Original Research

No association between alleles of the bradykinin receptor-B2 gene and acute mountain sickness

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Abstract

The pathophysiological mechanism(s) of the development of acute mountain sickness (AMS) is still unclear. Although the chance of developing AMS and the severity of the condition are influenced by ascent rate and altitude attained, previous history is a reliable predictor of subsequent affliction, and some individuals and families are clearly predisposed, suggesting a genetic component to susceptibility. As the vasodilator bradykinin may be involved in acclimatization to altitude, we hypothesized that variants in genes encoding components of this pathway might play a role in AMS susceptibility. We tested this by looking for associations between two functional polymorphisms (the in/del polymorphism +9/−9 [rs5810761] and the single-nucleotide polymorphism C − 58T [rs1799722]) of *BDKRB2* (the gene encoding the bradykinin receptor B2) and susceptibility to AMS in an altitude-exposed Nepalese population. Lowland attendees ($n = 233$) at a religious festival at 4380 m in the Nepalese Himalaya were recruited and assessed for AMS by clinical evaluation and Lake Louise score (LLS). Those with a clinical diagnosis of AMS and an LLS ≥3 were designated AMS+ ($n = 100$) and those without a diagnosis of AMS and with an LLS <3 were categorized as AMS− ($n = 117$). DNA was prepared from buccal cells, genotyped for the two polymorphisms and allele frequencies compared between the two cohorts. No association was found between alleles at either polymorphism and susceptibility to AMS ($P > 0.50$), although C − 58T heterozygotes were significantly more common ($P < 0.001$, $\chi^2 = 49.6$) in the subjects than would be predicted if the population was in Hardy–Weinberg equilibrium. The results of our association study do not support the hypothesis that variants in *BDKRB2* influence altitude tolerance in a lowland Nepalese population; however, the deviation from Hardy–Weinberg equilibrium observed for the C − 58T polymorphism could be explained by self-selection for altitude tolerance in the festival attendees.

Keywords: bradykinin, altitude, hypoxia, bradykinin receptor, Nepalese

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Introduction

Acute mountain sickness (AMS) is the most common, but most benign of the altitude-related illnesses. The pathology, epidemiology and genetics of the condition have been discussed in several recent reviews.[1–7] Briefly, AMS is characterized by headache, anorexia, insomnia, fatigue and nausea developing soon after rapid ascent to moderate (e.g. 2500 m) to high altitude. The underlying pathophysiology is not fully understood, but may involve hypoxia-induced cerebral vasodilation or mild edema, although there is debate as to whether recent imaging data support these mechanisms. The high re-occurrence rate of AMS indicates a potential genetic contribution to the acclimatization to high altitude.[8] Innate susceptibility and familial clustering but lack of simple Mendelian patterns of inheritance is consistent with a polygenic trait involving the interaction of a number of genes. A number of candidate–gene association studies have looked for evidence of specific genetic variants in contributing to AMS susceptibility (e.g. refs.,[9–12] reviewed in ref.[4]); however, no strong, consistent associations have been found.

Bradykinin is a circulating vasoactive peptide that stimulates vasodilation, primarily via activating endothelial bradykinin B2 receptors.[13] These receptors also interact with endothelial nitric oxide synthase (eNOS), an enzyme that produces nitric oxide (NO) – a potent local vasodilating agent. Protein–protein interactions between BDKRB2 (the gene encoding the bradykinin receptor B2) and eNOS[14] suggest that bradykinin B2 receptors may be involved in the regulation of vascular homeostasis in acclimatization

to high altitude. High-altitude natives showed a level of exhaled nitric oxide that was 25–200% greater than lowlanders,[15] and greater levels of exhaled nitric oxide has also been demonstrated in mountaineers resistant to high-altitude illness.[16] Furthermore, bradykinin is regulated by angiotensin-converting enzyme (ACE), which has been implicated in a number of studies to contribute to altitude performance such as successful summiting (i.e. ref.[17]), which could reflect resistance to AMS.

