VIA MEDICA

PRACA ORYGINALNA

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Polymorphism of the *ACE* gene and the risk of obstructive sleep apnoea

Polimorfizm genu *ACE* a ryzyko wystąpienia obturacyjnego bezdechu w czasie snu

The study was financed by a research grant from the Medical University in Lublin.

Abstract

Introduction: Obstructive sleep apnoea/hypopnea syndrome (OSA) is characterized by obstruction of the upper airway during sleep, resulting in repetitive breathing pauses accompanied by oxygen desaturation and arousal from sleep. Among the candidate genes affecting the risk of OSA, genes whose polymorphisms influence the development of diseases with similar pathogenesis such as OSA could be listed: *APOE*, genes for leptin and leptin receptor, *TNFA1*, *ADRB2* and *ACE* (gene for angiotensin-converting enzyme). Until now there has been a confirmed relationship between *ACE* gene polymorphism and cardiovascular diseases, but its effect on the incidence of OSA is debatable.
The aim of this study was to investigate the effect of ACE gene insertion/deletion (I/D) polymorphism on the risk of OSA.
Material and methods: Fifty-five patients with confirmed diagnose of OSA and qualified to CPAP therapy entered the study. The control group included 50 subjects who did not complain of any sleep related symptoms. Diagnose of OSA was set on the basis of full overnight polysomnography together with Epworth Sleepiness Scale according to American Academy of Sleep Medicine guidelines. DNA was isolated from peripheral blood leukocytes with Qiagen DNA mini Kit. *ACE* gene polymorphism was determined in genomic DNA using allele specific polymerase chain reaction. Different sizes of PCR products were observed on agarose gel electrophoresis.
Results: There were non-significant differences in the frequency of *ACE* genotypes. However, allele D had significantly lower prevalence in the study group than in the control group. ($\chi^2 = 4.25$ $p = 0.04$). Moreover, I allele carriers had a threefold greater risk of developing OSA (HR = 2.748, 95% CI = 1.029–7.340, $p < 0.05$).
Conclusions: Analysis of ACE gene polymorphism might be useful to determine the risk of developing OSA in clinically predisposed patients.

Key words: *ACE* polymorphism, obstructive sleep apnoea, polysomnography, PCR

Pneumonol. Alergol. Pol. 2013; 81: 207–213

Streszczenie

Wstęp: Zespół obturacyjnego bezdechu w czasie snu (obturacyjny bezdech śródsenny, OBŚ) charakteryzuje się pojawianiem nawracających epizodów bezdechów lub spłyceń oddychania. Wśród genów kandydatów, wpływających na ryzyko wystąpienia OBŚ wymienia się geny, których polimorfizmy wpływają na rozwój chorób o podobnej patogenezie co OBŚ: *APOE*, geny dla leptyny i receptora leptyny, *TNFA1*, *ADRB2* oraz *ACE* (gen dla enzymu konwertującego angiotensynę). Dotychczas opisano zależność pomiędzy polimorfizmem genu *ACE* a chorobami sercowo-naczyniowymi, a jego wpływ na zachorowanie na OBŚ jest dyskusyjny. Celem pracy było zbadanie wpływu polimorfizmu insercyjno/delecyjnego (I/D) genu *ACE* na ryzyko zachorowania na OBŚ.
Materiał i metody: Badaniem objęto grupę 55 chorych, którzy zgłaszali się w celu rozpoczęcia terapii CPAP. Grupę kontrolną stanowiło 50 osób, które nie zgłaszały zaburzeń snu. Rozpoznanie ustalano na podstawie wyniku polisomnografii (wg klasyfikacji AASM) i wartości punktowej Skali Senności Epworth. DNA izolowano z leukocytów krwi obwodowej za pomocą zestawu Qiagen DNA mini Kit. Polimorfizm genu *ACE* oceniano na podstawie długości fragmentów produktów reakcji PCR w żelu agarozowym.

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Praca wpłynęła do Redakcji: 21.07.2012 r.

ISSN 0867–7077

Wyniki: Nie wykazano statystycznie istotnych różnic w rozkładzie poszczególnych genotypów genu *ACE* w grupie chorych z OBŚ i w grupie kontrolnej. Wykazano natomiast, że allel D występował istotnie rzadziej w grupie chorych na OBŚ niż w grupie kontrolnej ($\chi^2 = 4{,}25$, $p = 0{,}04$). Ponadto, nosiciele allela I mieli trzykrotnie większe ryzyko wystąpienia OBŚ (HR = 2,748, 95% CI = 1,029–7,340, $p < 0{,}05$).
Wnioski: Analiza polimorfizmu genu *ACE* może być przydatna w ocenie ryzyka wystąpienia OBŚ u osób klinicznie predysponowanych do rozwoju tej choroby.

