

***ACE, NOS3 and EPAS1* Associations with Susceptibility to Acute Mountain Sickness**

Odell A, Ashmore T, Cross E, Inman J, and Baugher PJ

Department of Biology, College of Arts and Sciences, Pacific University, Forest Grove, OR

Brief Background

Acute mountain sickness (AMS) is a medical condition that results from exposure to high altitude environments, especially those above 8000 ft (2500 m). There is wide individual variation in the extent and time of physiological response to hypoxia, which makes the onset of AMS symptoms relatively unpredictable (MacInnis et al 2010). Several factors thought to influence AMS predisposition have been investigated, but their association with AMS is not clear (MacInnis et al, 2010). It appears, therefore, that individual variation is a major determinant of AMS. Because individual genes influence individual physiology, pathological consequences likely result from the interactions between genetic factors and the hypoxic environment (Hackett and Roach 2001). Therefore, genetic studies can increase the understanding of factors that can predict or contribute to AMS.

Numerous studies show that several different genes and their variants have been implicated in influencing susceptibility to AMS (MacInnis and Koehle 2016). In this current study, we explored the association of a subset of these genes with AMS in a population of mountaineers in the Pacific Northwest. For our study, we chose to investigate polymorphisms of *ACE*, *NOS*, and *EPAS1* (Luo et al 2012, Wang et al 2009, Buroker et al 2012). *ACE* (angiotensin converting enzyme) encodes a central component of the renin-angiotensin system (RAAS), a system that controls blood pressure by regulating the volume of body fluids. Specifically, the I-allele of *ACE* has been associated with high-altitude mountaineering success and lack of AMS development (Droma et al 2008, Wang et al 2016). *NOS3* (endothelial-derived nitric oxide synthase) encodes an enzyme involved in the biosynthesis of nitric oxide, a strong vasodilator. The G894T polymorphism of this gene has been associated with susceptibility to acute mountain sickness (Wang et al 2009). *EPAS1* (endothelial Per-Arnt-Sim domain protein 1) encodes hypoxia-inducible-factor-2 alpha (*HIF2A*), a transcription factor that is involved in the response to hypoxia. The *EPAS1* SNP [ch2: 46441523 (hg18)] was found to correlate with occurrence of altitude illness (Buroker et al 2012, Yang 2013). Therefore, we designed and performed a study to investigate the association of *ACE*, *NOS3*, and *EPAS1* genotypes in a population of mountaineers attempting to summit Mt. Rainier, a 14,441 ft. glaciated volcanic peak located in Washington State.

Proposed Goals

1. Identify a population of mountaineers appropriate for this study.
2. Collect data from subjects at altitude.
3. Genotype individuals and identify specific gene polymorphisms.
4. Perform statistical analysis to determine correlations between AMS symptoms, demographic data, and genotypic data.

Achieved Goals

1. **Identify a population of mountaineers appropriate for this study.** The population of mountaineers we have chosen from which to recruit volunteers were those attempting to summit Mt. Rainier from the Disappointment Cleaver route. Typically, mountaineers attempting this route start from the trailhead at Paradise, WA (1645 m), and ascend to Camp Muir (3080 m) to acclimatize for 12-48 hours before attempting the summit (4394 m). This location and population are ideal for this study due to the elevation, relative ease of access, and consistent rate of ascent experienced by all climbers.
2. **Collect data from subjects at altitude.** IRB approval was attained from the Pacific University Internal Review Board (PUIRB) prior to the collection of data (PUIRB 873094-1 and PUIRB 873094-2), see attached Informed Consent). Additionally, scientific research permits were obtained from the National Park Service prior to data collection (MORA-2016-SCI-0024 and MORA-2017-SCI-0023). Data reported here were collected over two summers in July of 2016 and July of 2017. During these two months, selected members of my research team (Pacific University undergraduate students Tyler

Ashmore, Elizabeth Cross, Tailor Dolgin, Jonathan Inman, Gwen O'Brien, Amanda Odell) and I ascended to Camp Muir from Paradise, set up a research camp, and collected data from a total of 205 study subjects. We collected demographic data (see attached demographics questionnaire), self-reported AMS data (see attached Lake Louise score sheet), and performed buccal swabs to collect genomic DNA. Subjects were offered \$10 REI (Recreational Equipment Incorporated) gift cards as incentive for participation (funded by the Mazamas Mountaineering Organization).

