INFLUENCE OF GENETIC ANCESTRY ON THE RISK FOR OBSTRUCTIVE SLEEP APNOEA SYNDROME

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ABSTRACT

The present study aimed to evaluate the influence of ethnicity in the risk of developing OSAS, using genomic analysis methods to estimate ancestry.

DNA samples were obtained from 1010 individuals participating in the Epidemiologic Sleep Study of Sao Paulo, who underwent full-night polysomnography. A total of 31 genetic ancestry informative markers were selected to estimate individual admixture proportions.

Patients with a diagnosis of OSAS demonstrated a higher number of individuals self-reporting Caucasian ethnicity (65.3%), as well as increased percentage of European ancestry (78.2±16.7%) and lower percentage of West-African ancestry (16.1±15.3%) compared to individuals without OSAS (53.6%, 73.5±18.1% and 20.1±16.8%, respectively) (p<0.001). Moreover, after correcting for sex, age, BMI and socio-economic status, logistic regression demonstrated that European ancestry was significantly associated with increased risk of manifesting OSAS (OR= 2.80; 95%IC= 1.11-7.09). Conversely, West-African ancestry was associated with a reduced risk for the OSAS phenotype (OR=0.26; 95%IC= 0.09-0.72).

This is the first study to incorporate genomic analysis methods to measure the influence of ethnicity on the risk of OSAS. Because genetically determined ancestry may not capture non-measured cultural and lifestyle differences, the contribution of environmental factors to the current findings should not be discarded.

KEYWORDS: Ancestry Informative markers, Ethnicity, Genetic ancestry, Obstructive sleep apnoea syndrome,
INTRODUCTION

Obstructive sleep apnoea syndrome (OSAS) is a prevalent condition and has become a growing cause of health concern due to its myriad associated co-morbidities related to repeated exposure to hypoxia and adverse physiologic stresses such as hypertension, cardiovascular disease, metabolic dysfunctions, hypersomnia and cognitive impairment. The manifestation of OSAS is known to be a result of a complex interaction between developmental, genetic, and environmental factors [1]. Recognised risk factors include male gender, increasing age, obesity, craniofacial structure, ventilatory control dysfunction, and alcohol and drug use [2].

Population-based surveys, mainly of Caucasian populations from Europe, North America and Australia, and more recently from individuals living in Asia, have demonstrated that the adult prevalence of OSAS lies between 3-7.5% in males and 2-3% in females [3-6]. In general, data outside these populations are surprisingly scarce, and inter-ethnic comparisons are difficult due to divergences in methodological designs and objective criteria for OSAS diagnosis. Nevertheless, few studies have described a similar prevalence of OSAS in middle-aged African-American individuals when compared to Caucasian Americans [2,7]. Alternatively, OSAS appears to be more prevalent and more severe in both younger and elderly African-Americans [7,8], suggesting that ethnicity may be an additional risk factor for the development of OSAS.

Differences among the major ethnic groups in a number of health-related traits have been reported in clinical and epidemiological investigations [9,10]. However, the definition of ethnicity is not always clear in the medical literature, and the majority of studies involving different ethnic groups have relied on self-reported information regarding the national origin of the family or on the assumptions of the researcher, normally based on physical appearance. These criteria for racial/ethnic definitions have, in the last few years, been the subject of much debate [11,12]. Ethnic classifications, such as African American, European American, Latino and Hispanic, are a product of both environmental and genetic components, which may differently influence the estimated prevalence of the disorder under study [13]. Therefore, in order to characterise the factors involved in health disparities among ethnically different groups, study designs must incorporate techniques capable of precisely discriminating the different sources of possible variations.

