

ACE Insertion / Deletion polymorphism and human performance at high altitude

MD(Res) Thesis

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Abstract

The hypoxia of high altitude ('hypobaric hypoxia') presents a profound physiological challenge to the human body and at extreme high altitude the human body nears the limits of its tolerance for oxygen deprivation. Physical performance in this environment is limited by two major physiological elements: 'acclimatisation' to hypobaric hypoxia sufficient to allow strenuous physical exertion, and the avoidance of Acute High Altitude Illnesses (AHAI). The rate and effectiveness of acclimatisation and the susceptibility to AHAI varies markedly between individuals, suggesting a possible genetic influence on high altitude performance.

A polymorphism of the human Angiotensin Converting Enzyme (ACE) gene has been identified in which the deletion (D-allele), rather than the insertion (I-allele), of a 287 base pair sequence is associated with higher circulating and tissue ACE activity. This polymorphism has also been associated with physical performance phenotypes, the ACE I-allele being associated with elite endurance performance. An excess frequency of the ACE I-allele has also been identified in a small sample of elite UK high altitude mountaineers.

This thesis set out to test the hypothesis that the ACE I-allele is indeed associated with successful physical performance at high altitude, and to explore the mechanism by which such an advantage may be mediated. I conducted a series of prospective gene-environment interaction studies to assess whether the ACE I-allele is associated with successful ascent to high altitude and, if such an advantage exists, whether this is mediated by reduced susceptibility to Acute Mountain Sickness (AMS) or improved oxygen saturations. To further define the underlying mechanism, I explored the association of bradykinin 2 receptor genotype with high altitude performance to

investigate whether the ACE I allele contribution is mediated by increased kinin activity. Additionally I extended these observations to those who have faced the most extreme hypoxic challenge - a successful ascent of Mount Everest.

“I am nothing more than a single narrow gasping lung, floating above the mists and
summits.”

Reinhold Meissner, the first human to reach the summit of Mount Everest without the
use of supplemental oxygen.

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Abbreviations

| | |
|-------------------------------|---|
| ACE | Angiotensin Converting Enzyme |
| AHAI | Acute High Altitude Illness |
| AMS | Acute Mountain Sickness |
| ATP | Adenosine Triphosphate |
| BK ₂ R | Bradykinin 2 Receptor |
| D | Deletion |
| HACE | High Altitude Cerebral Edema |
| HAH | High Altitude Headache |
| HAPE | High Altitude Pulmonary Edema |
| HIF | Hypoxia-Inducible Factor |
| HPVR | Hypoxic Pulmonary Vasoconstriction Response |
| HVR | Hypoxic Ventilatory Response |
| I | Insertion |
| LLS | Lake Louise Score |
| NO | Nitric Oxide |
| RAS | Renin Angiotensin System |
| S _a O ₂ | Arterial oxygen saturations |
| VO ₂ max | Maximal systemic exertional oxygen uptake |

CHAPTER 1: Introduction

- 1.1 Humans at high altitude
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1.1 Humans at high altitude

1.1.1 Hypobaric hypoxia of high altitude

“We live submerged in an ocean of air, which by unquestioned experiments is known to have weight.” Evangelista Torricelli (1608-1647)(1)

As altitude above sea level increases, the barometric pressure falls, resulting in a decreased atmospheric partial pressure of oxygen. Oxygen is critical to normal cellular function: acting as the ultimate proton acceptor of the mitochondrial electron transport chain, it is essential to aerobic cellular ATP synthesis. As such, the hypoxia of high altitude (‘hypobaric hypoxia’) presents a profound physiological challenge to the human body. At extreme high altitude the human body nears the limits of its tolerance for oxygen deprivation. An arterial blood gas taken from a healthy individual at 8400m near the summit of Mount Everest recorded a partial pressure of arterial oxygen (P_aO_2) of 2.55 kPa (2) and acute exposure to this degree of hypoxia would be rapidly fatal (3). However, with time, adaptive change (‘acclimatisation’) does allow some individuals to survive such extreme hypoxia.

1.1.2 High altitude performance

High-altitude mountaineers perform extreme endurance exercise in a hypoxic environment. Altitude regions have previously been defined as high altitude (1500-3500 m), very high altitude (3500-5500 m), and extreme altitude (>5500 m) (4). In addition to the isolated hypoxic physical challenge, safe and successful ascent and descent of mountains can be threatened by a diverse range of hazards that include extremes of temperature and weather, trauma, hypothermia and high altitude illness (5, 6). If the objective dangers are avoided, survival and performance at high altitude are limited by two major physiological elements: acclimatisation to hypobaric hypoxia sufficient to

allow strenuous physical exertion, and the avoidance of Acute High Altitude Illnesses (AHAI). The rate and effectiveness of acclimatisation and the susceptibility to AHAI varies markedly between individuals, suggesting a genetic influence on high altitude performance (7, 8).

1.1.3 Acclimatisation to hypobaric hypoxia

Acclimatisation is the set of beneficial processes whereby lowland humans respond to a reduced partial pressure of oxygen (PO_2) in inspired air. The systemic changes of acclimatisation, as classically described, include hyperventilation, polycythemia, changes in oxygen affinity of haemoglobin, and angiogenesis in peripheral muscle and have been extensively described (9). These processes tend to increase convective oxygen delivery to the tissues and take weeks or perhaps even months to optimize hypoxic acclimatisation. The importance of some of these classical mechanisms have been challenged, with the benefit of increased 2,3-DPG in shifting the haemoglobin oxygen dissociation curve to the left having little effect in vivo (10) and the increases in capillarisation and mitochondrial density appearing only marginal (11).

The Ventilatory Response to Hypoxia.

Exposure to hypoxia results in stimulation of the peripheral chemoreceptors, principally the carotid bodies, which sense the low PO_2 in arterial blood (12). Acute hypoxia stimulates an immediate increase in minute ventilation that peaks within minutes and then declines towards pre-hypoxic levels over the following minutes-to-hours. At high altitude, sustained hypoxia causes ventilation to rise again over subsequent hours, surpassing acute hypoxic levels and continuing to rise for several days. It is believed that oxygen-regulated alterations in gene expression cause changes in carotid body and ventral medullary function (13). This progressive increase in ventilation is known as

ventilatory acclimatisation to hypoxia, and the resultant elevation in arterial PO₂ mitigates the impact of environmental hypoxia.

The Cardiovascular Response to Hypoxia.

Acute hypoxia stimulates a progressive increase in cardiac output over several days, associated with an elevation in heart rate rather than stroke volume. Cardiac output gradually normalises as individuals acclimatise, although heart rate may remain high accompanied by a lower stroke volume (14, 15). Prolonged hypobaric hypoxia is associated with sustained, but reversible, changes in cardiac mass, stroke volume, function, and energy metabolism (16). Maximal cardiac output decreases with acclimatisation (both maximal stroke volume and maximal heart rate fall) and this is not explained by hypovolaemia, acid-base status, polycythaemia, autonomic changes or impaired systolic function. Pulmonary artery pressure remains persistently elevated and rises further with exercise (14).

Polycythaemia in Response to Hypoxia.

On arrival at high altitude, haemoconcentration occurs secondary to insensible losses, third space fluid redistribution, hormonal induced diuresis and altered thirst (17). However an increase in the secretion of erythropoietin is detectable within 90 minutes of exposure to acute hypoxia (18) and induces a true increase in haemoglobin production that develops slowly, taking several days to initiate and continuing to rise for up to eight months. This haemoglobin increase is unlikely to be an important factor in acclimatisation over the first days to weeks at altitude but subsequently the combination of increased haemoglobin and ventilatory acclimatisation can result in a blood oxygen content that may even exceed that at sea level (2).

Cellular mechanisms of acclimatisation.

Increasingly well characterized but incompletely elucidated are the molecular and cellular changes that are associated with acclimatisation (19-21). Supporting the concept of cellular changes in acclimatisation are studies that demonstrate that maximal systemic exertional oxygen uptake ($VO_2\text{max}$), cardiac output and skeletal muscle metabolism alter in response to hypobaric hypoxia and that these changes are not reversed by normoxia (16, 22, 23). Some authors have attributed the reduction in $VO_2\text{max}$ to a redistribution of blood flow to non exercising tissue (22) however others have proposed that hypoxia drives a coordinated transcriptional response that minimises hypoxic injury, redresses oxygen debt and achieves cellular acclimatisation by decreasing cellular oxygen demand (3). This concept of a reduced energy state is supported by hypoxia induced alterations in skeletal muscle metabolic substrate patterns and mitochondrial biogenesis that may optimise energy metabolism and reduce oxidative stress (7, 21, 24-26). Cellular hypoxia tolerance has been demonstrated in skeletal muscle with changes in glucose metabolism from oxidative to more anaerobic ATP production providing reduced oxidative stress and acute protection of myofibres against lethal ischemia (16, 26, 27).

Hypoxia-inducible factor and oxygen sensitive control of gene transcription

The hypoxia-inducible factor (HIF) family of transcription factors is now known to play a central regulatory role in these homeostatic changes at both the systemic and cellular levels; HIF directly or indirectly regulates several hundred genes, coordinating the response to hypoxia and initiating a wide spectrum of physiological responses that include changes in mitochondrial energy utilization, anaerobic metabolism,

erythropoiesis, angiogenesis and ventilation via the function of the carotid body chemoreceptor (28).

HIF-1 is a heterodimer consisting of HIF-1 β dimerised to an alpha subunit (one of HIF-1 α , 2 α or 3 α). HIF-1 α and HIF-2 $\alpha\beta$ heterodimers activate transcription of oxygen regulated target genes. Under normoxic conditions, HIF- α is continuously expressed and rapidly degraded whereas under hypoxic conditions, prolyl hydroxylation and proteasomal degradation are slowed, resulting in stabilisation and accumulation of HIF- α . The HIF- α subunit is then translocated to the nucleus where it dimerises with HIF- β , binds to the hypoxia response elements (HRE) of HIF-target genes and activates their transcription. The HRE is present in more than 70 genes (28).

1.2 Renin Angiotensin Systems

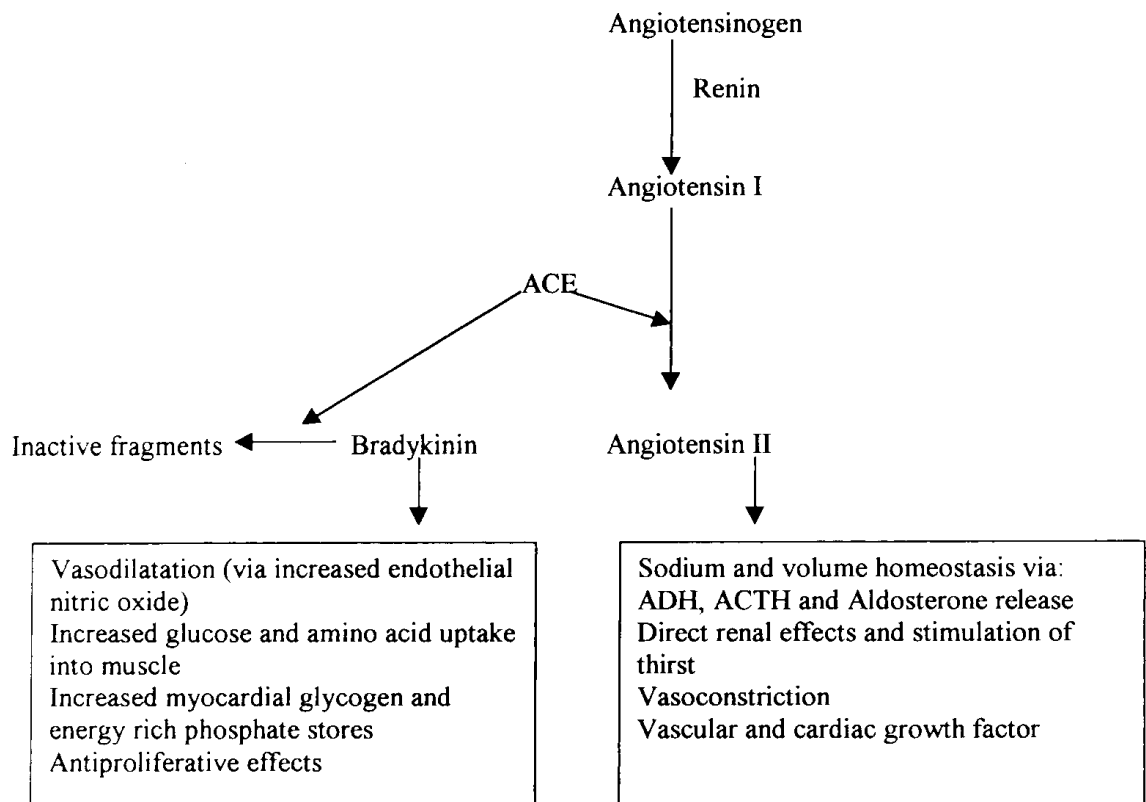
1.2.1 Circulating RAS

The endocrine Renin Angiotensin System (RAS) is a key homeostatic regulator of fluid balance and blood pressure. In response to decreased afferent arteriolar pressure, decreased filtered sodium load in the nephron ultra-filtrate or to sympathetic nervous stimulation, the renal juxtaglomerular apparatus releases the aspartyl protease, renin (29). Renin cleaves hepatically-derived angiotensinogen to yield angiotensin I. Angiotensin-converting-enzyme (ACE) is a key component of the RAS and hydrolyses Angiotensin I to generate octapeptide Angiotensin II (30). Two receptors, AT₁R and AT₂R, mediate the effects of Angiotensin II, although the roles of the AT₂R are less well defined. Angiotensin II binding to AT₁R causes an elevation in arterial blood pressure through both salt and water retention provoked by adrenal aldosterone release, and direct arterial vasoconstriction. Via this vasoconstrictor action, Angiotensin II has a role in mediating hypoxic pulmonary vasoconstriction. (31-33). Angiotensin II is degraded by vascular and erythrocyte angiotensinases to Angiotensin III and Angiotensin IV, which have similar circulatory effects to that of Angiotensin II. Angiotensin IV also has its own specific functional receptor (34).

ACE also cleaves the vasodilator bradykinin, a 9-amino acid peptide member of the kinin-kallikrein system (35). Bradykinin is a potent endothelium-dependent vasodilator that has a brief duration of action with a plasma half-life of approximately 15–30 seconds due to its rapid degradation by ACE (36, 37). As such, ACE efficiently breaks down approximately 95% of bradykinin in a single passage through the pulmonary circulation (38). It also causes contraction of non-vascular smooth muscle in the bronchus and gut and increases vascular permeability. Bradykinin acts upon the

bradykinin type-1 receptor (BK₁R), induced by tissue injury, and the constitutively expressed BK₂R, which stimulates a variety of inflammatory and vascular responses and which may engage in cross-talk with other RAS components (39-43). Bradykinin levels are inversely related to ACE activity (41) with increasing circulating ACE activity diminishing hypotensive responses due to reduced vasodilation via BK₂ receptor activation.

Figure 1.1 Circulating Renin Angiotensin System (adapted from Woods and Montgomery 2001).



In addition, a second angiotensin converting enzyme (Angiotensin converting enzyme 2, or ACE2), not only generates Angiotensin II from Angiotensin I but also acts upon Angiotensin II to synthesise Angiotensin (1-7), an angiotensin I heptapeptide (44, 45). Angiotensin (1-7) acts through the Mas receptor (46), and produces vasodilation via

activation of bradykinin and nitric oxide. ACE2 therefore negatively regulates the RAS and acts as an endogenous ACE inhibitor through alterations in formation of angiotensin II and angiotensin (1-7).

1.2.2 Local tissue RAS

In addition to the classical circulating RAS, a local RAS has now been identified in tissues as diverse as the heart (47), kidney (48), vasculature (49), adipose tissue (50), liver (51), nervous tissue/brain (48), adrenals (52), gonads/reproductive system (53), the gastrointestinal system (54) and the pancreas (49, 55). Such local RAS are believed to have paracrine, autocrine and intracrine roles that include the regulation of cell growth, differentiation, apoptosis, and proliferation; metabolism and generation of reactive oxygen species and free radicals; tissue inflammation and fibrosis; local haemodynamics; and hormonal secretion and reproduction (56-58) (49, 59-62).

1.2.3 RAS and altitude

The central role of the classical RAS in circulatory homeostasis has long suggested a role in the acclimatisation to hypobaric hypoxia (63-69). Early studies demonstrated that ACE activity changed during the acclimatisation response with circulating ACE activity falling on exposure to high altitude (69), and more so amongst ‘good acclimatisers’ (66).

There is a range of proposed mechanisms by which RAS may influence the processes involved in acclimatisation to high altitude discussed above. Angiotensin II is known to modulate *hypoxic pulmonary vasoconstriction* and this response is attenuated by AT₁ receptor antagonism (70) and ACE inhibition (71). Furthermore, pharmacological inhibition of ACE activity reduces pulmonary artery pressures in patients with high

altitude pulmonary hypertension (72).

Angiotensin II may additionally have a role in the regulation of *hypoxic respiratory drive*, through modulation of carotid body chemoreceptor activity (73) and its central transduction (74).

The RAS has been implicated in the *control of erythropoiesis* by observations that ACE inhibitors have proved effective for the treatment of secondary polycythaemia following renal transplant (75). Proposed mechanisms for this effect include lowering of erythropoietin production by increasing renal blood flow; a fall in sodium reabsorption and, thus, in oxygen consumption, which leads to increased oxygen availability for erythropoietin-producing cells; and interruption of a direct effect that angiotensin II might have on erythropoiesis (76), possibly mediated by an up-regulation of angiotensin type 1 receptors on erythroid precursors (77). Furthermore, ACE inhibitors have been demonstrated to ameliorate the polycythaemia of chronic mountain sickness (78).

Meanwhile, exercise-related sodium and fluid retention occur at HA and may contribute to AMS and pulmonary interstitial edema. A potential beneficial adaptation of RAS is that the plasma renin-induced release of aldosterone is blunted at altitude, which in turn may encourage natriuresis and diuresis (67, 79).

Contributing to successful adaptation to high altitude may be the RAS influence on metabolic efficiency and muscle fibre type that is described later and which is argued to contribute to its role in sea level athletic performance (80-83). It has been proposed that metabolic efficiency is likely to be increasingly important in the hypoxic environment of high altitude and that RAS may influence the cellular and molecular elements of hypoxic acclimatisation (3).

1.3 RAS polymorphisms associated with physical performance

1.3.1 ACE Insertion / Deletion polymorphism

Several functional polymorphic variants of the genes encoding various RAS components have been identified, but the most extensively studied is the ACE Insertion / Deletion polymorphism. The presence (insertion, I) rather than absence (deletion, D) of a 287 base-pair fragment Alu repeat sequence within intron 16 of the ACE gene is associated with lower circulating ACE activity and the accounts for 47% of the total phenotypic variance of serum ACE in Caucasians (84). Similar findings exist in cardiac tissue and in monocytes (47, 85).

In 1998 this ACE I/D polymorphism was the first gene variant to be associated with human physical performance (86). In these early studies, the I-allele was demonstrated to be strongly associated with fatigue resistance. Maximum duration of a standardized repetitive elbow flexion exercise (using a 15 kg barbell) was recorded in 78 Caucasian military recruits before and after 10 weeks of identical military training. Baseline performance was independent of ACE genotype, unlike improvements in exercise duration with training, which were strongly genotype-dependent; gains of 79.4 +/- 25.2 and 24.7 +/- 8.8 seconds were seen in those of I/I and I/D genotype, respectively ($p = 0.005$ and 0.007), but not in D/D homozygotes (7.1 +/- 14.9 seconds; $p = 0.642$). The I/I homozygotes thus showed an 11-fold greater improvement than those of D/D genotype (87). This association with endurance performance was supported by Myerson et al. who demonstrated in a group of 91 elite British runners that I-allele frequency increased with competitive distance, from 0.35 to 0.53 and 0.62 for the three distance groups 200m, 400–3000 m and 5000 m, respectively ($p = 0.009$ for linear trend) (88). In part, this seems to be related to enhanced training-related oxygen economy (89) and

metabolic efficiency (80).

Strong ACE I/D genotype-dependent associations were seen in other responses to physical training with an exaggerated left ventricular growth response association with the ACE D-allele in 140 Caucasian male military recruits following a 10 week physical training programme (87).

Since these landmark studies, a significant body of literature has developed to support these associations and this has been recently reviewed by Puthuchery et al. (90). The mechanisms underlying the association with physical performance have not been fully elucidated but ACE I/D genotype dependent differences in cardiac muscle growth (87, 91), skeletal muscle (92) and metabolic efficiency (80, 93-95) have been demonstrated. Although conflicting data exist, the I allele appears to be associated with elite endurance events (88) and the D allele with strength and power performance in Caucasians. This association is ethnicity/race dependent and not seen in subjects of black African origin (96), amongst whom ACE I/D genotype has little influence on ACE activity (97).