3. **Genotype individuals and identify specific gene polymorphisms.** Genomic DNA was isolated from individual buccal swabs according to manufacturer's instructions (QuickExtract™ DNA Extraction, Epicenter®, Madison, WI). Isolated genomic DNA was then subjected to polymerase chain reaction (PCR) to determine individual *ACE*, *NOS3* or *EPAS1* genotypes as described in (Rupert et al, 1999, Wang et al 2009, Buroker et al 2012). *ACE* PCR products were subjected to electrophoresis on a 2% agarose gel for analysis. *NOS3* and *EPAS1* PCR products were further subjected to restriction digests followed by gel electrophoresis.
4. **Statistical Correlation of Data.** Exclusionary criteria included use of medications intended to prevent AMS, medical conditions that mimic AMS, or lack of reported data, and resulted in the exclusion of 21 subjects. Statistical correlations comparing AMS cases with pre-exposure to altitude were calculated using Relative Risk (RR) ratios. Statistical correlations between demographic data and AMS occurrence were performed using a student's t-test for continuous data (height, weight, BMI) or χ^2 analysis for categorical data (sex, age, race, alcohol use, tobacco use). Due to the small sample size, race was analyzed as Caucasian/non-Caucasian, but percentages of subjects are reported out. An AMS score of 0-2 was considered not AMS; a score of 3 or greater with the presence of a headache was considered positive for AMS (Sutton et al 1992, see attached AMS questionnaire). With respect to the genotypic data, χ^2 analysis was used to calculate Hardy-Weinberg equilibrium (HWE), as well as genotype and allele frequency correlations with AMS occurrence rate. Statistical significance was indicated by a p value of ≤ 0.05 .

Results/Conclusions

Numerous studies exist that report AMS incidence at altitude. One report shows that 22% of individuals visiting altitudes of 2100-2750 m (7000-9000 ft) develop AMS, while 42% of those ascending above 3000 m (9800 ft) developed symptoms ("Altitude Hypoxia," altitudereseach.org). Maggionrini et al. show that incidence of AMS in trekkers were 9%, 13%, 34%, and 53% at 2850 m (6069 ft), 3050 m (10,007 ft), 3650 m (11,975 ft), and 4559 m (14,957 ft), respectively (1990). Kayser et al, report AMS rates of between 34-60% at altitude (2012). Clearly, reports of AMS incidence vary. These variances result from differences in locations and altitudes of data collection, time spent at altitude, population sampled, and rate of ascent. Therefore, understanding the rate of AMS frequency at a specific location and within a specific population is essential to preparing resources for potential medical needs to avoid possible emergency situations and to recommend specific preventative behaviors.

In this study, we collected data from 205 subjects at Camp Muir (10,188 ft, 3105 m, Mt. Rainier National Park) who had hiked up from Paradise, WA (5,400 ft, 1646 m) and been at Camp Muir at least overnight. Subjects were asked to fill out the self-reporting Lake Louise Score sheet for the diagnosis of AMS (Sutton et al 1992, see attached). Of the 205 subjects, 24 were excluded due to use of prophylactic medications, existing medical conditions that mimic AMS symptoms, and/or lack of reported data. AMS occurrence rates are reported in Table 1. Of the included subjects, 35.9% (65/181) reported symptoms of mild or severe AMS. Of those reporting AMS, 73.8% (48/65) reported mild AMS and 26.2% (17/65) reported severe AMS. Because pre-exposure to altitude has been shown to decrease the prevalence of AMS (Schneider et al 2002, Weng et al 2013, Zafren 2014), we analyzed AMS prevalence based on exposure to altitudes over 8000 ft (2438m) within the past two weeks of ascent to Camp Muir. AMS rates for those not pre-exposed to altitude were 38.1% (37/97) while AMS rates for those exposed to altitude within 2 weeks of ascent were 33.3% (28/84). Even though pre-exposure appeared to decrease AMS rates, the data were not significant (RR=0.8739, 95% CI 0.5890-1.2966, p=0.5031).

With regard to those subjects pre-exposed to altitudes of over 8000ft within 2 weeks of their ascent to Camp Muir, 67.8% (19/28) cases were reported as mild while 32.2% (9/28) were severe. With regard to the those not pre-exposed to altitude within 2 weeks, 78.4% (29/37) were reported as mild, while 21.6% (8/37) were reported as severe. Interestingly, pre-exposure to altitude appeared to increase the risk of severe AMS, but those data were not significant (RR=1.4866, 95% CI 0.7071-3.6521, p=0.3410).

Correlations between several factors and AMS occurrence have been explored, but few have been clearly associated with symptom onset (MacInnis et al, 2010). For example, physical fitness has not been shown to be an independent risk factor for AMS (Roach et al, 2000). Furthermore, demographic categories such as age, sex, height, weight, and body mass index (BMI), or lifestyle factors such as alcohol and tobacco use have not been found to be associated with risk of AMS development (Lawrence and Reid 2016). To evaluate a correlation between demographic and lifestyle factors and AMS onset, we requested information regarding sex, age, height, weight, ethnicity/race, tobacco and alcohol use from study subjects. We expected to find no correlation between demographic categories of lifestyle factors and AMS onset. Results are reported in Table 2.

