



## Original Article

## Reciprocal interactions of obstructive sleep apnea and hypertension associated with ACE I/D polymorphism in males

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## ABSTRACT

**Background:** The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism gene contributes to the genesis of hypertension (HTN) and may help explain the relationship between obstructive sleep apnea (OSA) and HTN. However, ACE is a pleiotropic gene that has several influences, including skeletal muscle and control of ventilation. We therefore tested the hypothesis that ACE polymorphism influences OSA severity.

**Methods:** Male OSA patients (apnea-hypopnea index [AHI] > 5 events/h) from 2 university sleep centers were evaluated by polysomnography and ACE I/D polymorphism genotyping.

**Results:** We studied 266 males with OSA (age = 48 ± 13y, body mass index = 29 ± 5kg/m<sup>2</sup>, AHI = 34 ± 25events/h). HTN was present in 114 patients (43%) who were older ( $p < 0.01$ ), heavier ( $p < 0.05$ ) and had more severe OSA ( $p < 0.01$ ). The I allele was associated with HTN in patients with mild to moderate OSA ( $p < 0.01$ ), but not in those with severe OSA. ACE I/D polymorphism was not associated with apnea severity among normotensive patients. In contrast, the only variables independently associated with OSA severity among patients with hypertension in multivariate analysis were BMI (OR = 1.12) and II genotype (OR = 0.27).

**Conclusions:** Our results indicate reciprocal interactions between OSA and HTN with ACE I/D polymorphism, suggesting that among hypertensive OSA males, the homozygous ACE I allele protects from severe OSA.

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### 1. Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of upper airway collapse during sleep, triggering apneas and hypopneas that are associated with oxyhemoglobin desaturation, persistent inspiratory efforts against the occluded airway, and arousal from sleep [1]. OSA and hypertension (HTN) often co-exist in the same patient. In patients with OSA, the prevalence of HTN varies from 30–70% [2]. OSA is now regarded as a risk factor for HTN. However, the relationship between OSA and HTN is not completely understood. In a previous study comparing patients with and without HTN in a population of OSA patients, who were paired for OSA severity, the main risk factors for HTN were similar to those in the general population and included increasing age, obesity, and diabetes [2]. In this study, HTN was also associated with a

previous family history of HTN, suggesting the interaction may also be modulated by genetic factors [2].

One potential candidate gene which may help to explain the relationship between OSA and HTN is the angiotensin converting enzyme (ACE) gene. The ACE gene contains an insertion/deletion (I/D) polymorphism characterized by a 287-bp DNA sequence in the intron 16 [4]. ACE plays an important role in the conversion of angiotensin I into the potent vasoconstrictor angiotensin II [3]. Plasma and tissue levels of ACE activity in II homozygote is approximately two times lower than that found in DD individuals [4–6]. However, large epidemiological studies that have not controlled for gender, have shown that ACE gene I/D polymorphism is associated with HTN only in the sub-group of patients with moderate OSA [7–9], but not in patients with no OSA or severe OSA [7–9]. However, the studies have shown remarkably contradictory results. While two studies found that the D allele is associated with HTN in OSA patients [7,8], two other studies found the opposite association, i.e., a decreased D allele frequency in OSA patients with HTN [9,10].

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The conflicting results between ACE gene I/D polymorphism with OSA and HTN suggest that a confounding variable not previously recognized could be involved. ACE is a key component of the local renin–angiotensin systems, which have been identified in diverse tissues including adipose tissue [11], skeletal muscle [12], and chemoreflex sensitivity [13]. Several of these factors may also play a role in the propensity to upper airway collapsibility and may modulate OSA severity [14]. In addition, the association between OSA and blood pressure control may be more complex than generally assumed. For instance, evidence suggests that blood pressure elevations increase pharyngeal collapsibility [15]. Moreover, recent studies have indicated that edema, particularly at the level of the upper airway, may greatly influence upper airway patency [16]. Despite this evidence, the hypothesis that ACE gene I/D polymorphism may influence OSA severity has not been previously investigated.

In the present study we aimed to investigate the association between OSA, ACE I/D polymorphisms and HTN. To this end, we restricted our sample to male patients with OSA, avoiding the confounding differences associated with gender. The question was not restricted to the hypothesis of a direct association between ACE I/D polymorphisms and HTN. We also asked a novel question: Are ACE I/D polymorphisms associated with OSA severity?

