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A preliminary perusal of ACE I/D polymorphism with adiposity traits and blood pressure among the AO NAGAS: Does gender-dependent gene expression matter?

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ABSTRACT

This study aims to evaluate the association of gender-dependent expression of angiotensin converting enzyme gene polymorphism (I/D) with adiposity markers and blood pressure among AoNagas.57AoNagas[Males (n) =26; Females (n) = 31; Mean Age: 30.56±7.5 and 31.9 ±8.3 1]residing in Delhi were included in this cross sectionalstudy. Anthropometric measurements and blood pressure were taken using standardized techniques. Adiposity indices viz., BMI, WHR and WHtR were computed. Body fat percentage was assessed by bioelectric-impedance technique using Tanita Body composition analyzer (T-6360). Venous blood samples were withdrawn for DNA extraction and genotyping of ACE gene (I/D) polymorphism was established by polymerase chain reaction (PCR). In female participants with DD homozygote, risk of both general and central obesity as depicted by BMI, body fat percentage, WC, WHR and WHtR were higher than ID heterozygote. Risk of hypertension was found to be greater among males with DD homozygote rather than females with DD homozygote. In males, obesity was not found to be associated with hypertension in either DD or ID genotypic variants of ACE. Whereas, in females obesity was significantly and positively correlated with hypertension in both DD and ID genotype. DD homozygous form of ACE is linked with both obesity and blood pressure in females and only with blood pressure in males. This genotype-by-gender interaction gives us a facet in understanding the complex genetic basis of adiposity and blood pressure phenotypes.

Keywords: ACE polymorphism, AoNagas, Adiposity indicators, Blood pressure

INTRODUCTION

Angiotensin converting enzyme (ACE) is an important component of Renin-angiotensin system (RAS) which regulates the function of blood pressure. ACE gene modulates the relationship between adiposity and blood pressure leading to increased risk of hypertension and cardiovascular diseases.[1]

Along with gender differences in physiology, anatomy and behavior, studies of disease-associated quantitative traits in humans suggests that genetics also plays a pivotal role in disease risk .The heritability estimates of disease risks also differ between males and females. Genotype-specific effects differ between males and females as can be commonly seen in model organisms. The genetic structure and frequency of polymorphisms on autosomes are same in both sexes except the regulatory genome, therefore sex-specific differences in gene regulation, rather than gene content, underlie gender-specific effects on disease risk. Hence, studies that do not consider gender-specific genotype effects could miss a significant proportion of genes contributing to complex disease risk.[2]Recently, some studies have implicated sex-specificity of ACE gene in hypertension genetics. Significant linkage and association of ACE (I/D) polymorphism and hypertension were observed only in males in Caucasian populations. This could partially explain the conflicting results for such an association in a number of previous studies in which the subjects were not stratified according to sex. Thus, the association of the ACE I/D polymorphism and blood pressure seem to be gender and ethnicity dependent.

As such, this study aims to evaluate association of gender-dependent expression of angiotensin converting enzyme gene polymorphism (I/D) not only with blood pressure but also with adiposity indicators among AoNagas. Probably, this could be the first report on gene association studies in this ethnic population which would provide some insight into the contribution of the gender aspect in the relationship between ACE polymorphism, obesity and hypertension when such data are sparse in the blooming era of lifestyle diseases.

MATERIALS AND METHODS

Population sample Fifty seven AoNagas(Males(n)=26;Females(n)= 31;Mean Age:30.56±7.5 and 31.9 ±8.31) residing in Delhi, India volunteered for the study.

An urban, neo-liberal agglomeration like Delhi has an influx of economic migrants from different parts of India. Migrants from the state of Nagaland account for a significant part of northeast migrants to Delhi. The Aos/AoNagas are one of the major Naga tribe of Nagaland. They ethnically belong to the Mongoloid stock and linguistically to the Tibeto-Burman group. They follow endogamous, patriarchal, and patrilineal system .Considering the increased risk of hypertension and obesity in migrant tribal populations with rapid nutritional and lifestyle behavioral transition in the host society, a young group of Aos were approached and studied.

