Sleep Breath
DOI 10.1007/s11325-012-0795-y

ORIGINAL ARTICLE

Angiotensin-converting enzyme gene polymorphism in north Indian population with obstructive sleep apnea

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Received: 4 August 2012 / Revised: 8 November 2012 / Accepted: 24 December 2012

Abstract
Background A deletion of 287-bp *Alu* repeat of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene is associated with hypertension.
Purpose The aim of this study is to determine the frequency of ACE (I/D) polymorphism in patients with obstructive sleep apnea (OSA).
Methods Genotyping of ACE (I/D) gene polymorphism and estimation of serum angiotensin-converting enzyme (SACE) activity were done in 813 subjects who underwent polysomnography. Of these, 395 were apneics and 418 were non-apneics.
Results The frequencies of II genotype (OR = 1.8, 95 % CI 1.26–2.60, p=0.001) and I allele (OR = 1.4, 95 % CI 1.13–1.69, p=0.001) of ACE gene were found to be significantly increased in patients with OSA as compared to patients without OSA. Frequency of II genotype was significantly decreased (OR = 0.46, 95 % CI 0.28–0.77, p=0.003) in OSA patients with hypertension. In contrast, the frequencies of ID (OR=1.80, 95 % CI 1.08–2.99, p=0.024) and DD genotypes (OR=2.15, 95 % CI 1.30–3.57, p=0.003) were significantly increased in this group. The activity of SACE was significantly decreased in the apneic group as compared to the non-apneic group (OR=0.99, 95 % CI 0.98–1.00, p=0.04).
Conclusions The findings suggest that II genotype confers susceptibility towards development of OSA whereas DD genotype confers susceptibility towards hypertension irrespective of OSA.

Keywords Angiotensin-converting enzyme (ACE) · Insertion/deletion (I/D) polymorphism · Polysomnography · Obstructive sleep apnea (OSA) · Hypertension · North Indian population

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Introduction

Idiopathic obstructive sleep apnea (OSA), a sleep-related breathing disorder of unknown etiology, is known to promote endothelial damage, atherosclerosis, hypertension, metabolic syndrome, and neurocognitive dysfunction [1–3]. The prevalence of OSA differs in each population according to their obesity-promoting sedentary lifestyle, geographical location, and racial differences. The Wisconsin study estimated a prevalence of 24 % in males and 9 % in females [4]. Population-based studies in Delhi found the prevalence of OSA to be 9.4–13.7 % and that of OSAS to be 2.7–3.8 % [5, 6].

Several studies have shown an association between OSA and hypertension [6, 7]. In addition, a homozygous deletion of 287 bp of *Alu* repeat of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene is also known to be associated with hypertension. Therefore, a potential mechanistic link between OSA and ACE (I/D) polymorphism cannot be ruled out.

The ACE gene is located on the long arm of chromosome no. 17 (17q23.3). It is an exopeptidase, which cleaves the end of angiotensin I (decapeptide) to angiotensin II (octapeptide). Angiotensin II degrades bradykinine (a potent vasodilator) and acts as a powerful vasoconstrictor. This conversion makes a phenomenal impingement on the renin–angiotensin–aldosterone–system; that is of hormonal disposition and controls blood pressure by maintaining the fluid–electrolyte balance. Thus, the expeditious formation of

Published online: 31 January 2013

angiotensin II results in vasoconstriction and high blood pressure.

A number of studies have shown that the homozygous deletion of this gene results in the increased activity of serum angiotensin-converting enzyme (SACE) enzyme [8, 9].

Studies have also shown a strong association of insertion allele of ACE with OSA, although a few have contradicted these findings [9–17]. The present study premises the hypothesis that ACE (I/D) polymorphism is associated with OSA.

Materials and methods

Study design

The study was approved by the institutional ethics committee and written informed consent was taken from each participant. Consecutive patients (from outpatient department, sleep-related breathing disorder clinics as well as community) were screened for overnight polysomnography (PSG) studies for the diagnosis of OSA [5, 6, 18] at the All India Institute of Medical Sciences Hospital, New Delhi. Relevant anthropometric assessment preceded the PSG study. Subjects between 18 and 65 years of either gender were enrolled for the assessment. Subjects having valvular or coronary heart disease, congestive heart failure, tracheostomy or recent upper airway surgery, airway cancers, hypothyroidism, chronic renal failure, acromegaly, pregnancy, females on hormone replacement therapy, and subjects on systemic steroids were excluded from the study.

