Polymorphisms in the 5-HTR2A gene related to obstructive sleep apnea syndrome

Vânia Belintani Piatto¹, Thiago Bittencourt Ottoni de Carvalho², Nely Silva Aragão De Marchi³, Fernando Drimel Molina⁴, José Victor Maniglia⁵

Abstract

Obstructive sleep apnea syndrome (OSAS) is one of the most complex disorders of sleep; it involves several genetic factors that contribute to the phenotype. Serotonin (5-HT) regulates a variety of visceral and physiological functions, including sleep. Gene 5-HTR2A polymorphisms may change the transcription of several receptors in the serotoninergic system, thereby contributing to OSAS.

Aim: To investigate the prevalence of T102C and -1438G/A polymorphisms in the 5-HTR2A gene of patients with and without OSAS.

Material and Method: A molecular study of 100 index-cases and 100 controls of both genders. DNA was extracted from blood leukocytes samples and the regions that enclose both polymorphisms were amplified with PCR-RFLP.

Study design: A cross-sectional case study.

Results: There was a significant prevalence of males in index cases compared to controls (p<0.0001). No significant genotypic differences between cases and controls were found in T102C polymorphisms (p=1.000). There were significant differences between the AA genotype of -1438G/A polymorphisms and patients with OSAS (OR:2.3; CI95%:1.20-4.38, p=0.01).

Conclusion: Serotonergic mechanisms may be related to OSAS. There were no differences in the prevalence of T102C polymorphisms in patients with OSAS and the control group. There is evidence of an association between the -1438G/A polymorphism and OSAS.

Keywords: polymorphism genetic, receptor serotonin 5-htr2a, serotonin, sleep apnea obstructive.
INTRODUCTION

The obstructive sleep apnea syndrome (OSAS) is a common disorder of sleep affecting from 2% to 4% of adult males. It may be characterized by recurring sleep-induced pharyngeal airway collapse resulting in hypoxemia and hypercapnia. Several genetic factors are probably involved, since there are also many phenotypic components.

Changes in central nervous control of upper airway muscles are considered an important component of this syndrome. The genioglossus muscle is innervated by neurons that originate in the hypoglossal nucleus within the brainstem; contractions of this muscle during inspiration help ventilate the lungs and keep upper airways open during wakefulness and sleep. Groups of serotonergic and noradrenergic cells may generate parallel patterns by stimulating directly the motor cells of the hypoglossal nerve, thereby regulating these dilating muscles. Thus, activation of these cell groups increases the activity of the genioglossus muscle.

It is evident that serotonin (5-hydroxytryptamine or 5-HT), a central nervous system neurotransmitter, is involved in regulating several visceral and physiologic functions such as sleep, appetite, thermoregulation, pain perception, hormone secretion, and sexual behavior. Changes in the serotonergic system have been implied in several human diseases such as depression, headaches, epilepsy, obsessive-compulsive behaviors, temporomandibular dysfunction, and affective disorders.

Serotonin also has a relevant role in upper airway patency, as it excites airway motor neurons and intrinsically excites the motor neurons in the brainstem. The activity of neurons that provide 5-HT to motor neurons declines with sleep; most of the functional studies have shown that 5-HT and serotonergic neurons exert a significant excitatory effect on respiratory motor neurons in vivo and in vitro. The activity of these neurons is maximal during wakefulness, and minimal during REM sleep. The sleep-related behavior of 5-HT on motor neurons results in upper airway collapse and the ensuing respiratory obstruction.

Serotonin operates by means of a large family of 5-HT receptors; these include the 5-HT2 receptors that comprise three subtypes (5-HT2A, 5-HT2B, and 5-HT2C) that have a similar molecular structure, pharmacology, and transduction signal patterns. The 5-HT2A and 5-HT2C subtypes have an important role in keeping upper airways stable and maintaining normal breathing in obese subjects. One of the receptor subtypes for serotonin - 5-HT2A - is mostly excitatory for the motor neurons of the hypoglossal muscle; the 5-HT2C subtype is excitatory for neurons in several areas of the brain. Although few excitatory responses have been analyzed with selective agents for receptor subtypes, evidence suggests that in some cases 5-HT2A mediates the responses, while in others, 5-HT2C is involved.