Collection of data

Statistics of the Aos residing in Delhi were taken from the tribal Union and Church registry. Purpose and procedure involved in the research was explained to each individual through telephone and those who volunteered to participate in the study were requested to come tothe Physiological Anthropology Laboratory, University of Delhi after an overnight fast. Ao individuals with no debilitating illness or physical deformity and who have married within the Ao Naga tribe were included in the study. Pregnant and lactating mothers, offsprings of inter-tribal marriages and those individuals who have become a member of theAo Naga tribe through adoption were excluded from the study.The protocol of the study was reviewed and approved by the Institutional Ethical committee, University of Delhi, India. Informed written consent was taken from each subject before commencement of the study.

Anthropometric measures Anthropometric measurements including height, weight, waist and hip circumference were obtained using standardized protocols given by Weiner and Lourie (1981).[3] Measurements were conducted by a trained anthropologist. All the instruments used were calibrated and verified before each assessment. Height was taken with the help of anthropometer to the nearest 0.1 cm in the standard arm hanging position. Body weight was measured by using weighing machine to the nearest 0.1 kg, with minimum clothing. Waist circumference (WC) and hip circumference (HC) were measured with a flexible steel tape to the nearest 0.1 cm. Body fat percentage was recorded by bioelectric-impedance technique using Tanita Body composition analyzer (T-6360).

Blood pressure assessment Blood pressure was assessed using standard mercury sphygmomanometer.

Adiposity indices Body mass index (BMI) was calculated as weight(kg) divided by the square of the height (m^2)and categorized as underweight(<18.5),normal(18.5-24.9) and overweight(25.0-29.9).[4]Waist-to-Hip ratio was calculated by dividing waist circumference by hip circumference. Waist-to-height ratio (WHtR) was computed as ratio of waist to height. WHR cutoff points of 0.80 and above for females and 0.90 for males were taken respectively.[5]WHtR cutoff points were taken at 0.50[6].Abdominal obesity as defined by WC was taken as 80 cm for women and 90 cm for men.[7]Cutoff points for fat percentage was taken at ≥25% for males and ≥30 % for females.[8]Based on the seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (2003),normotensive was defined as less than 120/80 mmHg, prehypertension as 120-139/80-89 mmHg and hypertension as ≥140/90mmHg. [9]

DNA isolation and genotyping of ACE I/D polymorphism

DNA was extracted from 2 ml venous blood samples using salting-out method as given by Miller et al,1988. [10] The samples were genotyped with the variant of ACE by PCR using New England Biolab (NEB) PCR kit (primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and 5'-GAT GTG GCC ATC AAT TCG TCA GAT-3' were used to amplify 287 bp DNA fragment in intron 16). Using gel electrophoresis, the PCR products were distinguished as a 190 bp fragment in the absence and a 490 bp fragment in the presence of the insertion. Genotypes are described as DD-190 bps,ID-490 + 190 bps and II-490 bps respectively.

Statistical analysis

The results are expressed as mean ± standard deviation (SD). A p value of < 0.05 was considered statistically significant. The Statistical Package for the Social Sciences (SPSS version 17.0) was used to analyze the data.

Student's t test was used to compare the means of adiposity measures and blood pressure among DD and ID variants of ACE gene. Categorization of adiposity indicators and hypertension was cross tabulated with respect to DD and ID genotypic variant. Pearson's correlation (two-tailed) was used to evaluate the strength and direction of linear relationship between hypertension and adiposity indicators according to DD and ID variant.

RESULTS

Table 1 highlights the frequency of the genotypic variants of ACE, viz., homozygous DD, heterozygous ID and homozygous II in the population. The frequency of DD, ID and II in males was35.1%, 8.8% and 1.8% respectively. In females, the frequencies of the genotypic variants were 49.1% DD, 5.3% ID with none in the II genotype. Since the frequency of II genotype in females was void, this particular genotypic variant has been excluded in the preceding analysis in both the genders.