Sex. Of the subjects surveyed, 76.2% (138/181) were male and 23.8% (43/181) were female. 47 males and 17 females reported AMS; 91 males and 26 females reported no AMS. No significant correlation was found between sex and reported AMS ($\chi^2=0.4303$, p=0.512).

Age. 35.4% (64/181) of subjects were between ages 18-30, 42% (76/181) were between 31-40, 13.8% (25/181) were between 41-50, 6.1% (11/181) were between 51-60, and 2.7% (5/181) were between 61-70. For age group 18-30, 32.5% reported AMS (21/64); for age group 31-40, 40.8% reported AMS (31/76); for age group 41-50, 36% reported AMS (9/25); for age group 51-60, 18.2% reported AMS (2/11); and for age group 61-70, 40% reported AMS (2/5). No significant correlation between age and reported AMS was found ($\chi^2=2.5916$, p=0.628).

Height, Weight, and BMI. The average height, weight, and body mass index (BMI) of all subjects were 69.22 inches (1.76 m, SD=3.199), 166.61 lbs (75.57 kg, SD=24.955), and 24.95 (SD=2.664) respectively. The average height, weight, and BMI of those reporting AMS were 69.31 inches (1.76 m, SD=3.157), 164.92 lbs (74.81kg, SD=24.342), and 24.66 (SD=2.890) respectively; the average height, weight, and BMI of those reporting no AMS were 69.17 inches (1.76 m, SD=3.236), 167.54 lbs (75.99 kg, SD=25.343), and 25.10 (SD=2.530) respectively. No significant correlation was found between height, weight, or BMI and reported AMS (p=0.775, p=0.497, p=0.308).

Race/Ethnicity. In this study, 86.8% (157/181) were Caucasian, 5.5% (10/181) were Latino, 5.5% (10/181) were Asian, 1.1% (2/181) described themselves as Other, and 0.55% (1/181) were each Native American or African American. Due to the small sample size of non-Caucasian study subjects, the data were analyzed as Caucasian and non-Caucasian. 54 Caucasians reported AMS, 103 reported no AMS; 11 non-Caucasians reported AMS; 13 non-Caucasians reported no AMS. No statistical correlation was found between race and AMS onset ($\chi^2=1.1835$, p=0.277).

Alcohol Use. 67.9% (123/181) of subjects reported light drinking, 14.9% (27/181) reported moderate drinking, 1.2% (2/181) reported heavy drinking, and 16.0% (29/181) reported being non-drinkers. 13 non-drinkers reported AMS, 16 reported no AMS; 43 light drinkers reported AMS, 80 reported no AMS; 9 moderate drinkers reported AMS, 18 reported no AMS; 0 heavy drinkers reported AMS, 2 reported no AMS. No significant correlation between alcohol use and AMS onset was found ($\chi^2=1.3498$, p=0.509).

Tobacco Use. 80.1% (145/181) of subjects were never-smokers, 16.6% (30/181) were past smokers, and 3.3% (6/181) were current smokers. 52 never-smokers reported AMS, 93 reported no AMS; 12 past smokers reported

AMS, 18 reported no AMS; 1 smoker reported AMS, 5 smokers reported no AMS. No significant correlation between tobacco use and AMS onset was found ($\chi^2=1.1836$, $p=0.553$).

As expected, we found no correlation between demographic or lifestyle factors and reported AMS. This lack of correlation increases evidence in favor of our hypothesis that there exists a genetic component to the development of AMS symptoms for individuals at altitude. To test our hypothesis that there is a correlation between certain gene polymorphisms and susceptibility to AMS, we determined the genotypes of all included study subjects for three genes: *ACE*, *NOS3*, and *EPAS1*. We then investigated a statistical correlation between genotypes and susceptibility to AMS.

ACE. The association of angiotensin converting enzyme (*ACE*) gene polymorphisms with AMS susceptibility and successful high altitude performance has been previously investigated (Luo et al 2012, Puthucherry et al 2011). The human *ACE* gene is located on chromosome 17q23, where an insertion/deletion polymorphism (I/D, dbSNP rs4646994) in intron 16 has been identified (Rigat et al 1990). This polymorphism is based on the presence (insertion, I) or absence (deletion, D) of a 287bp DNA fragment. A number of studies report a positive association with the presence of the I-allele, high altitude mountaineering success, and lack of AMS development (Puthucherry et al 2011, Droma et al 2008). However, other studies report no association between allele distributions (Luo et al, 2012). Therefore, it is important to understand the relationship between these polymorphisms and AMS occurrence within specific populations to determine the efficacy of this factor to predict AMS onset.

