of most DNA-reading enzymes. Gene structure is highly diverse and this figure is only one example of potential intron/exon and regulatory region configurations.

TABLE 2. Potential Effects of Single Nucleotide Polymorphisms (SNPs) Occurring in Protein-Coding Sequences

SNP	Result	Example
Silent (synonymous)	No change in amino acid sequence due to the degenerate genetic code—no effect on protein structure.	TCT and TCA both encode serine.
Missense (nonsynonymous)		
Conservative	One amino acid is substituted for an amino acid with similar chemical properties—lesser effect on protein structure[+].	CTT and ATT both encode hydrophobic amino acids (leucine and isoleucine respectively).
Nonconservative	One amino acid is substituted for an amino acid with different chemical properties – greater effect on protein structure[+].	GAG encodes a hydrophilic amino acid (glutamic acid), GTG encodes a hydrophobic amino acid (valine).
Frameshift	The three-base (triplet) reading frame is altered by the addition of n+1 or n+2 bases (where n is 0, 3, 6, etc) so that the reading frame for all following triplets is changed.	The insertion of an A changes GTG GAG CCA to GTA GGA GCC, altering the amino acid sequence from Val-Glu-Pro. . . to Val-Gly-Ala. . ..
Nonsense	A triplet encoding an amino acid is changed to a stop codon that terminates translation prematurely.	TAT encodes tyrosine, TAA is a stop codon.

*Examples show changes in three-base DNA codons and the resulting changes in the amino acid structure of the protein.

regulation. Importantly, many variants appear to have no phenotypic consequence (these are termed "nonfunctional" as compared with "functional" alleles, which result in a change in the phenotype).

DNA mutations occur at a single location relative to other variants in, or near, the gene. Over generations, meiotic recombination between chromosomes separates the alleles; nevertheless, until the alleles are segregating independently in the population, they are said to be in linkage disequilibrium (LD) and move between generations as a "haplotype." This is highly significant in gene association studies as it provides an explanation as to why an ostensibly nonfunctional variant can be associated with a condition—it may be in LD with the functional variant. Researchers can take advantage of LD to increase the efficiency of association studies by selecting maximally informative variants that represent large haplotypes (eg, "TagSNPs," [http://hapmap.ncbi.nlm.nih.gov/])—essentially, one genotype serves as a marker for all genotypes on the haplotype (if LD is complete). As haplotypes vary between population, results based on LD can also vary, which can account for inconsistent findings (ie, a strong association of a nonfunctional variant with AMS in whites may not replicate in Africans despite the fact that the gene is involved). By using tagSNPs specific to the population, the entire gene can be interrogated for association with the trait; nevertheless, if an association is found, additional experiments may be needed to determine which variant(s) is/are causal variant(s). Most gene association studies of AMS have not addressed issues of linkage LD.[29]

Acute mountain sickness is an excellent example of an environmentally triggered phenotype. Although all people will develop AMS if they rapidly ascend to extreme altitudes, clearly, some individuals are more susceptible than others, developing the condition at moderate altitudes and/or with conservative ascent rates. Individual susceptibility without obvious Mendelian patterns of inheritance is consistent with the condition being a complex, environmentally mediated, polygenic trait. Genetic evidence supporting this postulate can be grouped into the three broad categories discussed next: individual susceptibility, familial clustering, and genotype associations.

Individual Susceptibility

An individual's previous history of AMS is the best predictor of AMS on subsequent ascents. On ascent to 4559 m, regardless of recent exposure and rate of ascent, individuals with a history of AMS were twice as likely to develop AMS as those with a history of altitude tolerance.[11] Similarly, those who reported suffering from a previous altitude illness were twice as likely to develop AMS on ascent to 4419 m compared with those with no history of altitude illness.[30] These studies alone are not necessarily evidence for a genetic etiology to AMS, as individual differences could be due to developmental effects (eg, exposure as a child); nevertheless, that some individuals are susceptible while others are resistant is consistent with an innate, and therefore possibly genetic, factor.