Overnight polysomnography

Overnight PSGs were conducted in the Sleep Laboratory of the Department of Medicine at All India Institute of Medical Sciences by trained laboratory technicians using a Rambrandt 7.3 version PSG machine (Medicare Technologies, USA) as described previously [5, 6]. Apnea was defined as cessation of nasal airflow for ≥10 s. Hypopnea was defined according to the Chicago Criteria (1999) as also described elsewhere [5, 6, 19]. Sleep stages classification was done according to guidelines of American Academy of Sleep Medicine (AASM 2007) [20]. OSA was defined as AHI ≥5 events/h. The severity of OSA is graded on the basis of AHI as mild (AHI ≥5 and <15), moderate OSA (AHI ≥15 and <30), and severe OSA (AHI≥30).

Anthropometry and body composition analysis

A detailed physical examination was performed in which height, weight, BMI (kg/m^2), neck length, neck circumference, and waist and hip circumference were measured and the waist–hip ratio was calculated as describe previously [5, 6, 18].

Blood pressure was measured with a mercury sphygmomanometer to the nearest 2 mmHg, in sitting position, after at least 5 min of rest. The mean of three measurements done at 1-min intervals were taken. Subjects were considered hypertensive if they were currently receiving antihypertensive medication or if they fulfilled the Joint National Committee 7 criteria for hypertension [21].

Estimation of serum angiotensin-converting enzyme (SACE)

SACE activity was measured in all the subjects using the method described by Cushman and Cheung as described previously [13, 22–24]. Briefly, production of hippuric acid from hippuryl-L-histidyl-L-leucine by ACE activity present in the serum sample was measured using a spectrophotometer (CARY 100 Bio UV–visible spectrophotometer; VARIAN). Running reference ACE activity in our laboratory during study period was 12–35 U/L.

Genotyping of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism

Genomic DNA was isolated from peripheral blood leukocyte using the modified salt precipitation method [22]. Amplification of ACE (I/D) gene was performed on a thermocycler (Eppendorf AG 22331, Hamburg, Germany). The polyacrylamide gel electrophoresis (PAGE)-purified primers (Bio Basic Inc., East Markham, Canada), DNA Taq polymerase, and deoxynucleoside triphosphate used in the study were commercially procured (Bangalore Genie, India). The following primer sequences were used for the amplification of the gene: forward primer, 5′-CCACTCCCATCCTTTCTCC-3′; reverse primer, 5′-GGCCATCACATTCGTCAGA-3′ as reported elsewhere [25].

The PCR was performed using cycling conditions of initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, and extension at 72 °C for 1 min 30 s, with a final 5-min extension at 72 °C as described elsewhere [25].

The amplification product was resolved on 2.5 % agarose gel (Bangalore Genie) having 3 % ethidium bromide at 80 V for 1 h and visualized using an ultraviolet transilluminator [MultiImage^{TM}, UV transilluminator, Alpha Innotech (Johannesburg 1715, South Africa)].

Statistical analysis

Statistical analysis was performed using STATA 11 for Windows software (intercooled version; Stata Corporation, Houston, TX, USA). After assessing for approximate normal distribution, all continuous variables were summarized as mean ± SD and categorical variables were expressed as *n* (%). Comparison between two groups was done with Student's *t* test for continuous variables

