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Gender-specific Association of ACE gene insertion/deletion polymorphism with Schizophrenia

Medical Science

KEYWORDS : Angiotensin converting enzyme , Insertion/deletion polymorphism, Schizophrenia, Andhra Pradesh

Dr. V. Lakshmi	(Corresponding author) Assistant Professor, Department Of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India
D. Sandhya Deepthi	Research Scholar, Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India.
N. Priyambika	Research Scholar, Department of Human Genetics, Andhra University, Visakhapatnam, Andhra Pradesh, India.
K. Uma Jyothi	Senior Research Fellow, Biological anthropological, Unit Indian Statistical Institute, Hyderabad, Andhra Pradesh, India .

ABSTRACT

Angiotensin converting enzyme (ACE) is a key enzyme in the renin angiotensin system which also modulates dopamine, a neurotransmitter and is considered as a candidate gene for schizophrenia. There is a controversy regarding the association of ACE I/D polymorphism with schizophrenia. The present case control study was conducted to examine the association between ACE I/D polymorphism and schizophrenia in the population of Andhra Pradesh. No association was found between ACE I/D polymorphism and schizophrenia. However when the samples were segregated on the basis of sex, significant over representation of D allele was observed in unaffected females which suggests its protective role.

Introduction

Schizophrenia is a complex psychiatric disorder with a life time risk of around 1%. High heritability estimate of 81% [1] indicates the significant role of genetic variants in its etiology. Several genome wide association studies have been conducted to localize genes for schizophrenia.

Renin-angiotensin system (RAS) plays an important role in regulating blood pressure and maintaining water homeostasis. All components of RAS have been demonstrated in different regions of brain[2]. The existence of independently regulated local RAS in the central nervous system[3] renewed the interest in RAS beyond its classical function[4].

Angiotensin I converting enzyme (ACE, EC 3.4.15.1) plays a key role in the renin- angiotensin system (RAS). It catalyses the conversion of angiotensin I to angiotensin II and inactivates bradykinin[5]. Angiotensin II is a neurotransmitter that interacts with dopamine[6] and potentiates dopamine release[7,8]. *ACE* is a candidate gene for schizophrenia because of its role in modulation of dopaminergic activity in the brain[9] and its neuropeptide substrate (P substance) which is important in the etiopathology of schizophrenia[10].

ACE gene is located on chromosome 17q23 and consists of 26 exons. Insertion/deletion polymorphism in the sixteenth intron of *ACE* gene results in the presence or absence of 287 bp fragment, which is associated with ACE levels[11].The D allele is associated with higher ACE activity in the serum and tissue[12]. It is the most studied variant in *ACE* gene and has been associated with development of increased risk for hypertension, diabetic nephropathy, coronary artery disease, preeclampsia, encephalopathy, asthma etc[13].

Results obtained from several studies have been inconsistent with respect to the association of *ACE* insertion/deletion polymorphism (rs 4646994) and the risk of schizophrenia. In India only a single study has been carried out in North Indian population[14]. The present study was carried out to investigate whether the *ACE* insertion/deletion polymorphism is a risk factor for schizophrenia in the population of North Coastal Andhra Pradesh.

Materials and methods

The study sample consisted of 189 patients attending the Government Hospital for Mental Care in Visakhapatnam. They were diagnosed with schizophrenia by qualified psychiatrists in accordance with ICD 10 manual. Patients younger than 15 years and those with concomitant medical condition or intellectual disability were excluded. A total number of 197 age and sex matched healthy individuals with no family history of schizophrenia were recruited as the control group.

Samples were collected during the period of May 2013 to April 2015. The study was approved by the institutional ethical committee. Prior to the sample collection, informed consent was taken both from the cases and the controls. 5 ml of venous blood was collected in EDTA vials for genomic DNA extraction. DNA extraction was done by standard phenol chloroform method.