Table 2 summarizes the basic characteristics of the population under study showing the mean and standard error of the studied variables along with the t-value. In males, the mean value of all the variables except WHR, WHtR, SBP and DBP were higher among the individuals with DD genotype than the individuals carrying ID genotype. Only DBP showed statistical significance ($p<0.05$).Whereas, DD genotype among females showed higher mean value of all the adiposity markers and blood pressure than the ID genotype. However, no statistical significance could be seen in any of the studied parameters. II genotypic variant was excluded in the analysis due to shortage and non-availability of samples with the respective genotype in both the genders.

Table 3 depicts the distribution of adiposity marker and blood pressure in relation to DD and ID genotype. Among males with DD genotype 24% were overweight as compared to 8% overweight males with ID genotype. Whereas, 29.0 % overweight females were found in DD genotype only. Central adiposity according to waist circumference was found among 4% males with ID genotype only. 80% DD genotype males were found to be in non-risk category as compared to 16% ID genotype males. Among females with DD genotype,64.5% and 25.8% were in non-risk category and risk category respectively as compared to 9.7% in risk category among ID females. According to WHR,20% and 25.8 % were centrally obese in DD males and DD females respectively. Both DD and ID genotype males were 4% obese as compared to 41.9% DD and 3.22% ID obese females according to body fat percentage. According to SBP, 36.0% and 16.0% were prehypertensive and hypertensive among DD males as compared to 12.0% and 8.0% prehypertensive and hypertensive ID males. While, 16.2% and 12.9 % hypertensive and pre-hypertensive females were found among carriers of DD genotype only. According to DBP, 20.0% and 36.0% were prehypertensive among DD males in comparison with only 8.0% ID prehypertensivemales.35.4% and 12.9 % were prehypertensive and hypertensive among DD genotype females as compared to 6.5% and 3.22% ID genotype females respectively. Except the distribution of males according to waist circumference ($p<0.05$) ,all the other categories of adiposity indicators and blood pressure in both the gender in relation to DD and ID genotypic variants of ACE were found to be statistically non-significant .

Among females with DD genotype, positive correlations were found between DBP with WC, WHR and WHtR. Amongst ID genotype, SBP was found to be significantly correlated with BMI, body fat % and WC,while DBP with body fat % and WHtR respectively. No statistically significant correlations were found in any of the adiposity indicators with blood pressure in both the genotypic variants of ACE among males (Table 4).

DISCUSSION

The present study is population-based and examines the ethnic group of AoNagas residing in the same geographical area, i.e., Delhi and both parents belonging to the same ethnic tribe, thereby reducing the potential effect that may arise due to admixture.

We observed that the females of DD genotype had higher mean values in all the studied adiposity indicators viz., BMI, body fat percentage, WC, WHR and WHtR than the females carrying ID genotype, although the difference was statistically non-significant. The mean values of SBP and DBP was also found to be higher in the former genotype variant than the latter. Thus, DD genotype may reflect greater susceptibility of adult females to hypertension with obesity.

It also seems that allele I is associated both with lower body fat percentage and for better use of fatty acids for energy generation.[11]In a Mongolian population, interaction between the ACE DD (deletion/deletion) and ID polymorphism and BMI has been found.[12]Jimenez et al (2007) and Mehri et al. (2012) also reported strong associations between the DD genotype and the risk of hypertension in their study populations. [13] [14]The risk allele DD genotype happens to be playing a favorable role with respect to adiposity indicators in case of males in the

population; however SBP and DBP were found to be higher among the individuals with DD genotype as compared to individuals with ID genotype with DBP showing a significant difference($p<0.05$) between the two genotypic variants.

Similarly, in different ethnic population it has been observed that the DD genotype of ACE gene was associated with essential hypertension. In a study of a Sikh population by Mastana and Nunn (1997), the frequency of D allele of ACE gene was observed to be greater in hypertensive subjects than in the normotensive subjects, thereby suggesting that a variation in or near the ACE gene may contribute to the development and severity of hypertension. [15]Das eat al (2008)found that individuals with DD genotype were more likely to have hypertension than individuals with ID or II genotypes among adult Asians in Calcutta. [16]Sameer *et al*. (2010) observed that the frequency of DD genotype was greater than the ID and II genotypes among hypertensive cases in Kashmir.[17]Tchelougou et al (2015) found the association of DD genotype with essential hypertension in the population of Burkina Faso, West Africa. The increased risk of hypertension with the D/D genotype could be due to the conversion of angiotensin-I to the angiotensin II and inactivation of a potent vasodilator known as bradykinin. [18] Moreover, angiotensin is a proinflammatory and pro-oxidant, thereby it causes cellular toxicity and apoptosis and studies have demonstrated that chronic low grade systemic inflammation can predict the future risk of hypertension.[19]Agachan et al (2003) reported that DD genotype and the D allele of the ACE gene are strongly associated with hypertension compared to healthy individuals, and they confer increased risk of hypertension. [20]