## 2. Material and methods

### 2.1. Patients

The present study included a sample of 266 male patients with a recent diagnosis of OSA recruited at two distinct sleep centers: Department of Psychobiology, Federal University of São Paulo ( $n = 192$ ) and the Sleep Laboratory at the Heart Institute, University of São Paulo ( $n = 74$ ). The protocol was approved by the local ethics committee, and written informed consent was obtained from all participants. We excluded patients with neuromuscular, chronic pulmonary disease, stroke, other sleep disorders, and OSA patients already receiving treatment. The patients answered two questionnaires about their medical history and ethnic background. Body mass index (BMI) was calculated after body weight and height were measured with subjects in light clothing without shoes. HTN was defined as systolic blood pressure of  $\geq 140$  mm Hg and/or diastolic blood pressure of  $\geq 90$  mm Hg after two or more measurements obtained on separate occasions [17]. We also considered current use of antihypertensive therapy for the HTN diagnosis [2,17].

### 2.2. Polysomnography

All participants in the two sleep centers underwent standard overnight polysomnography (EMBLA – Flaga hf. Medical Devices, Reykjavik, Iceland), including oxymetry, thermistor and pressure canula airflow measurements, and measurements of rib cage and abdominal movements during breathing. Sleep apnea was defined as complete cessation of airflow for a least 10 s associated with oxygen desaturation of  $\geq 4\%$ . Hypopnea was defined as a reduction in respiratory signals for at least 10 s associated with oxygen desaturation of  $\geq 4\%$ . The apnea/hypopnea index (AHI) was defined as the average number of episodes of apnea and hypopnea per hour of objectively measured sleep [1,18]. OSA was defined by AHI  $> 5$  events/h and categorized as mild to moderate (AHI: 5–29.9) and severe OSA (AHI  $\geq 30$  events/h).

### 2.3. ACE I/D polymorphism genotyping

A blood sample was collected from each subject and leukocyte deoxyribonucleic acid (DNA) was extracted according to standard

protocols [19]. Genomic DNA samples were amplified with PCR by using 10 mM of each primer described by Rigat et al. [5] in a solution containing 1.0 mM  $MgCl_2^{2+}$ , 2.5 mM Tris–HCl (pH 9.0), 1.0 mM each of dNTP, and 1 U of Platinum Taq DNA polymerase (Invitrogen, São Paulo, Brazil).

The genotyping assay was performed using DHPLC (Denaturing High Performance Liquid Chromatography). We have developed a new assay to genotype large INDELS in which we use system software (Transgenomic Navigator Software version 1.5.4, Transgenomic, Inc. USA) to calculate the buffer gradients to analyze both sequences separately (I or D) at 50 °C [20]. Briefly, 8  $\mu$ L of the PCR product for each sample was applied twice to a DNasep column (Transgenomic-Wave 3500A DHPLC system, ref DNA 99 3510, 4.6 mm  $\times$  50 mm, Transgenomic Inc., USA) in two different gradient buffer conditions in order to detect the short sequence (D allele) and/or the large sequence (I allele). Elution of DNA in the column was detected by 260 nm UV absorbance and the chromatograms were analyzed by the presence or absence of amplicon at 50 °C with the two gradients and flows (for details see [20]).

### 2.4. Statistical analysis

Statistical analysis was performed using a statistical software package (StatSoft, Inc., USA). Differences in proportions were tested using the  $\chi^2$ -test. The  $\chi^2$ -test was used to compare genotype distribution with the expectation of the alleles being in Hardy–Weinberg equilibrium. The Hardy–Weinberg Equilibrium states that both allele and genotype frequencies in a population remain constant or are in equilibrium from generation to generation unless specific disturbing influences are introduced. Continuous variables with normal distribution were evaluated by *t*-test and are presented as mean  $\pm$  SD. Variables with skewed distribution are presented as medians (interquartile range). The comparisons between groups were made by unpaired *t*-test and Mann–Whitney, when appropriate. All variables with a *p* value  $< 0.1$  in the univariate analysis were selected for multivariate regression. A value of  $p < 0.05$  was considered significant.

