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Angiotensin converting enzyme insertion allele in relation to high altitude adaptation

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SUMMARY

Angiotensin converting enzyme (*ACE*) gene I/D polymorphism has been associated with high altitude (HA) disorders as well as physical performance. We, however, envisage that the polymorphism may be associated with adaptation to the hypobaric hypoxia of altitude, thus facilitating physical performance. For this purpose, three unrelated adult male groups, namely (1) the Ladakhis (HLs), who reside at and above a height of 3600 m, (2) lowlanders, who migrated to Ladakh (MLLs), and (3) resident lowlanders (LLs), have been investigated. The HLs had significantly ($p < 0.001$) greater numbers of the II homozygotes and the ID heterozygotes than the DD homozygotes, the genotype distribution being 0.46, 0.43 and 0.11 for II, ID and DD genotypes respectively. The MLLs comprised 60% II homozygotes, which was higher ($p < 0.001$) than the HLs (46%). In the LLs, the heterozygotes were greater ($p < 0.001$) in number than the II and DD homozygotes. The I allele frequency was 0.72 in the MLLs, 0.67 in the HLs and 0.55 in the LLs. Polymorphism study suggested that the II genotype could be associated with altitude adaptation, which might influence physical efficiency.

INTRODUCTION

People living at sea level experience various levels of physical discomfort at high altitude (HA) due to hypobaric hypoxia and cold. The effects of hypoxia on cardio-pulmonary function (Sutton *et al.* 1988; Mirrakhimov & Winslow, 1996) are most evident during hard physical work when the already taxed oxygen transport system needs to meet increased metabolic demands. This results in somewhat reduced levels of performance of individuals when faced with tasks demanding high levels of physical and mental performance. However, long-term inhabitants of HA (> 3000 m) demonstrate improvement in endurance performance (Chen *et al.* 1997), due to a process of adaptation, which enables large communities to settle in mountainous areas (Hochachka *et al.* 1996).

During the last few decades, a significant amount of work has been done to understand the human biology of HA adaptation and disorders (Mirrakhimov & Winslow, 1996; Semenza, 1999; Lahiri, 2000). Because of their intimate involvement, two main physiological systems have been targeted; namely, the sympathetic–adrenal-medullary system (Mazzeo *et al.* 1994; Lahiri, 2000; Prabhakar, 2000) and the renin-angiotensin-aldosterone system (Milledge & Catley, 1987; Morrell *et al.* 1999). In recent years, attention has shifted towards candidate genes, notable among them being the *ACE* gene (Rupert *et al.* 1999; Morell *et al.* 1999) and its insertion/

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deletion (I/D) polymorphism in particular. This has been the primary choice because of the clinical importance of the enzyme in the regulation of blood pressure (BP), even at altitude (Milledge & Catley, 1987; Morrell *et al.* 1999). Investigations of the I/D polymorphism in relation to physical performance in mountaineers and athletes (Montgomery *et al.* 1998; Gayagay *et al.* 1998) have given further impetus to similar studies on HA residents. Indeed, the *ACE* gene I/D polymorphism may contribute towards altitude adaptation. In view of this we decided to investigate whether there is an association of the *ACE* gene I/D polymorphism with HA adaptation, which in turn would influence physical performance.

MATERIALS AND METHODS

Subjects

The study groups consisted of unrelated males comprising of 131 Ladakhis (HLs), who reside at and above a height of 3600 m, 20 lowlanders, who migrated to Ladakh (MLLs), and 126 resident lowlanders (LLs). A detailed questionnaire about demography, ethnicity, relationship, habits and health was administered. The HLs and LLs were permanent residents of their respective lands from ancient times (Bhasin *et al.* 1994) and the MLLs were first generation settlers, who moved to the highlands in search of a livelihood. The Ancient Indian literature mentions Himalayan settlements of Indo-Aryan and Mongoloid origins, but it became more focused from the mediaeval age. The LLs and MLLs are from the same racial background. The genetic relationship between the high and low land populations remains unknown, although evidence suggests close bio-cultural affinity (Bhasin *et al.* 1994). The age of the subjects was between 19–25 years, and BP (supine) was $\leqslant 140/90$ mm Hg. Other characteristics such as body weight, height, percent oxygen saturation of arterial haemoglobin (SaO_2), and pulse were also recorded and are presented in Table 1. An Automatic Digital Blood Pressure Monitor (Omron MX2, Japan) was used to record BP and pulse. SaO_2 was measured with a Finger Pulse Oximeter 503 (Criticare Systems Inc., USA). The latter equipment also measured the pulse. Prior to blood collection, subjects were appraised of the study and written consent was obtained.