Sagnella *et al*.(1999) observed a significant association between the D allele and hypertension in women of African descent. [21] Borah et al (2012) gave evidence that in the Northeastern state of Assam and Mizoram, India, ACE polymorphism is linked to isolated systolic hypertension. [22]In contrast, no association between ACE gene polymorphism and hypertension were found in Dutch,[23]Belgian, [24]Bangladesh population,[25], Italian, [26]Hellenic, [27] Haryana rural population, [28]and Lebanese population[29]. Nevertheless, a strong association of the I allele was found in an Australian population with familial hypertension.[30]Ismail et al (2004) also found association of I/I genotype with early onset of essential hypertension among the Pakistanis. [31]These reported discrepancies reflect ethnic, genetic and geographical variation within the studied population. The negative associations found may be due to these differences and possible bias in data sampling or the marker studied could be linked to other genes with which they have an impact on the incidence of hypertension in the population or on any other disease. This leads to the importance of studying several polymorphisms in different ethnic groups. [32]

In males, risk of central adiposity as depicted by waist circumference was found among ID individuals(4%) as compared to DD genotype which was statistically significant ($p<0.05$) indicating the association of obesity with the ID variant only. While, in females 25.8% were centrally obese amongst DD genotype individuals as depicted by WC. In female participants with DD homozygote, risk of both general and central obesity as depicted by BMI, body fat percentage, WC, WHR and WHtR were higher than ID heterozygote. Similarly, DD genotype was associated with high body fat among Korean ballerinas.[33]Whereas, the risk of hypertension was found to be greater among males with DD homozygote rather than females with DD homozygote. Morise et al(1994) have shown that ID polymorphism is strongly associated with increased blood pressure in males. [34] The D allele has been associated with hypertension in some studies in white American and Japanese men but not in women.[35] Among Mongolian people,ACE gene ID+DD genotype was found to be the risk factor of hypertension in men.[36]

Table 1: Distribution of Ao Naga males and females according to genotypic variants of ACE gene

Gender	Genotype of ACE gene		
	DD n (%)	ID n (%)	II n (%)
Male(n=27)	20 (35.1)	5 (8.8)	1(1.8)
Female (n=31)	28 (49.1)	3 (5.3)	-
Total (n=57)	48 (84.2)	8 (14)	1 (1.8)

Table 2: Adiposity measures and blood pressure among DD and ID variants of ACE gene

	Male(n=26)		t-value	Female(n=31)		t- value
	DD	ID		DD	ID	
Height (cm)	165.39±1.23	168.52±2.21	1.152	157.33±0.85	160.06±3.54	0.959 ns
Weight (kg)	62.59±1.99	68.92±6.94	1.218	56.94±2.57	52.13±7.05	0.586 ns
BMI (kg/m2)	22.87±0.63	24.56±2.91	0.900	22.50±0.98	20.19±1.87	0.909 ns
Body fat (%)	19.29±1.07	19.62±3.97	0.115	29.00±1.56	23.55±6.55	0.924 ns
WC (cm)	76.53±1.71	77.26±5.67	0.165	70.02±2.54	65.70±3.69	0.542 ns
WHR	0.85±0.01	0.82±0.03	0.884	0.76±0.01	0.74±0.01	0.337 ns
WHtR	0.46±0.01	0.46±0.03	0.103	0.44±0.01	0.40±0.01	0.721 ns
SBP (mmHg)	129.47±2.35	120.40±5.49	1.691	122.25±2.10	115.33±3.71	0.230 ns
DBP(mmHg)	86.21±1.94	78.0±2.75	2.014*	83.06±1.63	75.33±3.71	0.761 ns

ns indicates non-significant

Table 3: Distribution of Ao Naga males and females according to adiposity indicators and blood pressure with respect to DD and ID genotypes of ACE