Familial Clustering

The family linkage study is a common tool in genetics; nevertheless, family studies of AMS are challenging for a number of reasons. Experimental studies would require transporting entire families to relatively extreme environments, while opportunistic samples are rare, presumably because most families will leave the highlands as soon as one member became sick (especially if that member is a child). Working with families in hypoxic chamber studies could overcome these problems but would be very time consuming because most chambers can accommodate only a small number of individuals. An alternative to families would be to use smaller cohorts of close genetic relatives (such as twins) as subjects; nevertheless, with the exception of a single study in which AMS was assessed in 17 twin pairs of preverbal children and their parents,[31] there have been no twin studies of altitude illness susceptibility. Of the seven children who developed AMS in the study by Yaron et al[31], six were sibling pairs. Zygosity of the twins was not reported in the article; nevertheless, two of the affected siblings were monozygous twins, one was dizygous, and the seventh affected child was one of a discordant pair of monozygous twins (M. Yaron, MD; written personal communication, 2006). The probability of six of seven cases being sibling pairs by chance alone is less than 10%; but, as the authors point out, a greater shared environment could account for the observed concordance between siblings. One other study measured AMS in parent-offspring dyads;[32] nevertheless, whether there was a concordance between the parent's and the child's responses to altitude was not reported.

Candidate Gene Association Studies

Candidate gene association studies are commonly used to investigate AMS. The genes selected usually encode proteins that are involved in physiological pathways thought to be associated with acclimatization to altitude. To date, 16 genes have been tested in a variety of populations and ascent conditions (Table 3).

Eight of these genes, components of a variety of biological pathways, were shown to have variants associated with AMS. The rationale underlying the candidacy of the gene and the overall results are discussed next. The genes are grouped into broad physiological categories; nevertheless, there is likely substantial overlap between categories, both at the genetic level (ie, gene to gene interactions) and the physiological level.

Blood Flow and Pressure

Endothelial NO synthase gene

Nitric oxide is a potent vasodilator with important roles in acclimatization[46] and adaptation[47,48] to hypoxia. In the vasculature, NO is produced by NO synthases (NOSs), including endothelial NOS, which is encoded by the *NOS3* gene, and acts locally by relaxing smooth muscles and thereby causing vasodilatation.[49] Acute hypoxia inhibits NO synthesis, and native lowlanders show decreased NO exhalation at altitude.[50] Several *NOS3* gene variants affect the expression and activity of the endothelial NOS enzyme,[51] levels of circulating NO,[48] and expired NO,[52] indicating that *NOS3* variants could affect high-altitude acclimatization. Brown et al[53] did not report any relationship between NO exhalation and AMS, but participants were subjected to a relatively short duration of hypoxia (~3 hours) and the AMS incidence was relatively low (11 participants, ~32%), limiting interpretation of their results.

Seven *NOS3* SNPs were selected for a study of AMS in Nepalese trekkers,[43] one of which (G894T) results in an amino acid substitution (Glu298Asp) in the enzyme. The distribution of genotypes of the G894T polymorphism was significantly different between the AMS and control groups; nevertheless, the allele and genotype frequencies from the other six *NOS3* gene polymorphisms were not significantly different between the groups. This study used tagSNPs specific to Han Chinese (a bioethnic group close to Nepalese) to interrogate variation across the entire gene. The G/G genotype was also more common in a highland Andean Quechua population compared with a lowland Mayan population, further supporting a role in altitude tolerance.[54]

Angiotensin I–Converting Enzyme, Angiotensin II Receptor Type I, and Bradykinin Receptor β_2 Genes

The *ACE* gene encodes angiotensin I–converting enzyme (ACE), which has an important role in the renin-angiotensin-aldosterone system (RAAS) that is responsible for maintaining fluid balance. Angiotensin I–converting enzyme degrades the vasodilator bradykinin and catalyses the conversion of inactive angiotensin I to angiotensin II, a potent vasoconstrictor that acts on the angiotensin II type I receptor (encoded by *AGTR1*). Angiotensin II also stimulates