The genes in the family 5-HT2 receptors (named HTR2 genes) have two introns (for genes 5-HTR2A and 5-HTR2B) or three (5-HT2C gene) in the coding sequence, which are all coupled positively to phospholipase C, and which mobilize intracellular Ca++. The 5-HTR2A gene is on chromosome 13 (13q14-q21); it has a relatively high amino acid sequence, identical to 5-HTR2C, although lower compared to the 5-HTR2B gene. The 5-HTR2C gene is located on chromosome X (Xq24).

A functional promoting variant of the 5-HT2A gene may differentially alter transcription, thereby changing the number of receptors. Polymorphisms in the 5-HT2 gene are associated with several diseases, including the OSAS; it affects the serotonergic system, thereby contributing to airway collapse during sleep. The excitatory effects of the 5-HT2C receptor are small; although the 5-HT2C gene polymorphism is functional, the effect of an active polymorphic allele may not be sufficient for this polymorphism to be associated with the apnea/hypopnea index (AHI) because this subtype of receptor is less dominant in the nucleus of the hypoglossus. Conversely, a 5-HT2A gene polymorphism has recently been found in patients with OSAS: it is defined by a T>C substitution in the nucleotide position 102, and a G>A substitution in position -1438 of the promoting region of the gene.

OBJECTIVE

The purpose of this study was to investigate the prevalence of T102C and -1438G/A polymorphisms in the 5-HT2A gene with the polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) test in a sample of patients with and without the OSAS.

MATERIAL AND METHOD

The institutional review board approved this study (Opinion no. 342/2006) based on the regulating norms for research on human beings - Resolution 196/96 of the Ministry of Health.

A cross-sectional cohort study was carried out from 1 July 2008 to 30 June 2010 of 100 index cases with the OSAS. There were 73 male and 27 female subjects with ages ranging from 23 to 70 years. The control group comprised 100 patients (40 male and 60 female) aged from 17 to 70 years. A full medical history was taken to investigate the presence of agitated sleep, night apnea, daytime drowsiness, medication use, arterial hypertensi-
sion, and depression. A systemic and otorhinolaryngo-
logical physical examination was carried out to assess
the body mass index (BMI), the neck diameter, neck
tumors, craniofacial malformations, and the nasal and
oral cavities. Subjects then underwent general and spe-
cific tests for OSAS - cephalometry, nasofibroscopy with
the Müeller maneuver, and polysomnography.

The following inclusion criteria were applied:
a) meeting criteria A or B, plus C for the diagnosis
of the syndrome.
A. excessive daytime drowsiness not explained
by other factors;
B. two or more of the following findings not ex-
plained by other factors:
gasping during sleep;
recurring awakenings from sleep;
restless sleep, daytime fatigue;
poor concentration.
C. nighttime monitoring demonstrating more than
five episodes of respiratory obstruction per hour during
sleep. These events may include any combination of
obstructive apnea/hypopnea or respiratory efforts with
awakening.
b) absence of other somatic or laboratory findings
in the general physical examination and tests;
c) absence of craniofacial dimorphism or tempo-
romandibular conditions, as seen in the specific physical
examination;
d) absence of drug dependency, alcoholism,
depression, or dementia, as investigated in the clinical
history and psychiatric assessments;
e) absence of apparent genetic syndromes, as
investigated by the clinical/genetic physical examination;
f) presence or absence of other cases in the family;
g) maximum age - 70 years.
h) BMI ≤ 35.
Exclusion criteria:
a) Patients aged over 70 years, as the prevalence
of central sleep apnea increases with age;
b) presence of psychiatric disorders;
c) altered laboratory tests, and findings on the
general and specific physical examination;
d) not presenting the minimal criteria for diagno-
sing the syndrome;
e) BMI > 35.
Patients for the control group were selected based
on the same criteria above for patients with the OSAS,
except for the AHI/hour, which for this group was ≤ 5.
The following data were gathered: age at the mo-
moment polysomnography was done, the BMI, and the AHI
from the recordings of the polysomnography equipment
(Stellat System QC, Harmonie TM, Canada). Patients
were allocated to the following age groups: adolescent
(11 to 17 years), young adult (18 to 40 years), adult (41
to 65 years), and elderly (> 65 years). Patients were
selected based on the following classification based
on the AHI: mild obstructive sleep apnea hypopnea syn-
drome (OSAHS) - AHI = 5 to 15.9 events/hour, moderate
OSAHS - AHI = 16 to 30 events/hour, severe OSAHS
- AHI > 30 events/hour. Patients were also grouped
according to the BMI based on the WHO classification:
ideal weight (18.5-24.9 kg/m²), overweight (25.0-29.9 kg/
m²), obesity grade I (30.0-34.9 kg/m²), and obesity grade
II (35.0-39.9 kg/m²). A BMI ≤ 35 limit was established
to avoid effects of higher obesity grades on the AHI.