The *ACE* I/D polymorphism was determined by polymerase chain reaction (PCR) amplification. The forward and reverse primers were 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT -3' respectively. The PCR conditions were 10 min of initial denaturation at 94°C followed by 40 cycles of denaturation, annealing and extension at 94 °C for 1 minute, 58°C for 1 minute and 72°C for 2 minutes respectively with a final extension of 3 minutes at 72°C. The PCR products were run on 2% agarose gels and visualized by ethidium bromide staining. The I allele was detected by the presence of a 490 bp band and the D allele by a 190 bp band (Fig 1).

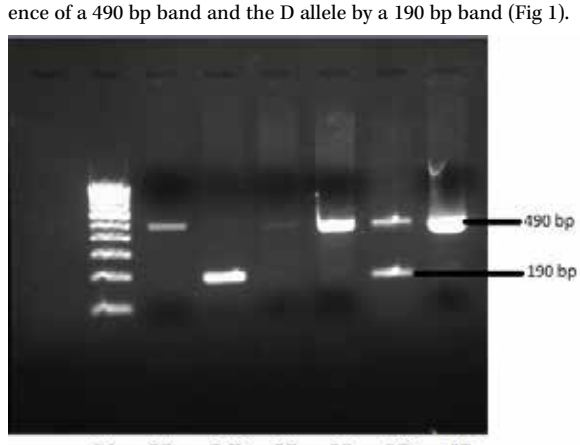

Fig.1 Agarose gel picture showing the three genotypes II,ID and DD along with the 100 bp Marker.

To avoid mistyping of *ACE* I/D heterozygotes due to preferential amplification of the D allele[17], PCR amplification was repeated for DD homozygotes using a second pair of insertion specific primers (Forward 5'-TGGGACCACAGCGCCCGCCACTAC-3' and Reverse 5'-TCGCCAGCCCTCCCATGCCCATAA-3'). Conditions for PCR were initial denaturation at 94°C for 10 min , 40 cycles of denaturation at 94°C for 10 sec, annealing at 68°C for 30 seconds, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes. A 335bp band was present only in the presence of an I allele (Fig 2).

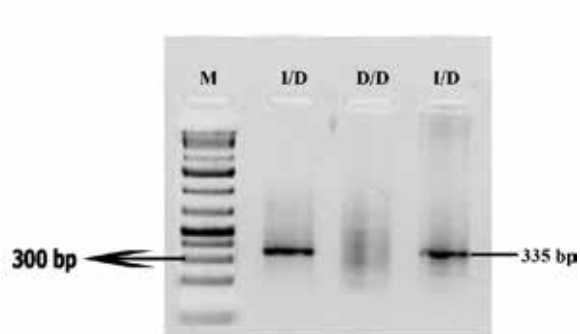

Fig 2. Agarose gel picture showing *ACE* ID and DD genotypes by using insertion-specific primers M=100 bp DNA Ladder

The chi-square (χ^2) goodness of fit test was used to identify departures from the Hardy-Weinberg equilibrium among the two study groups. The data were analysed using SPSS 20 statistical software package (SPSS Inc, Chicago, IL, USA). Comparison of the genotypic and allelic frequencies between the case and control groups was performed using χ^2 test. Odds ratio was calculated with 95% confidence interval using logistic regression. P value <0.05 was considered significant. The most common allele (I) in the subjects was used as the reference allele.

Results

The mean age of the cases and controls was 33.88± 9.98 and 34.06 ± 9.87 years, respectively. The percentage of males and females was 66 and 34 in both cases and the controls. Table 1 shows the summarized genotype and allele frequencies of *ACE* I/D polymorphism. The *ACE* I/D genotypes were in Hardy-Weinberg equilibrium in controls. Though the DD genotype frequency is relatively higher in controls when compared to cases, no significant difference was observed. Allele frequencies were found to be similar in the two groups.