TABLE 3. Candidate Gene Association Studies of Acute Mountain Sickness Susceptibility: Genes, Subjects, and Results

Gene*	Location	Polymorphism	Population†	Altitude	Association Results	Reference
ACE	17q23.3	Alu insertion/deletion; intron 16	European (104:47)	4,599 m; 3,611 m	No association	33
ACE	-	Alu insertion/deletion; intron 16	White (244:40)	3,817 m	Insertion/deletion genotype on day 1	34
ACE	-	Alu insertion/deletion; intron 16 A(-240)T; promoter A(2350)G; silent; exon 17	Nepalese (59:44)	4,380 m	No association	35
ACE	-	Alu insertion/deletion; intron 16	White‡	2,700–5,895 m	No association	36
ACE	-	Alu insertion/deletion; intron 16	Han Chinese (60:98)	3,670 m	D allele	37
ADRB2	5q31-q32	A/G; 5′ UTR A/G; 5; UTR G/A; 5′ UTR G/C; 5′ UTR A(285)G; arg16gly; exonic C(523)A; silent; exonic G(1053)C; silent; exonic	Nepalese (59:44)	4,380 m	No association	29
AGT	1q42-q43	T(704)C; met235thr, exon 2	Han Chinese (60:98)	3,670 m	T (met) allele	37
AGTR1	3q21-q25	A(1166)C; 3′ UTR	Nepalese (59:44)	4,380 m	no association	35
AGTR1	-	A(1166)C; 3′ UTR	Han Chinese (60:98)	3,670 m	no association	37
APOB	2p24-p23	A/G; silent; exon 2	Han Chinese (60:98)	3,670 m	no association	37
BDKRB2	14q32.1-q32.2	±9 base pairs; exon 1 C(-58)T; promoter	Nepalese (99:90)	4,380 m	no associations	38
GNB3	12p13	A(-350)G; 5′ UTR	Han Chinese (60:98)	3,670 m	A allele fixed in AMS cases	37
GSTM1	1p13.3	+/−	Chinese (80:43)	~3000 m	Negative (−/−) genotype	39
GSTT1	22q11.23	+/−	Chinese (80:43)	~3000 m	Positive (+/+, +/−) genotype	39
HSPA1A	6p21.3	C(+190)G; 5′ UTR	Chinese (173:56)	Not stated	no association	40
HSPA1A	-	G(+190)C; 5′ UTR	Han Chinese (100:56)	2,600–2,800 m	no association	41
HSPA1B	6p21.3	A(1267)G; silent; exon 1	Chinese (173:56)	Not stated	G/G genotype§	40
HSPA1B	-	A(1267)G; silent; exon 1	Han Chinese (100:56)	2,600–2,800 m	G/G genotype§	41
HSPA1L	6p21.3	G(2437)C‖; met493thr; exon 2	Han Chinese (100:56)	2600 m – 2800 m	B/B genotype¶	41
HIF1A	14q21-q24	C(1744T;) pro582ser; exon 2	Sherpa (59:45)	>>3200 m	no association	42
NOS3	7q36	G/A; intron 3 G(894)T; glu298asp; exon 7 A(1083)T; intron 14 A/C; intron 14 A/G; intron 21 T/G; intron 23 C/A;intron 24	Nepalese (70:22)	4380 m	no association G/T and T/T genotype no association no association no association no association no association	43
VHL	3p26-p25	C(589)T; exon 3 A/G; 5′ UTR T/C; intron 1 A/C; intron 2 A(1149)G; 3′ UTR	Sherpa (59:45)	>>3200 m	no associations	42
ABO blood type#	9q34.1-q34.2	A/B/O alleles	Not specified (666:374)	3952 m	no associations	44

ACE indicates angiotensin I–converting enzyme 1; ADRB2, adrenergic β_2 receptor; AGT, angiotensinogen; AGTR1, angiotensin II receptor–type 1; BDKRB2, bradykinin receptor $\beta 2$; GNB3, guanine nucleotide binding protein (G protein), beta polypeptide 3; GSTM1, glutathione S-transferase $\mu 1$; GSTT1; glutathione S-transferaseα; HSPA1A, heat shock 70 kDa protein 1A; HSPA1B, heat shock 70 kDa protein 1B; HSPA1L, heat shock 70 kDA protein 1-like; HIF1A, hypoxia-inducible factor 1; alpha subunit; NOS3, nitric oxide synthase 3, endothelial; VHL, von Hippel-Lindau tumor suppressor.