With respect to the *ACE* analysis, we performed PCR on 45 samples. Data is shown in Table 3. We report the following genotype distribution for the subject data: 12 II, 24 ID, 9 DD (26.7%, 53.3%, 20.0% respectively). We found this distribution to be in Hardy-Weinberg equilibrium (HWE, $\chi^2=0.23$, $p=0.6318$). Allele frequency was found to be 0.533 (I), 0.467 (D). With respect to genotypes of individuals reporting AMS occurrence ($n=19$), we report the following genotype distribution: 3 II, 11 ID, 5 DD. Allele frequency for this population was found to be 0.447 (I) and 0.553 (D). With respect to the genotypes of individuals reporting no AMS occurrence ($n=26$), we report the following genotype distribution: 9 II, 13 ID, 4 DD. Allele frequency for this population was found to be 0.596 (I) and 0.404 (D). No statistical significance was found regarding genotype distribution and AMS occurrence ($\chi^2=2.243$, $p=0.326$). However, statistical significance was found between allele frequency and AMS occurrence ($\chi^2=4.5113$, $p=0.0337$). These data agree with other studies that show a correlation between increased presence of the I *ACE* allele and decreased susceptibility to AMS.

NOS3. The association of *NOS3* gene polymorphisms with AMS susceptibility has been investigated (Ding et al 2011, Wang et al 2010, Wang et al 2009). However, reports conflict: some reports show a correlation between certain *NOS3* polymorphisms and AMS susceptibility, while others do not. Wang et al, 2009, show the rs1799983 (Glu298Asp) polymorphism is significantly correlated with susceptibility to AMS. This functional polymorphism has been shown to influence gene expression and NO production (Ahsan et al 2005, Dosenko et al 2006). NO acts as a vasodilator, increasing blood flow and oxygen delivery to body tissues. Because NO is associated with acclimatization to high altitudes, and eNOS (encoded by *NOS3*) is the primary source of NO in blood vessels, it is thought that *NOS3* polymorphisms increasing eNOS activity and/or expression could decrease susceptibility to AMS (Wang et al 2009).

With respect to *NOS3*, we performed PCR and restriction digest analysis on 181 samples (all included study subjects). In this study, we report the following genotype distribution: 87 GG, 71 GT, 23 TT (48.1%, 39.2%, and 12.7% respectively). Results are reported in Table 3. We found the distribution to be in Hardy-Weinberg equilibrium (HWE, $\chi^2=1.93$, $p=1.6476$). Allele frequency was found to be 0.68 (G) and 0.32 (T). With respect to genotypes of individuals reporting AMS ($n=65$), we report the following genotype distribution: 31 GG, 24 GT, and 10 TT. Allele frequency for this population was found to be 0.66 (G) and 0.34 (T). With respect to the genotypes of individuals reporting no AMS occurrence ($n=116$), we report the following genotype distribution: 56 GG, 47 GT, and 13 TT. Allele frequency for this population was found to be 0.69 (G) and 0.31 (T). No

statistical significance was found between either genotype distribution and AMS occurrence ($\chi^2=0.7123$, $p=0.700$) or allele frequency and AMS occurrence ($\chi^2=0.2051$, $p=0.651$). However, we do report an increase in the T allele in the AMS population as compared to the non-AMS population, although not significant.

EPAS1. The association of *EPAS1* gene polymorphisms with altitude illness susceptibility has been investigated (Guo et al 2015, Yu et al 2016, Buroker et al 2012, Yang et al 2013). *EPAS1* encodes a transcription factor involved in the induction of genes regulated by oxygen, and is induced as oxygen levels fall (hypoxia). It has been shown to be involved in a number of hypoxic responses such as erythropoiesis, angiogenesis, vascular regulation and anaerobic metabolism (Scortegagana M et al 2003 blood). The chr2:46441523(gh18) polymorphism has been associated with both susceptibility to chronic mountain sickness (CMS) as well as high altitude pulmonary edema (Yang et al 2013, Buroker et al, 2012). However, no association has been shown between this polymorphism and AMS. Therefore, we decided to test the association of this polymorphism with susceptibility to AMS in our study population.

With respect to *EPAS1*, we performed genetic analysis on 181 samples (all included study subjects). Subsequent to PCR amplification and restriction digest analysis, we found that 100% of our study population was homozygous for the CC polymorphism. Because there was no allelic variation found, we did not perform statistics to correlate allelic variation with AMS susceptibility. Interestingly, previous studies that have found correlations between the chr2:46441523(gh18) *EPAS1* polymorphism and altitude illnesses used study populations of Han and Tibetan Chinese (Buroker et al 2012, Yang et al 2013). One study reports that the G allele is more prevalent in Tibetan Highlanders, a population that has been adapted to high altitudes for generations, than in the general Han Chinese population (Yang et al 2013). Because of the relative racial homogeneity of our study population (86.8% Caucasian), it could be that the G allele of this *EPAS1* polymorphism is not evolutionarily beneficial to this population, so the C allele remains dominant.