1.3.2 Bradykinin receptor polymorphisms

The central role of ACE in the RAS and the biological plausibility that the ACE I/D functional polymorphism has a significant role in performance is strengthened by studies of other functional polymorphisms in the RAS. The role of ACE in cleaving bradykinin means that bradykinin levels are inversely related to ACE activity (41). The biological action of bradykinin is mediated through the activation of two G-protein-coupled kinin receptor subtypes, the inducible bradykinin type 1 (BK₁R) and constitutive type 2 (BK₂R) receptors. The vascular BK₁ receptor is normally expressed very weakly but is markedly upregulated in the presence of inflammation and

cardiovascular disease (98, 99). The endothelial cell associated BK₂R is constitutively expressed in most tissues, and is most highly expressed in the pulmonary vasculature. It is considered a much stronger mediator of vasodilation through increased production and release of nitric oxide at the endothelial level (100, 101).

In humans, the BK₂R gene has been mapped to chromosome 14q32, is more than 25 kb in size, and consists of three exons. It demonstrates a common functional polymorphism in which the absence (-9) of a 9 base pair repeat is associated with greater gene transcription (102) and higher mRNA expression of the receptor (103), while its presence (+9) is associated with increased vascular tone and systemic hypertension (104). The -9 allele has been shown to be associated with greater skeletal muscle metabolic efficiency (ratio of internal work performed to external work measured) (105). If part of the effect of the ACE I/D polymorphism on performance is mediated by increased bradykinin activity, the combination of the ACE I-allele and the BK₂R -9 allele should confer 'high kinin receptor activity' and potentiate the phenotypic advantage of this state. This ACE I / BK₂R -9 allele combination has been associated with endurance performance in a study of 81 Olympic-standard track athletes, and altered left ventricular growth in response to physical training (105, 106).

1.4 ACE I/D polymorphism and high altitude

1.4.1 ACE I/D polymorphism and high altitude performance

Given the association between the ACE I/D polymorphism and elite athletic performance, it appeared possible that an I-allele association with hypoxic performance might exist. Montgomery et al. demonstrated the first association between ACE I/D polymorphism and high altitude performance in a small group of elite high altitude mountaineers (86, 107). ACE I/D genotype was determined in 25 male mountaineers who had ascended to over 7000m without supplementary oxygen and compared with that of 1,906 healthy British males. ACE I/D genotype distribution and allele frequency differed significantly between climbers and controls ($P=0.02$ and 0.003 respectively), with a relative excess of II genotype and deficiency of DD genotype found in the climbers. Among the 15 climbers who had ascended beyond 8,000 m without oxygen, none was homozygous for the D allele (6 II and 9 ID: I allele frequency = 0.65). Although the association between the ACE I/D and elite high altitude mountaineers remained highly topical in subsequent years (81, 88, 89, 94, 107-109), no further studies attempted to confirm the finding of this small retrospective study.

The partial pressure of oxygen at an altitude of 5000m is approximately half that at sea level, and this presents a significant but lesser physiological challenge than that endured at extreme high altitude. The large cohort of recreational climbers and trekkers who undertake ascents to lesser altitudes may represent a different group to those attempting to reach 8000m (6). However, a prospective study of 284 climbers attempting to climb to the summit of Mt. Blanc (4,807 m) supported an association between the ACE I-allele and successful performance in the hypoxic environment. Success in reaching the

summit was genotype dependent (87.7% of DD, 94.9% of ID and 100% of II individuals; $p=0.048$); I-allele frequency for those reaching the summit was 0.47 compared to 0.21 for those who did not ($p=0.01$) (110).

Table 1.1 ACE I/D and ascent to altitude.

| Study | Subjects | Altitude | I/D association |
|-----------------------------|-------------------------------|-----------------|--------------------------------|
| Montgomery et al. 1998 (86) | 25 British elite mountaineers | >7000m | I allele and elite status |
| Tsianos et al. 2005 (110) | 284 climbers | Ascent to 4807m | I allele and successful ascent |

1.4.2 ACE I/D and acclimatisation

The findings of Montgomery et al. that the ACE I-allele was overrepresented in elite high altitude mountaineers (86) resulted in a series of studies to identify the mechanism by which the ACE I/D polymorphism exerts an influence over high altitude performance.

The ventilatory response to hypoxia is a critical element of successful acclimatisation. Woods et al. demonstrated that oxygen saturations (S_aO_2) in a group of 32 subjects ascending to 5000m over 12 days was significantly associated with the ACE genotype ($p = 0.01$) with a relatively sustained S_aO_2 in the II subjects. This association was not evident in a slower (18.5 day) ascent group (111). Further investigation of the mechanism underlying this observation was undertaken by Patel et al. who examined whether an altered ventilatory response to hypoxic exercise may contribute to this effect (112). Sixty subjects underwent incremental cardiopulmonary exercise testing in normoxic and subsequently hypoxic (12.5 \pm 0.5% oxygen) conditions and demonstrated that those of II genotype have an improved hypoxic ventilatory response (HVR) with increased minute volume and reduced end tidal CO_2 . Increased erythropoiesis represents another central element of the classical model of acclimatisation, however no association was demonstrated between ACE genotype and erythropoietin levels in 63 endurance athletes exposed to moderate altitude (2200m) for 48 hours (113).

Table 1.2 ACE I/D and acclimatisation.

| Study | Subjects | Exposure | Outcome measure | I/D association |
|----------------------------|---------------|-------------------|------------------|--------------------------------|
| Woods et al. 2002 (111) | 32 Caucasians | Ascent to 5000m | S_aO_2 | I and increased S_aO_2 |
| Patel et al. 2003 (112) | 60 Caucasians | Hypoxic CPEX test | HVR and S_aO_2 | I and increased HVR / S_aO_2 |
| Gonzalez et al. 2006 (113) | 63 Caucasians | 48 hours at 2200m | EPO levels | No association |

A recent review of ACE genotype and physical performance by Puthuchearry et al. discussed a range of mechanisms that may explain the association between the ACE I/D polymorphism and performance (90). ACE I/D dependent differences in sea level cardiac and skeletal muscle responses to training (86, 91, 92), differing metabolic efficiency (82) and muscle fibre type (83) may contribute to the impact of ACE genotype on high altitude performance. However, to date, these hypotheses have not been tested as determinants of successful acclimatisation to hypobaric hypoxia.

1.4.3 ACE I/D and Acute high altitude illness (AHAI)

Although the association between ACE I-allele and improved high altitude performance may be conferred by enhanced athleticism, it might also be accounted for by an association of the D-allele with illness at high altitude. Acute exposure to hypobaric hypoxia or an increase in altitude can induce the pathophysiological responses of AHAI. These conditions are Acute Mountain Sickness (AMS), High Altitude Pulmonary Edema (HAPE) and High Altitude Cerebral Edema (HACE) (114-116). Susceptibility to these debilitating and sometimes fatal conditions prevents successful performance at altitude. AHAI aetiology is incompletely understood and individual susceptibility highly variable with some individuals predisposed to developing altitude illness (8, 117): some individuals suffer the life threatening complications of high altitude cerebral and pulmonary oedema at altitudes as low as 3000m, whilst others have climbed all 14 of the world's 8000m mountains without supplementary oxygen.

1.4.4 ACE I/D and Acute Mountain Sickness

Acute mountain sickness (AMS) is the most common of the altitude diseases. AMS is characterised by headache following recent ascent to altitude associated with additional symptoms including loss of appetite, nausea, vomiting, fatigue or weakness, dizziness, or difficulty sleeping (114-116). The incidence of AMS ranges from 10% to 93%, depending on the highest altitude reached and the rate of ascent. It is thought that a continuum of cerebral oedema ranges from the mild symptoms of AMS to the extreme of coma and death in severe HACE (116). The Lake Louise Scoring (LLS) System can be used to quantify AMS and is an important tool in research studies of AMS (118, 119). Scores are based on patient reports of presence and severity of headache, gastrointestinal symptoms, fatigue or weakness, dizziness or lightheadedness and difficulty sleeping (Table 2.1).

Whilst the pathophysiology of AMS is not fully understood, increased levels of aldosterone and fluid retention have been found in persons suffering AMS (120, 121) which implicates the RAS in the development of the condition. A large recent study of 1,326 subjects travelling to an altitude of over 4000m identified low hypoxic ventilatory response and arterial oxygen desaturation on exercise as independent risk factors for developing severe high altitude illness (117). Previous findings that the ACE I-allele is associated with a brisk HVR and high S_aO_2 (111, 112, 122) may suggest a protective role against AHAI.

Conflicting data exist regarding the association of ACE I/D genotype with AMS development (8). Tsianos et al. demonstrated an association between the heterozygous ACE ID genotype and resistance to AMS ($p=0.003$) in 284 climbers assessed on arrival

from lower altitude at the Gouter Hut (3,807 m), Mt Blanc (110). Conversely no association between ACE I/D genotype and AMS was identified in studies of 159 climbers at 4559m (123) or 103 Nepalese pilgrims attending a religious festival at 4380m (124).

Table 1.4 ACE I/D and AMS.

| Study | Subjects | Altitude (m) | I/D association |
|----------------------------|-----------------|---------------------|------------------------|
| Koehle et al., 2006 (124) | 103 Nepalese | 4380 | No association |
| Tsianos et al., 2005 (110) | 284 Caucasians | 3817 | ID and reduced AMS |
| Dehnert et al., 2002 (123) | 159 Caucasians | 4559 | No association |

1.4.5 ACE I/D and High Altitude Pulmonary Edema (HAPE)

HAPE represents a potentially fatal form of non-cardiogenic pulmonary oedema in response to hypobaric hypoxia that may develop in otherwise healthy individuals on induction to altitudes above 2500m (114, 115). The disorder may be caused initially by an exaggerated hypoxic vasoconstriction leading to relative overperfusion of certain regions of the lung (125, 126), resulting in capillary endothelial shear stress failure and leakage in these pulmonary capillary beds (127). The hypoxic pulmonary vasoconstriction response (HPVR) may be partly ACE dependent: hypoxia increases pulmonary vascular ACE expression (128) and ACE inhibitors (71), and angiotensin II type 1 (AT₁) receptor blockers (70) attenuate the HPVR and, among patients hospitalized with HAPE, the D-allele is associated with a hyperresponsive hypoxia-induced increase in pulmonary vascular resistance (129).

Although environmental factors such as altitude and rate of ascent are central to developing HAPE, individual susceptibility to HAPE, a phenotype termed as HAPE susceptible (HAPE-s) rather than HAPE resistant (HAPE-r) is thought to have a genetic basis (8, 130). RAS plays a crucial role in regulation of pulmonary vascular tone and functional variants in this system, such as the ACE I/D polymorphism, may thus have a role in the exaggerated hypoxic vasoconstriction seen in HAPE-sensitive (HAPE-s) subjects when compared to HAPE-resistant (HAPE-r) controls (125, 127).

Several studies have addressed the association between ACE I/D and HAPE susceptibility, but with conflicting results (Table 4). Hotta et al. studied 49 Japanese HAPE-s subjects and 55 HAPE-r climbers and revealed no significant difference in ACE I/D polymorphism distribution between the two groups. However, in the 21 HAPE

patients who underwent right cardiac catheterization, the pulmonary vascular resistance (PVR), and PVR index (PVRI) were significantly higher in the HAPE-s subjects who were D-allele carriers than in the HAPE-s subjects who were I-allele carriers (PVR, $p = 0.015$; PVRI, $p = 0.028$) (129). A study of 19 HAPE-s and 20 HAPE-r Indian men demonstrated marked differences in RAS components including plasma renin, aldosterone and ACE between the 2 groups but no association with the ACE I/D polymorphism (131). This lack of association was also supported by Dehnert et al.'s study of 159 Caucasians at 4559m described earlier (123) and a further study in an Indian population (132).

Table 1.5 ACE I/D and HAPE

| Study | Ethnicity | HAPE-s | HAPE-r | Altitude (m) | I/D association |
|---------------------------|-----------|--------|--------|--------------|-----------------|
| Dehnert et al., 2002(123) | Caucasian | 25 | 51 | 4559 | No association |
| Hotta et al., 2004(129) | Japanese | 49 | 55 | 2758-3190 | D and PVR |
| Kumar et al., 2004 (131) | Indian | 19 | 20 | 3000-3800 | No association |
| Charu et al., 2006(132) | Indian | 64 | 53 | 3400-5600 | D and HAPE-s |

1.4.6 ACE I/D and High Altitude populations

The Tibetan and Andean populations have lived at high altitude for millennia and are regarded as well adapted to hypobaric hypoxia (133). Interestingly these two groups display different adaptive strategies with distinct patterns of genetic adaptation showing evidence of positive natural selection in different genes or gene regions (133, 134). Tibetans and Sherpas have lower haemoglobin concentrations at high altitude than do Han Chinese and Westerners (133, 135). These lower haemoglobin concentrations are consistent with the reduced prevalence of chronic mountain sickness in Tibetans compared with Andeans (136). Tibetans also exhibit a smaller degree of hypoxic pulmonary vasoconstriction compared with Andeans and other high-altitude populations (137). High altitude Andeans have lower resting ventilation and lower S_aO_2 when compared with Tibetans (133).

Despite these differences in adaptation, studies have assessed whether the ACE I/D allele has been positively selected in these high altitude populations. Rupert et al. determined the ACE I-allele frequency in high altitude native Andeans (>3000m) and found a greater frequency than that seen in Caucasians. However the I-allele frequency was not different from lowland native American populations suggesting that although the I-allele may have facilitated the migration of the ancestral native Andeans to the highlands, the ACE insertion allele has not been subsequently selected for in this high altitude population (138). The same author did not find any association between the ACE I/D genotype and cardiovascular disease in high altitude Andeans in a subsequent study (139).

Addressing the high altitude population of the Tibetan plateau, who include the Sherpas of Nepal and native inhabitants of Ladakh, India, Qadar Pasha et al. undertook a study of 3 groups: high altitude (>3600m) residents, lowlanders who migrated to live at altitudes >3600m, and lowlanders. The high altitude residents and migrants had significantly higher I allele frequencies than the lowlanders (140). A study of essential hypertensive patients in Tibet revealed an association of the D-allele with systemic hypertension in high-altitude Tibetan women, but not men (141).

Conversely to both the studies in other high altitude populations and the studies of HAPE susceptibility, a study of Kyrgyz high altitude residents demonstrated a strong association between the ACE II genotype and high altitude pulmonary hypertension ($p = 0.003$).

Table 1.6 ACE I/D and High Altitude populations

| Study | HA population | I/D association |
|--------------------------------|----------------------|---------------------------------|
| Rupert et al., 1999 (138) | Native Andean | I and Andean population |
| Qadar Pasha et al., 2001 (140) | Ladakhi | I and HA residents and migrants |
| Gesang et al., 2002 (141) | Tibetan | D and hypertension in women |
| Aldashev et al., 2002 (142) | Kyrgyz highlanders | II and HAPH |
| Rupert et al., 2003 (139) | Native Andean | No association with CVD |

1.5 Thesis rationale and aims

When this thesis was conceived in 2005, Montgomery et al. had demonstrated that the ACE I allele was overrepresented in 25 elite high altitude mountaineers (86) and, although a second study had confirmed an association of the I-allele with successful ascent at lower altitude (110), no further studies had tested the hypothesis that the ACE I-allele is associated with successful physical performance in the hypobaric hypoxic environment of extreme high altitude. Nor had the association been adequately explored across a range of altitudes. This study set out to test rectify these deficits, and to begin to elucidate the mechanism by which such an advantage may be mediated. The aims of my thesis were thus to:

1. Test the hypothesis that ACE I/D is associated with elite high altitude performance by comparing the ACE I/D genotype of mountaineers who succeed in climbing to extreme high altitude (8000m) with those who fail.
2. Investigate the mechanism that mediates the ACE I-allele associated improvement in high altitude performance by conducting a series of prospective gene-environment interaction studies. S_aO_2 and susceptibility to Acute Mountain Sickness were studied in individuals prospectively exposed to hypobaric hypoxia, and related to ACE I/D genotype.
3. To explore the association of bradykinin receptor-2 genotype (BK₂R), such as to investigate whether the ACE I/D contribution is mediated by alterations in bradykinin activity.
4. Extend these observations to those who have faced the most extreme hypoxic challenge - a successful ascent of Mount Everest (8848m).

To this end, Chapter 3 describes a study to assess whether the ACE I-allele is associated with successful ascent to 8000m. Chapters 4 and 5 describe two further prospective studies that assess whether the I-allele advantage may be conferred by resistance to AMS or increased S_aO_2 at altitudes of 2700m to 5895m. Chapter 6 assesses whether an ACE I-allele advantage may be mediated by increased kinin activity by testing whether there is an association between BK₂R +9/-9 polymorphism and performance at altitude as assessed by successful ascent, AMS incidence and arterial oxygen saturations at 4300m. Chapter 7 describes a retrospective study of 219 climbers who have reached the summit of Mount Everest (8848m) and assesses whether the ACE I/D and BK₂R +9/-9 allele frequencies observed in this elite hypoxia-tolerant cohort differ from those of published allele frequencies in race-matched controls.

CHAPTER 2: Generic Methods

2.1 Genotyping

2.2 Lake Louise Score for Acute Mountain Sickness

2.3 Pulse oximetry

Additional methodology specific to individual studies is described in subsequent chapters.

2.1 Genotyping

Genomic DNA was derived from buccal cells, collected using Whatman sterile, foam tipped applicators swabbed vigorously against both cheeks, dampened under the tongue, then pressed onto a Whatman FTA microcard (Whatman Bioscience, Abington, Cambridge, UK).

Genotyping was performed by staff at the Centre for Cardiovascular Genetics, University College London. 2.0 mm discs were punched from the Whatman FTA microcard for preparation. 200 µl of FTA Purification Reagent was added to the disc taken from the Whatman FTA microcard and incubated for 5 minutes at room temperature. Spent FTA Purification Reagent was removed and discarded using a pipette and this process was repeated twice further, for a total of three washes with FTA Purification Reagent.

200 µl of TE⁻¹ Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) were then added to the disc and incubated for 5 minutes at room temperature. Spent TE⁻¹ Buffer was removed and discarded using a pipette and this process was then repeated.

The disc was then allowed to dry at room temperature for about one hour (or at 56°C for 10 minutes) before performing Polymerase Chain Reaction (PCR).

ACE I/D genotyping was performed using PCR primer sequences: 5' Oligo : FH 76 Sequence: CAT CCT TTC TCC CAT TTC TC; 5' insertion oligo: FH77 Sequence: TGG GAT TAC AGG CGT GAT ACAG; 3' oligo: FH78 Sequence: ATT TCA GAG CTG GAA TAA AAT T. PCR was performed with amplification conditions as follows: initial denaturing temperature 95°C for five minutes, followed by 35 cycles of 95°C for 45 seconds, 54°C for 45 seconds and 72°C for 30 seconds and 1 cycle of 72°C for 5

minutes. All PCR mixes contained dNTP, 2.5mM final magnesium chloride, and 8µl of Taq polymerase in a 2000 µl final reaction volume. Reactions were overlaid with 20µl of mineral oil. The PCR products were run on 7.5% microtitre array diagonal gel electrophoresis (MADGE) for 30 minutes at 110V. Genotype was determined by identifying appropriate bands (Deletion allele 85bp; Insertion allele, 65 bp).

BK2R +9/-9 genotyping was performed using forward primer 5' TCTGGCTTCTGGGCTCCGAG 3' and reverse primer 5' AGCGGCATGGGCACTTCAGT 3'. PCR used annealing temperature 63°C (45 seconds) and 72°C (one minute). The PCR mix contained dNTP's, 1.5mM final magnesium chloride. PCR products were run on heteroduplex gels at 150V for 2 hours. Genotype was determined by identifying appropriate bands (+9, 109 bp; -9, 100 bp).

Genotyping accuracy and reliability. Known control ACE I/D and BK2R +9/-9 genotypes were run alongside study PCR samples to ensure that the PCR has amplified correctly. All genotypes were verified by two independent technicians, who were blind to subject data, and discrepancies were resolved by repeat genotyping. Although early ACE I/D genotyping techniques were prone to misreading ID as DD (84), the accuracy of the three primer PCR method used has been published as 100% (143, 144). The rate of failure of the genotyping technique is influenced by the quality of DNA sample utilized. In this series of studies, where DNA was derived from buccal cells stored on Whatman FTA microcards, the genotyping failure rate is described in each results section and ranges from 0 - 1.8% for ACE I/D and 0 - 4.4% for BK2R +9/-9.

2.2 Lake Louise Score for Acute Mountain Sickness

The Lake Louise Consensus Group defined AMS as the presence of headache in an unacclimatised person who has recently arrived at an altitude above 2500 m plus the presence of one or more of the following: gastrointestinal symptoms (anorexia, nausea, or vomiting), insomnia, dizziness, and lassitude or fatigue (119). Several scores exist for the diagnosis and assessment of the severity of AMS, including the Lake Louise Score (LLS) (119), the Environmental Systems Questionnaire (145) and visual analogue score (146). In this thesis, subjects were assessed using the LLS (Table 2.1). Data collection on Mount Kilimanjaro and Cerro Aconcagua was undertaken by teams of medical students from the Manchester University Medical School, Manchester, UK and the Norwegian University of Science and Technology, Tromso, Norway respectively.

The initial definition of AMS using the LLS was that of a score of 3 or more using the self reported symptom score (119). Subsequently there has been significant variation in both the threshold score for the diagnosis of AMS and the inclusion of the clinical assessment score (116, 118). This thesis presents the numerical Total LLS, which is the sum of the self reported Symptom score and the Clinical assessment score (Table 2.1), and uses a Total LLS of ≥ 4 as diagnostic for AMS.