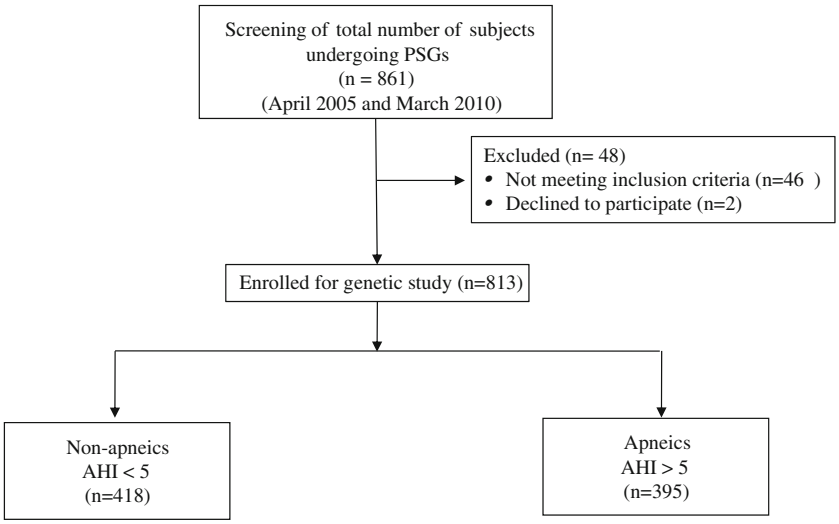

Fig. 1 Flow diagram showing recruitment of the study subjects

Table 1 Comparison of demographic and anthropometric characteristics between the apneic and non-apneic groups

Characteristic	Apneics (*N*=395)	Non-apneics (*N*=418)	*p* value	OR (95 % CI)
Age (years)	46.0±9.5	43.0±10.6	<0.001	1.02 (1.01–1.04)
Gender				
Female	86 (22)	167 (40)		1.00
Male	309 (78)	251 (60)	<0.001	2.39 (1.76–3.25)
Body mass index (BMI) (kg/m^2)	32.6±7.4	27.2±5.7	<0.001	1.15 (1.12–1.18)
% Body fat	32.3±12.1	29.2±10.7	<0.001	1.02 (1.01–1.04)
Waist–hip ratio (W-HR)[a]				
Normal	28 (7)	100 (24)		1.00
Abnormal	367 (93)	318 (76)	<0.001	4.12 (2.64–6.43)
Waist circumference[a]				
Normal	11 (3)	79 (19)		1.00
Abnormal	384 (97)	339 (81)	<0.001	8.14 (4.26–15.54)
Neck circumference (cm)	39.0±4.0	34.8±4.2	<0.001	1.23 (1.22–1.33)
SSFT (cm)	29.6±8.1	25.5±8.6	<0.001	1.06 (1.04–1.08)
SIFT (cm)	35.2±9.8	31.5±8.8	<0.001	1.04 (1.03–1.06)
BSFT (cm)	14.9±6.9	12.6±6.8	<0.001	1.05 (1.03–1.07)
TSFT (cm)	21.2±8.9	18.8±8.9	<0.001	1.03 (1.01–1.05)
Diabetes mellitus				
Absent	328 (83)	366 (88)		1.00
Present	67 (17)	52 (12)	<0.07	1.44 (0.097–2.13)
Hypertension				
Absent	179 (45)	266 (64)		1.00
Present	216 (55)	152 (36)	<0.001	2.11 (1.59–2.80)
SBP (mmHg)	135±16	130±15	<0.001	1.022 (1.013–1.032)
DBP (mmHg)	88±10	85±10	<0.001	1.034 (1.020–1.049)
Epworth sleepiness score	10.9±5.5	6.5±4.4	<0.001	1.19 (1.16–1.23)
Smoking status				
Non-smokers	314 (80)	371 (89)		1.00
Smokers	81 (20)	47 (12)	<0.001	2.04 (1.38–3.00)
Alcoholism				
Non-alcoholic	294 (74)	368 (88)		1.00
Alcoholic	101 (26)	50 (12)	<0.001	2.53 (1.74–3.67)

Data are presented as mean±SD or *n* (%)

MAC mid-arm circumference, *SSFT* subscapular skin fold thickness, *SIFT* subiliac fold thickness, *BSFT* biceps skin fold thickness, *TSFT* triceps skin fold thickness, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *OR* odds ratio, *CI* confidence interval

[a]Defining cut-offs were different for men and women—for W-HR >0.95 and >0.88; respectively; for abdominal circumference ≥90 cm and ≥80 cm, respectively

Table 2 Comparison of polysomnography parameters between apneic and non-apneic groups