Molecular study
A total blood sample (4.0 mL) was collected in a
Vacutainer® tube with anticoagulant (EDTA), after con-
sent was obtained from patients or caretakers. Genomic
DNA was extracted from blood samples using the Illusta
Blood GenomicPrep Mini Spin Kit (GE Healthcare™)
according to the manufacturer’s instructions.

Nuclear DNA fragments including the polymor-
phic region of the 5-HT2A gene were amplified by the
polymerase chain reaction (PCR) using the FidelITaq™
PCR MasterMix (2X) (GE Healthcare®) kit to detect both
coding polymorphisms (T102C and -1438G/A). In this reaction
two pairs of primers - synthetic oligonucleotides - were
used; the oligonucleotide sequence in the primers and
the PCR conditions were taken from the literature.8,9

A 342 pb fragment was amplified as product of the
PCR for the T102C polymorphism; it was then submitted
to the restriction analysis (restriction fragment length
polymorphism or RFLP) using the MspI enzyme (New
England Biolabs)TM for 2:30 h at 37°C. The
-1438G/A polymorphism (both mutant alleles) yields two fragments
(217 pb and 125 pb) because of recognition of the MspI
enzyme restriction site by substitution of the T->C nitro-
genated bases in the position 102 of the
5-HT2A gene. The heterozygote samples (TC) for this polymorphism,
after enzyme digestion, yielded three fragments: 342
pb (wild allele-T), 217, and 125 pb (mutant allele-C).
Samples with no polymorphism, therefore normal ho-
mozygotes (TT), contain only a 342 pb fragment, as the
enzyme site is not recognized.

Similarly, to detect the -1438G/A polymorphism,
a 469 pb fragment was obtained following PCR; it was
subjected to RFLP with the MspI enzyme (New England
Biolabs)TM for 2:30 h at 37°C. The
-1438G/A polymorphism yields two fragments (243 pb
and 226 pb) because of recognition of the MspI enzyme
restriction site. The mutant homozygotic samples (AA) for this polymorphism contain a single 469 pb fragment, as the enzyme site is not recognized by substitution of the G->A nitrogenated bases in the position -1438 of the 5-HTR2A gene; the heterozygotic samples (GA) contain three fragments: 469 pb (mutant allele-A), 243, and 226 pb (wild allele-G).

The products of both reactions (PCR/RFLP) were analyzed using 2% agarose gel electrophoresis in a TBE 1X buffer containing ethidium bromide at 0.5 mg/mL, under ultraviolet light, to confirm the success of the reaction; the gel was photographically documented.

Statistics
Descriptive statistics was applied to establish normalcy of results. Student’s t test (two-tailed) was applied for independent samples with a normal distribution; the Mann-Whitney test was used for samples with a non-normal distribution. If applicable, the chi-square test was used for comparisons among variables, and the odds ratio (95% confidence interval). The significance level was 5%. The Hardy-Weinberg equilibrium analysis was made for the genotype distribution.

The statistical tests were done using the GraphPad InStat version 3.00 software (GraphPad Software Inc; San Diego California USA; www.graphpad.com).

RESULTS
Of 100 patients with apnea (index cases), 73 (73%) were male and 27 (27%) were female. The age ranged from 23 to 70 years in males (mean - 50.1 years; SD ± 12.5) and 43 to 65 years in females (mean - years; SD ± 6.0). The BMI ranged from 21.2 to 35 kg/m² in males (mean - 29.5 kg/m²; SD ± 3.8), and 21 to 35 kg/m² in females (mean - 28.6 kg/m²; SD ± 3.5). The AHI ranged from 5.8 to 115 in males (mean - 37.5; SD ± 26.3) 5.4 to 79 in females (mean - 19.6; SD ± 19.3).

Of 100 patients in the control group, 40 (40%) were male and 60 (60%) were female. The age ranged from 17 to 66 years in males (mean - 46.1 years; SD ± 12.3) and 21 to 70 years in females (mean - 43.6 years; SD ± 11.7). The BMI ranged from 20 to 34.6 kg/m² in males (mean - 26.8 kg/m²; SD ± 3.9) and 20 to 35 kg/m² in females (mean - 27.1 kg/m²; SD ± 4.0). The AHI ranged from 0 to 4.9 in males (mean - 2.3; SD ± 1.5) and 0 to 4.9 in females (mean - 1.6; SD ± 1.4).