Słowa kluczowe: polimorfizm *ACE*, obturacyjny bezdech w czasie snu, polisomnografia, PCR
Pneumonol. Alergol. Pol. 2013; 81: 207–213

Introduction

Sleep apnoea is defined as the occurrence of multiple and recurring episodes of apnoea (breathing arrest) or hypopnoea (shallow breathing) during sleep. Obstructive sleep apnoea (OSA) is caused by closing of the airway lumen at the level of the pharynx despite normal function of the respiratory muscles [1–4]. This phenomenon is accompanied by hypoxaemia and hypercapnia, with resultant episodes of waking up, leading in turn to sleep fragmentation and worsening of sleep quality [1–4]. Major risk factors for OSA include cervical and abdominal obesity as well as structural abnormalities of upper airways, which decrease airway patency. Most affected patients are men of 45–65 years of age, many of them obese and overconsuming alcohol, and alternatively cigarette smokers [1–11]. Snoring is a sign that is easily detectable for persons within the patient's environment, and strongly suggesting OSA [1–4].

Diagnostics of OSA are based on results of polysomnographic investigations. Young et al. [1, 8] observed the occurrence of obstructive sleep apnoea in approximately 2% of women and 4% of men when investigating the presence of the clinically important syndrome of sleep apnoea consisting of apnoea-hypopnea index (AIH) value over 5 as assessed by polysomnography and signs of somnolence during the daytime. When only aberrant results of polysomnographic investigation were considered, the incidence of sleep apnoea was higher: up to 9% in women and 24% in men. Similar results were reported by Kim et al. [9], who performed polysomnographic investigations in 457 persons, of whom AHI ≥ 5 was found in 27% of men and 16% of women. These findings, alongside the occurrence of pathological signs during the daytime, result in an estimated OSA incidence of 4.5% in men and 3.2% in women. In Poland a similar study was performed by Pływaczewski et al. [10], who investigated 676 subjects. They identified 76 persons with signs of OSA and marked somnolence during the daytime, which represented approximately 11% of the studied population. It needs to be emphasised that incidence of sleep apnoea syndrome was 41% in the subpopulation of patients with body mass index (BMI) over 28 kg/m^2 [11].

Searching for causes of OSA is a multidirectional process since many pathogenetic factors play a role in this entity. Among candidate genes which influence the risk of OSA there are gene polymorphisms which were observed to occur in diseases of similar pathogenesis to OSA. These include apolipoprotein E gene (*APOEK)*, leptin and leptin receptor genes, proinflammatory cytokine genes including *TNFA1*, *IL-6*, *IL-1*, as well as β2--adrenergic receptor gene (*ADRB2*) and angiotensin converting enzyme gene (*ACE*) [5].

It is widely accepted that the renin-angiotensin-aldosterone (RAA) system plays a major role in pathogenesis of cardiovascular diseases. The role of ACE activity in the occurrence of excessive body weight and abdominal obesity has been demonstrated. A key role in this system is played by peptidase, an enzyme that converts angiotensin I (AT I) into angiotensin II (AT II), the latter being a potent vasoconstrictor, promoting hyperplasia of smooth muscle in vascular walls and having prothrombotic activity. Furthermore, ACE cleaves bradykinin, which is a potent vasodilator and has an anticoagulative effect [12–14]. Angiotensin II has also antidiuretic effect, which is related to increased secretion of antidiuretic hormone (ADH) by the pituitary gland. In renal tubules AT II causes increased sodium absorption. The hormone also activates the adrenergic system [12].

Numerous polymorphisms were identified recently in gene coding elements of the renin--angiotensin-aldosterone system. Correlations were also investigated between gene polymorphism and variegated expression of RAA genes. Polymorphisms of the angiotensin converting enzyme gene are attracting a great deal of researchers' attention currently [12–14].

Gene coding for angiotensin I converting enzyme is located on the long arm of chromosome 17, in region 23 (17q23); it has 21 kilobase pairs and consists of 26 exons and 25 introns [12].