Summary and Future Directions

Our study shows that 35.9% of the subjects in our study reported AMS occurrence at Camp Muir in Mt. Rainier National Park. Of those reporting AMS, 73.8% reported mild AMS and 26.2% reported severe AMS. As expected, exposure to altitudes over 8000 ft within 2 weeks of ascending to Camp Muir decreased the percentage of individuals reporting AMS, but this difference did not reach statistical significance in our study. Interestingly, subjects pre-exposed to altitudes reported a higher percentage of severe AMS than those subjects not exposed to altitude before ascent. Again, those data did not reach statistical significance. However, these data underscore the need for increased awareness of the prevalence of this condition at Camp Muir, a very popular destination for thousands of recreationalists and professionals every year. Increased awareness of this condition could lead to increased resource allocation for alleviation of symptoms, increased preparedness to anticipate symptoms, and increased proactive measures for the prevention of symptoms.

As expected, this study shows no statistical correlation between reported AMS occurrence and the demographic factors of height, weight, BMI, race, age, alcohol use, or tobacco use (Table 2). Furthermore, our study shows no correlation between the *NOS3* rs1799983 SNP genotype, allele distribution, and susceptibility to AMS (Table 3). *EPAS1* genetic analysis regarding the SNP chr2:46441523(gh18) revealed a homogenous population, with all subjects containing homozygous CC alleles. *ACE* I/D polymorphism analysis revealed no significant association between *ACE* genotype and AMS susceptibility. However, we did find a significant correlation between overrepresentation of the I allele and AMS susceptibility, suggesting a link between expression of this allele and altitude illness.

In the future, we propose to investigate the correlation of reported AMS with polymorphisms associated with other genes implicated in high altitude tolerance (Table 4). Although the *ACE* I/D polymorphism is the most well-studied genetic polymorphism with respect to high altitude tolerance and AMS susceptibility, studies in the literature report several other genes that have been investigated (Table 4). Investigation into the association of these gene polymorphisms and AMS occurrence is important to understand the presence and role of predictive

markers in a condition that affects such a large percentage of individuals at altitude. Because individual variation appears to be a major determinant of AMS, it is important to explore and understand the role of specific gene polymorphisms in AMS onset to better predict the occurrence of this condition for possible prevention.

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Informed Consent

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1. Study Title Associations between specific human gene polymorphisms and susceptibility to acute mountain sickness.

2. Study Personnel

Name	Paige Baugher	Amanda Odell
Role	Principal Investigator, Faculty Advisor	Student Investigator
Institution	Pacific University	Pacific University
Program	Biology Department	Biology Department
Email	paige.baugher@pacificu.edu	odel0057@pacificu.edu
Telephone	503.352.3165	530.276.5782

3. Study Invitation, Purpose, Location, and Dates

You are invited to participate in a research study about how individual genetics could influence the symptoms of acute mountain sickness. Acute mountain sickness (AMS) is a condition that results from exposure to altitudes of over 8000 ft. Over 10,000 people attempt to summit Mt Rainier (14,416) every year, so AMS can be a concern. We are trying to learn more about the risk factors for these symptoms so we can try to find ways to avoid or decrease them.

Even though most cases of AMS are mild (headache, slight nausea), some cases can progress and negatively affect the mountain experience. Unfortunately, it is not clear who will get AMS and who will not. People who are well-acclimatized and in great physical condition can still experience symptoms. That is why we think there may be a genetic link. We want to understand if there are variations of certain genes that make someone more likely to experience AMS symptoms than others. The genes we want to take a look at include those that regulate blood pressure and heart rate, and those that regulate responses to low oxygen and physical stress. Here is the list: ACE, AGT, EPAS1, EGLN1, NOS3, GNB3, GSTM1, GSTT1, HSPA1B, and HSPA1L.

We will conduct our study at Camp Muir (10,100 ft), the camp you will pass or stay at on your way up the Disappointment Cleaver, during the summer of 2016 and 2017. You will need to fill out a demographic survey, in which you will need to provide information about your age, height, weight, etc. You will also need to give us a DNA sample. This is very quick, painless, and easy -- it's just a simple cheek swab. Then, after you have summited or gotten as far as you can, you will come back to Muir and fill out our self-reporting AMS symptoms survey. Again, all very painless, easy, and quick. My students and I will then take all this data back to our lab and analyze it. Our hope is that we can understand better predictive markers of AMS symptoms and find ways to decrease them.

This study is approved by the Pacific University Institutional Review Board. This is the group that legally ensures no harm will come to you as a participant in our study, and that all genetic data will be in no way shared with others or identified with your name or your identity.