Characteristic	Apneics (*N*=395)	Non-apneics (*N*=418)	*p* value	OR (95 % CI)
AHI (events/h)	36.1±25.3	0.8±1.3	<0.001	–
Total sleep time (min)	397.2±75.7	372.6±72.4	<0.001	1.07 (1.04–1.10)[a]
Total wake time (min)	58.6±57.6	75.2±68.8	<0.001	1.03 (0.96–1.10)[a]
Sleep efficiency (%)	86.7±13.1	83.6±13.6	<0.001	1.02 (1.00–1.03)
Sleep stages (% of TST)[b]				
Stage N1	71.2±49.1	57.9±40.9	<0.001	1.12 (1.06–1.19)
Stage N2	217.3±67.6	190.1±68.1	<0.001	1.10 (1.07–1.13)
Stage N3	72.4±53.3	71.4±47.9	0.04	1.01 (0.96–1.06)
REM sleep	28.8±20.8	38.5±24.6	<0.001	0.70 (0.62–0.80)
Sleep latencies (min)				
Sleep onset latency	6.7±16.7	7.5±17.1	0.46	0.99 (0.98–1.01)
REM sleep onset latency	79.8±74.2	92.5±76.3	0.02	0.96 (0.93–0.99)[a]
Arousal index (arousals/h)	21.3±12.4	7.8±5.6	<0.001	1.19 (1.16–1.21)
Baseline SaO_2 (%)	97.3±5.2	97.6±2.5	0.24	0.97 (0.92–1.02)
Minimum SaO_2 (%)	72.6±14.0	94.9±64.0	<0.001	0.80 (0.78–0.83)
Average SaO_2 (%)	91.8±5.3	95.8±2.0	<0.001	0.68 (0.64–0.73)
SaO_2<90 % (%)TST (min)	79.9±90.4	2.7±14.8	<0.001	1.11 (1.09–1.13)

Data are presented as mean±SD

AHI apnea hypopnea index, *TST* total sleep time, *REM* rapid eye movement, *SaO_2* oxygen desaturation, *SD* standard deviation, *OR* odds ratio, *CI* confidence interval

[a]Per 15-min increase

[b]Sleep stages classification was done according to new guidelines of the American Academy of Sleep Medicine (AASM 2007) [20]

and χ^2 test for categorical variables; odds ratio with 95 % confidence interval (CI) were calculated with their respective *p* values. Multivariable logistic analyses were performed by multiple logistic regressions with OSA and hypertension as outcome variables.

Results

Comparative analysis of anthropometric and demographic characteristics of the study group

A total number of 861 patients were screened over 5 years (April 2005 to March 2010), and 813 of these were found suitable for enrollment as per inclusion criteria. Furthermore, 395 (48 %) of 813 patients had OSA (AHI ≥5 events/h). Figure 1 details this information. Comparison of demographic and anthropometric characteristics between subjects with and without OSA is provided in Table 1.

Comparative analysis of polysomnography parameters between the groups

By definition, OSA group had increased AHI, arousal index, and significantly decreased duration of REM sleep. Table 2 provides the comparison of polysomnography parameters between the two groups. Subjects with OSA were found to have significantly increased duration of stages N-1, N-2, and N-3 and significantly decreased duration of REM sleep, minimum SaO_2 (%), and average SaO_2 (%).

Genotypic and allelic frequency of ACE (I/D) polymorphism between the apneic and non-apneic groups

The comparative analyses of genotypic and allelic frequencies of ACE (I/D) polymorphism were done between the groups as depicted in Table 3. The frequency of II genotype (OR = 1.8 95 % CI 1.26–2.60, p=0.001) and I allele (OR = 1.4, 95 % CI 1.13–1.69, p=0.001) of ACE gene

Table 3 Distribution of genotypic and allelic frequency of ACE (I/D) polymorphism in apneic vs. non-apneic groups

ACE (I/D) polymorphism		Apneics (*n*=395)	Non-apneics (*n*=418)	χ^2	Overall *p* value	OR (95% CI)	*p* value
Genotypic frequency	DD	136 (34.4)	160 (38.2)			1.00	
	ID	132 (33.4)	175 (41.9)			0.91 (0.66–1.26)	0.580
	II	127 (32.2)	83 (19.9)	16.55	<0.001	1.81 (1.26–2.60)	0.001
Allelic frequency	D	404 (51.1)	495 (59.2)			1.00	
	I	386 (48.9)	341 (40.8)	10.35	0.001	1.39 (1.13–1.69)	0.001

was significantly higher in apneic group in comparison to non-apneics.