## 3. Results

Of the 266 male patients with OSA enrolled in the study, 114 (43%) had hypertension. The demographic and sleep characteristics of the population studied according to the presence or absence of HTN are presented in Table 1. In agreement with a previous study [2], hypertensive patients were older, more obese, and had more severe OSA than did normotensive patients. The lipid values, including total cholesterol, HDL, LDL and triglycerides were not different between groups (data not shown).

The ACE I/D polymorphism frequency distribution, in all samples or within groups, are in Hardy–Weinberg Equilibrium. In general, no significant differences were found in the genetic frequencies between normotensive and hypertensive OSA patients. In the normotensive group, frequencies for the three ACE genotypes (II, ID and DD) were 17.8, 49.3, and 32.9%, respectively, and in the hypertensive group 20.2, 54.4, and 25.4%, respectively. The allele frequencies were 0.58 versus 0.53 for the D allele in the normotensive and hypertensive groups, respectively. Race/ethnicity was not different between groups (Table 1). However, I/D polymorphism frequency analysis of normotensive and hypertensive patients stratified by OSA severity revealed significant differences. In patients with mild to moderate OSA, we observed increased I allele frequency in the group of patients with HTN compared with the normotensive group ( $\chi^2 = 5.14$ ;  $p < 0.05$ ). The same association was not found in the severe OSA patients (Table 2).

**Table 1**  
Characteristics of the sample of OSA patients with and without HTN.

Variables	All Sample n = 266	Normotensive n = 152	Hypertensive n = 114	p
Age (years)	48 ± 13	43 ± 12	54 ± 13	<0.01
BMI (kg/m <sup>2</sup> )	28 (26–31)	27 (25–30)	29 (27–33)	<0.01
Ethnicity				
Caucasian (%)	71	72	69	0.68
African descent (%)	7	6	9	0.51
Mulatto (%)	12	11	14	0.61
Others (%)	9	11	8	0.60
Current smoking (%)	14	16	15	0.30
Systolic blood pressure (mm Hg)	127 ± 17	119 ± 12	138 ± 16	<0.01
Diastolic blood pressure (mm Hg)	82 ± 12	77 ± 9	87 ± 12	<0.01
Mean Blood Pressure (mm Hg)	95 ± 18	89 ± 16	102 ± 18	<0.01
AHI (events/h)	25 (13–49)	22 (11–41)	33 (18–57)	<0.01
Mild to moderate OSA (%)	55.3	63.2	44.7	<0.05
Severe OSA (%)	44.7	36.8	55.3	<0.05
Mean oxygen saturation (%)	94 (92–95)	94 (93–95)	93 (92–95)	<0.01
Minimum oxygen saturation (%)	84 (76–87)	85 (80–88)	82 (75–87)	<0.01

BMI, Body Mass Index; AHI, Apnea Hypopnea Index; OSA, obstructive syndrome apnea. The values are median (lower and upper quartiles) or mean ± SD when appropriate. Some variables are present as percentage.

**Table 2**  
ACE I/D allele frequencies in normotensive and hypertensive groups according to OSA severity.

Variables	Normotensive		Hypertensive		p
	I (nc)	D (nc)	I (nc)	D (nc)	
Mild to moderate	0.40 (77)	0.60 (115)	0.54 (55)	0.46 (47)	0.02
Severe	0.46 (52)	0.54 (60)	0.42 (53)	0.58 (73)	0.50

D, deletion allele; I, insertion allele; nc, number of chromosomes; Differences in proportions were estimated by  $\chi^2$ -test.

To investigate association between ACE I/D polymorphism and OSA severity in the presence of HTN, we analyzed AHI according to genotype in patients with and without HTN (Table 3). According to data in Table 1, normotensive patients should have a lower AHI than hypertensive patients. Accordingly, hypertensive patients with ID or DD genotype had a significantly higher AHI than normotensives. In contrast, in patients with II genotype, the AHI was virtually identical in patients with and without HTN. Based on these results, showing that the presence of both I alleles is necessary to protect against severe OSA in hypertensive patients, we used a recessive model of genetic inheritance (II versus ID + DD) to analyze the genetic and phenotypic characteristics of the population. Using the genotypic association, we combined phenotypic and genotypic characteristics (Table 4). Within the normotensive group, no phenotypic differences were found between II and ID + DD groups (Table 4). In the hypertensive group, as expected, the II genotype was associated with lower levels of AHI compared with levels in the ID + DD group. According to Table 4, the protective association between II and OSA cannot be explained by pheno-

**Table 3**  
Apnea-hypopnea index in normotensive and hypertensive groups according to I/D genotype.

Genotype	Normotensive AHI (events/h)	Hypertensive AHI (events/h)	p
II	27 (14–41)	23 (10–43)	NS
ID	22 (10–50)	37 (22–57)*	<0.01
DD	19 (10–32)	42 (18–65)*	<0.01

The values are expressed as median (lower–upper quartiles).