In this context, Brazilians are ethnically, culturally and socio-economically diverse and provide a precious opportunity to dissect some of the risk factors involved in OSAS manifestation. In the early 16th century, when the Portuguese colonisation began, the territory of Brazil was inhabited by over 2.4 million
Native Americans. It is estimated that, between the years 1500-1808, approximately 500,000 Portuguese, mostly men, and over 4 million African slaves, mainly Angolan, Yoruban, Ewe and Bantu, arrived in Brazil. The intermixing between the three ancestral populations (Native American, European and African) was the origin of contemporary Brazilians, one of the most heterogeneous populations in the world, in which each person is, in the vast majority of the cases, highly admixed [14]. This complex ancestry is reflected in the individual's genetic background, which is composed of a variable proportion of the three parental populations with diverse patterns of admixture [14]. Recently, panels of Ancestry Informative Markers (AIMs) have become available as a tool to study the influence of ethnicity on the risk of multifactorial disorders [15-17]. AIMs are a specific type of genetic variation that, due to the large frequency differences among populations, carry information about population structure and can be used to estimate what proportion of ancestry is derived from a particular geographical region. To accurately estimate genetic ancestry, each individual is genotyped for a panel of AIMs located throughout the genome, which are normally unlinked to the genes potentially related to the disorder under study. The genotypic information is then used in an admixture model, which assumes that every individual inherited some proportion of his/her ancestry from each ancestral population and estimates the posterior mean of these proportions [18].

In this sense, when applied in an admixed population, genomic analysis methods may facilitate the interpretation of the putative genetic components of ethnic variation and enable the investigation of associations between genetic ancestry and disease-related phenotypes [15-17]. Thus, the aim of this study was to estimate the genetic ancestry of a large epidemiologically based sample from São Paulo, Brazil, and to correlate the findings with OSAS diagnosis, as measured by questionnaires and full night polysomnography (PSG), using a nasal cannula and thermistor.
METHODS

Subjects

The study was conducted with individuals participating in the Sao Paulo Epidemiologic Sleep Study, which was a population-based survey adopting a probabilistic three-stage cluster sample of Sao Paulo used to represent the population according to gender, age (20-80 years) and socio-economic status. The study was carried out in 2007 to establish the epidemiologic profile of sleep disorders in the adult population of Sao Paulo. Questionnaires, actigraphy, polysomnography (PSG) and blood samples were collected to investigate associations between sleep patterns and disturbances according to social-demographic status, activity/rest cycle, physical activity habits, mood disturbances, memory complaints, sexual dysfunction in males, drug addiction, genetic markers and anthropometric, clinical, biochemical, haematological, endocrine, immunologic and inflammatory indicators. More details on the rational design, sampling, and procedures utilized are provided elsewhere [19]

The study protocol was approved by the Ethics Committee for Research of the Universidade Federal de Sao Paulo (CEP 0593/06) and registered with ClinicalTrials.gov (number: NCT00596713; name: Epidemiology of sleep disturbances among adult population of the Sao Paulo City). All volunteers read and signed the informed consent form.

Clinical Assessment

Following the International Classification of Sleep Disorders (ICSD-2) [20], subjects were considered to have OSAS if they had an Apnoea-Hypopnea Index (AHI) between 5 and 14.9 and presented at least one of the following complaints: loud snoring, daytime sleepiness, fatigue and breathing interruptions observed during sleep. Subjects with an AHI equal to or higher than 15 were also considered to have OSAS, regardless of whether they had any of the aforementioned complaints.

Loud snoring was assessed using the second question of the Berlin Questionnaire for Sleep Apnoea [21], with a positive response being snoring “louder than talking” and “very loud – can be heard in adjacent rooms”. Daytime sleepiness was assessed using the Epworth Sleepiness Scale [22] and the eighth question of the Pittsburgh Sleep Quality Index [23]. Scores higher than nine in the Epworth Scale and/or frequencies greater than once a week for the eighth question of the Pittsburgh Index were considered positive. The criterion was chosen based on the previously published work, in which the scores in mild, moderate, and severe OSAS were found to be 9.5±3.3, 11.5±4.2, and 16±4.4, respectively [22]. Fatigue was assessed by the Chalder Fatigue Scale [24]. Scores higher than four were considered positive. Breathing interruptions
were assessed using the fifth question of the Berlin Questionnaire and were considered positive when the frequency was “higher than once a month”. A complete description of the clinical assessment and the respective outcomes are presented elsewhere [25].