Overall, without considering the genotypes, apneic group was found to have significantly decreased SACE activity in comparison to the non-apneic group (OR=0.99, 95 % CI 0.98–1.00, p=0.04). In addition, when subjects were stratified according to the genotypes, SACE activity was significantly higher in subjects with DD genotype as compared to subjects with II genotype in order of DD>ID>II. Similarly, subjects with DD genotype had significantly higher SACE activity as compared to II genotype even after stratifying the genotypes in accordance with the presence or absence of OSA as described in Table 4.

Analysis of the ACE genotypic frequencies by stratifying the subjects on the basis of presence or absence of hypertension along with polysomnography criteria was also done. Results revealed that the hypertensive apneic subjects had significantly higher frequency of DD genotype (39 %) and lower frequency of II genotype (26 %) in comparison to normotensive apneic subjects where increased frequency of II (40 %) and decreased frequency of DD (28 %) genotypes were observed, as shown in Fig. 2.

Analysis of the ACE genotypic frequencies by stratifying the subjects on the basis of their BMI and polysomnography criteria indicated a significant increased frequency of II genotype and decreased frequency of DD genotype in both the apneic groups (obese as well as non-obese) as compared to the non-apneic groups (obese and non-obese) (results not shown).

Stratification of subjects done on the basis of polysomnography criteria revealed that the increase in severity of OSA is associated with increased frequency of II genotype and decreased frequency of DD genotype (results not shown).

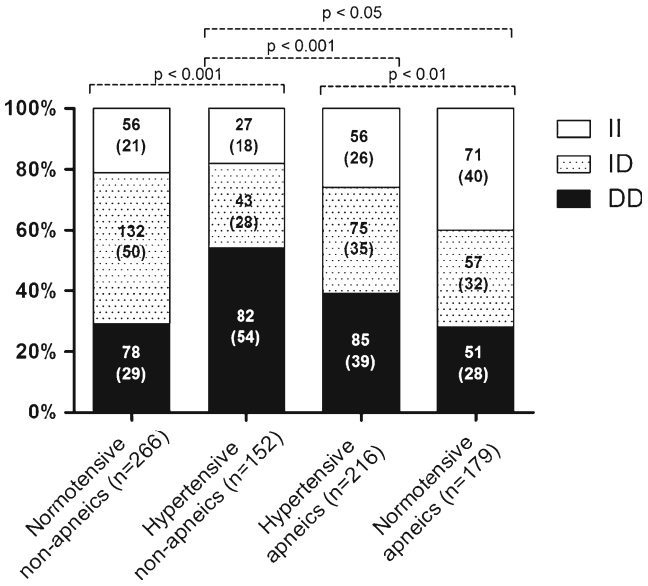

Fig. 2 Genotypic distribution of ACE (I/D) polymorphism n (%) (according to blood pressure and polysomnography criteria). The frequency of DD genotype is significantly higher (39 % vs. 28 %) and frequency of II genotype is significantly lower (26 % vs. 40 %) in hypertensive apneics in comparison to normotensive apneics

Multivariable analysis

A stepwise logistic regression was done to identify the independent risk factors of OSA. Some of these factors were interrelated such as BMI, fat mass, percent body fat, skin fold thicknesses, etc.; therefore, these variables were not considered in the multivariable analysis though they showed significant associations in the univariate analysis. The final model identified male gender, BMI, age, and II genotype of ACE gene as independent predictors of OSA (Table 5). Subjects having II genotype had 69 % more chances towards development of OSA independent of other various confounding factors. Similarly, a multivariable logistic regression analysis was done by keeping hypertension as an outcome variable in the study group (813 observations). BMI and age were the significant independent predictors, whereas ID and II genotypes were found to provide 51 % and 57 % protection, respectively, towards OSA which were significant (Table 6). In this analysis, DD was an independent risk factor for hypertension (OR=2.33, 95 % CI 1.59–3.42, p<0.001, results not shown).