Table 1 presents demographic data for the 100 index cases and 100 controls; Table 2 presents these data as means ± standard deviation.

Males predominated among index cases (73%) and females predominated in the control group (60%), which was significant ($p<0.0001$).

### Table 1. Distribution of demographic data of index cases (n=100) compared to the control group (n=100).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Index-cases n (%)</th>
<th>Controls n (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>73 (73)</td>
<td>40 (40)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female</td>
<td>27 (27)</td>
<td>60 (60)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ideal weight</td>
<td>13 (13)</td>
<td>32 (32)</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>44 (44)</td>
<td>41 (41)</td>
<td>0.0081</td>
</tr>
<tr>
<td>Obesity grade I</td>
<td>38 (38)</td>
<td>24 (24)</td>
<td></td>
</tr>
<tr>
<td>Obesity grade II</td>
<td>05 (05)</td>
<td>03 (03)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>0 (0)</td>
<td>01 (0)</td>
<td></td>
</tr>
<tr>
<td>Young adult</td>
<td>12 (12)</td>
<td>35 (35)</td>
<td>0.0007</td>
</tr>
<tr>
<td>Adult</td>
<td>80 (80)</td>
<td>61 (61)</td>
<td></td>
</tr>
<tr>
<td>Elderly</td>
<td>08 (08)</td>
<td>03 (03)</td>
<td></td>
</tr>
<tr>
<td>Data gathered during polysomnography n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>--</td>
<td>100 (100)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>40 (40)</td>
<td>--</td>
<td>NA</td>
</tr>
<tr>
<td>Moderate</td>
<td>15 (15)</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>45 (45)</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Distribution of demographic data of index cases (n=100) compared to the control group (n=100).

BMI - body mass index.
AHI - apnea/hypopnea index.
NA - Not analyzed.
*pChi-square test.

### Table 2. Clinical and polysomnographic parameters of index cases compared to controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Index-cases (n=100)</th>
<th>Controls (n=100)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (grade)</td>
<td>29.3 ± 3.7</td>
<td>27.0 ± 3.9</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.6 ± 11.1</td>
<td>44.6 ± 12.0</td>
<td>p=0.0004+</td>
</tr>
<tr>
<td>AHI</td>
<td>32.6 ± 25.7</td>
<td>1.9 ± 1.5</td>
<td>p&lt;0.0001+</td>
</tr>
</tbody>
</table>

Table 2. Clinical and polysomnographic parameters of index cases compared to controls.

Values are presented as the mean ± standard deviation.
BMI - body mass index.
AHI - apnea hypopnea index.
*Student’s t test (two-tailed) for independent samples.
+Mann-Whitney test.

The group of patients with OSAS had a higher prevalence of overweight and obesity grade I in the BMI classification -82% of cases; in the control group, ideal weight and obesity grade I were more prevalent (73%). This difference between groups was significant ($p=0.0081$).

The highest age prevalence in both groups (index
cases and controls) was in the young adult (92%) and adult (96%) ranges. There were no adolescents among the index cases. The statistical analysis of this variable in index cases and controls showed that it was significant ($p<0.0001$).

Table 2 presents the mean BMI values, the age, and the AHI, which were significantly higher among the index cases compared to the mean values in the control group.

Tables 3 to 7 present the molecular results and the comparison among variables.

**Table 3. Distribution of genotypes in index cases and controls for T102C and -1438G/A polymorphisms of the 5-HTR2A gene.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Index-cases n (%)</th>
<th>Controls n (%)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>23 (23)</td>
<td>20 (20)</td>
<td>0.8711</td>
</tr>
<tr>
<td>TC</td>
<td>66 (66)</td>
<td>69 (69)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>11 (11)</td>
<td>11 (11)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>4 (4)</td>
<td>10 (10)</td>
<td>0.0177</td>
</tr>
<tr>
<td>GA</td>
<td>61 (61)</td>
<td>71 (71)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>35 (35)</td>
<td>19 (19)</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test.