In 1990, Rigat et al. [12] described for the first time polymorphism consisting of insertion or deletion of 287 base pairs within one of the noncoding parts of the *ACE* gene. This alteration can result in three genotypes which affect activity of angiotensin converting enzyme (II, DD, ID). The presence of D allele is believed to correlate with 60% higher activity of serum ACE. Homozygotes DD have maximal ACE activity, heterozygotes ID have intermediate, and subjects with II genotype (lack of D allele) have the lowest serum ACE activity. Genotype DD of the *ACE* gene can therefore promote increased production of angiotensin II and increased inactivation of bradykinin [12–14]. Significant correlation was found between polymorphism of the *ACE* gene and cardiovascular morbidity and mortality. Subjects with DD genotype of the *ACE* gene had increased risk of myocardial infarction, hypertrophy of the left ventricle, postinfarct myocardial remodelling, and idiopathic arterial hypertension [12–14].

The aim of the study was to assess the incidence of insertion/deletion polymorphism of the *ACE* gene in patients with obstructive sleep apnoea and healthy subjects, as well as to analyse the influence of this polymorphism on the risk of OSA occurrence.

Material and methods

The study group consisted of 55 persons (46 men and 9 women, aged 32–80 years, with median age of 57 years) hospitalised in the Department of Pneumonology, Oncology, and Allergology of the Medical University in Lublin. Included were patients referred to the department for OSA diagnostics as well as persons with previously diagnosed obstructive sleep apnoea. Sleep apnoea was assessed using Johns' questionnaire, developed in Epworth, Australia (Epworth Sleepiness Scale, ESS). The questionnaire consists of eight questions concerning the patient's daily activity. Interpretation of the answers may disclose mild (< 10 points), moderate (10–16 points), or marked somnolence during the daytime (> 16 points), which requires further diagnostics.

Clinical somnographic investigations were performed using a Somnolab device (Weinmann, Germany) and Somnolab v1.31 SP1 software. Polysomnographic registration included parameters concerning respiration (chest and abdominal movements), airflow in the upper respiratory tract, snoring, body position, heart rate, ECG chart, oximetry, and EEG chart. Only polysomnographic registration lasting for at least four hours was included in the study. Respiratory events were identified automatically and then manually verified by an investigator. Diagnostic criteria for sleep respiratory aberrations were used as recommended by the American Academy of Sleep Medicine (AASM). The mean number of events (apnoea or hypopnoea) of at least five per one hour of registration (AHI ≥ 5) was one of the diagnostic criteria of apnoea syndrome. Sleep apnoea syndrome was diagnosed if AHI ≥ 5 coincided with marked somnolence or at least two signs or symptoms occurring during the daytime or during the night: feeling of choking during sleep, frequent waking up during sleep, feeling of unrest after sleep, feeling of tiredness, and decreased concentration and attention span. Diagnosis was also made when AHI ≥ 5 coincided with ESS ≥ 10 or when patients with no signs or symptoms had AHI ≥ 15 [1–4].

The apnoea-hypopnoea index (AHI) was also used for assessment of OSA severity, classified as mild for AHI 5–15 and falling asleep in situations that did not require much attention, moderate OSA at AHI 16–30 and patient falling asleep in situations requiring more attention, or severe OSA when AHI was > 30 and the patient was falling asleep during activities requiring maximal concentration [1–4].

Characteristics of patients with diagnosed OSA are presented in Table 1.

The control group consisted of 50 healthy persons aged 22–89 years (median age 60 years), in whom OSA was excluded using AASM criteria [1].

Each person from the study or control group completed a written informed consent and a venous blood sample was used for genetic testing. Blood samples were taken into tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at –20ºC. Isolation of DNA from peripheral blood was performed using QIAamp® DNA Blood Mini kit (QIAGEN, USA). Assessment of DNA quality was performed using a BioPhotometer spectrophotometer with cuvettes and UV/VIS filters (Eppendorf, Germany). Starter pairs of the following sequence were used for amplification of DNA fragments containing the investigated polymorphism site of the *ACE* gene (insertion/deletion): forward starter, 5' CTG GAG AGC CAC TCC CAT CCT TCT 3`; reverse starter (zero starter), and 5' GAC GTG GCC ATC ACA TTC GTC AGA TC 3'. Polymerase chain reaction (PCR) was performed in the TPersonal thermocycler (Biometra, USA) with the following sequence: initial denaturation at 96ºC for 15 min, followed by 30 cycles of denaturation at 95ºC for 30 s, hybridisation with starters at 61ºC for 30 s, and elongation at 72ºC for 2.5 minutes. After completion of all cycles, final elongation occurred at 72ºC for 10 minutes.