Methods

DNA was isolated from peripheral blood leukocytes using a modification of the salting-out procedure (Miller *et al.* 1988). The genotypes for the I/D polymorphism were determined by PCR amplification (Evans *et al.* 1994). Validation of the PCR product was carried out by Gene Scan analysis using an ABI Prism 377 Automatic DNA Sequencer (Perkin-Elmer, Applied Biosystem, USA).

Statistical analyses

Data are expressed as mean $\pm$ SD and differences between the groups were analysed by one-way analysis of variance. Differences in the distribution of genotypes and alleles were analysed by χ^2 test. A p value of < 0.05 was considered statistically significant.

RESULTS

Characteristics

Data are shown in Table 1. The subjects were young (19–25 years), all had normal BP; BMI was lowest ($p = 0.005$) in the MLLs, and heart rate highest ($p = 0.022$) in the MLLs. SaO_2, at an average of 97 %, was maximum ($p < 0.001$) in the LLs as expected, whereas the two HA groups showed 8–9 % decline. Between the HLs and the MLLs, the latter group had marginally higher SaO_2 levels.

Genotype distribution

The results of genotype distribution are presented in Table 2. The II homozygotes and the ID heterozygotes in the HLs were significantly ($p < 0.001$) greater in number than the DD homozygotes. In the case of MLLs, it was

Table 1. *Characteristics of Indian study sample at sea-level and high-altitude*

Subjects	LLs	HLs	MLLs	p
Altitude, m	Sea-level	> 3600	> 3600	
Age, years	23 ± 1.7	19.8 ± 0.7	22.5 ± 1.3	< 0.001
SBP, mmHg	116 ± 7	118 ± 8	124 ± 609	0.009
DBP, mmHg	69 ± 10	77 ± 9	86 ± 6	< 0.001
Weight, kg	60.0 ± 6.0	55.5 ± 4.7	56.1 ± 3.8	0.009
Height, cm	169.0 ± 4.5	164.3 ± 4.7	171 ± 3.6	< 0.001
BMI, kg/m^2	20.8 ± 1.4	20.5 ± 1.4	19.2 ± 1.2	0.005
SaO_2, %	97 ± 0.7	89 ± 3.0	90 ± 2.5	< 0.001
Heart rate, b/m	66 ± 8	66 ± 6	73 ± 9	0.022
Genotype	ID > II > DD	II & ID > DD	II > ID & DD	0.05

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; SaO_2, percent of oxygen saturation of arterial haemoglobin.

Table 2. *Genotype distribution in the three groups*

	Genotype			
Subjects	II	ID	DD	p
1. Highlanders				
a) natives, HLs (n = 131)	0.46 (60)	0.43 (56)	0.11 (15)	< 0.001
b) migrants, MLLs (n = 20)	0.60 (12)	0.25 (05)	0.15 (03)	0.006
2. Lowlanders, LLs (n = 126)	0.29 (37)	0.51 (64)	0.20 (25)	< 0.001

n denotes number of subjects.

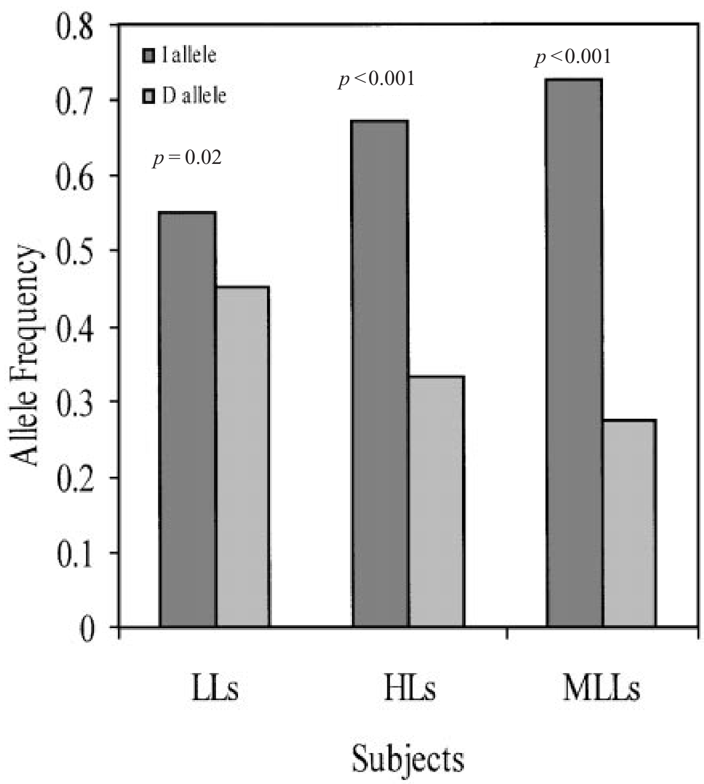

Fig. 1. *ACE* gene I/D polymorphism in the three Indian population groups i.e. LLs, HLs, and MLLs.

only the II homozygotes that were significantly ($p < 0.001$) greater in number than the ID heterozygotes and the DD homozygotes. The LLs departed from this trend, with the ID heterozygotes greater ($p < 0.001$) in number than the II and DD homozygotes. A comparison between the highlanders and the lowlanders revealed a significant difference in the genotype distribution. The HLs had a greater ($p < 0.05$) number of II homozygotes than the LLs. The situation was similar between the MLLs and the LLs. Between the MLLs and the HLs, the former had II homozygotes significantly ($p < 0.001$) greater in number.