*Positive associations are in bold.

†Controls (without acute mountain sickness): Cases (with acute mountain sickness).

‡Variable, depending on altitude.

§The G/G genotype is designated B/B in the article.

‖Listed as T(2437)C elsewhere,[45] and dbSNP.

¶Allele designation was unclear.

#Assessed by blood type, rather than genotype.

the release of aldosterone, a hormone that increases sodium and water reabsorption in the kidneys. Overall, the activation of the RAAS increases extracellular fluid and blood pressure. As increased aldosterone and fluid retention are linked to the development of AMS,[55] variants in genes encoding components of the RAAS pathway could contribute to susceptibility or resistance.

The most commonly studied *ACE* polymorphism is the insertion (I) or deletion (D) of a 287–base pair fragment (*Alu* repeat) in intron 16.[56] Several studies have shown that the I allele is associated with lower serum ACE,[56] as are alleles at two less-studied SNPs (A-240T and A2350G).[57] Exposure to hypoxia decreases serum ACE concentration, with the decrease being greatest in acclimated individuals,[58] suggesting that genetic variants that lower circulating ACE could be advantageous for acclimatizing to altitude.

Attempts to correlate the RAAS genes with the development and severity of AMS have produced mixed results. Development and severity was independent of the *ACE* I/D genotype in European white trekkers ascending to 3611 m or 4559 m[33] and 5895 m.[36] Consistent with these studies, the incidence of AMS in a study of Nepalese trekkers was independent of genotypes at the *ACE* I/D, A-240T and A2350G polymorphisms and the *AGTR1* A1166C polymorphism.[35] In the same study cohort, variants of two bradykinin receptor $\beta 2$ polymorphisms (C-58T SNP and ± 9–base pair insertion/deletion) were also not associated with the condition.[38] Conversely, the *ACE* D and *AGT* 235M alleles, but not variants of the *AGTR1* A1166C polymorphism, were associated with AMS in a population of Han Chinese patients suffering from AMS on ascent to approximately 3670 m[37]; nevertheless, the description of the case and control groups was limited and may have included other forms of altitude illness. The *ACE* I/D polymorphism was also associated with the development and severity of AMS on day 1 of altitude exposure (presummit attempt, 3817 m) but not on day 2 (postsummit attempt, 3817 m) in a group of Europeans.[34] The ACE I/D polymorphism has been a staple in altitude research since Montgomery et al[59] reported the I allele to be overrepresented among elite mountaineers. Climbing success at extreme altitude involves many factors, among which resistance to AMS is likely minor; nevertheless, the results of Montgomery et al[59] could support a role for the I allele in AMS resistance, albeit indirectly. Mountaineers are a self-selected population, and individuals who are genetically predisposed to feeling ill at moderate altitudes would likely leave the sport before achieving the climbing status of the study group of Montgomery et al.[59] The lack of association of the *ACE* I/D polymorphism in different populations may be due to differences in LD and is not necessarily evidence contrary to an association.

Guanine Nucleotide–Binding Protein, β Polypeptide 3 Gene

The β polypeptide 3 (*GNB3*) gene encodes the guanine nucleotide–binding protein β_3 subunit, a component of the heterotrimeric guanine nucleotide–binding protein that functions to modulate signals between receptor and effector proteins in transmembrane signaling systems. A polymorphism of the *GNB3* gene (C825T) has been associated with hypertension.[60] The *GNB3*-350A allele, a variant of a polymorphism that is in strong LD with the C825T polymorphism, was associated with AMS in Han Chinese patients who ascended to approximately 3670 m and developed AMS[37]; nevertheless, blood pressure was not significantly different between the case and control groups, leaving the mechanism of this association unclear.