Table 1. Characteristics of patients with obstructive sleep apnoea/hypopnea syndrome

Assessed parameter	Characteristics
Age (median value ± standard deviation)	57 ± 10.4 years
Sex	
Men (n, %)	46 (83.6%)
Women (n, %)	9 (16.4%)
Cigarette smoking	
Current smokers	15 (27.3%)
Past or non-smokers	40 (72.7%)
Body mass index (BMI, median value ± standard deviation)	33 ± 5.6
Concomitant diseases	
Arterial hypertension	44 (80%)
Type 2 diabetes	11 (20%)
Chronic obstructive pulmonary disease (COPD)	18 (32.7%)
Coronary heart disease	7 (12.7%)
Metabolic syndrome	33 (60%)
Obesity	40 (72.7%)
OSA severity	
Mild	7 (12.7%)
Moderate	17 (30.9%)
Severe	31 (53.4%)
Apnoea-hypopnoea index (AHI, median value ± standard deviation)	31 ± 19.1

The obtained PCR products were 190 base pairs (bp) for allele D or 480 bp for allele I. Separation of DNA was performed on 2% agarose gel with ethidium bromide. After completion of electrophoresis, DNA bands were visualised using transiluminator under UV light. Figure 1 presents separated products of PCR reaction for *ACE* gene polymorphism. For homozygous genotypes II and DD, a single band was present, representing 480 bp product (lane 1, 8, 9, 10) or 190 bp product (lane 3, 4, 11, 12, 15), respectively. Electrophoresis of PR products from heterozygous ID subjects produced two bands simultaneously: one of 480 bp and one of 190 bd (lanes 2, 5, 6, 7, 13, 14).

Distribution of respective genotypes and alleles in both investigated groups was assessed according to the Hardy-Weinberg principle. Differences in incidence of respective alleles and genes between the groups were analysed using the χ^2 test. The risk of OSA was assessed

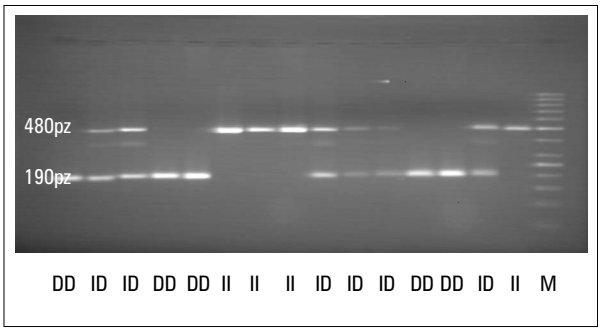

Figure 1. The representative electrophoregram of *ACE* gene I/D polymorphism. Lines by the order of the DNA marker: 1, 8, 10 — homozygous II; 2, 5, 6, 7, 13 and 14 — heterozygous ID; 3, 4, 11, 12 and 15 — homozygous DD

using the logistic regression test. Statistica 9.0 software was used for analyses. For evaluation of deviation from the Hardy-Weinberg equilibrium and of associations, a test available at the http://ihg.gsf.de/ihg/snps.html website was applied. Statistical significance level was adopted for $p < 0.05$.

Results

In the group of patients with OSA, II genotype was found in 17 persons (30.9%), ID genotype in 21 (38.2%), and DD genotype in 17 (30.9%). Incidences of respective genotypes in the control group were as follows: II genotype in 7 patients (14%), ID genotype in 25 (50%), and DD genotype in 18 (36%). The observed distribution of polymorphisms was concordant with the Hardy-Weinberg equilibrium.

Statistical analysis revealed no significant differences in incidence of respective *ACE* genotypes between the study and the control group (Fig. 2). However, it should be emphasised that the II genotype was observed more often in patients with sleep apnoea than in the control group ($\chi^2 = 4.31$; $p = 0.116$). Furthermore, D allele (both DD homozygotes and ID heterozygotes) was more often identified in healthy subjects (43 persons, 86%) than in patients with OSA (38 patients, 69.1%; $\chi^2 = 4.25$, $p = 0.04$) (Fig. 3).