Table 2.1 Lake Louise Scoring System for Acute Mountain Sickness

| | |
|---|---|
| Symptoms: | |
| 1. Headache: | |
| No headache | 0 |
| Mild headache | 1 |
| Moderate headache | 2 |
| Severe, incapacitating | 3 |
| 2. GI symptoms: | |
| No GI symptoms | 0 |
| Poor appetite or nausea | 1 |
| Moderate nausea or vomiting | 2 |
| Severe nausea and vomiting incapacitating | 3 |
| 3. Fatigue/weak: | |
| Not tired or weak | 0 |
| Mild fatigue/weakness | 1 |
| Moderate fatigue/weakness | 2 |
| Severe fatigue/weakness, incapacitating | 3 |
| 4. Dizzy/lightheadedness: | |
| Not dizzy | 0 |
| Mild dizziness | 1 |
| Moderate dizziness | 2 |
| Severe, incapacitating | 3 |
| 5. Difficulty sleeping: | |
| Slept well as usual | 0 |
| Did not sleep as well as usual | 1 |
| Woke many times, poor night's sleep | 2 |
| Could not sleep at all | 3 |
| Total symptom score: | |
| Clinical assessment: | |
| 6. Change in mental status: | |
| No change | 0 |
| Lethargy/lassitude | 1 |
| Disoriented/confused | 2 |
| Stupor/semiconsciousness | 3 |
| 7. Ataxia(heel to toe walking): | |
| No ataxia | 0 |
| Maneuvers to maintain balance | 1 |
| Steps off line | 2 |
| Falls down | 3 |
| Can't stand | 4 |
| 8. Peripheral edema: | |
| No edema | 0 |
| One location | 1 |
| Two or more locations | 2 |
| Clinical assessment score: | |
| Total score: | |

2.3 Pulse oximetry

Resting S_aO_2 was measured in subjects using a pulse oximeter (MCDEI 8500M, Nonin Medical Inc, Plymouth, MN). Pulse oximetry presents a rapid and non-invasive method for assessing S_aO_2 in a remote environment but their accuracy is reduced at lower S_aO_2 (147). Previous studies have demonstrated that in healthy volunteers, oximeters commonly have a mean difference (bias) of $<2\%$ and a standard deviation (precision) of $< 3\%$ when S_aO_2 is 90% or above (148). Accuracy of pulse oximeters deteriorates when S_aO_2 falls to 80% or less. In healthy volunteers under hypoxic conditions, bias of pulse oximetry using other similar devices varies from -15.0 to 13.1% while the precision ranges from 1.0 to 16.0% (147, 149).

Bias and precision data for the Nonin MCDEI 8500M pulse oximeter is not published, but the manufacturers state, through personal correspondence, that the device is accuracy tested by induced hypoxia studies on healthy subjects in an independent research laboratory over the S_aO_2 range of 70 - 100% (all S_aO_2 values recorded in this study fall within this range). In these tests, the S_aO_2 measured by the pulse oximeter is compared to arterial haemoglobin oxygen value, determined from blood samples with a laboratory co-oximeter. Accuracy data in these tests are calculated using the root-mean-squared (Arms value) and the pulse oximeter used in these studies conforms to the standards of ISO 9919:2005, Medical Electrical Equipment–Particular requirements for the basic safety and essential performance of pulse oximeter equipment for medical use (150).

To minimize the influence of movement, exertion and perfusion that may influence the accuracy of pulse oximeters (149), the measurement of S_aO_2 was standardised in this study. S_aO_2 was recorded in resting subjects in the sitting position and the pulse

oximeter was applied to the index finger. The accuracy of a finger probe, such as that used in this study exceeds the use of probes on other anatomical sites such as the ear lobe, forehead or nose (151). Digits were pre-warmed to avoid poor pulse signals due to low perfusion. SaO₂ values were accepted when the device displayed a stable result for several seconds.

CHAPTER 3: ACE I/D and Ascent to Extreme High Altitude

- 3.1 Background
- 3.2 Methods
 - 3.2.1 Ethics
 - 3.2.2 Subjects
 - 3.2.3 Subject Data
 - 3.2.4 Genetic Analysis
 - 3.2.5 Statistical Analysis
- 3.3 Results
- 3.4 Discussion

The data from this chapter have appeared in peer-reviewed published form, as:

Julian Thompson, James Raitt, Lynn Hutchings, Fotios Drenos, Eirik Bjargo, Are Loiset, Mike Grocott and Hugh Montgomery for the Caudwell Xtreme Everest Research Group. Angiotensin-converting enzyme genotype and successful ascent to extreme high altitude. *High Altitude Medicine and Biology*. Volume 8, Number 4, 2007, Pages 278-285.

3.1 Background

When this study was conceived in 2005, the ACE I-allele had been associated with elite endurance performance and was overrepresented in 25 elite high altitude mountaineers who had climbed regularly to over 7000m (86). Although a second study had confirmed this finding at moderate altitude (110), no further studies had tested the hypothesis that the ACE I-allele is associated with successful physical performance in the hypobaric hypoxic environment of extreme high altitude.

If the I-allele is indeed associated with enhanced performance in hypoxic environments, then the I-allele ought to be over-represented amongst those successful in ascending the very highest peaks - those of 8000 metres standing or above. I sought to test this hypothesis.

3.2 Methods

3.2.1 Ethics

The study was approved by the Joint UCL/UCLH Ethics Committee. Written informed consent was obtained from all study participants.

3.2.2 Subjects

Subjects were high altitude mountaineers who were participating in an expedition to climb one of the fourteen 8000m peaks in the world. I recruited participants at Everest North Side Base Camp, Tibet, China and mountaineers contacted through expedition organisers, mountaineering clubs, professional mountaineering associations and advertisements on mountaineering websites. No local mountaineering porters or guides were included in the study as their success or failure in reaching the summit of a mountain is frequently dependent upon the altitude reached by their client rather than their own ability.

3.2.3 Subject Data

Subjects were asked, either in person or by email, to complete a questionnaire of demographic data and high altitude history mountaineering experience (Questionnaire: Appendix 1). Following the attempt to climb an 8000m peak, self-reported maximum altitude reached, oxygen use, and reason for failed ascent (where appropriate) were recorded. Self-reported data were cross-referenced with the Himalayan Database (www.himalayandatabase.com) for consistency and peer confirmation.

3.2.4 Genetic Analysis

For those who consented to take part, DNA was collected using a buccal swab and was genotyped for ACE I/D polymorphism (see Chapter 2: Method and Materials for details).

3.2.5 Statistical Analysis

Genotype-dependent differences in high altitude performance were assessed by Chi-squared testing, Fisher's exact test and one-way ANOVA as appropriate. The influence of age, gender, race and ACE genotype on reaching 8000 metres were tested using binary logistic regression analysis. The level of statistical significance was set for $P \leq 0.05$.

3.3 Results

ACE genotyping failed in two of the 141 subjects initially recruited. Of the remaining 139, 125 were male and 14 female. One hundred and nine subjects were Caucasian and 30 were South Asian (28 Indian, 1 Nepalese and 1 Bhutanese). Age, weight and height data were available for 112: mean \pm sd age was 39.7 ± 9.5 years, mean height 175.6 ± 8.7 cm and weight 73.9 ± 10.1 kg. ACE genotype distribution was 33 (23.7%) vs 69 (49.6%) vs 37 (26.5%) for II vs ID vs DD respectively (I-allele frequency 0.49), and was consistent with Hardy Weinberg Equilibrium. Demographic data were independent of genotype.

Twelve individuals (8.6%) reported episodes of High Altitude Cerebral Edema (HACE) or High Altitude Pulmonary Edema (HAPE) in their past climbing history. A past history of HAPE or HACE was not associated with ACE genotype (Fisher's exact test $p=0.419$).

Table 3.1 ACE I/D genotype and mean maximum altitude achieved

| ACE genotype | Maximum altitude achieved |
|--------------|---------------------------|
| II | $8559 \pm 565\text{m}$ |
| ID | $8107 \pm 653\text{m}$ |
| DD | $8079 \pm 947\text{m}$ |

Mean maximum altitude in metres \pm standard deviation.

The maximal altitude achieved varied considerably, with a range of 4800-8848m, mean $8206.71 \pm 747\text{m}$, but was consistently associated with the I-allele ($F=5.112$, $df=2$, $p<0.002$) (Table 3.1). ACE genotype distribution differed significantly between those

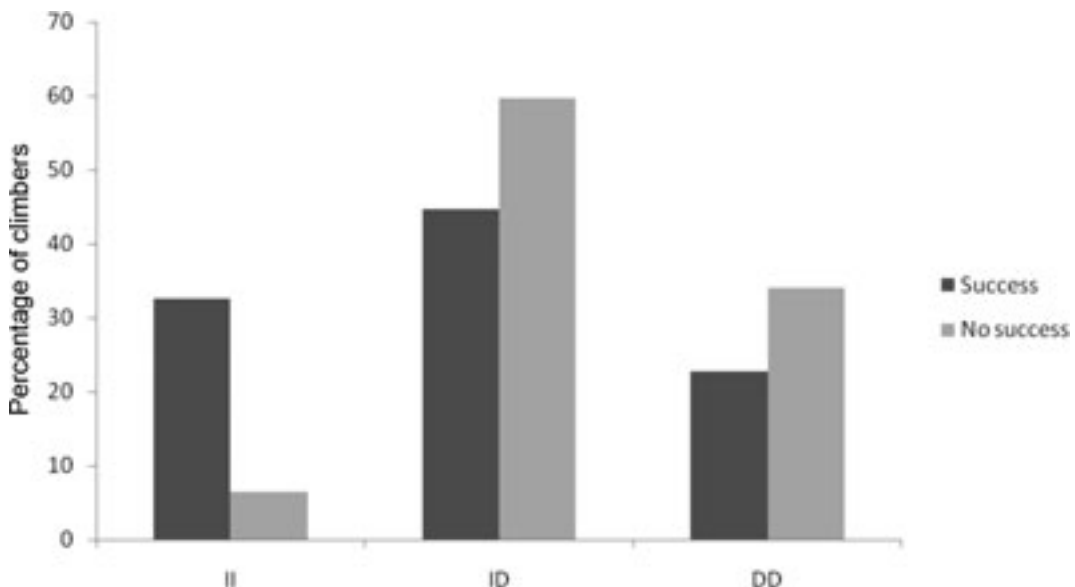
who successfully climbed beyond 8000m, and those who had failed in the attempt ($\chi^2=11.894$, $df = 2$, $p=0.003$; Fisher's exact test $p=0.001$), with a relative over-representation of the I-allele amongst the successful group (0.55 vs 0.36 in successful vs unsuccessful respectively). Overall, 32.6% (30) of individuals in the 'successful' group were of II genotype, but only 6% (3) in the 'unsuccessful' group (Table 3.2).

Table 3.2 ACE I/D genotype and success in ascent to 8000 metres.

| | Climbed >8000m | Not climbed >8000m | Total |
|--------------------|----------------|--------------------|------------|
| II | 30 (32.6%) | 3 (6.4%) | 33 (23.7%) |
| ID | 41 (44.6%) | 28 (59.6%) | 69 (49.6%) |
| DD | 21 (22.8%) | 16 (34.0%) | 37 (26.6%) |
| I allele frequency | 0.55 | 0.36 | 0.49 |
| D allele frequency | 0.45 | 0.64 | 0.51 |

Values are numbers of individuals (percentage of Group in brackets).

Figure 3.1 ACE I/D genotype distribution and success in 139 mountaineers attempting to climb beyond 8000 m



In the group who had successfully climbed to over 8000m, there was no statistical difference in ACE genotype frequency between those who climbed to over 8000m using oxygen and those who did not use oxygen (23 [34.8%] II, 26 [39.4%] ID, 17 [25.8%] DD vs 7 [26.9%], 15 [57.7%] and 4 [15.4%] respectively; $\chi^2=2.640$, $df = 2$, $p=0.267$).

Caution must be taken in the interpretation of data relating to samples in which race is mixed, given that genotype distributions may differ. Here, ACE genotype distribution was similar to those previously published (152), being 20 (18.3%) II, 56 (51.4%) ID and 33 (30.3%) DD for Caucasians, and 13 (43.3%), 13 (43.3%) and 4 (13.3%) respectively for South Asians ($\chi^2=9.029$, $df = 2$, $p=0.011$), with the I allele being overrepresented amongst the South Asian group (0.65 vs 0.44 respectively). However, such differences in genotype distribution between races could not have accounted for our findings: success at climbing to over 8000m was independent of race (66% of the Caucasian group and 67% of the South Asian groups successfully reaching this altitude ($\chi^2=0.004$, $df = 1$, $p=0.950$)). Further, when the two racial groups are analysed independently, the I-allele remained significantly associated with success in climbing to 8000 metres in the Caucasian group (Fisher's exact test $p=0.032$) and in the South Asian group (Fisher's exact test $p=0.003$).

Logistic regression analysis demonstrated a strongly significant association between reaching 8000 metres and the I-allele (Exp (B) = 8.554, $p=0.002$). None of the other confounding variables in the regression analysis were significantly associated with high altitude success, including age or race (Table 3.3).

Table 3.3: Logistic regression analysis of the categorical variables influencing success in ascent to 8000 metres.

| | P value |
|--------------|---------|
| Age | 0.620 |
| Sex | 0.184 |
| Race | 0.118 |
| ACE I-allele | 0.002 |

3.4 Discussion

This study demonstrates an association between the ACE I-allele and successful ascent to 8000m. At the time of publication this study was the first to confirm an association between elite high altitude performance and the ACE I-allele following the observation in a small number of elite British mountaineers (86).

This finding accords with studies that have demonstrated an association of the I-allele with prospective success in ascent of Mt. Blanc (4807 m)(110) and an increased I-allele frequency in high altitude residents in the Andes (138) and Himalayas (140).

As discussed in Chapter 1, several mechanisms have been proposed to underlie this association. Falling plasma aldosterone levels at altitude encourage natriuresis and diuresis, thus reducing the propensity to edema and possibly to acute mountain sickness (79). One might postulate that the D-allele, being associated with higher ACE activity, may inhibit this protective fall in aldosterone levels and increase the development of AMS. The association of ACE I/D and AMS is further explored in Chapter 4.

The I-allele has been associated with an enhanced ventilatory response to exertional hypoxia (112), which may result in better-maintained arterial oxygenation during rapid ascent to high altitude (111). However, against this supposition, arterial oxygen saturation seems independent of genotype when ascents are more graded (111), as is likely to be the case on ascents to 8000m and above.

The D-allele may be associated with an exaggerated hypoxic pulmonary vasoconstriction response and HAPE (129). The incidence of diagnosed HAPE reported in this study was low and seems unlikely to have accounted for success or failure in reaching 8000 m. However, it remains possible that increased pulmonary vascular

resistance or low-grade pulmonary edema may yet have accounted for the genotype dependence of success or its lack. Certainly, angiotensin II, generated by ACE activity, increases vascular permeability and might thus increase V/Q mismatch and offer a diffusion barrier to oxygen transport (153, 154). If the I-allele advantage is mediated by either of the mechanism of an altered ventilatory response or reduced diffusion barrier to oxygen transport it would be expected that the I-allele would be associated with enhanced oxygen saturations at high altitude. This is further investigated in Chapter 5.

Caution must be taken in studies such as this to identify possible confounding variables. The use of supplementary oxygen at extreme altitude is common and is generally regarded as an important determinant of success. This study demonstrated no significant difference in ACE I/D genotype between those who have climbed to above 8000m with or without the use of supplementary oxygen. This may be due to the number of climbers who successfully climbed above 8000m without oxygen being too small to demonstrate a difference. However, all mountaineers attempting to climb to 8000m spend several weeks at high altitude acclimatising to hypoxia.

Race was not associated with success in climbing to over 8000 m. Therefore, despite the difference in ACE genotype distribution in the Caucasian and South Asian groups seen in this and other studies (155), the inclusion of different racial groups in the study does not account for the increased frequency of the I-allele in the successful mountaineer group. Additionally, if the Caucasian group is addressed alone, the association of the I-allele with success remains.

Conclusions

This study demonstrates that the ACE I-allele is associated with successful ascent to 8000m.

**CHAPTER 4: ACE I/D genotype and Susceptibility to Acute Mountain Sickness
(AMS)**

- 4.1 Background
- 4.2 Study 1: ACE I/D and AMS in trekkers on Mt. Kilimanjaro (5895m)
 - 4.2.1 Methods
 - 4.2.1.1 Ethics
 - 4.2.1.2 Subjects
 - 4.2.1.3 Subject data
 - 4.2.1.4 Genetic analysis
 - 4.2.1.5 Statistical analysis
 - 4.2.2 Results
- 4.3 Study 2: ACE I/D and AMS in trekkers at 4300m on Cerro Aconcagua (6963m)
 - 4.3.1 Methods
 - 4.3.1.1 Ethics
 - 4.3.1.2 Subjects
 - 4.3.1.3 Subject data
 - 4.3.1.4 Genetic Analysis
 - 4.3.1.5 Statistical Analysis
 - 4.3.2 Results
- 4.4 Discussion

Data from this chapter have appeared in peer-reviewed published form, as:

NS Kalson, J Thompson, AJ Davies, MD Earl, S Stokes, AG Whitehead, I Tyrell-Marsh, H Frost, H Montgomery. The effect of the ACE I/D polymorphism on acute mountain sickness and summit success in trekkers attempting the summit of Mt Kilimanjaro (5895m). *European Journal of Applied Physiology*. 2009 Feb;105(3):373-379.

4.1 Background

ACE I/D polymorphism is associated with enhanced performance in hypoxic environments (107, 110, 156) but conflicting data existed regarding whether this enhanced performance is mediated by an association between the ACE I/D polymorphism and susceptibility to AMS (110, 123).

If resistance to AMS mediates the performance advantage at high altitude associated with the I-allele, then the I-allele should be associated with lower incidence and severity of AMS in those exposed to hypobaric hypoxia. I undertook 2 prospective studies to test the hypothesis that the ACE I-allele confers resistance to AMS.

4.2 Study 1: ACE I/D and AMS in trekkers on Mt. Kilimanjaro (5895m)

Mount Kilimanjaro (5895m) is the highest peak on the African continent and, as a volcano arising from lowland plains, is the highest free-standing mountain in the world. This status and the ease of ascent make it an attractive destination for trekkers. The geography of the mountain means that those who aspire to climb the mountain are largely altitude naïve and a combination of national park regulations and the commercial nature of expeditions to this mountain ensure that trekkers are required to participate in 4 or 5 day ascents from 2700m to the summit (5895m). These factors result in an incidence of AMS of up to 86% in those attempting to reach the summit (157). This study set out to recruit a large prospective cohort attempting to trek to high altitude on Mount Kilimanjaro (5895m), Tanzania with the aim of testing the hypothesis that the ACE I allele confers resistance to AMS.

The sample and data collection on Mount Kilimanjaro was undertaken by Manchester University undergraduate medical students. I collaborated with this team on study design, logistics, genotyping, data interpretation and writing up of this study.

4.2.1 Methods

4.2.1.1 Ethics

The study was approved by the Joint UCL/UCLH Ethics Committee. Written informed consent was obtained from all study participants. Additional approval was achieved from the Tanzanian National Institute for Medical Research, the Tanzanian Commission for Science and Technology (2005-261-NA-2005-62) and the Tanzanian National Parks Authority.

4.2.1.2 Subjects

Subjects comprised Caucasian tourist trekkers attempting to reach the summit of Mount Kilimanjaro (5895m) in Tanzania. Excluded were those exposed to altitudes >3000m in the past two weeks, those taking steroids or acetazolamide for AMS prophylaxis, those taking RAS antagonists (ACE inhibitors, or angiotensin II type 1 receptor antagonists) or those using supplemental oxygen.

All ascended by the Marango route, upon which subjects stay at fixed huts on their ascent: all stay for one night at 2700m, 3700m and 4700m, and some have an extra (second) overnight stop at 3700m. All then attempted the summit (5895m), and stopped at 3700m on descent. Researchers were stationed on the mountain at each of the three huts.

4.2.1.3 Subject data

All trekkers attempting the summit were asked to take part in the study at the first hut on their ascent (2700m). Written informed consent was obtained, as was a buccal swab to permit genetic analysis. Subjects were asked to report to a researcher each evening while on the mountain, during both ascent and descent, when the presence of AMS and its severity was determined using the Lake Louise Score (Table 1.3). As used by others, a score of ≥ 4 was used to define the presence of AMS (116, 118). AMS on the summit day was determined retrospectively at 4700m on descent.

4.2.1.3 Genetic analysis

For those who consented to take part, DNA was collected using a buccal swab and was genotyped for ACE I/D polymorphism (see Chapter 2: Methods for details).

4.2.1.4 Statistical analysis

Results were analysed using SPSS version 14. Chi squared test was used to confirm Hardy-Weinberg equilibrium. Differences in study population physiological parameters were assessed by ANOVA or Chi squared testing as appropriate. Differences between genotype groups for AMS incidence and severity were sought using chi squared or analysis of variance on ranks (Kruskal-Wallis). Differences in summiting success were tested using Fisher's exact test or chi squared as appropriate. Throughout, a p-value ≤ 0.05 was considered statistically significant. Normally distributed data are presented as mean \pm SD, and non-normal data as median (range). As greater speed of ascent is strongly associated with development of AMS, the two cohorts with different ascent profiles were, a priori, analysed separately.