To explore the independent association of ACE (I/D) genotypes with hypertension in apneic subjects, a multivariable logistic regression analysis was also performed by keeping hypertension as an outcome variable exclusively

Table 4 Serum ACE levels in apneic and non-apneic groups according to different ACE genotypes

	Apneics (n=395)					Non-apneics (n=418)				
Genotypes ACE (I/D)	Number	SACE levels mean±SD	F ratio 19.1	df 2	p value <0.001	Number	SACE levels mean±SD	F ratio 57.5	df 2	p value <0.001
DD	136	44.8±14.8				160	50.7±17.2			
ID	122	35.07±13.4				175	35.1±14.0			
II	127	37.2±12.2				83	34.2±10.8			

SACE serum ACE levels (U/l), *ACE* angiotensin-converting enzyme, *SD* standard deviation, *F ratio* variance of independent samples (Fisher–Snedecor), *df* degree of freedom

Table 5 Multivariable model with OSA as an outcome variable

Independent variable	Adjusted odds ratio (95 % CI)	*p* value
Gender		
Female	1.00	
Male	4.01 (2.75–5.85)	<0.001
Body mass index	1.17 (1.14–1.21)	<0.001
Age	1.03 (1.01–1.04)	<0.001
ACE (I/D)		
DD genotype	1.00	
ID genotype	0.98 (0.68–1.41)	0.92
II genotype	1.69 (1.13–2.53)	0.01

Body mass index and age were the continuous variables, whereas gender and ACE (I/D) were categorical variables

ACE (*I*/*D*) angiotensin-converting enzyme insertion deletion polymorphism, *OR* odds ratio, *CI* confidence interval

Table 7 Multivariable model with hypertension as an outcome variable in the apneic group

Independent variable	Adjusted odds ratio (95 % CI)	*p* value
Gender		
Female	1.00	
Male	1.04 (0.62–1.73)	0.89
Body mass index	1.04 (1.01–1.08)	0.004
Age	1.04 (1.01–1.06)	0.002
ACE (I/D)		
DD genotype	1.00	
ID genotype	0.84 (0.51–1.38)	0.48
II genotype	0.46 (0.28–0.77)	0.003

Body mass index and age were the continuous variables, whereas gender and ACE (I/D) were categorical variables

ACE (*I*/*D*) angiotensin-converting enzyme insertion deletion polymorphism, *OR* odds ratio, *CI* confidence interval

in OSA group (395 observations). In this analysis, BMI and age came out as significant independent predictors, whereas II genotype was found to provide 54 % (OR = 0.46, 95 % CI 0.28–0.77, p=0.003) protection towards OSA which was significant (Table 7). In this analysis, ID (OR=1.80, 95 % CI 1.08–2.99, p=0.024) and DD (OR=2.15, 95 % CI 1.30–3.57, p=0.003) genotypes were the independent risk factors for hypertension in OSA subjects (results not shown).

Discussion

Findings of the present study reveal an increased frequency of II genotype in patients with OSA. Several studies have investigated the association of ACE (I/D) polymorphism in patients with OSA and have yielded conflicting results (Table 8). Ogus et al. in a study done on a Turkish population have reported an increased frequency of II genotype in patients with OSA [26]. Similar findings were also reported in Chinese population [13, 17]. Various explanations have been put forward to explain the possible relationship between II genotype and OSA. The II genotype has been proposed to modulate the central factors regulating breathing and muscle tone during sleep [14]. Further, it has also been proposed that the inhibition of adipocyte differentiation occurs mainly due to angiotensin II which has an inverse relationship with II genotype [26]. Conversely, no association of II genotype with OSA has been reported in studies on Wisconsin Sleep Cohort [14], Spanish [10], Swedish [11], and Cleveland Family Study [15] populations. This disparity in results of aforementioned studies can be attributed to several factors including ethnic variations, differences in the study design, sample size, and selection bias.