4. Participant Characteristics and Exclusionary Criteria

- Participants must be sufficiently fluent in English language. This will be verified by checking "yes" in the box corresponding to the following statement, "I have read and understand the description of my participation duties."
- Participants must be at least 18 years old.
- Participants must be attempting the Disappointment Cleaver Route on their own and not because of this study.
- Participants will be excluded
 - if they are pregnant or at risk of becoming pregnant,
 - if they have pre-existing medical conditions with symptoms similar to AMS,
 - if they have a recent history of high altitude exposure or residency
 - if they are currently taking prophylaxis for AMS.
- There will be no restrictions based on gender, race, or ethnicity.

5. Study Materials and Procedures

We will need to collect the following from you:

- Signed Informed Consent Form (this form)
- Demographics questionnaire (height, weight, gender, etc)
- DNA sample
- AMS questionnaire

This should take no longer than 10-15 minutes, and there will be no cost to you. We will need to fill out the AMS questionnaire **after** your summit attempt, but while you are still at Camp Muir. The other information we can collect at any time. We have provided a brief description of each procedure below.

- Informed Consent Form. Please read this form and sign it before participating in this study.
- Demographics Questionnaire. This questionnaire asks basic information from you about your height, weight, gender, etc. Your name will not need to appear on this form. It will be identified only by a number.
- DNA collection. DNA will be collected by a cheek swab. This procedure is painless, quick, and easy. All you need to do is open your mouth, and we will gently rub the swab (kind of like a Q-tip) on the inside of your cheek for 5-10 seconds. We will take this swab back to the lab for analysis. All data will be completely confidential.
- AMS questionnaire. This asks you to report symptoms you may be experiencing that are common for AMS. They are rated on a 1 out of 3 scale or a 1 out of 4 scale.

6. Risks, Risk Reduction Steps, and Clinical Alternatives

a. Anticipated Risks and Strategies to Minimize or Avoid Risk

Any potential risks (e.g., emotional, financial, social, legal) due to participating are minimal, no greater than what you would face in normal daily life.

b. Unknown Risks

It is possible that participation in this study may expose you (or an embryo or fetus, if you are or become pregnant) to currently unforeseeable risks. It must be clear that at no time will the health or interest of the climbers be compromised for data collection purposes. Data collection will even be ceased if it is decreasing the odds of the participants from reaching the summit or detracting from their mountaineering experiment.

- c. **Advantageous Clinical Alternative** This study does not involve experimental clinical trial(s).

7. Adverse Event Handling and Reporting Plan

In the event that you become sick, injured, distressed, or otherwise uncomfortable as a result of your involvement in the research study, you may stop your participation immediately. If such an event occurs, promptly notify the principal investigator or the Pacific University Institutional Review Board. If the investigator(s) become aware of an adverse event, the IRB office will be notified by the next normal business day for minor events and within 24 hours for major events. If you experience or are directly affected by an adverse event, you will be given the opportunity to withdraw any data collected from you during the study up to the time of publication of this study.

8. Direct Benefits and/or Payment to Participants

a. Benefit(s)

Participants will be offered a gift card to Recreational Equipment Incorporated (REI) as an incentive to participate in this study.

b. Payment(s) or Reward(s)

None.

9. Promise of Privacy

The records of this study will be kept private and participants' identities will be protected by adhering to strict confidentiality procedures. In addition, as part of ongoing compliance efforts, the Food and Drug Administration may inspect any and all records pertaining to this study. FDA auditors maintain strict *confidentiality of all records reviewed*.

10. Medical Care and Compensation in the Event of Accidental Injury

During your participation in this project it is important to understand that you are not a Pacific University clinic patient or client, nor will you be receiving complete health care as a result of your participation in this study. If you are injured during your participation in this study and it is not due to negligence by Pacific University, the investigator(s), or any organization associated with the research, you should not expect to receive compensation or medical care from Pacific University, the investigator(s), or any organization associated with the study. If you are injured and it directly is related to your participation in this study as a research subject, please contact the Pacific University Institutional Review Board at 503-352-1478.

11. Voluntary Nature of the Study

Your decision whether or not to participate will not affect your current or future relations with Pacific University, local mountaineering organizations, mountain guiding services, or Mt. Rainier National Park. If you decide to participate, you are free to not answer any question or withdraw at any time without prejudice or negative consequences. If you choose to withdraw after beginning the study, you will be allowed to keep the participant compensation, and any data collected will be destroyed. If significant new findings develop (or are discovered) during the course of this research that could impact your decision to continue participation, such findings will be shared with you and you will be given the opportunity to withdraw from the study.

12. Contacts and Questions

The investigator(s) will be happy to answer any questions you may have at any time during the course of the study. If you are not satisfied with the answers you receive, please call the Pacific University Institutional Review Board at 503-352-1478 to discuss your questions or concerns further. If you have questions about your rights as a research subject, or if you experience a research-related injury of any kind, please contact the investigator(s) and/or the IRB office. All concerns and questions will be kept in confidence.