4.2.2 Results

During the study period, 343 subjects were approached of whom 56 declined to take part, 79 were taking prophylactic acetazolamide, and 32 were not altitude-naïve. This left a study population of 173 individuals (104 male), whose ACE genotype distribution was consistent with Hardy-Weinberg equilibrium (38 II, 91 ID, 43 DD, $p = 0.556$), and whose physical characteristics (height 173 ± 10 cm, weight 69.7 ± 13.5 kg, age 35.8 ± 11.6 years) were independent of ACE genotype.

Some fifty-seven subjects (34 male) were lost to follow-up during the study (likely due to non-communicated withdrawal from the study, or elective descent due to discomfort or illness) whose physical characteristics (height 1.74 ± 0.11 m, weight 69.9 ± 13.1 kg, age 35.2 ± 11.5 years) and ACE genotype distribution (14 II [24.6%], 30 ID [52.6%], 13 DD [22.8%]) did not differ from those followed up (24 II [20.7%], 61 ID [52.6%], 31 DD [26.7%], $p = 0.45 - 0.914$).

From 2700m, all subjects spent one night at 3700m ($n=165$). Of these, 107 spent an extra night at 3700 to aid acclimatisation (acclimatisation profile – A), whilst 42 continued to 4700 m (direct profile – D). Of the 107 attempting the summit via the acclimatisation profile (A), 92 were followed up at 4700m, then 82 on their summit attempt (Figure 4.1). Of these, 41 reached the summit. Of the 42 attempting the summit via the direct profile (D) 34 were followed up on their summit attempt, 20 of whom reached the summit. The physical characteristics (sex, height, weight and age) of those on route A did not differ from those on route D. However, there was an excess of ID genotype in the direct ascent group when compared with the A ascent group (7 II [12.2%], 38 ID [66.6%], 12 DD [21.0%] versus 29 II [27.1%], 48 ID [44.9%], 30 DD

[28.0%] respectively $p = 0.02$: I-allele frequency 0.46 in those on route D compared with 0.50 for those on route A, table 4.1).

Figure 4.1 Schematic diagram of subject progression up Mt. Kilimanjaro showing ACE genotype distribution of subjects followed-up during the study

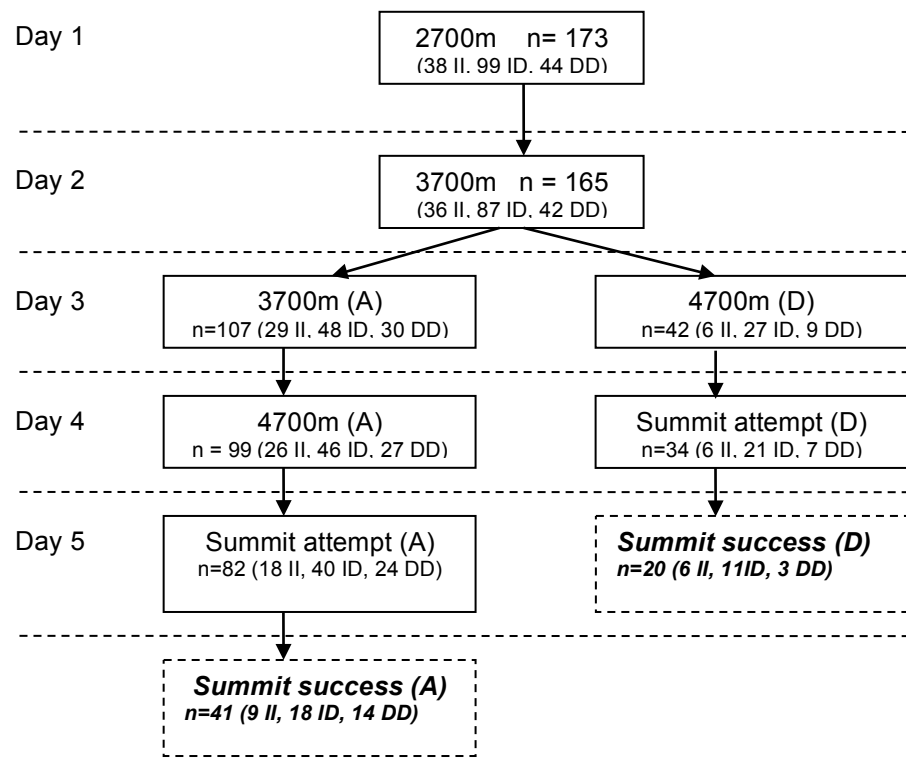


Table 4.1 Genotype frequencies in those completing the study and those dropping out for each altitude on ascent of Mt Kilimanjaro

| | 2700m | | | 3700m (D) | | | 3700m (A) | | | 4700m (D) | | | 4700m (A) | | | Summit (D) | | | Summit (A) | | |
|--|-------|----|----|-----------|----|----|-----------|----|----|-----------|----|----|-----------|----|----|------------|----|----|------------|----|----|
| | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD |
| Recorded | 38 | 91 | 44 | 7 | 38 | 12 | 29 | 48 | 30 | 6 | 27 | 9 | 26 | 46 | 27 | 6 | 21 | 7 | 18 | 40 | 24 |
| Total | 173 | | | 57 | | | 107 | | | 42 | | | 99 | | | 34 | | | 82 | | |
| Drop-out | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 11 | 3 | 3 | 2 | 3 | 0 | 6 | 2 | 8 | 6 | 3 |
| Total | 0 | | | 0 | | | 0 | | | 15 | | | 8 | | | 8 | | | 17 | | |
| (D) = Direct, (A) = Acclimatisation night at 3700m | | | | | | | | | | | | | | | | | | | | | |
| Total dropping out during the study = 57 (14 II, 30 ID, 13 DD, I-allele frequency 0.51) | | | | | | | | | | | | | | | | | | | | | |
| Total completing the study = 116 (24 II, 61 ID, 31 DD, I-allele frequency 0.47), p = 0.49 | | | | | | | | | | | | | | | | | | | | | |
| Total ascending by direct route (D) = 57 (7 II, 38 ID, 12 DD, I-allele frequency 0.46) | | | | | | | | | | | | | | | | | | | | | |
| Total ascending by acclimatisation route (A) = 107 (29 II, 48 ID, 30 DD, I-allele frequency 0.50 p = 0.02) | | | | | | | | | | | | | | | | | | | | | |

ACE genotype was not associated with presence of AMS per se at any altitude (Table 4.2). On arrival at most altitudes, there was a suggestion that AMS scores rose with increasing D-allele frequency. However, at no point did this reach statistical significance (Table 4.3).

Table 4.2 AMS presence, defined as LLS ≥ 4 , in subjects on the Direct and Acclimatisation profiles stratified according to ACE genotype

| | | 2700m | | | 3700m | | | 3700m (Acclimatisation night) | | | 4700m (D) | | | 4700m (A) | | | Summit (D) | | | Summit (A) | | |
|---|------------|-------------|----|----|-------------|----|----|-------------------------------------|----|----|-------------|----|----|-------------|----|----|---------------|----|----|---------------|----|----|
| | | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD |
| AMS presence | Yes | 0 | 0 | 1 | 5 | 9 | 6 | 4 | 10 | 8 | 2 | 13 | 7 | 9 | 19 | 13 | 5 | 19 | 6 | 12 | 32 | 15 |
| | No | 38 | 91 | 43 | 31 | 78 | 36 | 25 | 38 | 22 | 4 | 14 | 2 | 17 | 27 | 14 | 1 | 2 | 1 | 6 | 8 | 9 |
| | Total | 173 | | | 165 | | | 107 | | | 42 | | | 99 | | | 34 | | | 82 | | |
| | p = | 0.23 | | | 0.76 | | | 0.47 | | | 0.18 | | | 0.61 | | | 0.87 | | | 0.27 | | |
| (D = direct ascent profile, A = acclimatisation profile) | | | | | | | | | | | | | | | | | | | | | | |

Table 4.3 AMS severity (mean Lake Louise Score) in subjects on the Direct and Acclimatisation profiles stratified according to ACE genotype

| | 2700m | | | 3700m | | | 3700m (Acclimatisation night) | | | 4700m (D) | | | 4700m (A) | | | Summit (D) | | | Summit (A) | | |
|----------------------|---------------|---------------|---------------|---------------|---------------|---------------|-------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|---------------|---------------|---------------|---------------|---------------|
| | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD | II | ID | DD |
| n | 38 | 91 | 44 | 36 | 87 | 42 | 29 | 48 | 30 | 6 | 27 | 9 | 26 | 46 | 27 | 6 | 21 | 7 | 18 | 40 | 24 |
| Total | 173 | | | 165 | | | 107 | | | 42 | | | 99 | | | 34 | | | 82 | | |
| Mean AMS score | 0.24± 0.59 | 0.42± 0.73 | 0.73± 1.09 | 1.56± 1.46 | 1.65± 1.68 | 1.79± 1.59 | 1.86± 1.60 | 2.25± 2.32 | 2.13± 2.21 | 2.17± 1.94 | 3.67± 2.15 | 4.22± 1.77 | 2.77± 2.50 | 3.24± 2.16 | 3.37± 2.68 | 7.5± 5.01 | 6.81± 3.06 | 7.86± 3.98 | 6.67± 4.31 | 6.58± 3.56 | 6.04± 5.29 |
| P = | 0.07 | | | 0.76 | | | 0.83 | | | 0.14 | | | 0.51 | | | 0.93 | | | 0.59 | | |

At no altitude were AMS scores different between those dropping out and those followed-up ($p=0.43-0.87$).

There was no significant association between ACE genotype and successful ascent to the summit (Table 4.4) and, interestingly, there was no significant association between summit success and AMS presence at 4700m or on the summit attempt in either the Acclimatisation or Direct groups ($p = 0.09 - 1.00$, Table 4.5).

Table 4.4 Summit success in subjects on the Direct and Acclimatisation profiles stratified according to genotype

| | Summit Success | | | | | | | % success rate | |
|-----------|---------------------------|----|------------------------------|----------------|------------------|----|------------------------------|----------------|----------------|
| | Acclimatization route (A) | | | % success rate | Direct route (D) | | | | % success rate |
| | Y | No | Total | | Y | No | Total | | |
| II | 9 | 9 | 18 | 50 | 6 | 0 | 6 | 100 | |
| ID | 18 | 22 | 40 | 45 | 11 | 10 | 21 | 52 | |
| DD | 14 | 10 | 24 | 58 | 3 | 4 | 7 | 43 | |
| | 41 | 41 | 82 | 50 | 20 | 14 | 34 | 59 | |
| | | | $p = 0.54$ | | | | $p = 0.09$ | | |

Table 4.5 Summit success versus AMS presence (defined as $LLS \geq 4$) in subjects on the Direct and Acclimatisation profiles

| | | AMS presence | | | | | | | | | | | |
|-------------------|-------|------------------------------|-----|----|------------------------------|-----|----|------------------------------|-----|----|------------------------------|-----|----|
| | | 4700m (A) | | | 4700m (D) | | | Summit Day (A) | | | Summit Day (D) | | |
| | | No | Yes | | No | Yes | | No | Yes | | No | Yes | |
| Summit Success | Yes | 26 | 15 | 41 | 12 | 8 | 20 | 15 | 26 | 41 | 2 | 18 | 20 |
| | No | 20 | 21 | 41 | 7 | 7 | 14 | 8 | 33 | 41 | 2 | 12 | 14 |
| | Total | 46 | 36 | 82 | 19 | 15 | 34 | 23 | 59 | 82 | 4 | 30 | 34 |
| | | $p = 0.18$ | | | $p = 0.73$ | | | $p = 0.09$ | | | $p = 1.00$ | | |

4.3 Study 2: ACE I/D and AMS in trekkers at 4300m on Cerro Aconcagua (6959m)

Cerro Aconcagua (6959m), Argentina is the highest peak on the continent of South America and this status and the ease of ascent make it an attractive destination for trekkers. This study set out to recruit a large prospective cohort attempting to climb to the summit of Cerro Aconcagua (6959m) with the aim of testing the hypothesis that the ACE I-allele confers resistance to AMS.

The sample and data collection on Cerro Aconcagua, Argentina was undertaken by medical students from the Norwegian University of Science and Technology, Trondheim, Norway. I collaborated with this team on study design, logistics, genotyping, data interpretation and writing up of this study.

4.3.1 Methods

4.3.1.1 Ethics

The study was approved by the Joint UCL/UCLH Ethics Committee. Written informed consent was obtained from all study participants. Additional approval was obtained from the Regional Ethical Committee of Mid-Norway, the Norwegian Biobank Committee and the Norwegian Data Inspectorate.

4.3.1.2 Subjects

Subjects were Caucasian trekkers attempting to climb Cerro Aconcagua (6959m) and were recruited at Plaza de Mulas base camp (4300m). This base camp is reached by a 2-3 day approach via an intermediate camp at 3100m. Trekkers attempting to climb Cerro Aconcagua are required to register with the Park Rangers of Aconcagua Park on arrival

at base camp. The investigators were based at the rangers' hut where registration occurs with the aim of recruiting subjects on arrival to altitude. Excluded were those who had spent more than 48 hours at 4300m, those taking steroids or acetazolamide for AMS prophylaxis and those taking RAS antagonists (ACE inhibitors, or angiotensin II type 1 receptor antagonists).

4.3.1.3 Subject data

Subjects completed a questionnaire (in English or Spanish), and written informed consent was obtained from all volunteers. Age, sex, height, weight, duration of stay, medical history and recent medication were documented (Appendix 2). Presence and severity of current AMS symptoms were documented using the Lake Louise Score (LLS) (Table 4.1). As used by others, a score of ≥ 4 was used to define the presence of AMS (118). Success or failure in reaching the summit of Cerro Aconcagua (6959m) was self reported by the subjects as they returned to record their safe return to Base Camp with the Aconcagua Park Rangers.

4.3.1.4 Genetic Analysis

For those who consented to take part, DNA was collected using a buccal swab and was genotyped for ACE I/D polymorphism (see Chapter 2: Methods for details).

4.3.1.5 Statistical Analysis

Results were analysed using SPSS version 21. Chi squared test was used to confirm Hardy-Weinberg equilibrium. Differences in study population physiological parameters and genotype groups for AMS incidence and severity were assessed by ANOVA or Chi squared testing as appropriate. Throughout, a p-value ≤ 0.05 was considered statistically significant.

4.3.2 Results

During the study period, 110 (89 male) Caucasian subjects were recruited. ACE genotyping failed in two of the 110 subjects and in the remaining subjects genotype distribution was 23 (21%) versus 44 (41%) versus 41 (38%) for II versus ID versus DD, respectively (I-allele frequency 0.417), consistent with the Hardy-Weinberg equilibrium. In this cohort, physical characteristics (height 177.8 ± 9 cm, weight 74.5 ± 11 kg, age 35.2 ± 10.5 years) were independent of ACE genotype. At the time of assessment, the mean \pm SD duration of stay at 4300m had been 22.6 ± 9.1 hours and there was no association with duration of stay and ACE genotype ($p=0.394$).

AMS and LLS

LLS was assessed in all subjects and the mean \pm SD LLS was 2.2 ± 1.8 . AMS symptoms and clinical assessments are illustrated in Figures 4.3, 4.4 and 4.5. LLS score was not associated with age, sex, weight, height or with duration of time at 4300m prior to assessment.

Figure 4.3 AMS Symptoms at 4300m

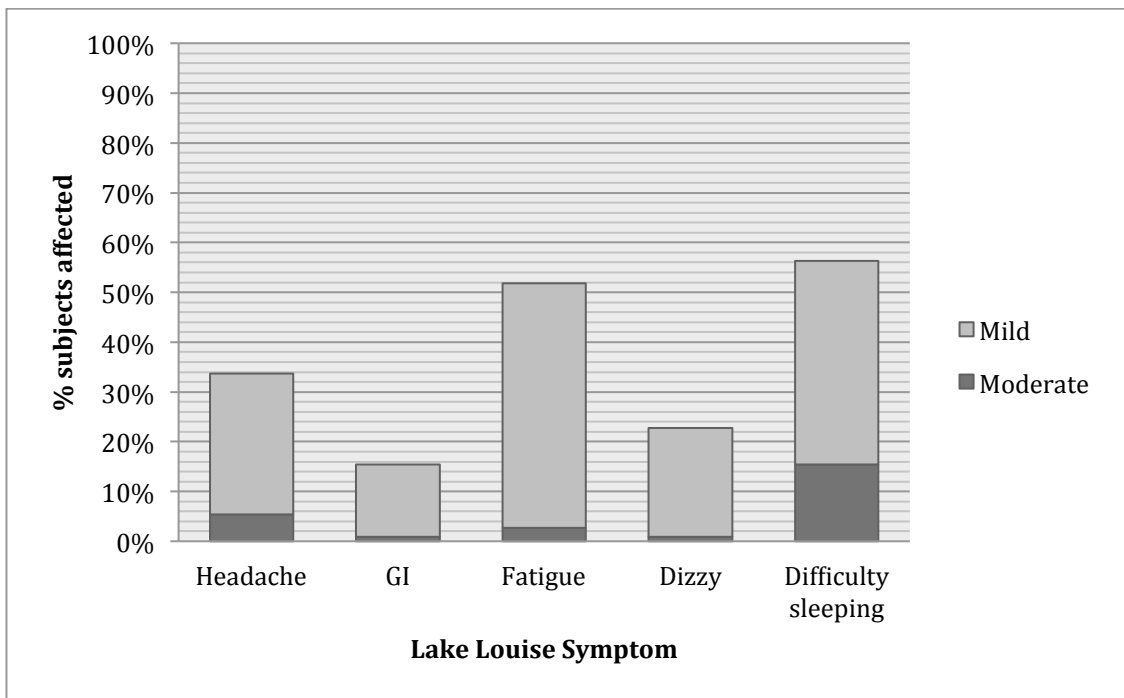


Figure 4.4 AMS Clinical Assessment at 4300m

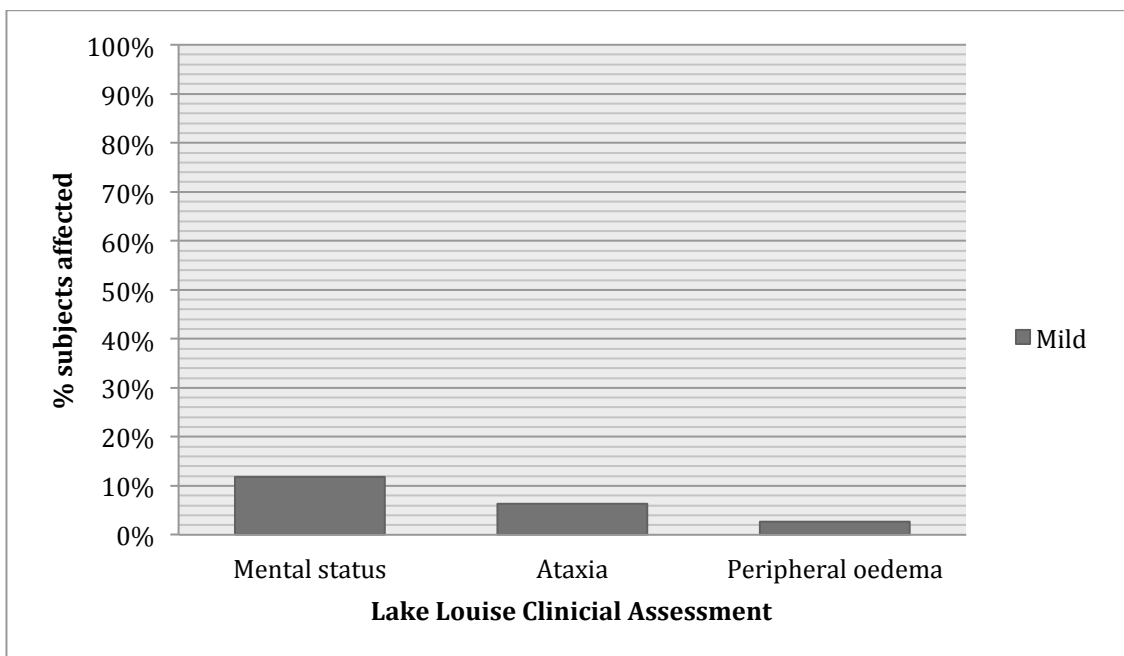
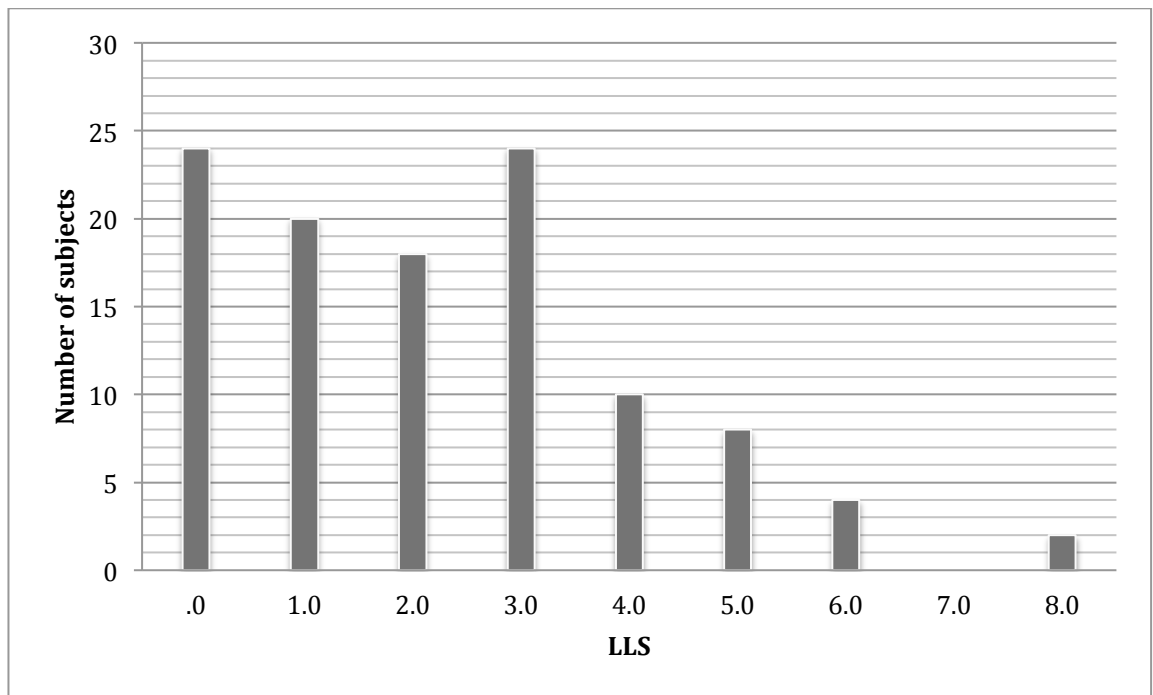