Subjects bearing the II genotype of the *ACE* gene had a significantly higher risk of developing OSA than subjects with ID genotype (HR [hazard ratio] = 2.891, 95% CI [confidence interval] = 1.007–8.297; $\chi^2 = 4.03$, $p = 0.0447$) and D allele carriers, including subjects with ID or DD genotype (HR = 2.748, 95% CI = 1.029–7.340; $\chi^2 = 4.25$, $p = 0.03932$).

There were no significant differences in incidence of respective *ACE* genotypes in patients with

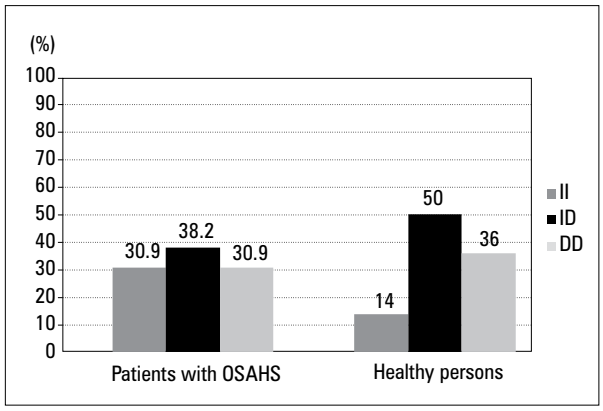

Figure 2. Distribution of ACE genotypes in patients with OSAHS and in healthy persons

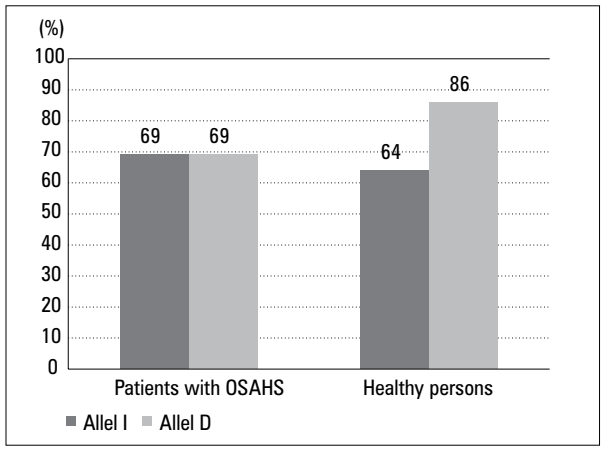

Figure 3. The frequency of allele I and allele D of *ACE* gene in patients with OSAHS and in healthy persons

OSA and various concomitant diseases, including arterial hypertension, metabolic syndrome, and chronic obstructive pulmonary disease (COPD). Polymorphism of the *ACE* gene had no impact on OSA severity, as the median AHI value was similar in subgroups of different genotypes. The lack of significant correlation between the presence of I allele and OSA severity may be linked to a high number of patients with severe OSA in the studied population and few subjects with mild OSA.

Discussion

Most studies published to date concern the relationships between ACE activity and *ACE* polymorphism and risk of OSA complications, including arterial hypertension, as well as the occurrence of other diseases coexisting with OSA. The authors of the presented study performed genetic testing in order to assess the incidence of insertion/deletion polymorphism of the *ACE* gene in patients with OSA and in healthy control subjects presenting no respiratory disturbances during sleep. Distribution of II, ID, and DD genotypes did not differ significantly between the two groups, although the II genotype was slightly more common in subjects with OSA compared to healthy subjects. These results are in accordance with data published by other researchers.

Analysis of *ACE* polymorphism in Polish patients was previously performed by the group of Renata Rubinsztajn and Ryszarda Chazan in a population of 62 persons with OSA [15]. These authors reported a similar distribution of *ACE* polymorphism, with II genotype identified in 23.8% of patients, ID genotype in 47.6%, and DD genotype in 28.6%. Patient stratification according to concomitant cardiovascular diseases and family tendency for OSA development did not significantly alter genotypic distribution.

One of the first reports concerning the incidence of respective polymorphisms of the *ACE* gene in patients with OSA was published by Ogus et al.[17]. This study included a population of 101 Turkish persons, including 64 patients with OSA and 37 subjects in the control group. Distribution of *ACE* genotypes was as follows: 27 patients had ID genotype, 27 had DD genotype, and 10 had II phenotype. There were no significant differences in genotypic distributions between the study and the control group. Barcelo et al. [17] reported similar observations based on analysis of the *ACE* genotype in 44 patients with OSA and 32 healthy subjects.