\* Difference statistically significant when comparing genotypes between groups.  $p < 0.05$  by Mann–Whitney test.

typic traits. Using AHI as a dependent variable and categorizing mild to moderate vs. severe OSA, we observed that BMI, II genotype, and diuretics correlated with OSA severity in the univariate analysis. In the multiple regression analyses, the only variables associated with OSA severity were BMI (odds ratio [OR] 1.12 [95%CI 1.03–1.23];  $p = 0.007$ ) and II genotype (OR 0.27 [95% CI 0.09–0.80];  $p = 0.017$ ).

#### 4. Discussion

The present work is in line with previous studies showing that in the population with OSA, HTN is associated with increasing age, obesity, and more severe OSA [2]. We also confirmed the association between I allele with HTN only in the group of patients with mild to moderate OSA [9,10]. The new findings were achieved when we explored the hypothesis that ACE I/D polymorphism is associated with OSA severity. We observed that among patients with OSA and HTN, ACE II genotype is independently associated with a lower AHI and oxygen desaturation than is the ACE DD genotype. In multivariate analyses, the only variables independently associated with OSA severity were BMI (OR = 1.12) and II genotype (OR = 0.27). Our results suggest that the ACE II genotype protects from severe OSA in hypertensive males.

OSA and HTN are tightly linked. The predominant paradigm so far has been to investigate and prove the causality role of OSA on HTN. The reverse causality (i.e., HTN causing or worsening OSA) has been much less explored and in fact neglected. However, few studies have shown that this pathway may exist. For instance, evidence from experimental studies suggests that increased blood pressure could influence upper airway patency [15]. It is recognized that acute blood pressure elevation caused by phenylephrine infusion reduces genioglossus electromyographic activity in men during wakefulness. This suggests that hypertensive patients, who frequently have an increased sympathetic tonus, have an increased collapsibility of the upper airway [21]. Previous studies in humans that evaluated the impact of ACE inhibitors in hypertensive patients with OSA found not only a significant decrease in systolic and diastolic blood pressure but also a significant reduction in AHI, suggesting that the renin-angiotensin system activity could also play an important role in upper airway collapsibility [22–24]. Animal studies have also shown that experimentally induced HTN can lead to increased collapsibility of the upper airway [15]. Several other mechanisms may help explain the interaction be-

**Table 4**  
Age, BMI, Blood Pressure and sleep parameters in the normotensive and hypertensive OSA groups related to ACE II genotype.

Variables	Normotensive		Hypertensive	
	II (27)	ID + DD (125)	II (23)	ID + DD (91)
Age (years)	42 ± 11	43 ± 12	56 ± 15	53 ± 12
BMI (kg/m <sup>2</sup> )	27 (25–29)	27 (25–30)	28 (26–36)	29 (27–33)
Systolic blood pressure (mm Hg)	120 ± 9	119 ± 12	138 ± 19	138 ± 16
Diastolic blood pressure (mm Hg)	77 ± 8	77 ± 9	86 ± 12	88 ± 12
Mean Arterial Pressure (mm Hg)	85 ± 26	90 ± 12	103 ± 13	102 ± 20
Antihypertensive (%)	–	–	39	56
ACE inhibitors (%) <sup>a</sup>	–	–	26	25
Beta-blockers (%) <sup>a</sup>	–	–	4	18
Diuretics (%) <sup>a</sup>	–	–	4	24*
Angiotensin II Receptor Blocker (%) <sup>a</sup>	–	–	13	12
AHI (events/h)	27 (14–41)	21 (10–41)	23 (10–43)	39 (21–62)*
Mean oxygen saturation (%)	94 (92–95)	94 (93–95)	94 (93–95)	93 (92–95)*
Minimum oxygen saturation (%)	85 (81–88)	85 (80–88)	84 (76–87)	81 (74–87)*
Mild to moderate OSA (%)	63	63.2	69.6	38.5*
Severe OSA (%)	37	36.8	30.4	61.5*

The comparisons were only made within normotensive and hypertensive patients. Differences between normotensive and hypertensive groups are shown in Table 1. AHI, Apnea Hypopnea index; BMI, Body Mass Index. Values are represented as median (lower and upper quartiles). Age and blood pressure is presented as mean ± SD.