Figure 4.5 Lake Louise Scores at 4300m



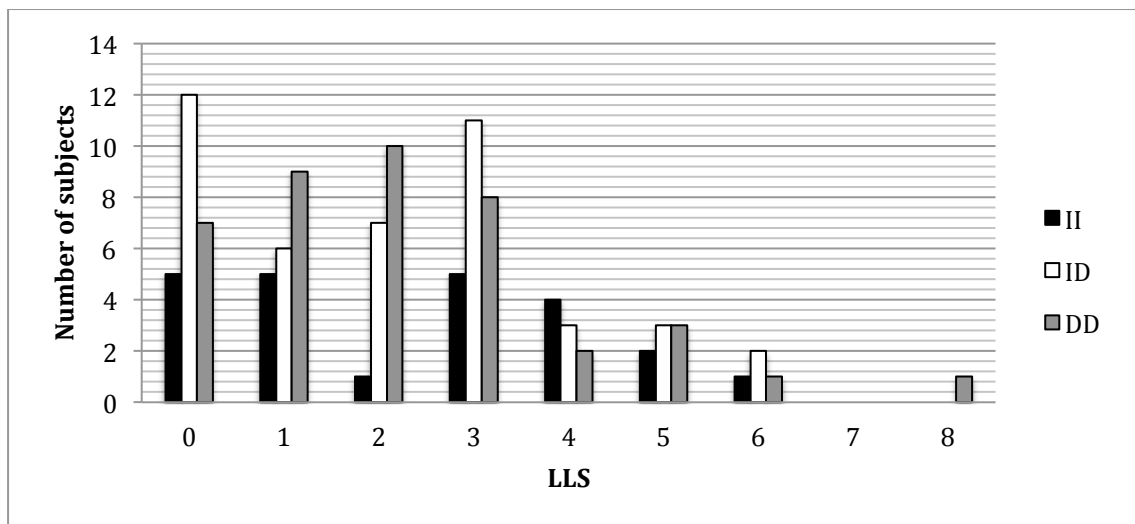
AMS score and ACE genotype

AMS scores by genotype are displayed in Table 4.7. Mean \pm SD AMS LLS by ACE genotype was 2.35 ± 1.87 , 2.091 ± 1.78 and 2.195 ± 1.0 for II, ID and DD respectively. There was no significant association between ACE I/D genotype and total LLS ($p=0.858$) or LLS symptom score ($p=0.875$) (Figure 4.6).

Table 4.6 ACE I/D genotype and LLS

| | ACE I/D genotype | | | Total |
|----------|------------------|---------------|---------------|---------------|
| | II | ID | DD | |
| 0 | 5 | 12 | 7 | 24 |
| 1 | 5 | 6 | 9 | 20 |
| 2 | 1 | 7 | 10 | 18 |
| 3 | 5 | 11 | 8 | 24 |
| 4 | 4 | 3 | 2 | 9 |
| 5 | 2 | 3 | 3 | 8 |
| 6 | 1 | 2 | 1 | 4 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 1 | 1 |
| Total | 23 | 44 | 41 | 108 |
| Mean LLS | 2.4 \pm 1.9 | 2.1 \pm 1.8 | 2.2 \pm 1.0 | 2.2 \pm 1.8 |
| p=0.858 | | | | |

Figure 4.6 ACE I/D genotype and LLS



AMS presence and ACE genotype

Using a LLS ≥ 4 to define the presence of AMS, 22 of the 110 subjects (20%) suffered from AMS at the time of assessment. Genotype distribution for those suffering from AMS was 7 (32%) vs 8 (36%) vs 7 (32%) (I allele frequency 0.5) and for those who did not suffer, 16 (19%) vs 36 (42%) vs 34 (39%) (I allele frequency 0.395) for II vs ID vs DD genotype respectively. There was no association between ACE genotype and the presence of AMS ($p=0.406$). Interestingly 30% of the ACE II subjects suffered from AMS by comparison with 18% and 17% of the ID and DD subjects respectively, but this did not reach statistical significance ($p=0.24$).

Table 4.7 ACE I/D genotype and presence of AMS (LLS ≥ 4)

| | | ACE genotype | | | Total |
|--------------|-----|--------------|-----|-----|-------|
| | | II | ID | DD | |
| AMS | No | 16 | 36 | 34 | 86 |
| | Yes | 7 | 8 | 7 | 22 |
| AMS presence | | 30% | 18% | 17% | 20% |
| | | p=0.406 | | | |

Successful summit ascent

Data on success or failure in reaching the summit of Cerro Aconcagua was available on 104 of the 110 subjects. Sixty-seven subjects with available data were successful in reaching the summit. There was no association between summit success and sex, weight or height (Table 4.11) although there was a strong association between age and summit success ($p=0.008$) with a mean \pm SD age of 32.9 ± 8.8 years in those who reached 6963m and 38.1 ± 10.8 years in those who did not. In those who succeeded in reaching the summit the mean \pm SD LLS at assessment was 2.00 ± 1.8 compared to 2.74 ± 1.9 in those who failed ($p=0.05$).

Table 4.8 Characteristics of subjects successful in reaching the summit of Cerro Aconcagua (data are presented as mean +/- SD)

| | Success (68 subjects) | Failure (38 subjects) | p= |
|----------------|--------------------------|--------------------------|-------|
| Age (years) | 32.9±8.8 | 38.1±10.8 | 0.008 |
| Sex | 84% male | 76% male | 0.348 |
| Height (cm) | 177.6±9.7 | 178.3±8.3 | 0.687 |
| Weight (kg) | 73.5±10.8 | 77.0±12.3 | 0.131 |
| Hours at 4300m | 23.1±9.8 | 21.6±8.1 | 0.404 |
| LLS | 2.00±1.8 | 2.74±1.9 | 0.05 |

ACE genotype and successful ascent

ACE genotype distribution for those who succeeded was 15 (22%), 26 (39%) and 26 (39%) and those who failed 7 (19%), 17 (46%) and 13 (35%) for II, ID and DD respectively (Table 4.12). There was no association demonstrated between ACE I/D genotype and success in climbing to the summit of Cerro Aconcagua (p=0.99).

Table 4.12 ACE I/D genotype and success in reaching 6963m.

| | ACE I/D genotype | | | Total |
|----------------|------------------|-----|-----|-------|
| | II | ID | DD | |
| Summit Success | 15 | 26 | 26 | 67 |
| Summit Failure | 7 | 17 | 13 | 37 |
| Success rate | 68% | 60% | 67% | 64% |
| | p=0.99 | | | |

4.4 Discussion

These 2 studies did not demonstrate an association between ACE I/D polymorphism and AMS in Caucasian mountaineers at altitudes between 2700m and 5895m on Mount Kilimanjaro, Tanzania and at 4300m on Cerro Aconcagua, Argentina. Moreover, the ACE I-allele was not associated with successful ascent to the summit of Mt Kilimanjaro (5895m) or Cerro Aconcagua (6959m) in the 2 large cohorts of high altitude trekkers assessed. An association between successfully reaching the summit of Cerro Aconcagua and younger age and lesser severity of AMS at assessment was demonstrated.

As described previously, while several plausible physiological mechanisms exist by which ACE I/D polymorphism may be hypothesized to influence AMS susceptibility, at the time of these studies conflicting data existed regarding the association of ACE I/D genotype with AMS development (110, 123, 124). Of the 2 other studies addressing this question in Caucasian subjects, Dehnert et al. demonstrated no association between the ACE I/D polymorphism and AMS susceptibility at 4559m (123) whereas Tsianos et al. demonstrated an association between the heterozygous ACE ID genotype and resistance to AMS ($P=0.003$) in 284 climbers assessed on arrival from lower altitude at the Gouter Hut (3,807 m), Mt Blanc (110). Subsequent studies have continued to deliver discordant results with Buroker et al. comparing the genotype of 98 AMS patients with 60 Han controls and finding the ACE D-allele to be significantly associated with AMS (158).

In 2012, Luo et al. undertook a meta-analysis of the published studies to assess the association between the ACE I/D polymorphism and AMS. A fixed effects model was applied and study quality was assessed in duplicate. Five studies with a total of 333 AMS cases and 373 healthy controls were assessed and revealed no significant

differences in risk for AMS between ACE I/D genotype (159). This meta-analysis included the results of Study 1 (160) but not the currently unpublished results of Study 2.

It may be proposed that some of these discordant results reflect the clinical imprecision of the diagnosis of AMS and the lack of diagnostic modalities or physical findings to reliably confirm the presence of this disease entity (114). The Lake Louise Consensus Committee definition of AMS is headache in the setting of recent arrival to high altitude and one or more of the following: anorexia, nausea, or vomiting; fatigue or weakness; dizziness or lightheadedness; or difficulty sleeping (119). Other diagnostic scoring systems also exist with variously reported sensitivity and specificity (118). Furthermore, different biological mechanisms may be driving different symptoms independently of one another.

AMS may progress to the life threatening HACE or HAPE and recognition and appropriate action is critical (116). However the differential diagnosis of these symptoms are broad in typically physically exhausted individuals functioning in austere environments. Differential diagnoses may range from the common complaints of exhaustion and dehydration to life threatening conditions that may present with similar symptoms including carbon monoxide poisoning, central nervous system infection, diabetic ketoacidosis, hypothermia, toxins, or viral and bacterial infections (114). Clinical examination may help narrow this differential but in larger studies using self reporting questionnaires it may be that various disease entities are grouped together and that it may be unrealistic to expect a clear association with a single polymorphism.

Some other points are worthy of note with regard to Study 1 on Mount Kilimanjaro. First, both AMS incidence and severity increased with altitude. The higher AMS scores

seen in subjects during their acclimatisation day at 3,700 m were likely due to a lag in the development of AMS and it is well established that slower ascent rates lead to lower incidence of AMS (161). Interestingly there was no difference in AMS scores between the subjects who spent an extra day acclimatising at 3,700 m and those who did not; it is possible that only one extra day on the mountain does not provide sufficient acclimatisation on such a rapid ascent to extreme altitude.

A strength of Study 1 was the chosen mountain and route. The Marango route on Mt. Kilimanjaro was selected because the rapid ascent schedules produce a high incidence of AMS and the ascent profile allows researchers to follow subjects at evenly graduated intervals on ascent. Additionally the population had little previous acclimatisation; only 10.2% of screened subjects had visited high altitude in the previous 2 weeks.

An important limitation of Study 2 was that, whilst subjects were excluded if they had spent more than 48 hours at 4300m, the study did not account for the speed of ascent to this altitude from sea level and recent history of altitude exposure. Although trekkers attempting to climb Aconcagua are principally participants on commercial single peak expeditions and guides and porters were excluded from the study, residual acclimatisation in some participants could be a confounding variable and subjects with different ascent profiles might be expected to be at different points in their acclimatisation process.

The inevitable logistic problems of such fieldwork added to the limitations in both studies. In Study 1, 57 subjects (34 male) were lost to follow-up during the study and there is a possibility that some of this group descended without presenting to the investigators due to AMS. However, this seems an unlikely confounder: the physical characteristics and ACE genotype distribution of these individuals did not differ from

those followed up, whilst the incidence of AMS and the last recorded AMS scores at each altitude were no higher in those lost to follow-up than in those who continued. Importantly, AMS scores at any altitude were unrelated to loss to follow-up. AMS scores immediately prior to the summit attempt were also independent of both ACE genotype and of summit success (Tables 4.2, 4.5). In Study 2, despite the regulation requiring trekkers to report to the Aconcagua Park rangers on arrival at 4300m there was variation in the time taken for climbers to report and hence to be assessed for this study (22.6 ± 9.1 hours). Interestingly, the variation in duration of time at 4300m prior to assessment was not associated with presence or severity of AMS. With a single assessment station at 4300m, Study 2 cannot account for AMS preventing some reaching this altitude at all and this may lead to some selection bias.

The lack of association between the ACE I-allele and successful ascent to the summit of Mt Kilimanjaro (5895m) or Cerro Aconcagua (6963m) is perhaps surprising given the results of Chapter 3 (156) and previous studies at both lower and higher altitudes (86, 110, 156).

It may be that such an association does exist but was not detected in these 2 studies. For instance in Study 1, although there was no association demonstrated between ACE genotype and AMS or successful ascent, there was a non-significant ($p=0.09$) trend for D-allele association with failure using the most rapid ascent profile. In this group, lack of true statistical significance may have been due to powering issues: post-hoc power calculations suggest that with the sample size of 34, with 20 successful and 14 unsuccessful subjects (100% II subjects successful, 52% of ID and 43% of DD, giving an effect size of 0.156) the study only had 52% power to detect a difference in summit success. Similarly whilst no significance was demonstrated, there was a trend for higher

mean AMS scores amongst ID and DD climbers (Table 4.3). This lack of statistical significance may again be due to lack of statistical power. The study was underpowered to detect a difference—given the difference observed in mean AMS scores between genotype groups at 4,700 m on the D route 106 subjects would be needed to achieve 80% power, rather than this sample of 42 subjects.

An alternative explanation for the lack of association of the I-allele with summit success in these studies may be that the populations assessed in these studies on Mt Kilimanjaro and Cerro Aconcagua differ from the previous studies of elite mountaineers and are representative of a non-elite trekking population. These mountains are not technical in nature and thus attract large numbers of amateur trekkers who aspire to reach great altitude. These peaks were chosen for exactly this popularity with tourist trekkers with the aim of generating large numbers of study participants who were being exposed to hypobaric hypoxia. At sea level, elite athletic endurance performance has been associated with the I-allele (162-164) but discordant results in groups of mixed ability and sporting discipline (90, 165). The diverse challenges and novel skills confronting amateur trekkers at altitude may confound the single limiting effect of hypobaric hypoxia when compared to experienced and elite high altitude mountaineers (86, 156). Moreover, whilst altitudes of up to 6959m do represent a profound physiological challenge, they do not approach the cusp of human survival that the hypoxia of peaks of 8000m or more present (2). It may be that the effect of the ACE I-allele becomes more evident at the phenotypic extremes and that the impact is not evident at lesser altitudes. However this is not consistent with the findings of Tsianos et al. who demonstrated an I-allele association with successful ascent of Mt Blanc at the lower altitudes of 4807m (110).

Conclusions

The ACE I/D genotype was compared between individuals who did or did not develop AMS, as defined by Lake Louise Score (LLS), during the ascent of Mount Kilimanjaro (5895m) and after arriving at Plaza de Mulas basecamp (4300m), Cerro Aconcagua, Argentina. No association was found between the ACE I/D genotype and AMS incidence or severity or success in reaching the summit of either mountain.

The lack of association between ACE I/D genotype and AMS demonstrated in these studies suggest that any advantage conferred by the ACE I-allele in previous studies is unlikely to be mediated by reduced susceptibility to AMS.

CHAPTER 5: ACE I/D Polymorphism and Arterial Oxygen Saturations at 4300m

- 5.1 Background
- 5.2 Methods
 - 5.2.1 Subjects
 - 5.2.2 Subject data
 - 5.2.3 Genetic Analysis
 - 5.2.4 Statistical Analysis
- 5.3 Results
- 5.4 Discussion

5.1 Background

ACE I/D polymorphism is associated with enhanced performance in hypoxic environments (107, 110, 156) but the mechanisms underlying this association remain unclear. Prior to this study, previous publications had demonstrated an association between the ACE I-allele and improved arterial oxygen saturation (S_aO_2) at altitude (111) and an increased hypoxic ventilatory response in laboratory conditions of hypoxia and exercise (112). I thus sought to undertake a large prospective study to test whether the ACE I-allele is associated with enhanced tolerance to high altitude as assessed by improved SaO_2 .

This study was conducted in the same cohort of Caucasian mountaineers attempting to climb Cerro Aconcagua described in detail in Chapter 4 Study 2.

5.2 Methods

5.2.1 Subjects

Subjects were mountaineers attempting to climb Argentina's Cerro Aconcagua (6963m) and were recruited at Plaza de Mulas base camp (4300m). Excluded were those who had spent more than 48 hours at 4300m, those taking steroids or acetazolamide for AMS prophylaxis, those taking RAS antagonists (ACE inhibitors, or angiotensin II type 1 receptor antagonists).

5.2.2 Subject data

Subjects were asked to complete a questionnaire of demographic data and high altitude history mountaineering experience (Appendix 2). A buccal swab was obtained for genetic analysis, subjects completed a questionnaire (in English or Spanish), and written informed consent was obtained from all volunteers.

Resting SaO₂ was measured in all subjects using a pulse oximeter (MCDEI 8500M, Nonin Medical Inc, Plymouth, MN) placed on the index finger. SaO₂ was measured with the subject in the sitting position and the value accepted when a stable result was displayed. Digits were pre-warmed to avoid poor pulse signals due to low perfusion.

5.2.3 Genetic Analysis

For those who consented to take part, DNA was collected using a buccal swab and was genotyped for ACE I/D polymorphism (see Chapter 2: Methods for details).

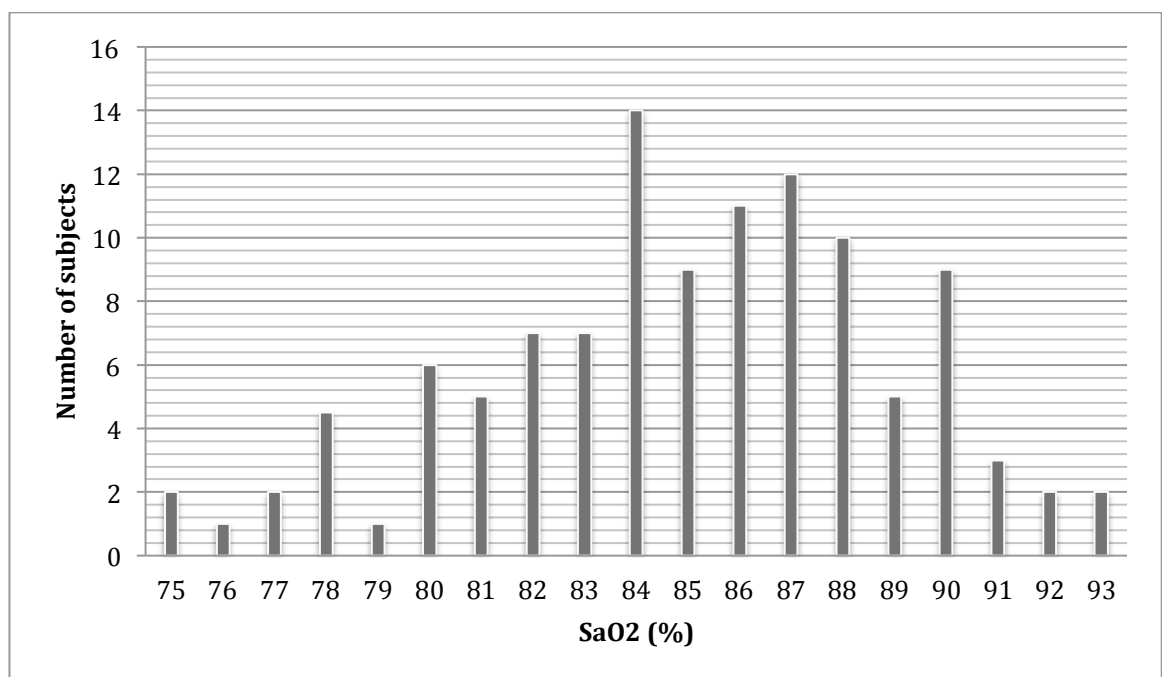
5.2.4 Statistical Analysis

Results were analysed using SPSS version 21. Differences in study population physiological parameters, AMS, summit success and genotype groups were assessed by ANOVA, Chi squared or linear regression analysis as appropriate. Throughout, a p-value ≤ 0.05 was considered statistically significant.