**Polysomnography**

A complete full-night PSG was performed on a digital system (EMBLA® S7000, Embla Systems, Inc., Broomfield, CO, USA) at the sleep laboratory using the subject's habitual bedtime. The following physiological variables were monitored simultaneously and continuously: four channels for the electroencephalogram (EEG); two channels for the electrooculogram; four channels for the surface electromyogram (submentonian region, anterior tibialis muscle, masseter region, and seventh intercostal space); one channel for an electrocardiogram; airflow detection via two channels through a thermocouple (one channel) and by nasal pressure (one channel); respiratory effort of the thorax (one channel) and of the abdomen (one channel) using inductance plethysmography; snoring (one channel) and body position (one channel); and oxi-hemoglobin saturation (SaO2) and pulse rate. All PSGs were performed, and sleep stages visually scored, by four trained technicians according to standardised criteria for investigating sleep [26]. EEG arousals and leg movements were scored in accordance with the criteria established by the AASM Manual for Scoring Sleep and Associated Events [27]. Apnoeas were scored and classified following the recommended respiratory rules for adults suggested by the AASM Manual, and hypopnoeas were scored in accordance with the alternative rules [27].

**Socio-Demographic characteristics**

General physical measurements were made immediately before preparation for the PSG hook up, following recommended procedures and utilising precise instruments. Measurements were taken by trained professionals and included body weight (kg), height (m), calculation of body mass index (BMI) using the formula weight/height$^2$ and circumferences (cm) of the neck, waist and hip. Socioeconomic classes were defined as high, middle or low according the Brazilian Economic Classification Criteria (www.abep.org) for household incomes greater than $15,961 (US dollars), between $4,561 and $15,960, and lower than $4,560 per year, respectively. We assessed 96 districts of the 1500 in which the São Paulo city is divided and proportionally selected individuals from the four homogeneous socio-economical regions of the city in an attempt to ensure the representativeness of different levels of wealth.

**Characterisation of population structure and admixture**
There were two types of ethnical definitions in this study. Initially, individuals responded to questionnaire in which ethnicity was coded according to one of the five following official classifications used by the Brazilian Institute of Geography and Statistics (IBGE): white, black, brown (mulattos), yellow (Asiatic), indigenous or others, for individuals that preferred a term not mentioned above. After all the response categories were read to the responder, a self-classification based solely on the personal understanding of the subject regarding his ethnic background was performed. The second approach was based on genetic information, independently from the previous chosen ethnic group or migration history of the family. In this analysis, the proportion of the genetic contribution of three founder populations of the modern Brazilians (European, West-African and Native American) was estimated for each individual. A total of 1010 individuals from the 1042 volunteers who underwent PSG recordings at a Sleep Institute had high quality DNA available for genetic analyses. We selected a set of 31 ancestry informative markers (AIMs) that exhibit a high level of allele frequency difference among the three founder populations of the Brazilian individuals (Europeans, West-Africans and Native Americans) [28] (Supplemental Table 1). The AIMs were all Single Nucleotide Polymorphisms (SNPs) genotyped using allele-specific PCR with universal energy transfer labelled primers, under contract by Prevention Genetics (Marshfield, Wisconsin) [29]. PCR reactions were carried out with ArrayTape instrumentation, and allele calls were generated based on the clustering of fluorescent signals. Only genotypes with a level of confidence ≥90% were included in the analysis. Moreover, a total of 30 duplicate samples were included in an attempt to check for genotyping errors. Primers and PCR conditions are available upon request. Using the genotypic data, the number of ancestral populations (K) among the “Sao Paulo Epidemiologic Sleep Study” cohort and individual admixture proportions were estimated using the Bayesian Markov Chain–Monte Carlo (MCMC) method implemented in the STRUCTURE 2.1 program [18]. The program was run under the admixture model, using correlated allele frequencies and no prior population information with a burn-in of 100,000 iterations and 1,000,000 iterations after burn-in, for a different number (1–5) of parental populations (K).