The present study also reveals that the activity of SACE activity was lower in subjects having II genotype and higher in individuals with DD genotype. It has been earlier proposed that the decreased activity of SACE may increase the local concentration of nitric oxide which helps in improving the mitochondrial respiration and contractile functions of the muscle during physical activities [27]. It has also been proven that the blockade of nitric oxide synthesis decreases adiposity and improves insulin resistance in high fat-induced obese mice [28]. These findings are important as accumulation of nitric oxide improves respiratory efficiency; however, it also promotes accumulation of abnormal fat deposition during sedentary lifestyle. Moreover, obesity is a risk factor for the development of OSA, and the latter is known to be independently associated with insulin resistance

Table 6 Multivariable model with hypertension as an outcome variable in the study group as a whole

Independent variable	Adjusted odds ratio (95 % CI)	*p* value
Gender		
Female	1.00	
Male	1.36 (0.98–1.88)	0.07
Body mass index	1.08 (1.05–1.10)	<0.001
Age	1.04 (1.02–1.05)	<0.001
ACE (I/D)		
DD genotype	1.00	
ID genotype	0.49 (0.35–0.69)	<0.001
II genotype	0.43 (0.29–0.63)	<0.001

Body mass index and age were the continuous variables, whereas gender and ACE (I/D) were categorical variables

ACE (*I*/*D*) angiotensin-converting enzyme insertion deletion polymorphism, *OR* odds ratio, *CI* confidence interval

Table 8 Association studies of the ACE insertion/deletion polymorphism with obstructive sleep apnea

Reference	Population	Sample size and categorization	Results
Zhang et al. 2000 [17]	Chinese	MSO=34 MO=27 HT=45 NC=68	•The frequencies of I allele and II genotype were significantly higher in MSO group ($p<0.05$) than in other groups
Barceló et al. 2001 [10]	Spanish	OSA=63 Controls=32	•Allelic and genotypic frequencies were not significantly different between patients and controls •ACE activity was significantly higher in OSAS patients than in healthy controls ($p<0.01$)
Lin et al. 2004 [14]	Wisconsin Sleep Cohort	OSA=474 Controls=626	•The polymorphism was not associated with the occurrence of sleep-disordered breathing (SDB) •SDB and polymorphism interacted significantly to modulate blood pressure independently of age, sex, ethnicity, and body mass index •In the absence of SDB, deletion allele alone may not be sufficient to increase blood pressure and at severe levels of SDB, the effect of sleep apnea on blood pressure overwhelms any association of the deletion allele with hypertension
Li et al. 2004 [13]	Chinese	OSA with Htn=30 OSA without Htn=30 Controls=30	The frequencies of II genotype and I allele were significantly higher in OSAHS with Htn group in comparison with other two groups
Patel et al. 2007 [15]	Cleveland Family Study	OSA=638 Controls=334	DD genotype is associated with a 37 % reduction in the odds of hypertension as compared with II genotype ($p=0.03$)
Boström et al. 2007 [11]	Swedish (Skara)	Cases=157 Controls=181	•ACE polymorphism was not associated with hypertension in either sex •Significant interaction has been found between OSA and D-allele of ACE gene ($p=0.010$)
Koyama et al. 2009 [12]	Brazilian	OSA with Htn=114 OSA without Htn=152	•Among hypertensive OSA males, the homozygous ACE I allele protects from severe OSA
Yakut et al. 2010 [16]	Turkish	OSA=64 Controls=37	•Allelic and genotypic frequencies were not significantly different between patients and controls
Ogus et al. 2010 [26]	Turkish	OSA=97 Controls=79	•An increase in I allele frequency in OSAS patients was observed ($p=0.02$) •Carrying I allele (genotype II or ID) increases OSAS risk by 2.41 times ($p=0.006$) •ACE activity was significantly lower in II genotype and OSAS patients
Present study, 2010	North Indian	AHI<5, OSA ($n=395$); AHI≥5, normal ($n=418$)	•The frequencies of I allele and II genotype were significantly higher in patients with OSA ($P<0.001$)

This table is a summary of results adapted from references [10–17, 26]

OSA obstructive sleep apnea, *Htn* hypertension, *MSO* moderate to severe OSA group, *MO* mild OSA group, *HT* isolated hypertension group, *NC* normal control

[29]. Further exploratory studies are required to confirm the significance of these pathways.