5.3 Results

During the study period, 110 (89 male) Caucasian subjects were recruited. S_aO_2 data were available on all subjects and mean \pm SD S_aO_2 on assessment at 4300m were $85.2 \pm 3.9\%$ (Table 5.1). S_aO_2 were not associated with age, sex, height or weight and there was no significant correlation between S_aO_2 and hours spent at 4300m prior to assessment ($r^2=0.005$).

Table 5.1 S_aO_2 at 4300m in 110 Caucasian trekkers



S_aO₂ ACE I/D genotype

S_aO_2 by ACE genotype were $85.8 \pm 3.3\%$, $85.5 \pm 4.2\%$, and $84.4 \pm 3.9\%$ for II, ID and DD respectively and no significant association was demonstrated ($p=0.284$).

S_aO₂ and AMS

There was a strong association between S_aO_2 and LLS score ($p=0.009$). If a $LLS \geq 4$ was used to define AMS presence the mean \pm SD S_aO_2 in those who were suffering from AMS was $83.7 \pm 3.1\%$ and in those did not have AMS, $85.5 \pm 4.0\%$ ($p=0.021$).

S_aO₂ and summit success

In those who succeeded in reaching the summit the mean \pm SD SaO₂ at assessment was 85.1 \pm 4.0 compared to 85.4 \pm 3.9 (p=0.65) in those who failed.

5.4 Discussion

This study did not demonstrate an association between resting S_aO_2 and ACE I/D genotype or summit success in subjects assessed at 4300m on Cerro Aconcagua. However it did identify an association of resting S_aO_2 with AMS presence and Lake Louise Score when assessed at 22.6 ± 9.1 hours following arrival at this altitude.

Previously the ACE I-allele has been associated with an enhanced ventilatory response to exertional hypoxia (112) and better-maintained arterial oxygenation during rapid ascent to high altitude (111). In support, the I-allele is more prevalent, and S_aO_2 higher, amongst highlanders (rather than lowlanders) living in Ladakh (140).

Patel et al. exposed subjects to a fractional inspired oxygen concentration (FiO_2) of 0.125 during exertion in the laboratory and showed that the hypoxia-induced rise in exertional minute ventilation was significantly greater among those of II genotype ($39.6 \pm 4.1\%$ versus $27.9 \pm 2.0\%$ versus $28.4 \pm 2.2\%$ for II versus ID versus DD, respectively). However, similarly to the results in this study on Aconcagua, this laboratory study did not show a significant association of ACE genotype with S_aO_2 . Conversely, Woods et al. measured S_aO_2 over many days in trekkers ascending to over 5000m in the Himalaya. Subjects of ACE II genotype who ascended to above 5000 m in 12 days were able to maintain higher S_aO_2 , especially as altitude increased. A similar trend was evident, but not significant, in a slower ascent group who trekked over 18.5 days (111). An explanation for this discordance between the two rate of ascent groups was proposed that the I-allele benefit is short lived and more important during early-stage acclimatisation to high altitude. According to Woods et al., this could be due to the normalization of the plasma aldosterone concentration to plasma renin activity ratio (PAC/PRA) that occurs after 12 to 20 days at high altitude.

More recently Bigham et al demonstrated an association of ACE II genotype with higher resting and submaximal exercise S_aO_2 in two groups of Peruvians, one high altitude residents and one lowland residents, who were tested at 4338 m altitude ($p = 0.008$). In both study groups, individuals of ACE II genotype maintained a ~2.3 percentage point higher S_aO_2 , explaining about 4% of the total variance in resting and exercise values (122). This study suggests that the I-allele effect on increasing S_aO_2 is present in high altitude residents and is therefore not an acclimatisation or short-lived phenomenon. There is known to be a significant genetic influence upon S_aO_2 in high altitude residents (111, 140, 166, 167) and previous studies have suggested a role for the ACE I/D polymorphism (140).

Proposed mechanisms underlying the ACE I-allele influence have included the increased HVR (hypoxic ventilatory response, by which hypoxia stimulates a rise in minute ventilation) to increase S_aO_2 (112) and which is supported by animal studies that have shown that the peripheral chemoreceptors contain angiotensin receptors with potential effects on the regulation of ventilation (74, 168). Bigham et al. did not find an ACE I-allele associated with HVR and speculated that the explanation may be another central cardiopulmonary effect of the ACE gene, such as angiotensin II modulating hypoxia-induced pulmonary vasoconstriction (70) or differences in circulating ACE affecting ventilation–perfusion (V/Q) relationships within the lung (130).

The lack of association between the ACE I-allele and increased S_aO_2 seen in this study accords with Patel et al. (112) but not with the 2 other studies addressing this issue (111, 122). This study accounts for time spent at 4300m, but not for the speed of ascent to this altitude from sea level and recent history of altitude exposure. Residual acclimatisation in some mountaineers could be a confounding variable and mountaineers with different

speeds of ascent profile might be expected to be at different points in their acclimatisation process. The hypoxic ventilatory response (HVR) increases as part of the process of acclimatisation to altitude. As a result, S_aO_2 increases progressively with acclimatisation (169). Despite the probable uniformity imposed by commercial expeditions on Cerro Aconcagua, this study could not control for and did not record the ascent profile prior to arrival at 4300m. Following arrival there was a delay and variance in the time of assessment and recording of S_aO_2 . Although there was no association between either ACE I and duration of time spent at 4300m or between duration of time spent at 4300m prior to assessment and S_aO_2 in this study, this potential variability in acclimatisation is an important confounding factor.

In this study it is confirmed that there is a significant association between both increasing AMS score and AMS incidence, and reduced S_aO_2 . This finding confirms the recognised relationship between AMS and hypoxemia (79, 170-172) and concords with previous studies (171, 173) that demonstrated that noninvasive pulse oximetry saturations are inversely correlated with the severity of AMS and may detect exaggerated hypoxemia in severe AMS (171).

A potential limitation in this study may be that the use of pulse oximetry in cold environments, at altitude and in patients with severe hypoxia may cause inaccurate recordings. To minimise the effect of cold, digits were warmed to avoid poor pulse signals due to low perfusion. However, pulse oximetry calibration inaccuracy increases below 80% and with 8 subject recording S_aO_2 below this threshold, there may be some uncertainty in the accuracy of these lowest values.

Conclusion

There is no association between resting S_aO_2 and ACE I/D genotype in Caucasian trekkers assessed at 4300m on Cerro Aconcagua. This study confirms the well recognized association between AMS and S_aO_2 .

CHAPTER 6: Bradykinin 2 Receptor +9/-9 Polymorphism and High Altitude

- 6.1 Background
- 6.2 Methods
 - 6.2.1 Subjects
 - 6.2.2 Subject data
 - 6.2.3 Genetic Analysis
 - 6.2.4 Statistical Analysis
- 6.3 Results
- 6.4 Discussion

6.1 Background

As a key component of the circulating RAS, ACE mediates its effects both through generation of vasoconstrictor Angiotensin II (AT II) and the degradation of vasodilator kinins. The role of ACE in bradykinin degradation means that bradykinin levels are inversely related to ACE activity with the low circulating ACE levels conferred by the ACE I-allele associated with increased bradykinin levels (41, 42). Bradykinin is a potent endothelium-dependent vasodilator and increases vascular permeability (35). Its effects are mediated through the inducible bradykinin type 1 (BK₁R) and constitutive type 2 (BK₂R) receptors. The BK₂R gene demonstrates a common functional polymorphism in which the absence (-9) of a 9 base pair repeat is associated with greater gene transcription (102), higher mRNA expression of the receptor (103) and lower vascular resistance (104).

If the influence of the ACE I/D polymorphism on successful ascent to high altitude is, in part, mediated by altered levels of bradykinin, it may be postulated that the BK₂R +9/-9 polymorphism might influence performance in a hypoxic environment. Supporting this hypothesis are data demonstrating that the +9/+9 genotype is associated with reduced metabolic efficiency during exercise in healthy subjects at sea level and the combined ACE I / BK₂R -9 'high kinin receptor activity' haplotype is significantly associated with endurance performance among elite athletes (105).

I tested the hypothesis that inter-individual variations in AMS, peripheral S_aO₂ and successful ascent to high altitude may be influenced by the BK₂R +9/-9 polymorphism.

6.2 Methods

6.2.1 Subjects

Subjects were the cohort of Caucasian mountaineers attempting to climb Cerro Aconcagua (6963m), and described in detail in Chapter 4. In brief, subjects were recruited soon after arrival at Plaza de Mulas base camp (4300m). Excluded were those who had spent more than 48 hours at 4300m, those taking steroids or acetazolamide for AMS prophylaxis and those taking RAS antagonists (ACE inhibitors, or angiotensin II type 1 receptor antagonists).

6.2.2 Subject data

A buccal swab was obtained for genetic analysis, subjects completed a questionnaire and written informed consent was obtained from all volunteers (Questionnaire: Appendix 2). Success or failure in reaching the summit of Cerro Aconcagua (6963m) was self-reported by the subjects as they returned to record their safe return to Base Camp with the Aconcagua Park Rangers.

Presence and severity of current AMS symptoms were documented using the Lake Louise Score (LLS) (Table 4.1). As used by others, a score of ≥ 4 was used to define the presence of AMS (118).

S_aO₂ was measured using a pulse oximeter (MCDEI 8500M, Nonin Medical Inc, Plymouth, MN) placed on the index finger. S_aO₂ was measured with subjects resting in the sitting position. The saturation value was accepted when a stable result was displayed. Digits were pre-warmed to avoid poor pulse signals due to low perfusion.

6.2.3 Genetic Analysis

For those who consented to take part, DNA was collected using a buccal swab and was genotyped for BK₂R +9/-9 polymorphism (see Chapter 2: Methods for details).

6.2.4 Statistical Analysis

Results were analysed using SPSS version 21. Chi squared test was used to confirm Hardy-Weinberg equilibrium. Differences in study population physiological parameters and genotype groups for AMS incidence and severity were assessed by ANOVA or Chi squared testing as appropriate. Throughout, a p-value ≤ 0.05 was considered statistically significant.

6.3 Results

During the study period, 110 (89 male) Caucasian subjects were recruited. BK₂R +9/-9 genotyping was successful in all subjects and the genotype distribution was 22 (20%) versus 59 (54%) versus 29 (26%) for +9/+9 versus +9/-9 versus -9/-9, respectively (+9-allele frequency 0.468), consistent with the Hardy-Weinberg equilibrium. Bradykinin genotype was independent of age, sex, height and weight. At the time of assessment, the mean +/- SD duration of stay at 4300m had been 22.4±9.1 hours and there was no association between duration of stay and bradykinin genotype (p=0.182).

AMS score and BK₂R genotype

There was a significant association between BK₂R -9/-9 genotype and increased LLS with LLS by BK₂R genotype of 2.00±1.54, 1.93±1.61 and 3.1±2.34 for +9/+9, +9/-9 and -9/-9, respectively (p=0.016) (Table 6.1).

Table 6.1 LLS by BK₂R genotype

| | BK ₂ R +9/-9 genotype | | | Total |
|----------|----------------------------------|----------|---------|----------|
| | +9/+9 | +9/-9 | -9/-9 | |
| 0 | 5 | 15 | 4 | 24 |
| 1 | 4 | 11 | 5 | 20 |
| 2 | 4 | 11 | 3 | 18 |
| 3 | 5 | 12 | 7 | 24 |
| 4 | 3 | 6 | 1 | 10 |
| 5 | 1 | 3 | 4 | 8 |
| 6 | 0 | 1 | 3 | 4 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 2 | 2 |
| Total | 22 | 59 | 29 | 110 |
| Mean LLS | 2.00±1.5 | 1.93±1.6 | 3.1±2.3 | 2.03±1.7 |
| | p=0.016 | | | |

AMS symptoms and signs and BK₂R genotype

Analysis of LLS breakdown demonstrated that this association in LLS was attributable to a strong association of BK₂R -9/-9 genotype with the cardinal AMS symptom of headache (p=0.007) that was present in 15/29 (52%) of subjects with the -9/-9 genotype by comparison with 17/59 (29%) and 5/22 (23%) of those with +9/-9 and +9/+9 genotype respectively (Table 6.2). The -9/-9 genotype was also significantly associated with difficulty sleeping (p=0.032). There was no association between BK₂R genotype and the clinical assessment scores (Table 6.3).

Table 6.2 AMS Symptoms by BK₂R genotype

| | Headache | | | Gastrointestinal | | | Fatigue | | | Dizziness | | | Difficulty Sleeping | | |
|----------------------------|--------------|-------|-------|------------------|-------|-------|--------------|-------|-------|--------------|-------|-------|---------------------|-------|-------|
| | +9/+9 | +9/-9 | -9/-9 | +9/+9 | +9/-9 | -9/-9 | +9/+9 | +9/-9 | -9/-9 | +9/+9 | +9/-9 | -9/-9 | +9/+9 | +9/-9 | -9/-9 |
| BK ₂ R Genotype | | | | | | | | | | | | | | | |
| Present (No of subjects) | 5 | 17 | 15 | 3 | 7 | 7 | 12 | 29 | 16 | 4 | 11 | 9 | 10 | 31 | 21 |
| Absent (No of subjects) | 17 | 42 | 14 | 19 | 52 | 22 | 10 | 30 | 13 | 18 | 48 | 19 | 12 | 28 | 8 |
| Symptom by genotype | 23% | 29% | 52% | 14% | 12% | 24% | 55% | 49% | 55% | 18% | 19% | 34% | 45% | 53% | 72% |
| % subjects with symptom | 34% | | | 15% | | | 52% | | | 23% | | | 56% | | |
| <i>p</i> = | 0.007 | | | 0.510 | | | 0.579 | | | 0.093 | | | 0.032 | | |

Table 6.3 AMS Clinical Assessment by BK₂R genotype

| BK ₂ R Genotype | Change in mental status | | | Ataxia | | | Peripheral oedema | | |
|-------------------------------|-------------------------|-------|-------|--------------|-------|-------|-------------------|-------|-------|
| | +9/+9 | +9/-9 | -9/-9 | +9/+9 | +9/-9 | -9/-9 | +9/+9 | +9/-9 | -9/-9 |
| Present | 5 | 4 | 4 | 1 | 4 | 2 | 1 | 1 | 1 |
| Absent | 17 | 55 | 25 | 21 | 55 | 27 | 21 | 58 | 28 |
| Sign by genotype | 23% | 7% | 14% | 5% | 7% | 7% | 5% | 2% | 3% |
| % subjects with clinical sign | 12% | | | 6% | | | 3% | | |
| <i>p</i> = | 0.428 | | | 0.750 | | | 0.870 | | |

AMS presence and BK₂R genotype

However if a LLS ≥ 4 was used to define the presence of AMS, there was not an association between BK₂R -9/-9 genotype and the presence of AMS ($p=0.159$) (Table 6.4). Genotype distribution for the 24 subjects who suffered from AMS was 4 (16%) vs 10 (42%) vs 10 (42%) (+9 allele frequency 0.375) and in the 86 subjects who did not suffer was 18 (21%) vs 49 (57%) vs 19 (22%) (+9 allele frequency 0.494) for +9/+9, +9/-9 and -9/-9 genotype respectively.

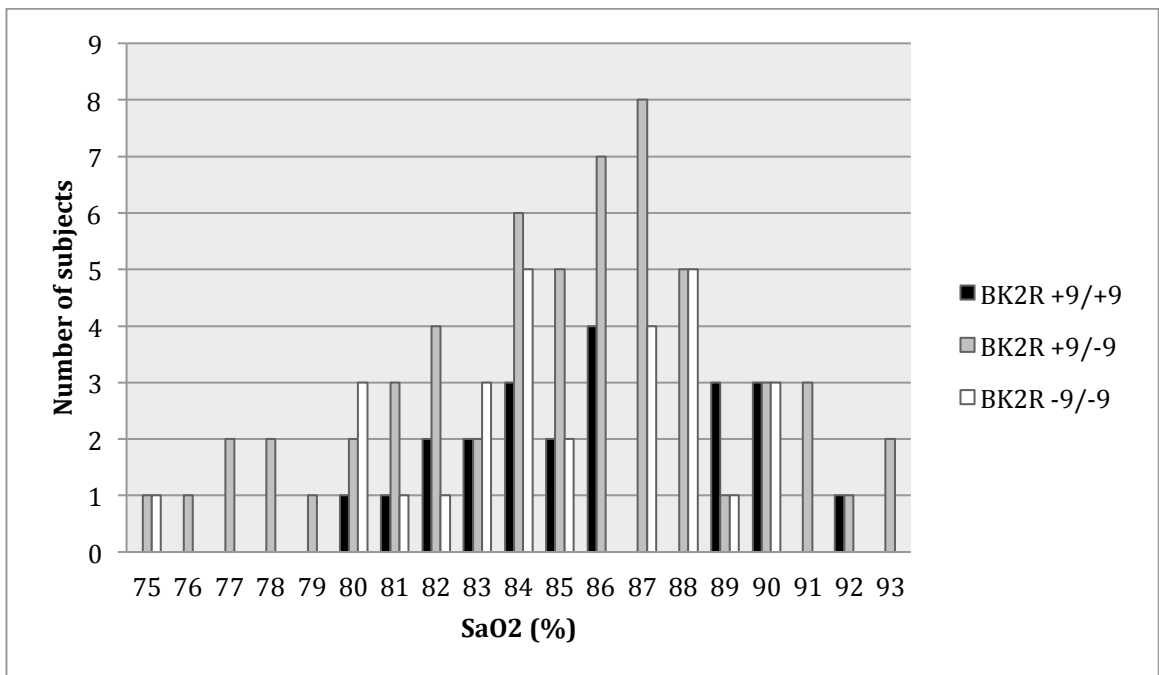
Table 6.4 AMS presence and BK₂R genotype

| | | BK ₂ R genotype | | | Total |
|--------------|----------|----------------------------|-------|-------|-------|
| | | +9/+9 | +9/-9 | -9/-9 | |
| LLS | 0-3 | 18 | 49 | 19 | 86 |
| | ≥ 4 | 4 | 10 | 10 | 24 |
| Total | | 22 | 59 | 29 | 110 |
| AMS presence | | 18% | 18% | 34% | 22% |
| | | $p=0.159$ | | | |

S_aO₂ and BK₂R genotype

SaO₂ was not associated with BK₂R genotype with mean±SD SaO₂ of 85.7±3.3, 84.9±4.2 and 85.0±3.6 for BK₂R +9/+9, +9/-9 and -9/-9 respectively (p=0.727). (Figure 6.1)

Figure 6.1 SaO₂ and BK₂R genotype



Summit success and BK₂R genotype

Interestingly, given the association of BK₂R -9/-9 with increasing AMS score, there was no consequent disadvantage associated with BK₂R genotype and success in reaching the summit of Cerro Aconcagua (6963m) (Table 6.5).

Table 6.5 Summit success by BK2R genotype

| | | <i>BK2R</i> genotype | | | Total |
|--------------|---------|----------------------|-------|-------|-------|
| | | +9/+9 | +9/-9 | -9/-9 | |
| Summit | Success | 13 | 37 | 18 | 68 |
| | Failure | 8 | 21 | 9 | 38 |
| Success rate | | 62% | 64% | 67% | 64% |
| p=0.520 | | | | | |

BK2R genotype and ACE interaction

When combined BK₂R +9/-9 and ACE I/D haplotypes (e.g. ACE II and BK -9/-9) were assessed there was no association between any haplotype and AMS presence, Lake Louise Score, S_aO₂ or summit success.

6.4 Discussion

This study is the first to demonstrate an association between the BK₂R -9/-9 genotype and increased LLS. In particular the BK₂R -9/-9 genotype is strongly associated with high altitude headache in subjects assessed after arrival at 4300m. There was no association demonstrated between BK₂R genotype and S_aO₂ at 4300m or success in reaching the summit of Cerro Aconcagua (6963m).

The finding that the BK₂R -9/-9 is associated with increased severity of AMS and high altitude headache is novel and perhaps surprising given the existing literature that predominantly supports the -9 allele as associated with increased metabolic efficiency and improved athletic performance. The underlying pathophysiology of AMS and high altitude headache is not fully understood, but is thought to involve hypoxia-induced cerebral vasodilation and mild oedema, which, given the vasomodulatory and capillary permeability effects of bradykinin, suggests a bioplausible role for bradykinin in AMS susceptibility. (114, 116, 174-176). Recent evidence has advanced understanding of the development of AMS and reveals that hypoxia-induced cerebral vasodilation and potentially cerebral venous outflow restriction have a central role (115, 116, 177-179). The only previous study that has assessed the role of BK₂R genotype in AMS susceptibility analysed 223 lowland Nepalese attendees to a religious festival at 4380 m in the Nepalese Himalaya and found no association with the BK₂R -9/+9 polymorphism and susceptibility to AMS (180). However the authors noted that the +9/-9 allele frequencies in the Nepalese subjects were significantly different from those of other populations and the phenotypic consequences of BK₂R genotype differ significantly between races (103, 104). They concluded that these variations limit the value of extrapolating their results to other populations and further association studies in other ethnic groups would be required to exclude the role of BK₂R polymorphisms.