When the cases and controls were divided based on gender (Table 2), high frequency of DD genotype was observed in females and the difference was found to be significant. Though the allele frequencies were found to be similar in both cases and controls in males, in case of females, D allele frequency was found to be significantly high in controls.

Discussion

The present study showed no significant association between *ACE* I/D genotypes and schizophrenia in the pooled sample of males and females. The lack of significant association of this marker with schizophrenia was also apparent in the eight earlier studies among populations of different countries.- Japan18,19, Taiwan20 , Israel21 , Finnland22, Russia 23, Turkey24, Croatia25 , China26, Brazil27 . The present study is consistent with the above studies reporting negative association between *ACE* I/D polymorphism and schizophrenia. On the other hand, Crescenti et al (2009)[28] reported positive association in Spanish population and the same observation was reported in Turkish population[29] and North Indian population[16]. Nevertheless, the variant allele which conferred protection was different in the above three populations. While the *I* allele was suggested to be protective in Turkish and North Indian populations it was *D* allele in case Spanish population that was suggested to provide protection against schizophrenia.

The gender specific analysis of our samples from Visakhapatnam suggests significant association of *ACE* I/D genotype DD in a protective role against schizophrenia in the females, not in males. Although genetic basis has not been precisely established, gender differences are well known in schizophrenia. A sex difference in the risk of developing schizophrenia has been conclusively shown in a systematic review[30]. A number of loci which show sex specific association with schizophrenia have been reported[31-34] but the same single nucleotide polymorphism has not been observed to replicate consistently in the same sex[27,29]. Further, till date, only a single study[31] has attempted to investigate sex differences with respect to the association between *ACE* I/D polymorphism and schizophrenia. While gender specific association was observed by them, it was with reference to II genotype, not DD, that was found to be protective against schizophrenia in females.

Conclusion

The present study reveals a negative association between *ACE* I/D polymorphism and schizophrenia, which is concurrent to the results based on meta analyses recently reported[36,37]. However, a positive association was observed in females with D allele as the protective one which is contradictory to the earlier finding of Mazaheri et al (2015)[35] who found I allele as the protective one. This inconsistency in the genetic variant associated may be due to small sample size of females, which is less than 100 in both the studies. It is necessary to test the nature of association in a relatively much larger sample size and in diverse ethnic and geographic populations within India and in other parts of the globe in order to reach unequivocal conclusions.

Table 1 Distribution of genotype and allele frequencies

Polymorphism *ACE* I/D	Cases (%) N=189	Controls (%) N=197	OR CI(95	p value
Genotypes				
II	70(37)	69(35)	0.9 0.6 – 1.5	0.8
ID	102(54)	105(53)		
DD	17(9)	23(12)	0.7 0.3 - 1.5	0.3
Allele				
D	0.36	0.38	0.9 0.6 – 1.2	0.5
I	0.64	0.62		

Allelic $\chi 2$ = 0.455, df=1, p value=0.500

Table 2 Gender wise distribution of genotypes and allele frequencies

Polymorphism *ACE* I/D	Cases (%)	Controls(%)	OR	CI(95%)	p value
Males	N=125	N=130			
Genotypes					
II	40(32)	47(36)			
ID	70(56)	74(57)	1.11	0.65 – 1.89	0.69
DD	15(12)	9(7)	1.95	0.77 – 4.90	0.15
Allele					
D	0.40	0.36	1.22	0.85 – 1.74	0.28
I	0.60	0.64			
Females	N=64	N=67			
Genotypes					
II	30(47)	22(33)			
ID	32(50)	31(46)	0.75	0.36 – 1.50	0.46
DD	2(3)	14(21)	**0.10**	**0.02 – 0.50**	**0.005**
Allele					
D	0.28	0.44	**0.40**	**0.29 – 0.83**	**0.008**
I	0.72	0.56			

Allelic $\chi 2$ for males=1.77, df=1, p value=0.183

Allelic $\chi 2$ for females=9.312, ,df=1, p value=0.002

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