\*  $p < 0.05$  compared with hypertensive II genotypes.

<sup>a</sup> Some patients took more than one medication.

tween OSA severity, HTN and I/D polymorphism. For example, chemoreceptor activity is regulated by angiotensin II in animals [25,26]. Recent genetic studies have also shown improved performance in extreme endurance exercise athletes in a hypoxic environment (like high altitudes), where maintenance of oxygen saturation becomes crucial, to be associated with the ACE I allele [27–30]. These data suggest that those with the ACE II genotype would have a protective effect on the respiratory control center in unfavorable conditions, including a low oxygen environment. The activation of AT1 receptor by angiotensin II in the carotid body induces a predominantly excitatory effect on afferent chemoreceptor activity [25,31]. Therefore, it is reasonable to speculate that the decreased ACE activity (observed in ACE II genotype carriers) can reduce local production of angiotensin II, leading to a less responsive carotid chemoreceptor. All these mechanisms may be particularly important to explain our results because it is now recognized that chemoreflex sensitivity plays a major role in the genesis of central and obstructive sleep apnea [32].

There is a general agreement that the association between ACE polymorphism and HTN is only found in patients with mild to moderate OSA [8,9]. These findings support the concept that the environmental effect (moderate OSA) interacts with genetic factors in these patients. In contrast, in patients with severe OSA it seems that the environmental effect overrides any possible genetic influence. Based on blood pressure control literature, HTN should be associated with DD genotype because in these patients ACE is more active [4]. How does one then explain the association found between II and HTN in our study (Table 2) and others [9,10]? This contra intuitive association suggests that a relationship not previously tested may be present. In the present study we put forward a novel hypothesis of an inverse relationship, i.e., the association of ACE polymorphism and OSA severity. This association is first suggested when analyzing in conjunction Tables 1, 3 and 4. Table 1 showed OSA patients with HTN are significantly older, more obese and present a significantly higher AHI than normotensive patients. However, Table 3 shows that the difference in AHI severity between normotensive and HTN patients is not present in patients with II genotype. In addition, Table 4 shows that this “protection” of II genotype cannot be explained by phenotypic confounding variables, including age and BMI. The final piece of evidence comes from multivariate analysis that showed that the only significant variables associated with OSA severity were BMI and II genotype. The OR for II genotype was 0.27, suggesting that II protects from severe OSA.

Our study has some limitations. First, it involved an apparently small sample size for a genetic study. However, it must be stressed that we focused on male patients with OSA, the only subpopulation in which any relevant association between ACE genotype and HTN was previously reported [7–10]. The largest study reported so far involved 1100 participants from the general population, of which 474 presented AHI > 5 events/h [8]. Considering that approximately 50% were males [8], the sample size is similar to the present study ( $n = 226$ ). Furthermore, the association between I allele and HTN in patients with moderate OSA is in accordance with results from previous studies in Chinese [10] and American [9] populations, suggesting consistency in our sample. It is also important to mention that the genetic frequencies observed in our sample (54% of the D allele) did not differ from a sample of the Brazilian population (data not shown) and are consistent with previously reported values for the Caucasian population [8,33,34]. Despite the consistency of our sample, we believe that further studies should be performed in different populations in order to confirm our results. Another limitation is that we only included men, thus our study does not allow extrapolating the results to women. On the other hand, our study design allowed us to study a narrow population and avoided confounding factors, i.e., the influence of the hormonal cycle or genetic factors that may play a different role in men and women with OSA. For instance, Bostrom et al. found association between ACE gene I/D polymorphism and HTN in men but not in women [7].

In conclusion, our data suggest a complex interaction between OSA, HTN and ACE I/D polymorphism. We describe a reciprocal interaction between severity of OSA and HTN that is associated with the ACE I/D polymorphism. In addition to modulating blood pressure response, I/D polymorphism may modulate OSA severity in HTN patients.

#### Conflict of interest

No authors have potential conflicts of interest.

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