13. Statement of Consent

YES	NO	
_____	_____	I am 18 years of age or over.
_____	_____	All my questions have been answered.
_____	_____	I have read and understand the description of my participation duties.
_____	_____	I have been offered a copy of this form to keep for my records.
_____	_____	I voluntarily agree to participate in this study and understand that I may withdraw at any time without consequence.
_____	_____	I give permission for the investigator(s) to gather and use my genetic information only for the purposes specifically delineated in this consent form.

_____	_____
Participant Signature	Date

_____	_____
Printed Full Name	Participant Study Role

_____	_____
Investigator Signature	Date

_____	_____
Paige J. Baugher Printed Full Name	Investigator Study Role*

*This individual must be trained in obtaining informed consent and have authorization from the principal investigator and/or faculty advisor to do so.

DEMOGRAPHICS QUESTIONNAIRE

Please circle the answer that best fits your response.

1. What is your gender? Female Male
2. What is your age? 18-30 31-40 41-50 51-60 61-70 71+
3. How tall are you? _____ feet and _____ inches
4. How much do you weigh in pounds? _____ pounds
5. To which racial or ethnic group do you most identify?
African-America (non-Hispanic)
Asian/Pacific Islander
Caucasian (non-Hispanic)
Latino or Hispanic
Native American or Aleut
Other
6. Please characterize your use of alcohol.
non-drinker (I do not drink alcohol)
light drinker (1-6 drinks per week for women, 1-12 drinks per week for men)
moderate drinker (1 drink per day for women, 2 drinks per day for men)
heavy drinker (more than 1 drink per day for women, more than 2 drinks per day for men)
7. Please characterize your use of tobacco smoking. never smoker present smoker past smoker
8. Have you been exposed to altitudes above 8000 ft (2500 m) in the past 2 weeks? yes no
9. Do you have a history of high altitude residency (above 8000 feet or 2500m)? yes no
10. Are you currently taking acetazolamide or steroids for acute mountain sickness prophylaxis? yes no
11. Please list any medical conditions you are currently experiencing below:

LAKE LOUISE SCORE (LLS) for the Diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on

1. A rise in altitude within the last 4 days
2. Presence of a headache

PLUS

3. Presence of at least one other symptom
4. A total score of 3 or more from the questions below

SELF-REPORT QUESTIONNAIRE

Add together the individual scores for each symptom to get the **total score**.

Headache	No headache	0	
	Mild headache	1	
	Moderate headache	2	
	Severe headache, incapacitating	3	
Gastrointestinal symptoms	None	0	
	Poor appetite or nausea	1	
	Moderate nausea &/or vomiting	2	
	Severe nausea &/or vomiting	3	
Fatigue &/or weakness	Not tired or weak	0	
	Mild fatigue/ weakness	1	
	Moderate fatigue/ weakness	2	
	Severe fatigue/ weakness	3	
Dizziness/lightheadedness	Not dizzy	0	
	Mild dizziness	1	
	Moderate dizziness	2	
	Severe dizziness, incapacitating	3	
Difficulty sleeping	Slept as well as usual	0	
	Did not sleep as well as usual	1	
	Woke many times, poor sleep	2	
	Could not sleep at all	3	

Total Score _____

Total Score of

- 3 to 5 = mild AMS
- 6 or more = severe AMS

Table 1. AMS Occurrence Rates

	TOTAL STUDY POPULATION	NO ALTITUDE PRE-EXPOSURE	PRE-EXPOSED TO ALTITDUE
AMS	35.9% (65/181)	38.1% (37/97)	33.3% (28/84) RR=0.8739, 95% CI (0.5890-1.2966), p=0.5031
MILD AMS	73.8% (48/65)	78.4% (29/37)	67.8% (19/28)
SEVERE AMS	26.2% (17/65)	21.6% (8/37)	32.2% (9/28) RR=1.4866, 95% CI (0.7071-3.6521), p=0.3410

Table 2. Demographic Correlations with AMS Occurrence Rate

	Total	AMS	Non-AMS	χ^2 value	df	p-value	Significance ($p \leq 0.05$)
Total No. Subjects	181	65	116				
Baseline Demographics Data							
Sex				0.4303	3	0.512	NS
Male	138	47	91				
Female	43	17	26				
Height (m)	1.76	1.76	1.76			0.775	NS
Weight (kg)	75.57	74.81	75.99			0.497	NS
BMI	24.95	24.66	25.10			0.308	NS
Age				2.5916	7	0.628	NS
18-30	64	21	43				
31-40	76	31	45				
41-50	25	9	16				
51-60	11	2	9				
61-70	5	2	3				
Race				1.1835	3	0.277	NS
Caucasian	157	54	103				
Non-Caucasian	24	11	13				
Alcohol Use				1.3498	5	0.509	NS
non	29	13	16				
light	123	43	80				
moderate	27	9	18				
heavy	2	0	2				
Tobacco Use				1.1836	5	0.553	NS
Past User	30	12	18				
Never User	145	52	93				
Present User	6	1	5				