**Statistical Analyses**

Statistical analyses were performed using SPSS statistical software (version 15.0 SPSS Inc, Chicago, IL). Hardy Weinberg equilibrium (HWE) values were determined by calculating a χ² statistic and corresponding p value. Fisher’s Exact Test was used to compare sex, socio-economic status, age and BMI strata. Because the distribution of the admixture proportions was not normal, Mann-Whitney U Test was used to compare European, West-African and Native American ancestry between OSAS and non-OSAS
groups, while Kruskal-Wallis, followed by the Mann-Whitney U Test and Bonferroni correction, was used to compare the same variables among the different self-reported ethnic groups. OSAS was the outcome of interest, and logistic regression analyses were performed for the identification of independent risk factors. A p value < 0.05 was established as statistically significant.
RESULTS

The sample (1010 subjects) was 44.3% male with a mean age of 42.4±14.4 years. A total of 28.4% of the participants were from a high socio-economic class, while 61.9% and 9.7% were classified as middle and low socio-economic class, respectively. Of the 1010 subjects, 337 (33.4%) were diagnosed with OSAS. Demographic characteristics of study participants, according to OSAS status, are presented in Table 1. In brief, OSAS was associated with male gender, increasing age, higher socio-economic status and obesity (p<0.01).

Admixture analysis

The average call rate for the 31 genotyped markers was 97% and the error rate was <0.5%. Using a Hardy Weinberg Equilibrium threshold of p< 0.01, six AIMs (19.3%) deviated from expected HWE proportions (rs2814778; rs2077681; rs1369290; rs719776; rs2341823; rs1487214). This rate is higher than would be expected under the null distribution; and for all of the markers, there was a decrease in heterozygosity, probably as a consequence of population substructure attributable to variable degrees of individual admixture. Using the genotypic data of the 31 AIMs, the STRUCTURE 2.1 program estimated the smallest log probability for K=3 populations, suggesting that there was a greater likelihood that the cohort descended from three ancestral populations rather than one, two or four ancestral population(s). For the sample as a whole, we estimated the mean proportions of ancestry to be (75.1±17.7%) European, (18.8±16.4%) West-African, and (6.3±9.1%) Native American (Figure 1).

The correlation between self-reported ethnicity and ancestry proportions was highly significant (p<0.0001). Graph 1 illustrates the proportions of genetic determined ancestry in the self-reported ancestry groups. As expected, “Caucasian” individuals have a higher proportion of European ancestry (84.0±11.3%) and a lower proportion of West-African ancestry (11.3±9.4%) in comparison to the “Black” and “Mulatto” populations (60.1±17.0% and 35.2±17.9%, respectively; p<0.0001). Individuals self-reporting “Asian” or indigenous backgrounds showed higher levels of Native American ancestry (24.9±22.3%) compared to both the “Caucasian” (4.8±5.5%) and the “Black”/“Mulatto” groups (4.9±4.8%) (p<0.0001). Moreover, when compared to Asian or indigenous ethnic groups, European ancestry and West-African ancestry proportions were also higher in “Caucasian” and “Black”/“Mulatto” individuals, respectively (p<0.0001). These results remained highly significant even after Bonferroni correction for multiple tests.
Correlation between ancestry and OSAS

The prevalence of OSAS in the total population was 33.4%. In the group of patients diagnosed with OSAS, a total of 65.3% reported “Caucasian” as their ethnic background, compared to 53.6% of the individuals without OSAS (p=0.009). Alternatively, the frequency of individuals reporting “Mulatto” or “Black” ethnicity was significantly higher in the non-OSAS (29.3%) than in the OSAS group (19.8%). The same trend was observed when considering the genetically determined ancestry proportions. Patients with a diagnosis of OSAS demonstrated a higher percentage of European ancestry (78.2±16.7%) and a lower percentage of West-African ancestry (16.1±15.3%) compared to individuals without OSAS (73.5±18.1% and 20.1±16.8%, respectively) (p<0.0001 for both comparisons). The percentage of Native American ancestry did not differ between groups (p=0.35).