Exploring the potential contribution of bradykinin to the development of AMS, bradykinin is a potent endothelium-dependent vasodilator and has been demonstrated to have a role in the modulation of vascular resistance in humans, mediated in part by nitric oxide and endothelium-derived hyperpolarizing factor (37, 38, 181-183). Furthermore the BK₂R +9/-9 polymorphism has been associated with differences in peripheral, systemic and pulmonary vascular tone (184-186). NO has been proposed as having a central role in high-altitude headache (187) and the effect of bradykinin as a powerful inducer of NO and further in the post translational regulation of eNOS (188) supports a bradykinin contribution to the development of AMS. High-altitude natives have shown a level of exhaled nitric oxide 25 to 200% greater than lowlanders (189), and greater levels of exhaled nitric oxide has also been demonstrated in mountaineers resistant to high-altitude illness (190).

Whilst a plausible mechanism exists for a bradykinin influence on AMS and High Altitude Headache (HAH) development, the BK₂R -9 allele has been associated with enhanced performance phenotypes and increased metabolic efficiency in previous studies, rather than the strong association of the -9/-9 genotype with AMS and HAH seen in this study (105, 106, 184, 191, 192). The mechanisms suggested for the influence of bradykinin and the BK₂R +9/-9 polymorphism on physical performance include muscle blood flow and vascular resistance, skeletal muscle glucose uptake, nitric oxide (NO) mediated mitochondrial regulation and muscle fibre type (105).

Bradykinin promotes glucose uptake in muscle (193, 194), the absence of the BK₂R gene is associated with insulin resistance in mice (195) and the action of ACE inhibitors to increase whole-body insulin sensitivity is attenuated by BK₂R blockade (196) and

may influence skeletal muscle glucose uptake and muscle blood flow (193). Through the BK₂R, bradykinin enhances insulin-stimulated tyrosine kinase activity of the insulin receptor, with subsequent GLUT-4 translocation in skeletal muscle tissue during exercise (197). BK₂R activation can lead to transient rises in inositol 1,4,5-trisphosphate (198), which is involved in excitation coupling of skeletal muscle (199) via increases in cytoplasmic calcium (200). This process is enhanced both by insulin and by inhibition of ACE (199, 201, 202).

Bradykinin-induced nitric oxide (NO) generation may also modulate mitochondrial respiratory control (203). NO reversibly inhibits cytochrome-*c* oxidase in competition with oxygen and thus reduces VO₂ in skeletal muscle and heart mitochondria (204). Tissue and whole animal studies have shown that kinins can suppress oxygen consumption via endogenous NO production in skeletal and cardiac muscle, an effect mimicked by ACE inhibition and prevented by blockade of BK₂R (205, 206).

It may also be that BK₂R genotype influences skeletal muscle fiber type. The relative proportion of type I (slow twitch, oxidative) to type IIA (fast oxidative) and type IIB (fast glycolytic) skeletal muscle fibers has a strong influence on propensity to endurance or sprint performance (207) whereas ACE I/D genotype has been associated with fiber-type distribution (83).

However in this study no association was seen between BK₂R -9/-9 genotype and physical performance, as assessed by summit success. It is important to note that AMS susceptibility is not associated with sea level performance (116) and that it would not be physiologically inconsistent for a polymorphism that is associated with enhanced endurance performance to also be associated with increased susceptibility to the hypobaric hypoxia induced and unrelated condition of AMS. Furthermore it is plausible

that the 'high kinin' phenotype associated with the -9 allele characterised by increased gene transcription (102), higher mRNA expression of the receptor (103) and lower vascular resistance (104) may accentuate the cerebral vasodilation and oedema responsible for AMS, and in particular the cardinal feature of high altitude headache, that is so strongly associated with the BK₂R -9/-9 genotype in this study.

Plausible mechanisms by which the BK₂R polymorphism may influence S_aO₂ include altered capillary permeability and pulmonary vascular resistance (186) that may be hypothesised to predispose to pulmonary oedema and an increased diffusion barrier for oxygen. This study did not demonstrate any association between the BK₂R +9/-9 polymorphism and S_aO₂ at 4300m. Further evidence against such a hypothesis is provided by a study that found no association between the BK₂R polymorphism and HAPE in 140 HAPE patients and 144 controls during the construction of Qinghai-Tibet railway (208).

The 'high kinin' phenotype conferred by the ACE I and BK₂R -9 haplotype has been associated with enhanced physical performance (105). This study did not demonstrate an association between any combined ACE I/D and BK₂R +9/-9 haplotype. The lack of any association between either the ACE I/D or BK₂R +9/-9 polymorphisms and high altitude performance, as assessed by successfully reaching the summit of Cerro Aconcagua (6963m), means that a haplotype association would be unlikely. Contrary to expectations of the -9 allele conferring an advantage at altitude, this allele has been demonstrated to be associated with increasing AMS score and high altitude headache which may counter any beneficial effect of increased metabolic efficiency. Finally it is unlikely that analysis of the 9 haplotype subgroups of the total group of 110 subjects would be sufficiently powered to demonstrate a significant difference.

As discussed in previous chapters this study has limitations, primarily imposed by the lack of control over the acclimatisation profile of subjects prior to their arrival at 4300m. It is also noted that although an association exists between the BK₂R -9/-9 genotype and AMS score and previously between AMS score and SaO₂, that no association is demonstrated between BK₂R genotype and SaO₂. This may be due to insufficient subjects to demonstrate a significant difference, although against this explanation there is no trend evident between genotypes, with mean±SD S_aO₂ of 85.7±3.3, 84.9±4.2 and 85.0±3.6 for BK₂R +9/+9, +9/-9 and -9/-9 respectively (p=0.727). Alternatively it may be that both BK₂R -9/-9 genotype and low oxygen saturations are associated with increased AMS scores but in unrelated ways.

Conclusion

This is the first study to demonstrate an association between the BK₂R -9/-9 genotype and AMS LLS and the presence of high altitude headache at 4300m on Cerro Aconcagua. This finding is consistent with current understanding of the development of AMS. No association with summit success or S_aO₂ was demonstrated.

CHAPTER 7: ACE I/D and BK₂R +9/-9 genotype in Everest Summiteers

- 7.1 Background
- 7.2 Methods
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7.1 Background

Reaching the summit of Mount Everest (8848m) represents the world's greatest environmental hypoxic challenge and nears the limit of human survival (2). A genetic influence on successful ascent to such extreme high altitude is supported by previous studies of elite mountaineers (86, 156) and by inter-individual differences in both acclimatisation to hypobaric hypoxia and susceptibility to AHAI (7, 8).

Many environmental and logistical factors independent of individual performance influence success and survival in climbing the highest mountains in the world (5, 6). However, common to every successful mountaineer who has reached the summit of Mount Everest is a maintained ability to perform extreme physical exertion following a prolonged period of increasingly severe hypoxia (2). Acclimatisation to this degree of hypobaric hypoxia conventionally takes many weeks of residence at extreme high altitudes of >5500m with several successful forays to >7000m without supplemental oxygen prior to an attempt to climb to the summit. During the final ascent to the summit of Mount Everest (8848m), supplemental oxygen is used by over 96% of successful mountaineers and is commenced at altitudes between 7100m and 8300m at flow rates of 0.5-4 litres per minute (2, 209).

In 2006, a total of 1768 non-Sherpa climbers had reached the summit of Mount Everest (8848m) (5). If a genetic influence over performance at extreme high altitude exists, then polymorphisms that are associated with enhanced tolerance of hypobaric hypoxia ought to be over-represented in this unique group of elite hypoxic mountaineers. This study recruited mountaineers who had successfully climbed Mount Everest and tested the hypothesis that the allele frequency of ACE I/D and BK₂R +9/-9, two candidate

polymorphisms believed to be associated with high altitude tolerance, should be over-represented in this group when compared with race-matched non-mountaineer controls.

7.2 Methods

7.2.1 Ethics

The study was approved by the Joint UCL/UCLH Ethics Committee. Written informed consent was obtained from all volunteers.

7.2.2 Subjects

I attempted to recruit as many successful Everest summiteers as possible using two principal approaches: firstly email contact followed by postal pack and, secondly, recruitment of mountaineers attempting to climb Mount Everest at the North and South Base Camps.

Email contact with Everest summiteers was achieved by several methods.

1. A list of all Everest summiteers and limited contact details were available from online databases and mountaineering websites such as the Himalayan Database (www.himalayandatabase.com), Everest News (www.everestnews.com) and Explorersweb (www.explorersweb.com).
2. Many of these summiteers identified were contactable by personal websites, professional mountaineering websites or through their public speaking agencies.
3. To generate wider interest in the Everest community I placed advertisements and articles in the international mountaineering press and websites.
4. I requested assistance from over 30 international mountaineering associations and clubs. Several organisations emailed their membership or forwarded contact details for all Everest summiteer members.
5. I contacted all the commercial Everest expedition companies operating in 2006 and requested assistance in contacting their previous successful Everest summiteers.

6. Through the successful recruitment of subjects and ongoing personal involvement in high altitude mountaineering I established a growing network of Everest community contacts who helped recruit further subjects.

When an Everest summiteer agreed to participate in the study, a postal pack was sent to the subject's address containing a questionnaire, consent form, and background information regarding the study (Appendix 3). Enclosed in the pack was a buccal swab collection kit consisting of a Whatman foam tipped applicator, Whatman FTA microcard, desiccating sachet, sealable pouch and instructions for use (Whatman Bioscience, Abington, Cambridge, UK). An international mail delivery envelope was enclosed to allow the subjects to return the completed consent form, questionnaire and sample without cost.

Secondly, I personally recruited subjects at Everest North Base Camp in 2005 and arranged further recruitment by medical mountaineers who were visiting Everest North and South Base Camp in 2006 and 2007. An overview of subject recruitment is presented in Figure 7.1.

Included in this study were mountaineers who have successfully reached the summit of Mount Everest and who, in response to a free text question requesting 'Race', self-defined themselves as 'white', 'white European' or 'white Caucasian'.

7.2.3 Subject Data

Subjects were asked, either in person or by post, to complete a questionnaire of demographic data, high altitude history mountaineering experience (Appendix 3) and details of date, route and oxygen use for the successful ascent of Everest. Self-reported

data were cross-referenced with the Himalayan Database for consistency and peer confirmation (www.himalayandatabase.com).

7.2.4 Genetic Analysis

DNA was collected using a buccal swab and was genotyped for ACE I/D and BK₂R +9/-9 polymorphisms (see Chapter 2: Methods for details).

7.2.5 Controls

Published race-matched control allele frequencies were sought for the ACE I/D and BK₂R +9/-9 polymorphisms.

BK₂R +9/-9 controls: The largest and closest race matched published control population who have been genotyped for BK₂R +9/-9 identified was the cohort of 2541 unrelated healthy Caucasian subjects recruited from nine UK general practices (184).

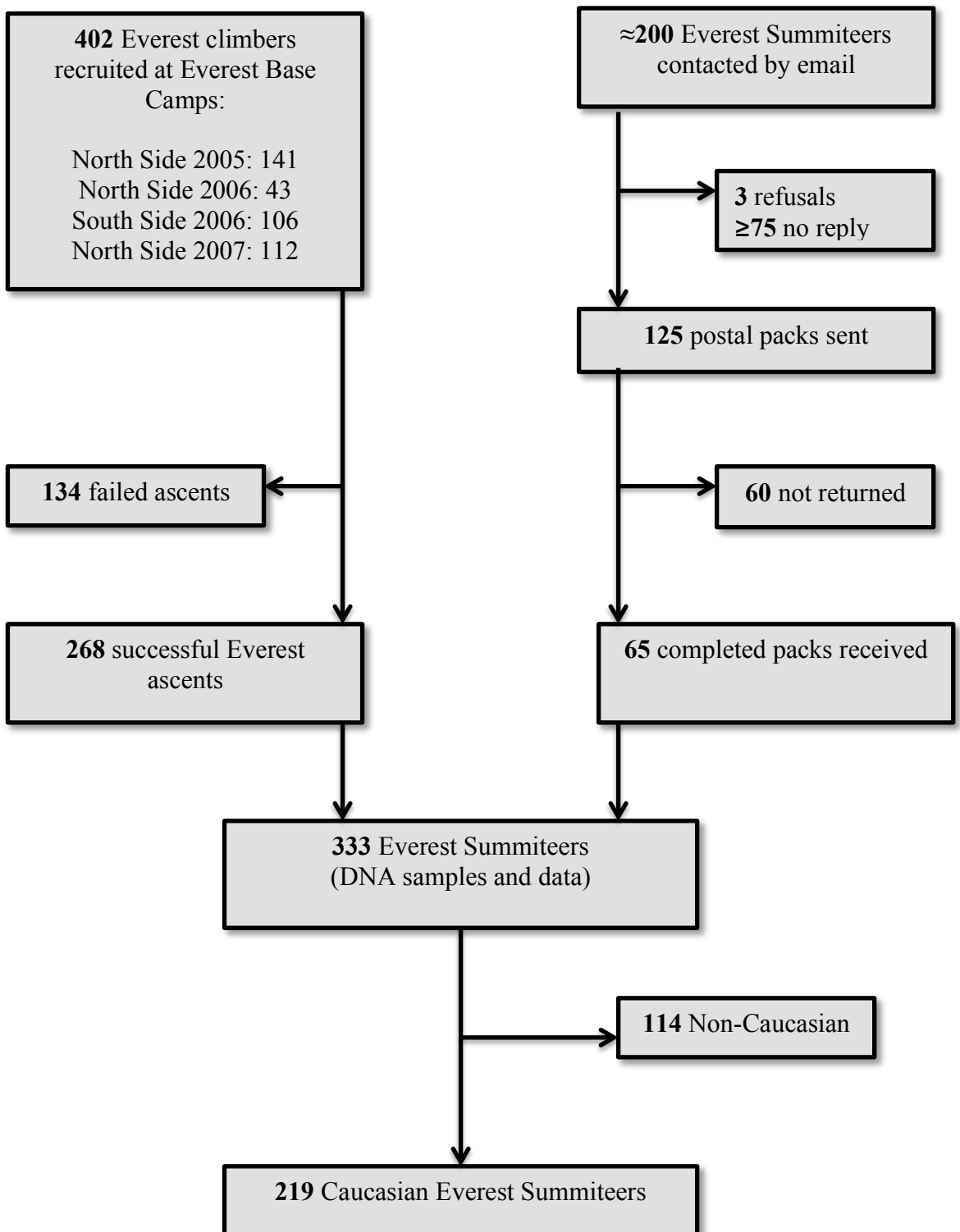
ACE I/D controls: Although larger ACE I/D genotyped control cohorts have been previously published, these studies have not been restricted to Caucasian subjects (210). ACE I/D allele frequency differs significantly between racial groups (152) and consequently the control population used in this study was a cohort of 462 white Caucasians who were selected by stratified random sampling of general practice lists in London (155).

7.2.6 Statistical Analysis

Results were analysed using SPSS version 21. Chi squared test was used to confirm Hardy-Weinberg equilibrium. Differences in study population physiological parameters and genotype groups were assessed by ANOVA, Chi squared or Fisher's Exact testing as appropriate. Throughout, a p-value ≤ 0.05 was considered statistically significant.

7.3 Results

Figure 7.1 Flow diagram of Everest Summiteer recruitment



Everest Summiters

Two hundred and nineteen Caucasian mountaineers who had reached the summit of Mount Everest (8848m) were successfully recruited to this study (Figure 7.1), of whom 197 (90%) were male. Only 6 subjects (2.7%) had reached the summit of Everest without oxygen. The mean±SD age at the time of reaching the summit was 39.4±9.1 years, with height 178.7±7.8cm and weight 76.8±10.0kg.

High altitude mountaineering history

There was a wide variation in the reported high altitude mountaineering history. Everest Summiters included in this study had climbed between 1 and 40 peaks of 7000-7999m altitude (mean±SD 3.8±5.6 ascents) and between 1 and 20 of >8000m (mean±SD 2.2±2.4 ascents) (Figure 7.1 and 7.2).

Figure 7.2 Number of mountains 7000-7999m climbed by Everest Summiteers

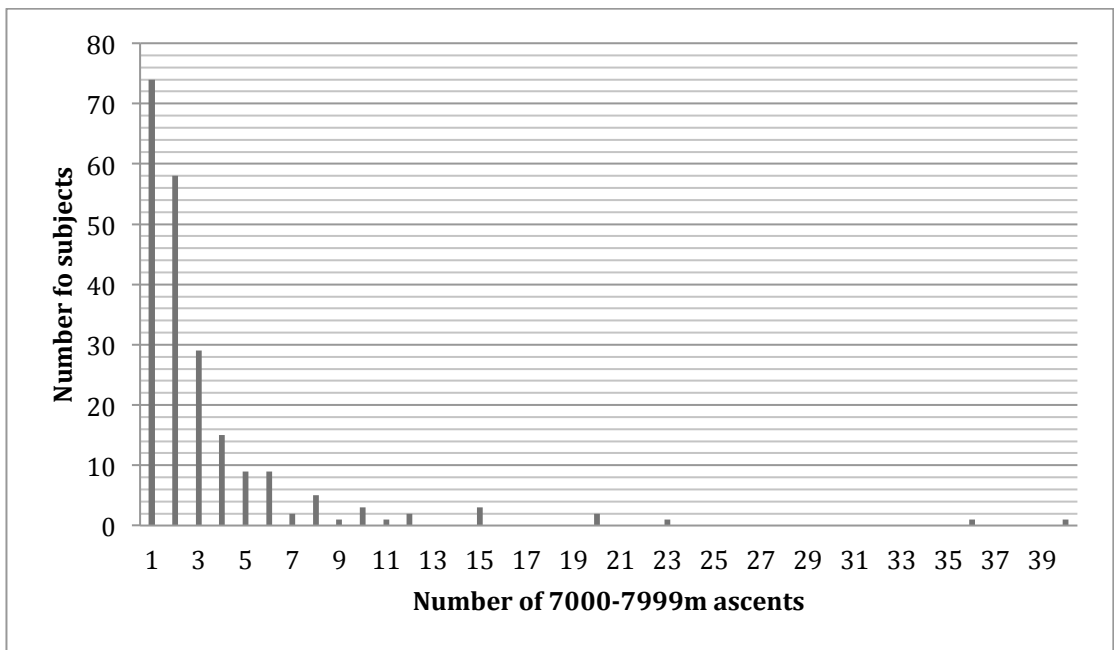
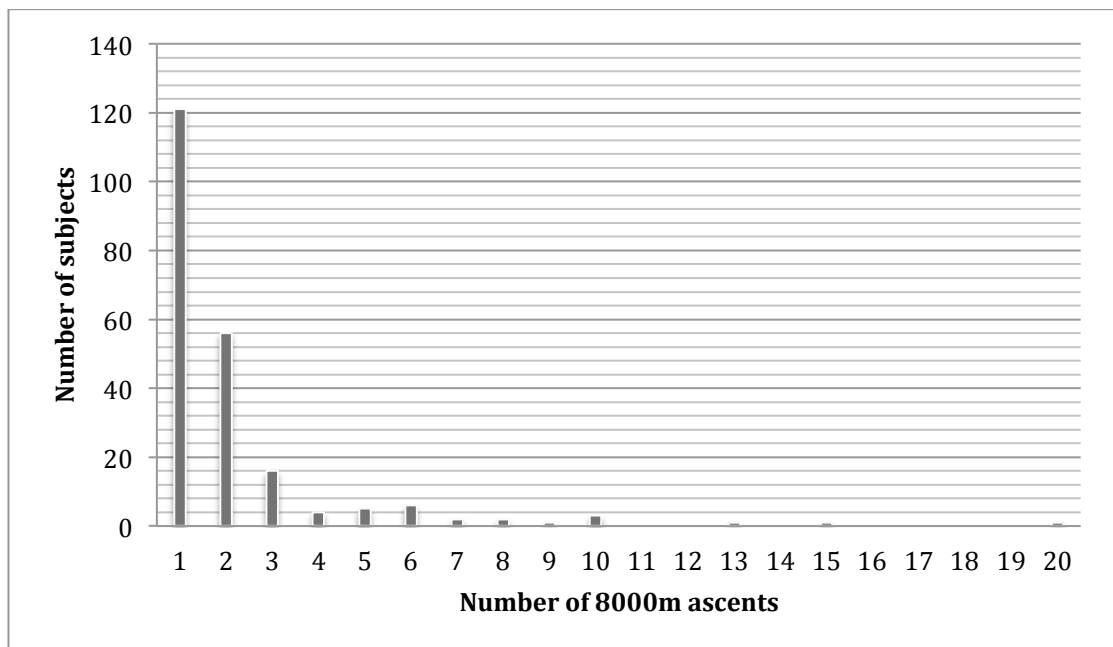


Figure 7.3 Number of mountains >8000m climbed by Everest Summiteers



ACE I/D genotype in Everest Summiteers

ACE genotyping was successful in all of the 219 subjects and genotype distribution (58 [26.5%] II, 102 [46.6%] ID, 59 [26.9%] DD; I-allele frequency 0.498) consistent with Hardy-Weinberg equilibrium. Height, weight and age were independent of ACE genotype.

