Original Article

Garo Population of Bangladeshi Hilly Region Possesses Higher Frequency of ACE I/I Genotype in Angiotensin-converting Enzyme-1 (ACE-1) Gene

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ABSTRACT: A common polymorphism in the Angiotensin-converting Enzyme-1 (ACE-1) gene I/D variant have been shown to be associated in the context of physical performance. The present study was undertaken, to determine the frequency of ACE genotype polymorphism in Bengali, Garo and Rakhain populations of Bangladesh. A total of 338 healthy subjects were included in this study, among them 128 were Bengali, 96 were Garo and 114 were Rakhain. DNA was extracted and ACE I/D polymorphism was determined. Data were analyzed using SPSS for windows version 12. BMI of the Garo subjects was significantly lower than the Bengali and Rakhain subjects. Blood pressure of the Garo subjects was significantly higher than the Bengali subjects but lower than the Rakhain subjects. Serum triglyceride and total cholesterol of the Garo subjects were significantly lower than the Bengali subjects. Wild genotype (II) of ACE-1 gene in Garo subjects was significantly higher than the Bengali and Rakhain subjects. Variant genotype in hypertensive subjects of Bengali and Rakhain subjects were significantly higher. From the above results it can be concluded that Garo populations are associated with higher frequency of II genotype in ACE-1 gene and variant genotypes (II and ID) of ACE gene are associated with hypertension in Bengali and Rakhain subjects.

KEYWORDS: ACE I/D polymorphism; Aboriginal population.

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INTRODUCTION

Angiotensin-converting enzyme-1 (ACE-1) plays a major role in the renin-angiotensin system, a system that regulates blood pressure and water balance in mammals. Although ACE has been studied for more than four decades, use of new technologies continues to give new insights into the important role plays in mammalian physiology. ACE is a zinc metallopeptidase that typically cleaves the last two amino acids from its peptide substrates. [1] Bradykinin is one of the well-known substrate of ACE. Accumulations of bradykinin are thought to be responsible for a persistent cough sometimes observed with ACE-inhibitor therapy.[2] The ability of ACE to degrade bradykinin positions this enzyme as a common player in both the kinin-kininogen and renin-angiotensin systems.

Several polymorphisms have been reported in genes of the renin angiotensin system and represent genetic factors that affect both circulating and tissue RAS. These include polymorphisms in the angiotensinogen, ACE, and Angiotensin Receptor genes. The most studied polymorphism is of the insertion/deletion type; the two ACE alleles differ in size because of the insertion of a 287-bp DNA sequence in intron 16 of the ACE gene (Rigat *et al.,* 1990). This

polymorphism is also associated with overall plasma ACE levels.[3] The ACE DD genotype is associated with increased circulating ACE levels, which are generally two times as high as those found for II genotypes; ID heterozygotes are associated with intermediate ACE levels.[3] This relationship was repeatedly confirmed by other studies, for both circulating and cellular ACE.[4-8] However, because the ACE I/D polymorphism is intronic, the mechanism of ACE overexpression in subjects with DD genotype is unclear; it is possible that this relationship is the result of tight linkage to another locus involved in the regulation of ACE gene expression.[9]

Some studies reported a positive association between the D allele and high blood pressure [10-12). There was a significant relationship between the D allele and hypertension in women and in Asians [13]. But no correlation has been found between plasma ACE levels and hypertension or between ACE DD genotype and hypertension, in multiple studies.[5,8,14-19]

It has been reported an association between the ACE polymorphism and physical performance [20] which has been replicated when it was tested with 64 Australian national rowers[21], 91 British Olympic caliber runners[22], 217 Russian athletes[23], 60 Spanish elite athletes[24], 120 swimmers from the European and Commonwealth championships[25], and 35 swimmers from the World Championships particularly in very long distance athletes[26].