**Table 4. Genotype relation between index cases and controls for T102C and -1438G/A polymorphisms of the 5-HTR2A gene.**

<table>
<thead>
<tr>
<th>Genótipo (Casos x Controles)</th>
<th>Odds ratio (OR)</th>
<th>IC 95%</th>
<th>Chi-square</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT and TC (89 x 89)</td>
<td>1.0</td>
<td>0.41-2.43</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>CC (11 x 11)</td>
<td>1.0</td>
<td>0.41-2.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG and GA (65 x 81)</td>
<td>0.44</td>
<td>0.23-0.83</td>
<td>5.71</td>
<td>0.0169</td>
</tr>
<tr>
<td>AA (35 x 19)</td>
<td>2.3</td>
<td>1.20-4.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI - 95% confidence interval.

**Table 5. Gender distribution of index cases and controls relative to the genotypes for T102C and -1438G/A polymorphisms of the 5-HTR2A gene.**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Index-cases</th>
<th>Controls</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n (%)</td>
<td>Female n (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male n (%)</td>
<td>Female n (%)</td>
<td></td>
</tr>
<tr>
<td>TT and TC</td>
<td>64</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>CC</td>
<td>9</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>GG and GA</td>
<td>40</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>AA</td>
<td>33</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

*Chi-square test.
cases in adult males; only 8% of cases were aged over 65 years.

The T102C polymorphism appears to be unrelated with OSAS, as the control group had the same frequency as index cases for the TT/TC and CC genotypes; this finding concurs with other published results. Although the 5-HT2A receptor is mostly related with serotonin (5-HT) excitation within the representative motor nucleus of upper airways, lack of common polymorphisms or epigenetic changes that alter messenger RNA levels for the 5-HTR2A gene may explain the absence of association between the T102C polymorphism and OSAS in this population; it is also considered a silent polymorphism and was present in a similar proportion in the control group.

The genotype AA was significantly higher in index cases, whereas the genotypes GG/GA were higher in controls, meaning that relative to the -1438G/A polymorphism, the genotype AA may be associated with OSAS in the study population; this finding concurs with other published results.

There was a significant gender difference between index cases and controls in relation to the -1438G/A polymorphism genotypes, which was not the case with T102C polymorphism genotypes. The comparison of genotypes with gender was done because of a higher prevalence of OSAS in males, as shown in the literature.

There was no correlation between the AHI (obtained during polysomnography) and the genotypes of both polymorphisms in index cases, which again concurs with the literature.

This condition may suggest that other mechanisms besides 5-HTR2A gene polymorphisms may be involved in the pathophysiology of OSAS. It is evident that OSAS is a phenotype of several related or unrelated disorders, and that it results from a complex association between genes and environmental modifying factors. This may explain the statistically significant absence of association between both polymorphisms and the polysomnographic findings in the present study.

**Table 6.** Distribution of the BMI (body mass index) of index cases and controls relative to the genotypes for T102C and -1438G/A polymorphisms of the 5-HTR2A gene.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Index-cases n (%)</th>
<th>Controls n (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 ≤ BMI &lt; 25 Kg/m²</td>
<td>25 ≤ BMI ≤ 35 Kg/m²</td>
<td></td>
</tr>
<tr>
<td>TT and TC</td>
<td>12 (12)</td>
<td>77 (77)</td>
<td>0,7542</td>
</tr>
<tr>
<td>CC</td>
<td>1 (1)</td>
<td>10 (10)</td>
<td></td>
</tr>
<tr>
<td>GG and GA</td>
<td>6 (6)</td>
<td>59 (59)</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>7 (7)</td>
<td>28 (28)</td>
<td></td>
</tr>
</tbody>
</table>

*Chi-square test.

**Table 7.** Distribution of the AHI (apnea/hypopnea index) of index cases relative to the genotypes for T102C and -1438G/A polymorphisms of the 5-HTR2A gene.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Index-cases n (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 ≤ AHI ≤ 30</td>
<td>AHI &gt; 30</td>
</tr>
<tr>
<td>TT and TC</td>
<td>50 (50)</td>
<td>39 (39)</td>
</tr>
<tr>
<td>CC</td>
<td>5 (5)</td>
<td>6 (6)</td>
</tr>
<tr>
<td>GG and GA</td>
<td>38 (38)</td>
<td>27 (27)</td>
</tr>
<tr>
<td>AA</td>
<td>17 (17)</td>
<td>18 (18)</td>
</tr>
</tbody>
</table>

*Chi-square test.