When the genetic ancestry is compared using AIH scores, it is demonstrated a higher percentage of European ancestry in both groups of individuals, with AIH scores 5-15 (77.4±15.9%) and >15 (78.0±17.5%) in relation to individuals with AIH score <5 (73.4±18.3) (p=0.008 and p=0.001, respectively). Corroborating with the previous findings, African ancestry was significantly lower in the groups of individuals with mild and moderate OSA (16.8±14.9%) and severe OSA (15.9±15.6%), when compared to the group of subjects with AHI<5 (20.3±17.0%) (p=0.004 and p<0.0001, respectively). Moreover, no significant differences were observed between individuals with AIH scores between 5 and 15 versus the group of individuals with AIH score>15, for European and African ancestry (p>0.05).

Age, gender, BMI, socio-economic status and proportions of ancestry were tested in a stepwise forward logistic regression model to identify independent risk factors for the manifestation of OSAS. The best predictive model indicated that, in addition to male gender (OR= 3.88; 95%IC=2.76-5.46), age (OR= 1.07; 95%IC=1.06-1.09) and BMI (OR= 1.19; 95%IC=1.15-1.23), European ancestry was also significantly associated with the risk of manifesting OSAS (OR= 2.80; 95%IC= 1.11-7.09) (Table 2). Due to the high correlation between the genetic ancestry estimates, the effect of West-African ancestry was evaluated in a second regression model. It was demonstrated that West-African ancestry is associated with a reduced risk for the OSAS phenotype (OR=0.26; 95%IC= 0.09-0.72), when analyzed together with sex, age and BMI. The effect of socio-economic status was also investigated in both stepwise logistic regression models; however, it was not found to be significantly predictive of OSAS and was not entered in the final model.
DISCUSSION

In this study, we estimated ancestry proportions of 1010 Brazilians living in São Paulo, who participated in Sao Paulo Epidemiologic Sleep Study, a population-based survey adopting a probabilistic three-stage cluster sample of Sao Paulo used to represent the population according to gender, age (20-80 years) and socio-economic status. The study was carried out in 2007 to establish the epidemiologic profile of sleep disorders in the adult population of Sao Paulo. Sleep Apnoea Syndrome was diagnosed according to the International Classification of Sleep Disorders: Diagnostic and Coding Manual (ICSD-2): 1) subjects presenting with an AHI between 5 and 14.9 and at least one of the follow complaints (fatigue, insomnia, unrefreshing sleep, daytime sleepiness and breath hold, gasping or choking, bed partner reports loud snoring and/ or breathing interruptions); or 2) subjects with an AHI greater than or equal to 15, independent of the number of complaints [25]. In this previous study, it was demonstrated that a total of 29.5% of the individuals with 5≤AHI<15 did not have sleep complaints. On the other hand, 66.0% of the subjects with AHI<5 and 74.3% of the subjects with AHI>15 did report at least one of the previously mentioned symptoms.