Calculations based on excluded data. Df = degrees of freedom; significance indicated by a p value ≤ 0.05 . NS=not significant. Light alcohol use = 1-6 drinks per week for women, 1-12 drinks per week for men; moderate alcohol use = 1 drink per day for women, 2 drinks per day for men; heavy alcohol use = more than 1 drink per day for women, more than 2 drinks per day for men. Categorical data (sex, age, race, alcohol use, tobacco use) correlations with AMS occurrence were calculated using χ^2 analysis; continuous data (height, weight, BMI) were analyzed as a comparison between means using a student's t-test (two-tailed, unpaired p value ≤ 0.05 indicated statistical significance).

Table 3. Genotype and Allele Frequencies in Subjects with and without Acute Mountain Sickness

	AMS GENOTYPES	AMS ALLELE FREQUENCY	NON AMS GENOTYPES	NON AMS ALLELE FREQUENCY	GENOTYPE FREQUENCY P-VALUE	ALLELE FREQUENCY P-VALUE	HWE P-VALUE
NOS3 RS1799983 (GLU298ASP) N	65		116				
	31 GG	0.66 (G)	56 GG	0.69 (G)	0.700	0.651	1.6476
	24 GT	0.34 (T)	47 GT	0.31 (T)			
	10 TT		13 TT				
ACE (I/D) N	19		26				
	3 II	0.447 (I)	9 II	0.596 (I)	0.326	0.0337*	0.6318
	11 ID	0.553 (D)	13 ID	0.404 (D)			
	5 DD		4 DD				

Allele frequencies were established by gene counting, and the genotype distribution was tested for Hardy-Weinberg equilibrium (HWE) using χ^2 analysis. The association between genotypes and AMS occurrence rate was calculated using χ^2 analysis; the association between allele frequencies and AMS occurrence rate was calculated using χ^2 analysis. Statistical significance is indicated by a p value ≤ 0.05 (*).

Table 4. Gene Associations Studies of AMS Susceptibility (adapted from MacInnis et al 2011).

GENE	POLYMORPHISM	ALTITUDE	ASSOCIATION	REFERENCE
ACE	Alu insertion/deletion; intron 16	3,817 m 3,670 m	Insertion/deletion genotype day 1 D allele	Tsianos et al, 2005 Buroker et al, 2010
AGT	T(704)C; met235thr, exon 2	3,670 m	T (met) allele	Buroker et al, 2010
EGLN1	rs480902 (C/T)	3,670 m	C allele	Buroker et al, 2012
EPAS1	chr2:46441523 (hg18) (C/G)	3,670 m	C allele	Buroker et al, 2012
GNB3	A(-350)G; 5'UTR	3,670 m	A allele fixed in AMS cases	Buroker et al, 2010
GSTM1	+/-	3000 m	negative (-/-)	Jiang et al, 2005
GSTT1	+/-	3000 m	positive (+/+,+/-)	Jiang et al, 2005
HSPA1B	A(1267)G; silent, exon 1	- 2,600- 2,800 m	G/G G/G	Li et al, 2004 Zhou et al, 2005
HSPA1L	G(2437)C; met493thr, exon 2	2,600- 2,800 m	B/B	Zhou et al, 2005
NOS3	G(894)T, glu298asp, exon 7	4380 m	G/G	Wang et al, 2009

Gene abbreviations and functions: *ACE* (angiotensin I-converting enzyme), plays an important role in the renin-angiotensin-aldosterone system that maintains fluid balance; *AGT* (angiotensinogen), plays a role in the renin-angiotensin-aldosterone system that maintains fluid balance; *EPAS1* (endothelial PAS domain protein 1), is involved in the induction of genes regulated by oxygen; *EGLN1* (Egl-9 family hypoxia-inducible factor 1), contributes to the post-translational modification of hypoxia-inducible factor 1 (HIF-1); *NOS3* (endothelial nitric oxide synthase), acts to relax smooth muscle and plays a role in vasodilation; *GNB3* (beta polypeptide 3), acts to mediate signals in transmembrane signaling systems; *GSTM1* (glutathione S-transferase mu 1) and *GSTT1* (glutathione S-transferase theta 1), are members of the glutathione S-transferases, intracellular enzymes that play a role in detoxification (including detoxification of free radicals generated during hypoxia); *HSPA1B* (heat shock protein A1B), *HSPA1L* (heat shock protein A1L) are members of the heat shock family of proteins that are upregulated during cellular stress (including hypoxia).