A study done on Asian Rugby Players in National University of Singapore have shown that the I allele confers an advantage in aerobic capacity as measured by the $V0_2$max (maximum volume of oxygen uptake) and VT (ventilatory threshold).[27]

Although Bangladeshi nationals are made up by mainly homogeneous Bengali population there are a good number of aborigines (about one percent of total population) living in mainly in North-west, North-east and South-East part of the country. The Garo people first came to the Garo hill region of Bangladesh and Meghalaya (India) from Tibet about 400 years ago. The tribal society Rakhain is a small tribe of Arakan origin belonging to the Bhotbarmi community of the Mongoloids. In the eighteenth century, many Rakhain people migrated from their homeland in Arakan (Myanmar) and they gradually settled in different areas of Chittagong and Patuakhali district of Bangladesh. In keeping with the associations of ACE I/D alleles with different performance phenotypes, and since the tribal populations are mainly originated in the hilly region of the country, so it may speculate that there may be an association of ACE I/D polymorphism with this population but no data exists in this regard.

MATERIAL AND MATHODS

Subjects
The study included a total of 338 subjects of both sexes from Bengali (n=128), Garo (n=96), and Rakhain (n=114) population. The 128 Bengali subjects were recruited from different parts of Dhaka city. Garo and Rakhain subjects involved in the present study were recruited through a medical camp. Permission was obtained from the appropriate body and local community leader(s) were involved in the process. Request for participation was made through an open public announcement. The volunteers were informed about the study and written consent was obtained. Bengali subjects were collected through personal communication. Interested individuals were requested to come on a prescheduled morning in fasting condition.

Collection of blood samples
Overnight fasting (8-10 hours) blood was collected between 8.00-9.00 am. Venous blood (10 ml) was obtained by venipuncture following standard procedure. A portion of blood (5 ml) sample was taken into a tube containing EDTA (1 mg/ml), mixed thoroughly and preserved at -30°C for future DNA extraction and subsequent experimentation. The rest of the sample was taken into plain tube allowed to clot for 30 minutes and serum was separated by centrifugation for 10 min at 3000 rpm using a refrigerated centrifuge and preserved at -30°C for biochemical analyses.

Biochemical methods
Glucose was estimated by enzymatic colorimetric (GOD-PAP) method in the Hitachi 704 Automatic Analyzer, Hitachi Ltd., Tokyo, Japan using reagents of RANDOX Laboratories Ltd., UK. Serum Triglyceride, total cholesterol and Gutamate Pyruvate Transaminase were measured by enzymatic endpoint method (cholesterol Oxidase/Peroxidase) in Auto analyzer HITACHI 704, Hitachi Ltd Tokyo, Japan. Estimation of creatinine was done by alkaline-picrate methods using reagents from Randox Laboratories, UK.

DNA extraction

Extraction of DNA was performed using GenElute DNA extraction kit (QIAGEN, USA). The kit uses the principal of silica gel DNA isolation from whole blood adapted in spin column.

ACE gene ID polymorphism analysis

The ACE gene ID polymorphic marker was analysed by PCR using the following flanking primer set: Forward primer: 5´-CTGGAGACCACTCCCATCCTTTCT-3´Reverse primer: 5´-GATGTGGCCATCACATTCGTCAGAT-3.´ PCR was carried out in 10 µl reaction volume. Product size for the above-mentioned primer set is 490 bp.

PCR conditions

PCR was carried out using HotStart Taq polymerase. Conditions for the amplification of the above mentioned product include an initial step of denaturation at 105°C for 15 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 45 sec and elongation at 72°C for 30 sec followed by a step of final elongation at 72°C for 10 min.

The PCR products were resolved in 1% agarose gel and visualized using gel documentation system following ethidium bromide staining. The PCR products were detected as 490bp for homozygous wild (I/I), 490bp and 190bp for heterozygous variant polymorphism (I/D) and, 190bp for homozygous variant polymorphism (D/D) respectively.

Statistical Methods

Data were expressed as mean (±SD) and number (percentage) as appropriate. The difference between two groups was determined by unpaired Student's 't' test, Chi-square test and Z-test for two proportions where applicable. Data were analyzed using statistical package for social science (SPSS) for Windows Version 12.

RESULTS

Clinical and biochemical characteristics of the study subjects

In Bengali subjects, significantly higher BMI values were found compared to Garo subjects (p<0.001) but significantly lower values were observed when compared with Rakhain subjects ((p<0.001) **(Table 1)**. Bengali subjects have significantly lower systolic and diastolic blood pressure values compared to Garo (p<0.001) and Rakhain subjects (p<0.001) **(Table 1)**.