Pulmonary and Cardiovascular Function

β_2-adrenergic receptor gene

The β_2-adrenergic receptor (encoded by *ADRB2* gene) is a guanine nucleotide–binding protein–coupled receptor that acts as the primary catecholamine receptor in the lungs. At high altitudes, epinephrine increases acutely and acts on β_2 receptors to increase oxygen transport (via increased cardiac output, heart rate, stroke volume, and vasodilation) and to increase oxygen utilization efficiency.[61] The adrenergic tone (eg, increased peripheral vasodilatation and heart rate) of AMS patients was greater than controls,[62] implying a potential role for these receptors in AMS susceptibility. An analysis of seven *ADRB2* TagSNPs in a Nepalese population of trekkers did not show any associations with the development of AMS.[29]

Responses to Oxidative Damage

Glutathione S-transferase μ_1 and glutathione S-transferase υ_1 genes

Glutathione S-transferases (GSTs) are a group of intracellular enzymes that detoxify endogenous and exogenous substances via the addition of reduced glutathione. Glutathione S-transferase mu 1 (*GSTM1*) and glutathione S-transferase theta 1 (*GSTT1*) are two members of the GST family, and the deletion of these genes is common (~50% and 13%–20%, respectively).[63] A decrease in the activities of plasma GSTs was associated with AMS in Chinese soldiers.[64] In a follow-up study, the presence of the *GSTT1* gene (+/−, +/+ genotypes) and the absence of the *GSTM1* gene (−/− genotype) were independently associated with a higher prevalence of AMS.[39] Although the *GSTM1* null mutation association supports the previous study, the association of the *GSTT1*-positive genotype with AMS is difficult to explain. Subjects, who were both *GSTM1* and *GSTT1* null, were five times more likely to develop AMS than subjects who were *GSTM1* positive and *GSTT1* null, which seems to be in support of their initial study.

Heat Shock Protein (HSPA1A, HSPA1B, HSPA1L) Genes

Heat shock proteins (HSPs) are a group of intracellular proteins that are upregulated during times of stress (eg, heat, hypoxia, and oxidative stress). The HSP70 gene family, including *hsp70-1* (ie, *HSPA1A*), *hsp70-2* (ie, *HSPA1B*), and *hsp70-hom* (ie, *HSPA1L*), is involved in homeostatic adjustments to stress.[65] Polymorphisms in these genes are known to affect their proteins' functions and overall stress tolerance.[66] Two studies have associated variants of HSP gene polymorphisms with AMS in Chinese populations. Li et al[40] examined polymorphisms in the *HSPA1A* (b1/b2; G+190C) and *HSPA1B* (A/B[1]; A+1267G) genes. No association was found between the b1/b2 *HSPA1A* polymorphism and AMS, but the *HSPA1B* G/G genotype was overrepresented in individuals with AMS. Similarly, Zhou et al[41] examined the same polymorphisms in the *HSPA1A* and *HSPA1B* genes, as well as another polymorphism (A/B; G2437C) in the *HSPA1L* gene. This study reproduced the findings of Li et al,[40] and also showed that the *HSPA1L* B/B genotype was also overrepresented in the AMS group.

Hypoxia-Inducible Factor–1α Subunit Gene and Von Hippel-Lindau Tumor Suppressor Protein Gene

Hypoxia-inducible factor–1α (HIF-1α) in combination with the HIF-1β subunit forms the HIF-1 transcription factor that controls the expression of genes involved in maintaining oxygen homeostasis.[67] The HIF-1α subunit is constitutively expressed but is degraded under normoxic conditions and upregulated under hypoxic conditions.[68] The *VHL* gene encodes the von Hippel-Lindau tumor suppressor protein, which is part of a larger complex of proteins responsible for the ubiquitination and degradation of HIF-1α.[68] One SNP from the *HIF1A* gene and five SNPs in the *VHL* gene were studied in Sherpas with and without a history of AMS, but no associations were seen.[42]

