Variations in angiotensin-converting enzyme gene insertion/deletion polymorphism in Indian populations of different ethnic origins

M A QADAR PASHA*, AMJAD P KHAN, RATAN KUMAR†, REKH B RAM, SURINDER K GROVER†, KAUSHAL K SRIVASTAVA†, WILLIAM SELVAMURTHY† and SAMIR K BRAHMACHARI

Functional Genomics Unit, Centre for Biochemical Technology, Mall Road, Delhi 110 007, India

†*Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi 110 054, India*

**Corresponding author (Fax, 91-11-7667471; Email, qpasha@cbt.res.in).*

The pattern of angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism in the Indian population is poorly known. In order to determine the status of the polymorphism, young unrelated male army recruits were screened. The population had cultural and linguistic differences and lived in an environment that varied significantly from one region to another. Analysis of the genotype, showed higher frequency of the insertion allele in four of the five groups i.e. I allele frequency was significantly higher ($P < 0{\cdot}05$) in Dogras, Assamese and Kumaonese. The deletion allele frequency was comparatively higher in the fifth group that belonged to Punjab. A correlation was observed between the genotype and enzyme activity. Involvement of a single D allele in the genotype enhanced the activity up to $37{\cdot}56 \pm 3{\cdot}13\%$. The results suggested ethnic heterogeneity with a significant gene cline with higher insertion allele frequency. Such population-based data on various polymorphisms can ultimately be exploited in pharmacogenomics.

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1. Introduction

Angiotensin-converting enzyme (ACE, EC 3.4.15.1, dipeptidyl carboxypeptidase) is associated with the regulation of blood pressure and maintenance of salt and water homeostasis in the body (Ward 1995). Because of the enzyme's central role in the renin-angiotensin-aldosterone system, numerous association studies have been carried out. Though the human ACE gene contains a number of variable polymorphic regions that can be of potential use in genetic analysis of populations (Rieder *et al* 1999), the insertion/deletion (I/D) polymorphism present in intron 16, in particular has been extensively investigated. The D allele has been associated with hypertension and various organ disorders, although discord exists (Danser *et al* 1995; Zee *et al* 1999; Pontremoli *et al* 2000), and in recent years the I allele has been associated with high endurance (Gayagay *et al* 1998; Montgomery *et al* 1998; Qadar Pasha *et al* 2001). In the present study, we have made an attempt to investigate the association of the I/D polymorphism with respect to racial closeness and drift. It is also realized that polymorphism studies on a larger scale could be of greater help in relation to fitness or disorders and in designing the future medicines.

2. Subjects and methods

2.1 *Subjects*

Blood sample from 220 unrelated healthy Indian populace was obtained in acid citrate dextrose (ACD). The population namely: Sikh, Jat, Dogra, Kumaonese, and Assamese differed with respect to ethnic origin. The states they belonged to were Punjab, Haryana, Himachal

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Pradesh, Uttaranchal and Assam respectively. The former four were neighbours in the north and the latter was from the eastern part of the country. The age of the subjects varied between 19–40 years. Blood pressure (supine) was ≤ 140/90 mm Hg. The subjects being in the same employment had identical routines.

Prior to blood collection the subjects were apprised of the study. A questionnaire was administered about demography and a written consent was obtained from each donor.

2.2 *Methods*

Genomic DNA was isolated by a standard technique (Miller *et al* 1988). ACE gene polymorphism was analysed by the method of Evans *et al* (1994) on a Perkin-Elmer Thermal Cycler. Random gene sizing for confirmation was carried out on an ABI prism 377 automated DNA Sequencer (Perkin-Elmer, Foster City, USA).

ACE activity was determined by a modification of the macromethod (Manju *et al* 2000) to a micromethod (ELISA plate) on a high throughput SpectraMAX 190 spectrophotometer (Molecular devices, USA). Each assay was performed in duplicate and was repeated thrice on different days.

2.3 *Statistical analysis*

Results are expressed as mean ± SD. Differences in overall allele distribution were determined by c^2 test. Specific allele frequencies of the population were compared by Fisher's exact test. The Hardy–Weinberg equilibrium was examined by the Marker Chain method with a programme for population genetics data analysis (Epi Info version 7·0). A P value ≤ 0·05 was considered statistically significant.

3. Results and discussion

The present study investigated for the first time, ethnic variations in the frequency of the ACE gene I/D polymorphism and enzyme activity in a well-defined, multiethnic population of India. The study has several novel aspects in addressing genetic variations according to ethnic origin. It is population-based, with the groups having been studied within the same geographical area, thereby mitigating the potential effects of differences in environmental background.

Our results demonstrated a difference in the distribution of the genotype between the groups as is evident from table 1. Out of the five groups only the Sikhs showed marginally higher number of DD homozygotes over the II homozygotes. Among the remaining groups, the Dogras, Assamese and Kumaonese had the II genotype greater in number ($P < 0·05$) than the DD genotype.

A difference was visible in the allele distribution between the Sikhs and the rest of the groups with the D allele tending to be dominant in the former group. In contrast, there was a striking preponderance of the I allele in Jats, Dogras, Assamese and the Kumaonese as can be seen from figure 1. Our results on the Sikh population differed from a previous report (Mastana and Nunn 1997), where the I allele frequency was reported higher than the D allele in the normal subjects. The subjects in that study belonged to a select sect called the

Table 1. ACE genotype distribution in the five Indian population groups.