For the total sample, the mean proportions of European, West-African and Native American ancestry was estimated to be 75.1±17.7%, 18.8±16.4% and 6.3±9.1%, respectively. The high level of admixture found in our sample is consistent with the three-way admixture history of the Brazilian population due to the contribution of three main founder populations [14]. Several studies have shown that the level of contribution of the ancestral population to the genetic background of Brazilians may vary significantly in different regions of Brazil [30]. For the population as a whole, Salzano (1997) estimated 51% European, 36% African, and 13% Native American ancestries, whereas European ancestry was found to vary from 92% [31] in the South to 54% in the North of Brazil [32]. Similarly, the African contribution is distributed from 8% [31] in the South to 34% in the Northeast region [33], and the Native American contribution can be as low as 0% in the South of Brazil [31] and as high as 41% in the North Amazonian region [34]. As expected, the estimates obtained in this study show intermediate values compared to the regions with extreme European, African or Native American ancestry. Therefore, although our estimates of admixture in modern Brazilians should be seen only as approximations – because the non-admixed populations used to provide the ancestry-specific allele frequencies in this study were not the exact mix of European, African and Native American subpopulations that contributed to the modern Brazilian population – the results are likely to represent the real scenario of the admixture patterns in the Southeast region of Brazil.
According to the 2007 National Survey, the Brazilians have classified themselves as: “white” (49.7%), “mulatto” (42.6%), “black” (6.9%), “Asian” and “Native American” (<1%). Comparisons of ancestry estimation with classification of self-reported ethnicity are highly correlated. “Caucasian” individuals have higher proportions of European ancestry, “Black” and “Mulatto” individuals have higher proportions of West-African ancestry, and “Asian” and “Indigenous” individuals have increased levels of Native American ancestry. Although the correlation is significant, individuals classified as “Caucasian” have a substantial contribution of West-African ancestry, and individuals classified as “Black” and “Mulatto” have a substantial contribution of European ancestry. These results agree with those of other studies demonstrating the complexity of the genetic ancestry of Brazilians and the risk of relying on self-reported ethnicity to classify individuals in genetic studies [14].

It has been suggested that younger and elderly African-Americans exhibit significantly greater risk for OSAS and are diagnosed later with more severe OSAS [7,8]. Currently, the biological cause of such disproportion has not been elucidated, but it is likely due to multiple, potentially interacting gene and environmental factors. In this study, we reported that European ancestry, estimated by genetic markers, increases the risk for OSAS, even after correction for potential risk factors such as age, gender and BMI. In addition, West-African ancestry was also found to be significantly associated with the risk for OSAS manifestation, however, as a protective factor. One possible explanation for these contradictory findings is simply that the ancestral genetic composition of African-Americans is distinct from that observed for the Black and Mulatto groups in Brazil. While we found that in the latter the overall West-African ancestry contribution is approximately 35±18%, a recent study indentified 83±9% African Ancestry in a sample of 4,464 African-Americans [35]. As a result of historical factors, the contribution of European genetic ancestry is much more significant among Brazilians, and this may have a direct impact on the influence of genetic factors modulating the interethnic differences in the risk of OSAS. Moreover, it is evident that each population is unique. Brazilians and Americans have been shaped by different historical, socio-economical and other non-measured environmental sources of variation. Interactions between biological and environmental factors may also modify their individual contribution to the disorder and consequently modulate risk and severity in a population-specific manner.

It is recognised that African-Americans have a high prevalence of obesity, type 2 diabetes and cardiovascular disease when compared to non-Hispanic Whites [9,10]. To date, studies using ancestry proportions to model genetic contributions in order to correlate ethnic differences with risk have produced conflicting results [15,17]. Williams and colleagues demonstrated that European ancestry is inversely associated with BMI and fasting glucose measurements in a sample of non-diabetic Pima Indians [17]. After
adjusting for age and socioeconomic status, a significant association was also observed between African admixture and obesity among African-American females [15]. Alternatively, Lorenzo et al.[36] reported that a Spanish genetic admixture was not a risk factor for hypertension in Mexican-origin populations. Furthermore, a recent study examining ~ 1000 individuals from the Boston Puerto Rican Health Study has demonstrated that African ancestry is inversely associated with type 2 diabetes and cardiovascular disease, and positively correlated with hypertension [16]. In a different context, Salari and Burchard (2007) [37] showed that, among individuals of higher socio-economic status, the risk for asthma increased with African ancestry. Conversely, asthma risk increased in individuals of European ancestry and of a lower socio-economic class. Taken together, these data suggest that the genetic and environmental factors influencing risk are likely to be contextual and that ancestry acts as a modifier. Nevertheless, the relationship between risk of OSAS and ancestry demonstrated in this study is consistent with an additive genetic model with population-specific alleles influencing the ethnic difference in risk.