Ventilation and the Hypoxic Ventilatory Response

Several studies have examined the role of genetics in the hypoxic ventilatory response (HVR), which is the compensatory increase in ventilation (rate and depth) elicited by a decrease in arterial oxygen pressure. A relatively low HVR is a plausible risk factor for AMS, as a low HVR indicates a blunted increase in ventilation in hypoxia, leading to reduced oxygen delivery and possibly AMS; nevertheless, studies linking HVR to AMS have been conflicting possibly because of the wide variation in methodologies for the measurement of HVR).[69,70]

There is evidence that HVR is genetically determined,[71] and three studies have assessed the potential role of specific genes. Subjects with an *ACE* I/I genotype had a greater increase in minute ventilation and a greater decrease in end-tidal carbon dioxide in response to exertional hypoxia compared with those with the I/D and D/D genotypes.[72] In contrast, none of the selected alleles from succinate dehydrogenase subunit (*SDHB*, *SDHC*, *SDHD* [subunits B, C, and D, respectively]), *ACE*, and *HIF1A* genes was associated with the magnitude of the HVR,[73] nor was there an association between *ACE* genotype and isocapnic HVR.[74]

Oxygen Management

Acute mountain sickness was rare in individuals at 4380 m who had an oxygen saturation (measured by pulse oximetry [SpO_2], a noninvasive proxy for arterial oxygen saturation [SaO_2]) above 86% (ie, a 92% negative predictive value).[75] Also, low S_aO_2 values while sleeping were associated with AMS.[76] S_aO_2 had a relatively high heritability (the contribution of genotype to phenotypic variability) in two separate Tibetan highland populations, with a major autosomal dominant gene likely contributing large portions of the total variance.[77,78]

Several studies have investigated the role of genes on S_aO_2 at altitude. *ACE* genotype was independent of S_aO_2 in a group of whites ascending slowly (18.5 days) from 2800 to 5180 m, but among those who ascended rapidly (12 days), subjects with the *ACE* I/I genotype had a higher S_aO_2 than those with the I/D and D/D genotypes.[79] Overall, these data suggest that any role that the *ACE* I/D genotype plays in AMS may be secondary to its effect on S_aO_2, although the study of Wood et al illustrates the confounding gene: environment effect of ascent rate that complicates the reconciliation of data from diverse studies. A polymorphism from a second gene in the RAAS, *AGT*, was also associated with S_aO_2 at 3670 m in patients with AMS,[37] demonstrating that variants of multiple genes could contribute to individual differences in S_aO_2.

Other Systems

ABO blood group gene

The ABO blood group (*ABO*) gene has three main alleles (A, B, and O) that determine a person's blood type (eg, A, B, AB, or O). Alleles of the *ABO* gene were associated with mean ACE activity in hypertensive Han Chinese patients, but the physiology behind this association is unclear.[80] In a large epidemiological study, blood type was not a significant determinant of AMS development in a population of trekkers who ascended 3952 m on Yushan (Jade Mountain) in Taiwan.[44] No other AMS studies have ascertained subjects' ABO blood types.

Apolipoprotein B gene

Apolipoprotein B gene (*APOB*) encodes apolipoprotein B, the primary apolipoprotein in chylomicrons and low-density lipoproteins. Apolipoprotein B binds to the low-density lipoprotein–receptor on hepatic cells and triggers the uptake of LDL into the liver, where it is degraded. There is a strong genetic component in the determination of plasma apolipoprotein B concentration.[81] No association was found between variants of an *APOB* SNP and AMS in a Han Chinese population.[37]