Variables	Category	Males			Females		
		DD N (%)	ID N(%)	x^2 value	DD N (%)	ID N(%)	x^2 value
BMI (kg/m^2)	Underweight	2 (8.0)	-	0.625	7 (22.6)	1 (3.2)	1.337
	Normal	12 (48.0)	3 (12.0)		12(38.7)	2 (6.5)	
	Overweight	6 (24.0)	2 (8.0)		9 (29.0)	-	
WC (cm)	Risk	-	1 (4.0)	4.167*	8 (25.8)	-	1.155
	Non-risk	20 (80.0)	4 (16.0)		20(64.5)	3 (9.7)	
WHtR	Risk	3 (12.0)	2 (8.0)	1.563	7 (22.6)	-	0.969
	Non-risk	17 (68.0)	3 (12.0)		21(67.7)	2 (6.5)	
WHR	Risk	5 (20.0)	-	1.563	8 (25.8)	-	1.155
	Non-risk	15 (60.0)	5 (20.0)		20(64.51)	3 (9.7)	
Body Fat (%)	Risk	1 (4.0)	1 (4.0)	1.223	13 (41.9)	1 (3.22)	0.188
	Non-risk	19 (76.0)	4 (16.0)		15 (48.4)	2 (6.45)	
SBP (mmHg)	Normotensive	7 (28.0)	-	1.648	19(61.3)	3 (9.7)	1.350
	Prehypertensive	9 (36.0)	3 (12.0)		5 (16.2)	-	
	Hypertensive	4 (16.0)	2 (8.0)		4 (12.9)	-	
DBP (mmHg)	Normotensive	6 (24.0)	3 (12.0)	3.571	13 (41.9)	-	0.683
	Prehypertensive	5 (20.0)	2 (8.0)		11 (35.4)	2 (6.5)	
	Hypertensive	9 (36.0)	-		4 (12.9)	1 (3.22)	

**indicates p <0.05*

Table 4: Correlation of blood pressure and adiposity markers among DD and ID genotypic variants of ACE

Adiposity markers	Males				Females			
	DD		ID		DD		ID	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
BMI	-.00	-.14	-.38	.27	.20	.29	1**	.94
Body fat %	.00	-.03	-.47	.22	.15	.20	1**	1**
WC	-.10	-.03	-.46	.07	.28	.39*	.99*	.93
WHR	-.21	-.00	-.32	-.15	.33	.43*	.99	.91
WHtR	-.06	-.61	-.38	.13	.31	.38*	.97	.86**

**p<0.05*
***p<0.01*

Cardoso et al (2008) have also reported that the D allele might increase the risk of cardiovascular disease by facilitating the development of left ventricular hypertension, especially among men.[37]The mechanism of gender-specific association with hypertension remains unclear. It might be the estrogen playing a protective role against hypertension as it acts as negative feedback mechanism in controlling renin secretion and ACE secretion in renin-angiotensin system.[38]Sex differences exist in the regulation of arterial pressure and renal function by RAS The ACE/AngII/AGTR1 pathways in males are enhanced, but in females, the balance is shifted towards the ACE2/Ang(1- 7)/MasR (Mas receptor) and angiotensin type 2 receptor (AT2R) pathways.[39]

In males, obesity was not found to be associated with hypertension in both the genotypic variants of ACE. Whereas, in females obesity was significantly and positively correlated with hypertension in both DD and ID genotype. In a study by Vitor et al (1997), older women with ID genotype of the ACE gene had higher cardiorespiratory fitness and a lower body fat percentage. [40]

CONCLUSION

Since the samples were drawn from a Mendelian population with a common gene pool, the results are unlikely to be affected by unmeasured confounding factors of population stratification. The present study provides a baseline data among AoNagas. Subsequent population-based research on a larger population sample would be explicit in explaining the discrepancy of gender-dependent expression of ACE polymorphism in the obesity-hypertension syndrome.

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