Population (State)	Genotype II	ID	DD	*P*
Sikh (Punjab) (n = 45)	0·25 (11)	0·48 (21)	0·27 (12)	0·777
Jat (Haryana) (n = 30)	0·20 (06)	0·70 (21)	0·10 (03)	0·150
Dogra (HP) (n = 52)	0·36 (19)	0·40 (21)	0·24 (12)	0·043
Assamese (Assam) (n = 52)	0·31 (16)	0·54 (28)	0·15 (08)	0·033
Kumaonese (Uttaranchal) (n = 33)	0·39 (13)	0·39 (13)	0·22 (07)	0·015

n = number of subjects studied in each group; numbers in parentheses under genotype denote absolute numbers of subjects. HP, Himachal Pradesh.

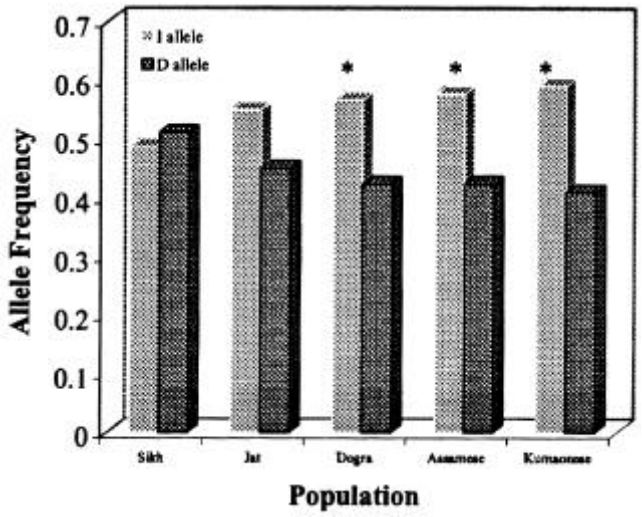

Figure 1. Insertion (I) and deletion (D) allele distribution of ACE gene in each population. *$P < 0·05$.

Table 2. ACE activity distribution with respect to the genotype of each population.

Population	Total ACE activity[a] Units/L	ACE genotype[b]		
		II	ID	DD
		ACE activity, units/L[c,d,e]		
Sikh (n = 45)	38·85 ± 11·08	27·47 ± 3·55	38·14 ± 4·42	54·18 ± 4·83
Jat (n = 30)	38·72 ± 11·64	29·86 ± 4·28	40·06 ± 4·88	55·77 ± 8·62
Dogra (n = 52)	36·48 ± 8·68	27·26 ± 3·24	36·98 ± 4·36	52·88 ± 5·67
Assamese (n = 52)	35·56 ± 8·20	26·94 ± 3·66	36·12 ± 2·27	49·48 ± 6·47
Kumaonese (n = 33)	39·24 ± 12·98	28·40 ± 4·40	39·85 ± 4·66	56·41 ± 8·30

The enzyme activity depicts mean ± SD. The enzyme activity in each sample was estimated in duplicate on three different days. [a]Average activity of each group; [b]genotype of each population presented in table 1; [c]activity according to the genotype; [d]37·56 ± 3·13% increase in activity identified as DD > ID > II; [e]$P < 0·05$. n, number of subjects.

Lobanas, who were originally nomads but had now settled at one place as agriculturists (Mastana and Nunn 1997). With regard to our data, at present there is no explanation for the higher frequency of the D allele in the Sikhs and the I allele in rest of the four populations. The reasons for this difference could be the genetic drift as is found in many other polymorphisms such as that of blood groups. The influence of some unknown sampling bias such as community bias cannot be excluded. Most of the populations under investigation and for that matter most Indians in general, marry within the community and caste and this may also influence the genotype. The present results thus suggests an ethnic heterogeneity with a significant gene cline having a higher insertion allele frequency. Majumder *et al* (1999), in a different context, also reported higher frequency of ACE insertion allele in various ethnic groups.

Several investigations have provided a substantial database on genotype distribution in a number of population groups (Johanning *et al* 1995 and references therein). The ethnic background appears to influence the ACE gene I/D polymorphism globally. It demonstrates the importance of using a homogeneous population in the selection of the study samples, making possible the identification of more exact distributions of the ACE genotypes among racial populations. The higher frequency of I allele in the present study groups is in agreement with Asiatic and Mongoloid populations (Zee *et al* 1992; Higashimori *et al* 1993; Hong *et al* 1997) but differs from the Americans, Caucasians and Europeans, who had a greater frequency of D allele and were reported to have a high risk of hypertension (Johanning *et al* 1995; Morris 1996; Sagnella *et al* 1999).

In the present investigation, ACE activity was also estimated in the subjects and a correlation was observed between the circulating enzyme level and the genotype as can be seen from table 2. Higher levels of the enzyme activity was observed with the presence of the D allele. The average increase in activity observed was 37·56 ± 3·13% with a change from II to ID and ID to DD genotype. Such a correlation between genotype and phenotype has been reported earlier (Rigat *et al* 1990; Danser *et al* 1995). The circulating enzyme levels between subjects show wide variation that can easily be categorized into three ranges low, medium and high, although within a subject the level remains constant. It seems that the three levels correspond to II, ID and DD genotypes respectively (table 2). Varying levels of the enzyme in individuals will produce corresponding levels of Ang II, which is a potent vasoconstrictor (Ward 1995), stimulates Ca^{2+}, aldosterone pathways (Pratt *et al* 1989) and the vascular endothelial growth factor (Otani *et al* 1998). Low concentrations of Ang II could perhaps have long term benefits, especially in delaying the pathophysiological condition (Woods *et al* 2000).

The analysis of distribution of the ACE polymorphism and activity within and across the major human groups appears to be useful in identifying the mechanisms contributing to the emergence of common diseases such as hypertension, cardiovascular disease, diabetes and nephritis. Such comparative studies could be of significant clinical relevance and utility in the upcoming field of pharmacogenomics.

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