Although mean (±SD) fasting serum glucose (FG, mmol/l) of the Bengali, Garo and Rakhain subjects were within normal range but Bengali subjects have significantly lower fasting serum glucose values compared to Garo subjects (p=0.019) and Garo subjects have shown significantly higher fasting serum glucose values compared to Rakhain subjects (p=0.009) **(Table 1)**.

Mean (±SD) serum triglycerides (TG, mg/dl) and total cholesterol (T Chol, mg/dl) of Bengali subjects have significantly higher compared to Garo subjects (p=0.009, p=0.003repectively).

Both the GPT and creatinine in the serum of all the three groups were in normal range.

Wild (I/I) and variant (I/D+D/D) genotypes of ACE gene

Hardy-Weinberg distribution of ACE I/D genotype frequencies in the Bengali (χ^2=2.612; p=0.106), Garo (χ^2=0.122; p=0.726) and Rakhain (χ^2=0.056; p=0.812) subjects was found to be in equilibrium (data not shown in table).

Wild and variant genotypes in Bengali subjects were 39.8%, 60.2% respectively, in Garo subjects 53.1%, 46.9% and in Rakhain subjects 37.7%, 62.3% respectively. Garo subjects have shown significantly higher wild genotype compared to Bengali and Rakhain subjects in proportion test or in χ^2 test **(Table 2)**.

ACE I/D genotype frequencies of the study subjects on the basis of blood pressure

The frequency distribution of ACE I/D genotypes was reanalyzed on the basis of normotensive (NTN) and hypertensive (HTN) and it has been found that HTN group of both the Bengali and Rakhain subjects have shown the significantly higher proportion of variant genotype compared to wild genotype **(Table 3)**.

DISCUSSION

Angiotensin converting enzyme (ACE) is a rate-limiting enzyme of the renin-angiotensin system (RAS), a system that regulates blood pressure and water balance in mammals. ACE gene is highly polymorphic. The ACE gene localizes to chromosome 17q23 and a common variant (I/D) is the result of either an insertion (I) or deletion (D) of a 287bp fragment in intron 16. Although there are variations in different population but the ACE

Table 1: Clinical and biochemical characteristics of the study groups

Variables	Bengali (n=128)	Garo (n=96)	Rakhain (n=114)	t/p values B vs G	t/p values B vs R	t/p values G vs R
Age (Yrs)	43±10	50±15	49±14	*3.73/**<0.001***	*3.49/**0.001***	*0.56/0.573*
BMI (Kg/m^2)	24.0±2.9	19.9±3.4	29.0±5.7	*9.57/**<0.001***	*8.48/**<0.001***	*14.3/**<0.001***
SBP (mmHg)	113±11	131±23	132±12	*6.79/**<0.001***	*12.4/**<0.001***	*0.46/0.648*
DBP (mmHg)	76±7	83±12	86±8	*4.73/**<0.001***	*9.77/**<0.001***	*2.10/**0.037***
FG (mmol/l)	5.3±1.2	5.7±1.1	5.4±0.9	*2.35/**0.019***	*0.09/0.924*	*2.64/**0.009***
TG (mg/dl)	157±110	127±57	138 ±86	*2.62/**0.009***	*1.46/0.146*	*1.10/0.272*
T Chol (mg/dl)	204±71	173±40	182±35	*3.86/**<0.001***	*3.01/**0.003***	*1.74/0.084*
SGPT (U/L)	27.3±18.7	23.1±12.4	17.3±11.5	*1.93/0.055*	*4.99/**<0.001***	*3.44/**0.001***

Results were expressed as mean±SD. t/p values were calculated using unpaired Student's 't' test. n, Number; BMI, body mass index; MAC, mid-upper arm circumference; FG, fasting glucose; TG, triglyceride; T Chol, total cholesterol; SGPT, Glutamate Pyruvate Transaminase; SCreat, serum creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; B, Bengali; G, Garo; R, Rakhain.

Table 2: Frequency distribution of wild (II) and variant (I/D+DD) genotype of ACE gene in the study subjects

	Wild	Variant		χ2/p	OR (95% CI)
Bengali	0.398 (51)	0.602 (77)	*Bengali & Garo*	3.90/0.048	1.71 (1.002-2.921)
Garo	0.531* (51)	0.469 (45)	*Bengali & Rakhain*	0.115/0.735	1.094 (0.651-1.836)
Rakhain	0.377	0.623	*Garo &*	5.00/0.025	1.871 (1.078-

Results were expressed as frequency (number). Chi-square test was performed to calculate statistical association; *p<0.05 when ACE wild (II) genotype of Garo subjects was compared with Bengali or Rakhain subjects using Z-test for two proportions.