One limitation of this study is the number of markers used to estimate genetic admixture. Indeed, previous simulation studies were used to show that greater than 50 AIMs are needed to accurately estimate ancestral proportions in admixed populations [38]. Nevertheless, we acknowledge that the precision of the estimation depends not only on the number of AIMs used in the analyses but also their ability to discriminate among parental populations. In this sense, it has also been demonstrated that fewer markers are satisfactory when the average marker allele frequency difference between parental populations is > 60% [13,38,39]. In the present panel, a marker was deemed informative if there was at least a 30% allele frequency difference between any two parental groups [40]. From the total of 31 markers selected, 18 were informative to determine European/West-African contributions, with an average allele frequency differential of 74%. There were 19 and 27 markers useful in detecting European/Native American and Native American/West-African contributions, with a corresponding average allele frequency difference of 76% and 78%, respectively. Therefore, although the use of a greater number of markers would have increased the precision of the ancestral proportion estimate, the highly significant correlation between genetic ancestry estimates and self-report ethnicity and the fact that both classifications agree in suggesting that higher European ancestry is associated with a higher risk of OSAS argue that it is unlikely the direction of the association would be altered.

This analysis is the first study to apply genomic methods to measure the relationship between OSAS and ethnicity. The higher risk attributed to European and the lower risk attributed to West-African ancestries highlight the need to consider genetic information as an important factor in clinical and epidemiological studies of OSAS prevalence, especially in populations with high levels of ethnic admixture, such as the in
South and North America. Nevertheless, although the genetic informative markers were used in an attempt to dissect the influence of biological aspects of ethnicity on OSAS phenotype apart from cultural elements, it should be noted that the findings presented here might still be a result of a complex interaction between heritable and non-genetic factors such as diet, physical activity level, lifestyle and differing access to health care, to name a few. Therefore, the impact of environmental factors on the overall risk attributed to ethnicity in the susceptibility of OSAS should not be discarded, and future studies should incorporate statistical models capable of quantifying its influence and considering its interaction with the genetic/biological aspects contributing to this phenotype. Finally, the study highlights the Brazilian population and its wide variation in ethnic admixture and environmental exposure as a powerful resource of information to help explain the differences in OSAS and other sleep disorders prevalent among ethnic and racial groups.

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FIGURE AND GRAPH LEGENDS

Figure 1: Distribution of individual European, West-African and Native American ancestry proportions in the population of Sao Paulo, Brazil.

Graph 1: Mean proportions of genetically determined European, West-African and Native American ancestry in the self-reported ethnic groups of individuals participating in the Epidemiologic Sleep Study of Sao Paulo city, Brazil.
REFERENCES


Table 1: Frequency of gender, age groups, socio-economic and nutritional status in the Obstructive Sleep Apnoea Syndrome groups.

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<td>40.7</td>
<td></td>
</tr>
</tbody>
</table>

*Normal=Body mass index (BMI)<25 kg/m²; Overweight= BMI between 25 and 30 kg/m²; Obese= BMI>30 kg/m²

![Image](attachment:image.png)

Table 2: Multivariate logistic model for the effect of European ancestry on the risk of Obstructive Sleep Apnea Syndrome according to the best predictive model.

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95.0% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Ancestry</td>
<td>2.80</td>
<td>1.11</td>
<td>7.09</td>
</tr>
<tr>
<td>Sex</td>
<td>3.88</td>
<td>2.76</td>
<td>5.46</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.07</td>
<td>1.06</td>
<td>1.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1.19</td>
<td>1.15</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Only variables that met the .05 significance level for entry into the stepwise best predictive model are shown.