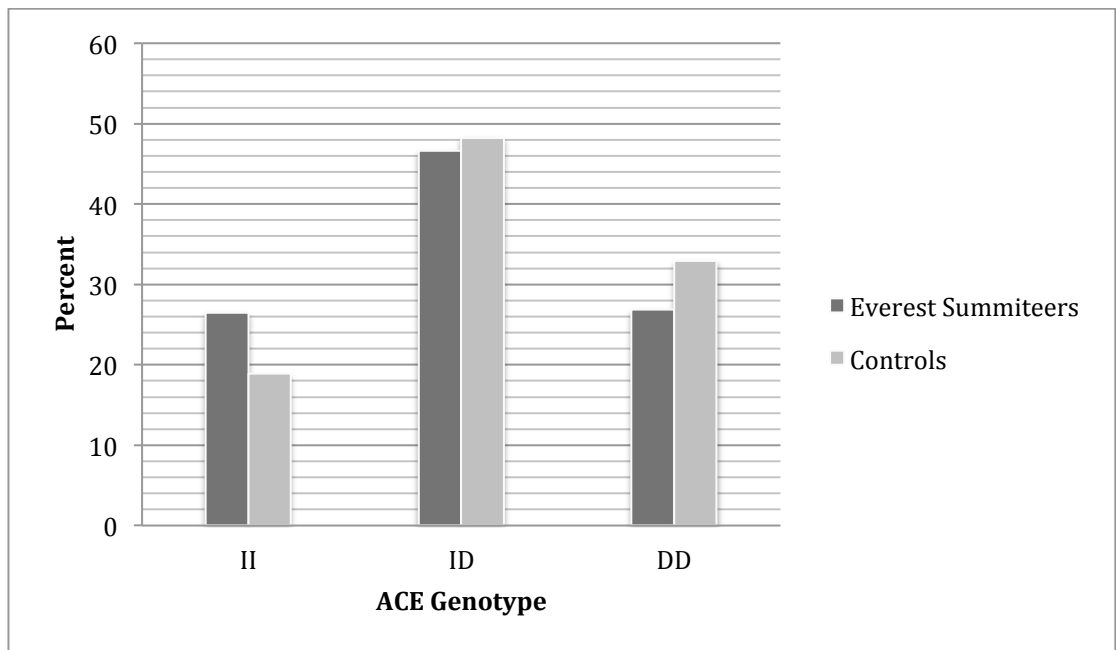
The I-allele was more prevalent in Everest Summiteers than in controls (0.498 vs 0.43, $p=0.0196$) (Table 7.1), the ACE II genotype being over-represented in this group ($p=0.01$).

Table 7.1 ACE I/D genotype distribution in Everest Summiteers and controls

| | Everest Summiteers | Controls |
|--------------------|--------------------|-------------|
| II | 58 (26.5%) | 85 (18.9%) |
| ID | 102 (46.6%) | 217 (48.2%) |
| DD | 59 (26.9%) | 148 (32.9%) |
| | $p=0.01$ | |
| I-allele frequency | 0.498 | 0.43 |
| D-allele frequency | 0.502 | 0.57 |
| | $p=0.019$ | |

Values are numbers of individuals unless stated (percentage in brackets).

Figure 7.4 ACE I/D genotype distribution (%) in Everest Summiteers and controls



BK₂R +9/-9 genotype in Everest Summiteers

BK₂R +9/-9 genotyping failed in 14 of the 219 subjects. In the remaining 205 subjects, genotype distribution (60 [29.3%], 102 [51.7%] and 39 [19.0%] for +9/+9, +9/-9 and -9/-9 respectively; +9 allele frequency 0.55) was consistent with the Hardy-Weinberg equilibrium. Height, weight and age were independent of BK₂R genotype.

BK₂R +9/-9 genotype distribution was not significantly different in the Everest Summiteer cohort compared to race matched controls (p=0.182) and similarly the +9 allele frequency was not significantly different between the two groups (p=0.108) (0.55 vs 0.51 in Everest Summiteers vs controls) (Table 7.2).

Table 7.2 BK₂R +9/-9 genotype distribution in Everest Summiteers and controls

| | Everest Summiteers | Controls |
|---------------------|--------------------|--------------|
| +9/+9 | 60 (29.3%) | 572 (26.0%) |
| +9/-9 | 106 (51.7%) | 1088 (49.6%) |
| -9/-9 | 39 (19.0%) | 535 (24.4%) |
| | p=0.182 | |
| +9 allele frequency | 0.55 | 0.51 |
| -9 allele frequency | 0.45 | 0.49 |
| | p=0.108 | |

Values are numbers of individuals unless stated (percentage in brackets).

Mount Everest without supplemental oxygen

Only 6 subjects reached the summit of Mount Everest without supplemental oxygen and the ACE and BK₂R genotype of this group are displayed in Table 7.3. In this very small group there was no significant difference between the distribution of either genotype and that seen in the control group.

Table 7.3 ACE I/D and BK₂R genotype in Everest Summiteers who did not use oxygen.

| ACE genotype | | BK ₂ R genotype | |
|--------------|---|----------------------------|---|
| II | 3 | +9/+9 | 1 |
| ID | 1 | +9/-9 | 4 |
| DD | 2 | -9/-9 | 1 |

7.4 Discussion

This is the first study to assess genotype distribution in Everest Summiteers and demonstrates that the ACE I-allele is over-represented in this group of successful high altitude mountaineers compared to race-matched controls. This finding in a large cohort of 219 Caucasians, provides powerful confirmatory support to the hypothesis that the ACE I-allele confers enhanced performance in the hypoxic environment. It accords with published data demonstrating that the I-allele was over-represented in 25 elite British high altitude mountaineers (86) and associations with successful ascent to extreme and moderate high altitude (90, 110, 156). Whilst this study demonstrates that the ACE I-allele is likely to confer an advantage in climbing to extreme high altitude, the strength of the association is less than that observed in a small group of elite British mountaineers who had climbed to over 8000m, amongst whom I-allele frequency was 0.65 (86). Moreover Montgomery et al. did not find any of the elite mountaineer group to be homozygous for the D-allele, whereas in this study 2 of the 6 mountaineers who climbed Mount Everest without oxygen were of ACE DD genotype, as were 4 of the 6 mountaineers who had climbed 10 or more 8000m mountains.

No significant difference in allele frequencies was observed for the BK₂R +9/-9 polymorphism between Everest Summiteers and race-matched controls. The BK₂R -9 allele has been associated with enhanced performance phenotypes and increased metabolic efficiency in previous studies at sea level (105, 106, 184, 191, 192) but the single published study assessing the influence of the BK₂R +9/-9 polymorphism at altitude demonstrated no association with the development of AMS in Nepalese pilgrims at 4380m (180). The BK₂R +9/-9 polymorphism may have no influence on performance in the hypoxic environment or it may be that the -9 allele associated enhanced metabolic efficiency observed at sea level is offset by deleterious associations

at high altitude. The strong association of the -9 allele with AMS score and high altitude headache observed in Chapter 6 may provide an explanation for the lack of association of the BK₂R +9/-9 polymorphism with high altitude performance if the -9 allele simultaneously confers both the advantage of metabolic efficiency and the disadvantage of high altitude headache. It may be proposed that the short lived duration of AMS on arrival to high altitude would be of little relevance to the 6-8 week period required to climb Mount Everest but mountaineers are sequentially exposed to higher altitudes during this period, culminating in a summit attempt, and may suffer from AHAI at each successive increase in altitude (2, 5).

Although this study examines a cohort that has achieved extreme physical success in a profoundly hypoxic environment, a confounding factor is the use of supplemental oxygen. Supplemental oxygen is conventionally used during the summit attempt on Mount Everest (2, 209, 211) and provides both subjective benefit and improves S_aO₂ in the resting state and during exercise (212). Only 6 subjects (2.7%) of this cohort had climbed Everest without oxygen. The remaining 213 subjects used supplemental oxygen and while they have not been exposed to a sustained P_aO₂ of 2.55kPa, as collected from an arterial blood gas at 8400m on Mount Everest (2), they have all demonstrated tolerance of a prolonged and profound but lesser degree of hypoxia. Whether this group would differ in genotype from a larger cohort of mountaineers who have reached the summit of Everest without oxygen is unclear but, if tolerance of hypoxia indeed has a genetic influence (7, 8, 184), the degree of hypoxic challenge that this cohort have successfully endured during the ascent of Everest would be expected to yield a group that is highly selected for this phenotype.

In a study of this kind, both cases and controls should be bio-geographically matched as closely as possible because the frequency of genetic variants, including the ACE I/D polymorphism (152), differ greatly across populations and can skew the results of a study. Bio-geographical matching of cases and controls was challenging in this study due to the international distribution and limited total numbers of Everest Summiteers. Subjects were asked to define their own race in this study and included in the analysis if they self-described as ‘white’, ‘white – European’ or ‘Caucasian’, and subjects of Asian or African descent were excluded. The term Caucasian was self-defined and although it is widely used in the scientific literature, some view this as a term that carries racial overtones, and that technically refers only to people from the area between the Black and Caspian seas. The control groups selected were both large UK cohorts recruited from general practice lists and used the same racial descriptor of ‘white Caucasian’ (155, 184). Larger published control groups for the ACE I/D polymorphism exist but do not include descriptions of the racial composition of the cohort and so were not used (210).

Conclusion

This study demonstrates that the ACE I-allele is over-represented in mountaineers who have successfully climbed Mount Everest (8848m) when compared with race-matched controls and supports data suggesting that the I-allele is associated with enhanced performance in hypoxic conditions.

CHAPTER 8: Discussion

The work in this thesis explored the association between the ACE I-allele and human performance at high altitude. Prior to the studies in this thesis, an over-representation of the I-allele had been observed in a small group of 25 elite British mountaineers who climbed regularly to over 7000m (86). No studies had attempted to replicate this finding in a larger group at extreme high altitude and the mechanism underlying such an association was unclear.

I have demonstrated that the ACE I-allele is indeed associated with successful extreme high altitude performance in both a prospective study assessing success in climbing to 8000m and with the observation that the I-allele is over-represented in mountaineers who have reached the summit of Mount Everest when compared to sea level, race-matched controls. Moreover I have explored the mechanism underlying this association and demonstrated in 2 independent studies at various altitudes that the I-allele advantage is not mediated by susceptibility to AMS or by increased S_aO_2 . These data regarding AMS accord with subsequent studies and meta-analysis (158, 159).

The ACE I-allele advantage might be mediated via the role of ACE in bradykinin metabolism, the ACE I-allele conferring a phenotype of high kinin activity at the BK₂ receptor. If true, a similarly advantageous association should be observed with the BK₂R -9 allele, and amplified by the combination of the ACE and BK₂R -9 allele. In exploring this hypothesis, I demonstrated a novel and strong association between the BK₂R -9/-9 genotype and the presence of high altitude headache and an increased AMS Lake Louise Score. This finding may be of importance in understanding the inter-individual variation in the development of AMS and high altitude headache. A role for

bradykinin activity as a potent vasodilator and increasing capillary permeability in the development of AMS seems plausible, particularly in the context of a growing understanding that cerebral vasomodulation is central to the genesis of high altitude headache (116, 158, 159, 177-179). No association was demonstrated between the BK₂R +9/-9 polymorphism and success in reaching the summit of Cerro Aconcagua (6963m) or any difference in allele frequency between Everest summiteers and race-matched controls.

Perhaps surprisingly given the strong association demonstrated between the I-allele and successful ascent to 8000m and a published study reporting an I-allele advantage at an altitude of 4807m (110), I did not demonstrate any association between the ACE I-allele and successful ascent to 5895m (Mount Kilimanjaro) or 6963m (Cerro Aconcagua). This lack of association in two independent studies at lower altitudes may be explained firstly by between-cohort differences between subject characteristics and, secondly, by differences between the physiological challenge posed by altitudes of 2700m to 6963m, and that experienced at altitudes of over 8000m. Both the Mount Kilimanjaro study and Cerro Aconcagua studies recruited subjects who were attempting to climb highly popular and non-technical mountains. It may be postulated that these subjects recruited on popular trekking peaks differ from mountaineers attempting to climb the highest mountains in the world, where I have demonstrated a clear association (156). This explanation is supported by a previous study where no association between ACE I/D polymorphism and performance was demonstrated when a large group of mixed ability and sporting disciplines were analysed together, but strong allele associations were revealed if outstanding athletes in specific disciplines were assessed (165). The authors concluded that associations with ACE I/D allele differ between disciplines and are most marked in elite performers. This interpretation may accord with lack of association

between the ACE I/D polymorphism and high altitude success in two cohorts of non-elite high altitude trekkers. Secondly, the discordance seen between the two studies at 8000m or higher that demonstrated association between the ACE I-allele and success, and the two studies at lower altitudes that did not, may be due to the extreme nature of the physiological challenge at 8000m that accentuates the influence of minor phenotypic advantages. There is clear precedence for phenotypic differences only being apparent at the extremes of human physiology in critical care research where interestingly the ACE I-allele has been associated with a lower mortality from acute respiratory distress syndrome (213), improved outcomes in childhood meningococcal septicaemia (214) and improved cardiorespiratory response to premature birth (215).

This thesis describes a series of candidate-gene association studies where a genetic polymorphism is usually selected for investigation based upon the known or predicted function of the gene product that is believed to influence the phenotype of interest. More recently a candidate gene may be selected if it lies in an area of interest identified by genome-wide association studies (GWAS) that are used to simultaneously interrogate regional associations across all chromosomes (216). The sensitivity of candidate gene association studies depends on both sample and effect size, and identification of any association does not prove causation. Further cautions with candidate-gene association studies are that errors in multiple testing are possible, especially when a large number of polymorphisms are tested. To avoid such errors, my thesis has focused primarily on the ACE I/D polymorphism and secondarily investigated the B₂KR +9/-9 polymorphism to explore whether the ACE I/D allele influence is mediated via kinin activity.

Although this thesis has investigated whether and how the ACE I/D polymorphism may directly influence high altitude performance, another explanation may be that the ACE I/D locus is either functionally related or in close linkage disequilibrium with a true causal locus that affects high altitude performance (217). It has been shown that polymorphic loci in the RAS act synergistically with one another in the pathogenesis of disease (208). Moreover, the ACE I/D polymorphism has been shown to act in such a manner with genes outside the RAS (218, 219).

As discussed in previous Chapters, other potential limitations in the studies in this thesis include the variation of ACE I/D allele frequencies in different populations and, similarly to the wider literature studying the ACE I/D polymorphism and human performance that have generated conflicting results, ethnic and genetic differences in study groups (90). The advent of ancestry-informative markers (AIMs) (220) may reduce this as a confounding factor in future studies and will greatly facilitate quantification of sample stratification as well as reduce the reliance on self-reports or anecdotal data when defining the genetic backgrounds of study cohorts.

The expedition environment introduces a number of confounding factors that are dissimilar to the controlled laboratory environment. Genetic associations may be obscured by factors such as cold, fatigue, dehydration, weather, logistics, accidents, concurrent illness, and environmental hazards and may influence the phenotypic traits of interest, such as summit success or AHAI (6). It is even possible that genetic associations with summit success may be determined by tolerance or avoidance of one or more of these expedition environmental factors rather than hypoxia, although the association of ACE I/D with altered physiological responses to hypoxia in the laboratory environment make this less likely (112).

In recent years, novel developments in genetic and statistical techniques have been employed on both AHAI subjects and high altitude populations that may supersede the candidate gene analysis assessed in this study. Analysis of multiple candidate genes (221), whole genome amplification (222) and gene-gene interaction studies using multifactor dimensionality reduction (223) have revealed multiple additional polymorphisms, predominantly HIF related, that may be central to future understanding of the human response and adaption to hypobaric hypoxia. I attempted to reflect these advances in technology by undertaking whole genome amplification on the Everest Summiters buccal swab samples. Unfortunately the buccal swab DNA collection and storage method used, the Whatman FTA microcard, did not yield sufficient DNA for analysis by this technique. Moreover there was insufficient DNA remaining to genotype for the B₂KR +9/-9 polymorphism on the samples in Chapters 3 and 4 which explains the lack of uniformity of analysis across the experiments presented in this thesis.

However, with the support of the novel findings that I have demonstrated in this thesis, I believe that there are several directions for future development of this work. More detailed physiological data such as that collected on the Caudwell Xtreme Everest expedition (224) would allow further elucidation of the role of the ACE I/D and B₂KR +9/-9 polymorphisms than I achieved in these studies. In particular it would be fascinating to explore a B₂KR +9/-9 association with the inter-individual variation in cerebral vasomodulation and AMS development identified previously (177, 178).

In the final experiment of this thesis I have analysed DNA from 219 Everest Summiters for ACE I/D and B₂KR +9/-9 but have consent and ethical approval to analyse these samples for other polymorphisms associated with hypoxic performance. This is a unique and valuable cohort of elite hypoxic adaptors and, as novel hypoxia

associated polymorphisms in the HIF pathway (222) are identified by other techniques, it represents an ideal confirmatory dataset for additional hypoxia related candidate genes.

APPENDICES

1. 8000m Questionnaire
2. Aconcagua Base Camp (4300m) Questionnaire
3. Everest Summiteer Questionnaire

Appendix 1. 8000m questionnaire

1. Name (printed) and email:

2. Race:

4. Nationality:

5. Age:

6. Sex:

7. Height:

8. Normal weight:

9. Mountaineering History

How many times have you been:

With Supplemental Oxygen

Without Supplemental Oxygen

6000-6999m

7000-7999m

8000-8848m

10. What is the highest that you have ever climbed to?

11. Have you suffered in the past from:

HAPE (High Altitude Pulmonary Edema)

HACE (High Altitude Cerebral Edema)

For completion after summit attempt

12. Maximum altitude reached:

13. Did you use oxygen and, if so, from what altitude:

14. If unsuccessful, reason for failure to reach summit:

Appendix 2. Aconcagua Base Camp (4300m) questionnaire

1. Name (printed) and email:

2. (Optional) Contact details: Address

3. Race:

4. Nationality:

5. Age:

6. Sex:

7. Height:

8. Normal weight:

9. Do you have any disease or medical condition: Yes No

If Yes, what diagnosis / medical condition do you have:

10. Are you taking any pharmaceutical drugs: Yes No

If Yes, what drugs are you taking:

11. Mountaineering History

How many times have you been:

With Supplemental Oxygen

Without Supplemental Oxygen

3000-3999m

4000-4999m

5000-5999m

6000-6999m

7000-7999m

8000-8848m

12. Have you suffered in the past from:

AMS (Acute Mountain Sickness)

HAPE (High Altitude Pulmonary Edema)

HACE (High Altitude Cerebral Edema)

13. How many hours have you spent at this altitude (4300m):

14. AMS Score at 4300m

| | | | |
|----------------------|--|--|---|
| Symptoms: | 1. Headache: | | |
| | No headache | | 0 |
| | Mild headache | | 1 |
| | Moderate headache | | 2 |
| | Severe, incapacitating | | 3 |
| | 2. GI symptoms: | | |
| | No GI symptoms | | 0 |
| | Poor appetite or nausea | | 1 |
| | Moderate nausea or vomiting | | 2 |
| | Severe nausea and vomiting, incapacitating | | 3 |
| | 3. Fatigue/weak: | | |
| | Not tired or weak | | 0 |
| | Mild fatigue/weakness | | 1 |
| | Moderate fatigue/weakness | | 2 |
| | Severe fatigue/weakness, incapacitating | | 3 |
| | 4. Dizzy/lightheadedness: | | |
| | Not dizzy | | 0 |
| | Mild dizziness | | 1 |
| | Moderate dizziness | | 2 |
| | Severe, incapacitating | | 3 |
| | 5. Difficulty sleeping: | | |
| | Slept well as usual | | 0 |
| | Did not sleep as well as usual | | 1 |
| | Woke many times, poor night's sleep | | 2 |
| | Could not sleep at all | | 3 |
| | Total symptom score: | | — |
| Clinical assessment: | 6. Change in mental status: | | |
| | No change | | 0 |
| | Lethargy/lassitude | | 1 |
| | Disoriented/confused | | 2 |
| | Stupor/semiconsciousness | | 3 |
| | 7. Ataxia (heel to toe walking): | | |
| | No ataxia | | 0 |
| | Maneuvers to maintain balance | | 1 |
| | Steps off line | | 2 |
| | Falls down | | 3 |
| | Can't stand | | 4 |
| | 8. Peripheral edema: | | |
| | No edema | | 0 |
| | One location | | 1 |
| | Two or more locations | | 2 |
| | Clinical assessment score: | | — |
| Total score: | | | — |

15. Summit success (for completion after summit attempt) Yes No

Appendix 3. Everest Summiteer questionnaire

- 1. Name (printed) and email:
- 2. (Optional) Contact details: Address
Tel
- 3. Race:
- 4. Nationality:
- 5. Age:
- 6. Sex:
- 7. Height:
- 8. Normal weight:

Mountaineering History

How many mountains have you successfully summited?

With Supplemental Oxygen Without Supplemental Oxygen

6000-6999m

7000-7999m

8000-8848m

Everest

On how many occasions did you attempt to climb Mt Everest, and by which routes?

With Supplemental O2 (Y/N) Successful? (Y/N) Date Your age then

1.....

2.....

3.....

4.....

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