Table 3: Frequency distribution of wild and variant genotypes in ACE gene on the basis of blood pressure

ACE I/D genotype	Bengali NTN, % (n)	Bengali HTN, % (n)	Garo NTN, % (n)	Garo HTN, % (n)	Rakhain NTN, % (n)	Rakhain HTN, % (n)
Wild (II)	0.426 (49)	0.167 (2)	0.509 (27)	0.611 (22)	0.420 (21)	0.316 (18)
Variant (ID+DD)	0.574 (66)	0.833 (10)	0.491 (26)	0.389 (14)	0.580 (29)	0.684(39)

NTN, normotensive; HTN, hypertensive; Results were expressed as frequency (number). Z/p values were calculated using proportion test.

I/D genotype frequencies in the general populations are approximately 25-30% DD, 45-50% ID and 20-25% II. In comparison with the I allele, the D allele is associated with a higher circulating ACE activity.[28,29] This higher ACE activity with the D allele is associated with lower bradykinin concentrations suggestive of less efficient substrate use and with greater angiotensin I conversion to angiotensin II, which serves to augment overload-induced hypertrophy of muscle.[30] Bradykinin influences glycogen and lactate levels, as well as the balance of availability of glucose/ free fatty acids[31], it also influences the expression of the GLUT4 glucose transporter during exercise[32] and thus insulin sensitivity. These findings contribute biologic plausibility for a role of the II genotype in enhancing energy efficiency and muscular endurance [33]. Indigenous populations in Bangladesh are thought to be needed more physical strength and muscular endurance to survive in the mountain region. So, in the present study a group of Garo, Rakhain and Bengali subjects in Bangladeshi populations were investigated to observe the ACE I/D polymorphisms.

In the present study ACE gene polymorphism was examined in 338 healthy subjects of both sexes from Bengali, Garo, and Rakhain population. Bengali and Garo subjects have shown significantly lower BMI values compared to Rakhain subjects (p<0.001) but Bengali subjects have significantly higher BMI values compared to Garo subjects (p<0.001). In hemodynamic status, Bengali subjects have shown significantly lower systolic blood pressure compared to Garo (p<0.001) and Rakhain subjects (p<0.001), and Bengali subjects have also shown significantly lower diastolic blood pressure values compared to Garo (p<0.001) and Rakhain subjects (p<0.001).
In this study, Garo population has shown significant higher frequency of variant genotype (I/D+D/D combined) compared to Bengali and Rakhain population. In the two indigenous (Garo, Rakhain) community, Garo populations were originated from Mountain region and still maintain their lives in the hilly regions of the country where it needs more physical strength and endurance to survive. On the other hand, Rakhain subjects in our study were from Patuakhali region of the country which is mostly plain and low land like general Bengali populations reside. In literature it has been found that II genotype is associated with muscle performance[21-26], aerobic capacity[27], longevity[34] and even with elite judo players[35]. The higher II genotype frequency in Garo subjects may have a role for their more physical activities needed to survive in the hilly region. Human physical performance characteristics are strongly influenced by the genetic inheritance and by gene-environment interactions. Therefore, the genotype frequency may be associated with the beneficial effect on performance may thus vary with the environment to which the population is exposed.

A hypertensive group of both the Bengali and Rakhain subjects have shown the significantly higher proportion of variant (I/D+D/D combined) genotype compared to wild (I/I) genotype which has been supported by previous studies [10-12. A recent case-control study found that subjects with the ACE DD genotype were 1.6 times more likely to be hypertensive than carriers of the I allele, and that 15% of all cases of hypertension could be attributed to the ACE DD genotype.[36]

From viewpoint of above discussion it may be concluded that a) Garo populations are associated with higher frequency of II genotype in ACE gene, b) Variant genotypes of ACE gene are associated with hypertension in Bengali and Rakhain subjects.

Conflict of interest:
Authors are declared that there is no conflict of interest for this manuscript to publish.

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