LIMITATIONS AND CONSIDERATIONS

As much of the data supporting a genetic contribution to AMS are candidate gene association studies, potential (and not uncommon) weaknesses in this experimental design should be considered. Chief among these is population stratification: the unequal distribution of alleles across different populations due to heterogeneous ancestry. This can result in spurious findings if by chance the allele of interest is differentially represented in the case versus control cohorts. To minimize this problem, cases and controls should be matched for ancestry,[82] or if the cohorts differ (as is often the case for samples of convenience such as trekkers sampled in the mountains), corrected for this variability. Dealing with this confounding factor has been greatly facilitated by the characterization of highly informative ancestry-specific markers (ancestry informative markers) that permit researchers to quickly assess the background composition of their study groups. A related issue is LD which refers to the physical proximity of alleles on chromosomes. A nonfunctional genetic marker may associate with a trait because of being physically close (ie, linked) to the functional allele (ie, on the same haplotype). As patterns of LD vary between populations, associations such as this can be population-specific. Although a noteworthy confounder, LD also contributes to the power of genetic studies, especially genome-wide association studies, as a limited number of highly variable, nonfunctional markers can be used to rapidly screen large regions of chromosomes for potentially functional variants.

Association studies are also highly dependent on the accuracy of phenotype characterization, which can be very challenging for an environmentally triggered condition with nonspecific symptoms. Attempts to compare and reconcile studies need to consider altitude attained, ascent rates, subject health, weather, latitude (which affects barometric pressure), and assessment tools (especially self-report vs clinical evaluation).

There are also statistical challenges inherent to candidate gene association studies. Sample size is a concern because a lack of an association may not reflect a true negative but rather lack of statistical power to detect a small effect. Alternatively, positive associations may be spurious as the large number of genes and polymorphisms tested increases the odds of obtaining false positives. To reduce this risk, corrections for multiple hypotheses testing such as the Bonferroni method are required.

The risk of false positives because of multiple testing or the inability to reproduce findings because of population stratification or phenotype mischaracterization are among the reasons that some genetics journals now require follow-up phenotype (ie, functionality) studies or replicated associations before accepting data for publication.

ADAPTATION AND HIGH-ALTITUDE POPULATIONS

There is substantial interest in the genetic basis of altitude adaptation of highland native populations, such as Tibetans, Ethiopians, and Andeans.[83–85] Although transient responses to acute hypoxia do not necessarily mimic evolutionary adaptive changes in response to chronic hypoxia (eg, the hypoxia-triggered erythropoiesis in sojourners compared with the blunted hematological response to hypoxemia in Tibetans), genetic variants that are shown to be more common in highlanders may be worth investigating in AMS for two reasons: (1) The advantage conferred by the variant may be beneficial under both acute and chronic hypoxia and (2) migrational selection may have favored AMS resistance in the founding population (ie, individuals prone to AMS may have self-selected not to colonize high-altitude environments because of discomfort). Although the

more serious altitude illnesses (high-altitude pulmonary edema and HACE) could have been selected because of differential mortality, AMS is likely too benign to exert much selective pressure beyond differential migration.

Four recent studies have used genomic approaches to interrogate the entire genome (or the entire expressed genome; the "exome")[86–89] for genetic variants that might contribute to high-altitude adaptation. Although this article is not a review of high-altitude adaptation per se, the major findings of each study are briefly discussed next, as these articles could guide future research on AMS (in terms of both specific hypotheses and general techniques).