A study published by Lee et al. [18] seems to be the most reliable as it was performed in a population of 1,227 patients with OSA and 1,227 healthy subjects. The authors did not observe any correlation between *ACE* gene polymorphism and risk of OSA development or disease severity. Risk of developing OSA was 0.92 for carriers of D allele (95% CI = 0.69–1.23). No significant correlation between the presence of D allele and risk of OSA was observed after stratification according to ethnic origin or occurrence of other diseases, particularly arterial hypertension. However, the population investigated by Lee et al. was dominated by subjects of Asian ethnicity.

Contrary to the results reported by Lee et al., the authors of the presented study observed a significantly lower incidence of D allele of the *ACE* gene in patients with OSA compared to healthy subjects. Furthermore, the presence of I allele positively correlated with the risk of OSA development.

Ogus et al. did not observe significant differences in the incidence of the respective *ACE* genotypes between patients with OSA and healthy persons in a Turkish population; however, the I allele was present more often in patients with sleep apnoea than in control subjects in their study ($p = 0.02$), and the risk of developing apnoea in group of I allele carriers was 2.41. These authors suggest that II and ID genotypes of the *ACE* gene may correlate with a higher risk of developing apnoea in the Turkish population [16].

However, the hypothesis of I allele increasing the risk of OSA development seems contradictory to publications concerning the relationships between *ACE* gene polymorphisms and modulation of skeletal muscle tone. It was observed that the endurance and strength of skeletal muscles during physical exercise in patients with COPD is higher in carriers of the I allele as compared to persons with DD genotype. This mechanism can possibly be protective against collapse and obturation of the upper respiratory tract in patients with OSA. This hypothesis warrants further investigation [19, 20].

As mentioned previously, most authors focused on correlations between *ACE* gene polymorphisms and the risk of developing cardiovascular diseases in patients with OSA. Koyama et al. [21] noted that I allele occurs more often in men with arterial hypertension and mild-to-moderate sleep apnoea but not with severe apnoea. These results led the authors to the hypothesis that the presence of I allele of the *ACE* gene in men suffering from arterial hypertension has a protective effect against severe OSA. This study included 266 Brazilian patients with OSA [16].

Zhang et al. [19] demonstrated that I allele of the *ACE* gene occurs more often in subjects with coexisting arterial hypertension and moderate-to--severe OSA as compared to healthy persons. No such correlation was observed for I allele in patients with arterial hypertension and mild OSA versus control persons. The authors also reported that central obesity in patients with OSA and hypertension correlate with the presence of the D allele in the *ACE* locus. However, polymorphisms of the *ACE* gene were not investigated in patients with OSA who did not have arterial hypertension. Furthermore, the study included 174 patients of Asian origin, coming from the Han population, thereby impeding direct result comparison with studies concerning Caucasian subjects, even though the results seem to be similar.

The biggest study concerning correlations between *ACE* gene polymorphisms and risk of arterial hypertension in patients with OSA enrolled 1,100 mostly Caucasian persons (Wisconsin Sleep Cohort Study). Lin et al. [22] point to the fact that the risk of developing arterial hypertension by patients with mild-to-moderate OSA is correlated with presence of the D allele. These authors found the highest values of increased arterial pressure in subjects with DD genotype, and the lowest for the type II genotype. Interestingly, this correlation was most significant for patients with mild OSA. Similar results were reported by Boström et al. [23, 24], who compared 157 patients with OSA and arterial hypertension with 181 persons suffering from OSA but with no hypertension. Irrespective of OSA severity, the presence of the D allele increased the risk of developing arterial hypertension in patients with sleep apnoea. These results are contradictory to those published by Patela et al. [24, 25], who studied 972 subjects during the Cleveland Family Study. Those authors reported a lower risk of arterial hypertension in carriers of the D allele in the *ACE* gene locus.

Such contradictory results of studies on the role of *ACE* polymorphism in development of arterial hypertension among patients suffering from OSA warrant further investigations and trials conducted in large patient populations.

Conclusions

1. The incidence of the respective *ACE* genotypes in patients with OSA and healthy subjects was similar; however, the D allele of this gene was less often present in persons with signs of OSA. The presence of I allele increased the risk of developing OSA, which may suggest that polymorphism of the *ACE* gene influences genetic predisposition to this disease.
2. Analysis of *ACE* polymorphism may be useful in the assessment of risk of OSA development in subjects with clinical signs suggesting predisposition for the disease.

Conflict of interest

The authors declare no conflict of interest.

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