The gene that encodes the bradykinin B2 receptors (*BDKRB2*) contains a number of polymorphic loci, including a nine-base insertion/deletion in the first exon of the gene (+9/−9, rs5810761) and C to T transition in the promoter region (C − 58T, rs1799722). The presence of the 9 bp segment (+9) has been associated with decreased expression of the gene[18,19] as well as greater vascular resistance and higher systolic blood pressure,[20] while the −58 T allele has been implicated as a risk factor for the development of hypertension.[21] *BDKRB2* genotype affects the response to ACE inhibitors[22] and also interacts with genotype at the gene (*NOS3*) that encodes eNOS.[23] Our previous studies on the same Nepalese cohort described in the paper found an association between the T allele of the functional polymorphism G298T (rs1799983) in *NOS3* and the susceptibility to AMS,[24] but no association with *ACE* polymorphisms and the condition.[9]

Given that variants in *BDKRB2* may influence blood flow through a number of central vasomodulating pathways and the potential role of cerebral blood flow in AMS, we tested the hypothesis that either (or both) of the alleles in *BDKRB2* that are associated with higher vascular resistance and blood pressure (+9 allele and −58T) will be over-represented in individuals with AMS compared with an AMS-resistant cohort. This hypothesis was tested using DNA samples collected from lowland Nepalese travelers to Gosainkunda, Nepal (4380 m), who were evaluated for AMS clinically and by Lake Louise score.

Methods and materials

Initial samples were collected in 2005. Those subjects, the experimental venue, recruitment strategy and DNA sampling methods have been described previously.[9] A second, independent set of samples were obtained using the same procedures in 2008 and combined with the original sample set for this analysis. Briefly, all subjects were lowland (<1800 m) Nepalese pilgrims attending the Janai Purnima Festival at Gosainkunda, Nepal (4380 m). Access to the site is by foot and most of the attendees ascend ~3000 m in 12–24 h. The time that our subjects had spent at altitude is in the 48–72 h range. Most of our subjects were first time visitors and none had been at an altitude in the three months before joining our study. Attendees did not take anti-AMS medications prophylatically and high instances of AMS have been reported at previous festivals.[25]

In this study, AMS was assigned to individuals who had a Lake Louise score of 3 or greater and were clinically diagnosed by physicians' experience in altitude medicine (AMS+, $N = 100$). Individuals who were diagnosed as being free of AMS and had Lake Louise scores less than 3 were considered AMS negative (AMS−, $N = 117$). Patients who were discordant for the two diagnostic criteria ($N = 14$) were not included in the analysis. Reaction conditions for genotyping the two polymorphic loci (+9/−9 and C − 58T) were as follows: DNA (100 ng) was amplified in a 25 μL reaction buffer containing 0.2 mmol/L deoxynucleotide triphosphates, 1.0 mmol/L $MgCl_2$, 20 mmol/L Tris/Cl (pH = 8.4), 50 mmol/L KCl, 0.015 nmol of each primer and 0.5 U *Taq* polymerase (Invitrogen Corporation, Carlsbad, CA, USA) for 40 cycles of one minute at 94°C, 30 s at 60°C (+9/−9) or 57°C (C − 58T) and 10 s at 72°C, followed by a five-minute soak at 72°C in a G-Storm Thermal Cycler (AlphaMetrix Biotech GmbH, Rödermark, Germany). The primers for the *BDKRB2* +9/−9 polymorphism were as follows: forward, 5′-TCC AGCTCTGGCTTCTGG-3′ and reverse, 5′-AGTCGCTCC CTGGTACTGC-3′, and the amplification products were 80 bp (−9) versus 89 bp (+9).[26] The *BDKRB2* C − 58T polymorphism was assayed using a pair of degenerate primers which were as follows: forward, 5′-AAGGTGGCCGCA GCCTTCC-3′ and reverse, 5′-CTCATCTTTCAAGGGCTGG CTA-3′. The reverse primer contained a G–C transversion (underlined) that generated a recognition site (5′-CTAG-3′) for the restriction endonuclease *Bfa*I (New England Biolabs, Ipswich, MA, USA) in the presence of the C allele. If the C allele was present, the 133 bp PCR product digested to 112 + 21 bp. All PCR or digestion products were size-separated by electrophoresis in 8% polyacrylamide gel electrophoresis gels run in 1 × tris-borate-EDTA buffer and visualized with ethidium bromide and ultraviolet light. Allele and genotype frequencies were compared by χ^2 analysis. When observed or expected values included a cell with a value less than 5, Fisher's exact test was used. In all cases, significance was accepted at $P < 0.05$.

Procedures were approved by the UBC Clinical Research Ethics Board and the Nepal Health Research Council.

Results

Neither alleles nor genotypes of either of the *BDKRB2* polymorphisms tested were over-represented in the AMS+ cohort (Table 1). Genotype frequencies were in Hardy–Weinberg equilibrium (HWE) at the *BDKRB2* +9/−9 polymorphism ($P = 0.99$, $\chi^2 = 0.0001$, $n = 209$) but not for the *BDKRB2* C − 58T polymorphism ($P < 0.001$, $\chi^2 = 49.6$, $n = 189$), due to a higher proportion of heterozygotes than expected. This was significant in both the AMS+ cohort ($P < 0.001$, $\chi^2 = 19.57$, $n = 90$) and the AMS− cohort ($P < 0.001$, $\chi^2 = 30.58$, $n = 99$).