When compared with a lowland Han Chinese population, variants of the *EPAS1* gene were associated with high altitude Tibetan populations residing at 3200 to 3500 m.[86] This association was reproduced in two other distinct Tibetan populations at 4200m and 4300m from the same study[86] and in Tibetan populations from three other studies at 4350 m,[88] > 4300 m,[89] and between 3000 and 4400 m.[87] *EPAS1* encodes HIF-2α, which like HIF-1α (explained previously), is a transcription factor that dimerizes with HIF-1β and turns on the expression of certain genes regulated by oxygen concentration under hypoxic conditions. A mutation in this gene is associated with polycytosis,[90] and interestingly, the major alleles of associated variants from the *EPAS1* gene were also associated with lower hemoglobin levels in both studies. This indicates that these alleles may also be protective against chronic mountain sickness, a disease more prominent in Andeans than Tibetans that is characterized by polycythemia.[91] Variants of egl nine homolog 1 (EGLN1) and peroxisomeproliferator-activated receptor alpha (PPARA) were also associated with lower hemoglobin levels in Tibetans.[88] EGLN1 is involved with cellular oxygen sensing, and certain *EGLN1* mutations lead to polycythemia.[92] PPARA agonists are known to cause lower hemoglobin concentrations in humans.[93] Additional genes from HIF and other pathways were associated with high-altitude Tibetans but not with hemoglobin levels.[88] Although variants of *EPAS1*, *EGLN1*, and *PPARA* may be responsible for lower hemoglobin production directly, it is possible that these variants act on other components of the phenotype, making lower hemoglobin levels artifacts of "better" acclimatization that requires less hemoglobin for individuals to survive at altitude.[88,89] A separate study by Bigham et al[87] reported variants of *EGLN1* to be associated with both Andean and Tibetan high-altitude populations indicating possible convergent evolution between the two populations.[87] Variants of two other HIF pathway genes, *PRKAA1* (protein kinase, adenosine monophosphate–activated, α_1 catalytic subunit) and *NOS2A* (NOS 2A) but not *EPAS1,* were associated with a high-altitude adaptation in an Andean population, but this study did not assess hemoglobin concentration. *NOS3* variants associated with resistance to AMS have also been shown to be more common in Andean highlanders.[54]

CONCLUSIONS AND FUTURE CONSIDERATIONS

Although AMS is an environment-dependent illness, current evidence is consistent with a multifactorial etiology including genetic factors. To date, 16 genes have been tested for an association with AMS, eight of which have variants that were reported to be significantly more common in people with AMS: *ACE*, *AGT*, *GNB3*, *GSTM1*, *GSTT1*, *HSP70A1B*, *HSP70A1L*, and *NOS3*. Although these results are correlative, the physiological function of each gene's product is congruent with a causal relationship. Positive associations were also found between variants of the *ACE* gene and HVR and between variants of the *ACE* and *AGT* genes and S_aO_2 (at altitude) but the significance of a low HVR or low S_aO_2 in the development of AMS remains controversial.

Although the genes that have been investigated represent a number of pathways, studies have focused primarily on proposed causal variants. Few studies have been done on genes that may contribute to the principal symptom of AMS—headache. A number of genes may be involved in primary headache[94] and these would be promising candidates for future association studies. Other categories of genes that warrant study are those involved in vascular permeability and vasogenic edemas (eg, the gene encoding aquaporin 4)[95] and balance.[96]

The recent studies on the genetics of highland natives discussed earlier demonstrate the power of genome-wide studies. The next generation of AMS studies will likely use array or high-throughput sequencing technology to investigate hundreds of thousands of polymorphisms in a single experiment. Such experiments require large sample sizes and the validity of the results is greatly affected by the homogeneity of the initial phenotypes (ie, AMS+ and AMS−). Unfortunately, most AMS studies are "opportunistic" cross-sectional studies, and while most researchers use the LLS system to assign AMS status, characterization of the condition could be improved if there were standardized exposure metrics (altitude and ascent rate). Large-scale genomic experiments would be facilitated if there was an AMS registry from which large numbers of well-characterized subjects could be recruited, although comparisons between cohorts of different bioethinic origins would have to take into consideration that patterns of LD could vary between the populations, which could lead to conflicting data.

Further investigations into the genetic etiology of AMS will increase our understanding of the pathophysiology of the condition, while simultaneously enhancing our capacity for prediction, prevention, and/or treatment. In addition, at-risk individuals, identified by screening on the basis of genetic markers or family histories, could be instructed to take extra precautions (eg, slow ascent or minimal altitude) or to take prophylactic medication. Ultimately, identifying hypoxia-tolerant or hypoxia-sensitive individuals may be possible. Integrating these individual "altitude phenotypes" with environmental factors could contribute to healthy, productive, and enjoyable travel in the world's highlands.

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