Numerous studies have shown association of OSA with hypertension [5, 6, 30]. The Sleep Heart Health Study has demonstrated a dose–response relationship of AHI with the development of hypertension [30]. Studies have also reported an association of DD genotype of ACE (I/D) gene with higher SACE activity which results in hypertension. The results of the present study also reveal that the frequency of DD genotype was higher in subjects of OSA with hypertension as compared to the normotensive OSA subjects. In agreement with the findings of the present study, Lin et al. have also shown a significant interaction between increase in blood pressure and D allele in patients with mild to moderate OSA [14]. However, activity of SACE was not measured in this study. On the other hand, Patel et al. in a study done on 972 subjects showed that DD genotype provides protection towards hypertension in OSA patients, which according to

authors could be due to the difference in the characteristics of cohort [15]. Their cohort included a younger population with higher proportion of African Americans from a region with increased susceptibility to OSA. Furthermore, the proportion of hypertensive subjects in their study was relatively lower (15 %) in non-apneics (AHI<5) as compared to apneic subjects (38 % in subjects having AHI range 5–30 and 62 % in subjects having AHI ≥30), and SACE activity was also not measured in this study. Whereas in the present study, 36 % of non-apneics and 55 % of apneics were hypertensive, and these differences could account for discordant results. Li et al. has reported an increased frequency of II genotype in Chinese patients with OSA and hypertension. These differences could be driven by race and ethnicity [13]. Our findings of association of DD genotype are in agreement with a study of hypertension by Sameer Syed et al. in north Indian patients. In contrast, another study by Srivastava et al. has found an association of I allele with essential hypertension. The authors did not measure SACE activity to validate the functional impact of these associations [31, 32]. Comparison of our findings with these two north Indian studies should be done cautiously as our patients had both OSA and hypertension. Further, control subject population in our study does not represent normal population as these were obese and were habitual snorers.

The DD genotype is known to increase the activity of SACE and therefore it is difficult to justify its protective role in the development of hypertension in OSA patients. However, studies supporting this assumption have not measured the activity of SACE. In the absence of measurement of SACE activity in these studies, it is difficult to pinpoint the role of DD genotype in hypertension. The activity of SACE could be substantially altered by environmental, biochemical, and genetic factors. Usage of antihypertensive drugs and dietary habits could also influence the activity of SACE [33]. Therefore, before concluding the association of DD genotype with hypertension in patients with OSA, it is important to measure the activity of SACE along with genotyping. The development of hypertension is also governed by catecholamines which are elevated in patients with OSA [34]. The interaction of catecholamines with SACE should be explored in OSA. Hypertension is one of the components of metabolic syndrome and the cause-and-effect relationship between OSA and metabolic syndrome (i.e., which occurs first) is not clear. In view of this, and based on the findings of the present study, a due consideration of Mendelian inheritance should be given before concluding such relationship.

The strength of the study includes robust sample size. Important limitation of the study includes enrollment of the obese subjects who were referred for PSG studies and hence could constitute a referral bias. It is difficult to avoid this bias as snoring is relatively common in obese subjects and it is inappropriate to invite non-snorers for PSG study from the ethical point of view unless they have some other sleep-related complaints. Therefore, the control subjects in the present study represent the control of OSA having AHI <5 and cannot perfectly represent the controls from the normal population. This could be the probable reason of not achieving the Hardy–Weinberg equilibrium of ACE (I/D) polymorphism in the present study. Additionally, our study groups include some hypertensive patients consuming ACE inhibitors which are evidently known to block the ACE activity.

In conclusion, the findings of the present study indicate that the presence of II genotype and decreased SACE activity might promote the development of OSA; however, the presence of DD genotype in patients with OSA could play an additive role in the development of hypertension.

Acknowledgments We thank all the patients and healthy volunteers for participating in this study. We thankfully acknowledge the residents of our Department of Medicine, All India Institute of Medical Sciences, New Delhi for their help in referring patients, the staff members of polysomnography laboratory (Mr. Hridesh Kumar Mishra, Mr. Jitendra Sharma, and Mr. Jitendra Kumar) for numerous sleepless nights they spent in performing polysomnography, and the support staff (Mr. Sandeep Gupta and Mr. Pankaj Mishra) who helped us to collect reliable and meaningful information about our study subjects.

Conflict of interest We declare that we have no conflict of interest.

Funding Supported by a grant from the Indian Council of Medical Research (ICMR), Ministry of Health and Family Welfare, Government of India (Project ID No. 2008-03070). HKM acknowledges the Council for Industrial and Scientific Research (CSIR) for his fellowship.

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