Discussion

Our study found no significant differences in allele frequencies between the AMS+ and AMS− cohorts for both of the *BDKRB2* polymorphisms tested and thus no association between these variants and susceptibility to AMS in a cohort of Nepalese pilgrims who had rapidly ascended to

Table 1 Genotype and allele frequencies of the *BDKRB2* C − 58T and +9/−9 polymorphisms in Nepalese pilgrims with, and without, AMS at Gosainkunda, Nepal (4380 m)

Polymorphism	Condition	Genotype	Frequency*	*P* value	Allele	Frequency	*P* value
BDKRB2 +9/−9	AMS+	+9/+9	2 (2.1%)	0.90	+9	25 (13.3%)	0.75
		+9/−9	21 (22.3%)		−9	163 (86.7%)	
		−9/−9	71 (75.6%)				
	AMS−	+9/+9	2 (2%)		+9	33 (14.3%)	
		+9/−9	29 (25%)		−9	197 (85.7%)	
		−9/−9	84 (73%)				
C − 58T	AMS+	C/C	7 (7.8%)	0.55	C	79 (43.9%)	0.70
		C/T	65 (72.2%)		T	101 (56.1%)	
		T/T	18 (20%)				
	AMS−	C/C	4 (4%)		C	83 (41.9%)	
		C/T	75 (76%)		T	115 (58.1%)	
		T/T	20 (20%)				

AMS, acute mountain sickness; *BDKRB2*, bradykinin receptor-B2 gene
*Discrepancy between genotype number and total sample size is due to failure of some samples to PCR amplification

4380 m. Given our sample sizes, we had the power to detect moderately higher frequencies of the hypothesized causal alleles in the AMS+ cohort (13.3–22.0% for +9; 56.1–69.0% for −58T), so our data suffice to exclude a substantial role for these alleles in AMS susceptibility. It must be noted, however, that our results do not exclude a genetic influence of *BDKRB2* in the acclimatization to high altitude in other populations. The genotype and allele frequencies of the +9/−9 polymorphism in Nepalese were significantly different from those of other populations (e.g. Caucasian, northeast Asian and African-American[19,20]) and the phenotypic consequences of the +9/−9 alleles varies between populations (e.g. systolic blood pressure and pulse pressure was significantly higher in +9 + 9 Caucasian but not in +9 + 9 African-Americans[20]). This phenotypic variation limits the value of extrapolating our results to other populations and further association studies in other ethnic groups would be required to exclude completely *BDKRB2* variants as potential factors contributing to altitude acclimatization.

Genotype frequencies for the +9/−9 polymorphism were in HWE, but the genotype frequencies of the C − 58T polymorphism were not, due to a significantly higher proportion of C/T genotypes than expected. This was true in both the AMS+ and AMS− cohorts (although more pronounced in subjects who were AMS−). The distribution of genotypes in a population rapidly comes to HWE if there is random mating, no population influx or efflux and no selection for, or against, genotypes.[27] One explanation for the deviation from HWE that we observed (i.e. the higher than predicted frequency of heterozygotes) is self-selection in festival attendees. If C/C and T/T individuals are prone to AMS, people carrying these genotypes may have been under-represented at our collecting site because they turned back when they began to feel discomfort during the ascent, left the festival early or did not attend because of AMS suffered in previous years. Any or all of these responses would result in an over-representation of C/T heterozygotes in attendance. The non-significant trend in our data for a greater representation of C/T individuals in the AMS− cohort supports this model of −58 C/T heterozygous advantage for AMS resistance. As we did not see an association between the C/C or T/T genotypes and AMS, other variables (genetic or environmental) would have to be ameliorating the effects of C/C and T/T genotypes on the pilgrims that we tested if this model is correct. We do not have genotype data from the source population for the cohort who traveled to Gosainkunda, so we do not know whether the deviation from HWE that we observed is limited to the pilgrims (in which case it could be due to self-selection for altitude tolerance discussed above), or whether it is characteristic of the source population in general.

In summary, we found no association between the *BDKRB2* +9/−9 and C − 58T polymorphisms and the susceptibility to AMS in Nepalese. Deviation from HWE for the C − 58T polymorphism suggests a protective effect of the C/T genotype which merits future investigation.

Author contributions: All authors participated in the design, interpretation of the studies and analysis of the data and preparation of the manuscript. MSK and JLR obtained the samples and were responsible for AMS assessment, and PW performed the genotyping experiments.

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