**DISCUSSION**

The serotonergic system is an important component of sleep and relaxation of airways during sleep. Thus, factors that affect this system may result in sleep and respiratory disorders. The 5-HT2A receptor is an essential component of the serotonergic system; it is under genetic control. Gene polymorphisms that code for the receptor may alter the functional state and serotonergic activity. Thus, our study aimed to investigate the prevalence of T102C and -1438G/A polymorphisms of the 5-HTR2A gene in patients with and without OSAS. Until this time there were no published Brazilian papers on this topic; only two studies in the literature had reported the frequency of these polymorphisms and their association with OSAS, which underlines the importance of the present study.

All subjects underwent polysomnography to obtain the AHI, through which the diagnosis of OSAS could be made to compose the groups (OSAS and controls). This criterion was followed strictly so that no patient with only a clinical exclusion diagnosis of OSAS is included in the control group, as was done in other published studies.

The OSAS is more frequent in men of all age groups, although the prevalence increases with age. As in the literature, we found a higher prevalence of index cases in adult males; only 8% of cases were aged over 65 years.

The T102C polymorphism appears to be unrelated with OSAS, as the control group had the same frequency as index cases for the TT/TC and CC genotypes; this finding concurs with other published results. Although the 5-HT2A receptor is mostly related with serotonin (5-HT) excitation within the representative motor nucleus of upper airways, lack of common polymorphisms or epigenetic changes that alter messenger RNA levels for the 5-HTR2A gene may explain the absence of association between the T102C polymorphism and OSAS in this population; it is also considered a silent polymorphism and was present in a similar proportion in the control group.

The genotype AA was significantly higher in index cases, whereas the genotypes GG/GA were higher in controls, meaning that relative to the -1438G/A polymorphism, the genotype AA may be associated with OSAS in the study population; this finding concurs with other published results.

There was a significant gender difference between index cases and controls in relation to the -1438G/A polymorphism genotypes, which was not the case with T102C polymorphism genotypes. The comparison of genotypes with gender was done because of a higher prevalence of OSAS in males, as shown in the literature.

There was no correlation between the AHI (obtained during polysomnography) and the genotypes of both polymorphisms in index cases, which again concurs with the literature.

This condition may suggest that other mechanisms besides 5-HTR2A gene polymorphisms may be involved in the pathophysiology of OSAS. It is evident that OSAS is a phenotype of several related or unrelated disorders, and that it results from a complex association between genes and environmental modifying factors. This may explain the statistically significant absence of association between both polymorphisms and the polysomnographic findings in the present study.
Airway size variations are probably defined by genetic influences on bone structure, and tongue and tonsil size; there may also be other factors that are acquired because of obesity. Although 5-HTR2A gene polymorphisms may have an important role in the activity of pharyngeal dilating muscles, other factors such as cephalometric characteristics and soft tissue structures may be determinant for opening the airways during sleep. Absence of craniofacial dimorphism and temporomandibular alterations and a BMI ≤ 35 kg/m² were two of the inclusion criteria for this study. These criteria were applied strictly to exclude these variables - and others - which affect upper airway patency. Thus, when the BMI was compared with the genotypes of both polymorphisms in index cases and controls, the BMI from 25 to 35 kg/m² was related significantly with the -1438G/A polymorphism in index cases; this result concurs with those of the literature, as it results from more cases in this BMI range rather than the effect of the polymorphism.

Therefore, the T102C polymorphism appears to be unrelated with OSAS in our sample, compared with the -1438G/A polymorphism. This in turn may be associated with OSAS in the study population, especially in males with a BMI from 25 to 35 kg/m², since the prevalence was higher among patients presenting these variables.

Genetic studies are being carried out to clarify some of the complexities of sleep and to open new therapeutic approaches for treating sleep disorders. Genes are probably less involved in changes of state (non-REM->REM) that are seen electrophysiologically during the sleep cycle; rather, genes are certainly related with the circadian rhythm and sleep homeostasis. The most common sleep disorders result from the interaction of several genes and environmental factors. Understanding the impact of genetic factors will help elucidate the pathophysiology of sleep disorders, and may provide support for therapeutic approaches.

CONCLUSION

Using molecular techniques (PCR/RFLP) we identified T102C and -1438G polymorphisms of the 5-HTR2A gene, thereby adding to the molecular investigation of the OSAS. Serotonergic mechanisms may be related with the OSAS. There were no differences in the prevalence of the T102C polymorphism among OSAS patients and controls. There is evidence that the -1438G/A polymorphism is associated with the OSAS.

REFERENCES

25. Riha RL. Genetics aspects of the obstructive sleep apnoea/hypopnoea syndrome - is there a common link with obesity? Respiration. 2009;78:5-17.