Allele frequency

As can be seen from Figure 1, the I allele frequency in comparison to the D allele was higher ($p < 0.05$) in all the three groups. A further comparison of the I/D frequencies of the three groups revealed that the MLLs had the highest ($p < 0.001$) frequency of I allele followed by the HLs ($p < 0.001$) and then the LLs ($p = 0.02$), the frequencies being 0.725, 0.67 and 0.55 respectively. It was observed that the I allele frequency was significantly ($p < 0.05$) higher in the highland groups as compared to the LLs. Between the highland groups, the MLLs had higher ($p < 0.05$) frequency of the I allele than the HLs.

DISCUSSION

The two Ladakhi groups in this study consisted of the HLs and the MLLs. The latter group had migrated to the highlands in search of employment and ultimately took to similar jobs and lifestyle as that of the HA natives. It was interesting to note that the majority of the subjects in this group were homozygous for the insertion allele. Accordingly, the percentage distribution of the I allele came out to 72.5 ($p < 0.001$), with marked I allele homozygosity ($p < 0.05$) when compared to that of the highland natives. Might such a finding relate to natural selection to the hypoxic environment? The LLs on the other hand deviate from the other two groups in the distribution of the genotype, as instead of the II homozygotes they have the ID heterozygotes greater in number. The genotypes of the highlanders, as found in the present study, seem to support our hypothesis of the association of the I allele with HA adaptation.

Genomic research on HA subjects in relation to physical performance or health and disorder is yet to gain momentum. With respect to *ACE* polymorphism only two reports can be cited, one for an association with high endurance (Rupert *et al*. 1999) and the other one with pulmonary hypertension (Morell *et al*. 1999). In the first report, the highland Quechua were reported to have higher frequency of the I allele but without any association with physical performance, since their lowland counterparts also had identical allele frequencies (Rupert *et al*. 1999). According to these investigators, the high frequency of the I allele might have helped during migration to the highlands. Contrary to this, Morell *et al*. (1999), in their findings on the two HA native groups of The Republic of Kyrgystan, observed high frequency of the D allele in controls and of the I allele in subjects with pulmonary hypertension.

ACE studies at the molecular level, especially genotyping in association with phenotypic characteristics, might provide relevant information on adaptation and physical performance especially in a newcomer. It is known that during exercise, a redistribution of cardiac output occurs such that blood flow is decreased to the renal and splanchnic circulations and increased to the heart and skeletal muscles. This increases activation of the renin-angiotensin system (Muller *et al*. 1997; Symons *et al*. 1999). *ACE* plays a pivotal role in this process; therefore, we also estimated ACE activity (Manju *et al*. 2000). Approximately 40% increase in activity was observed with a change from II to ID and ID to DD genotype (Rigat *et al*. 1990; Danser *et al*. 1995). Normal subjects show wide variation in the circulating enzyme levels that can be categorized into three ranges i. e. low, medium and high, correlating to II, ID and DD genotype respectively. It may be added that low enzyme activity in an individual could be beneficial in the long term, especially in disease states. The enzyme activity in an individual is an indication of corresponding level of Ang II (Woods *et al.* 2000), which is a potent vasoconstrictor (Ward, 1995), and stimulates vascular endothelial growth factor (Otani *et al* 1998) and the Ca^{++} and aldosterone pathways (Pratt *et al.* 1989).

Variations in the observed characteristics may reflect differences in adaptive response to hypobaric hypoxia (Zhuang *et al.* 1993; Heath & Williams, 1995). A correlation could not be observed between the genotype and the characteristics (Table 1); however, it seemed that body weight and BMI increased with the D allele, which was comparatively greater in number in the LLs, followed by the HLs and MLLs.

In conclusion, it may be said that the highland natives have the II genotype in greater numbers than the ID and DD genotypes and, as a consequence, have higher frequency of the I allele. The I allele may contribute towards routine physical activities by way of adaptation to the hypoxic environment at HA. The I allele may also influence physical performance, which is corroborated by our observation in the MLLs, who migrated and settled at HA.

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