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OSAHS

Evaluation of clinical and genetic factors in obstructive sleep apnoea

Valutazione dei fattori clinici e genetici nella sindrome da apnee ostruttive del sonno

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SUMMARY

Purpose. To evaluate the correlation between several presumed candidate genes for obstructive sleep apnoea (OSA) and clinical OSA phenotypes and propose a predictive comprehensive model for diagnosis of OSA.

Methods. This case-control study compared polysomnographic patterns, clinical data, morbidities, dental factors and genetic data for polymorphisms in *PER3, BDNF, NRXN3, APOE, HCRTR2, MC4R* between confirmed OSA cases and ethnically matched clinically unaffected controls. A logistic regression model was developed to predict OSA using the combined data.

Results. The cohort consisted of 161 OSA cases and 81 controls. Mean age of cases was 53.5 ± 14.0 years, mostly males (57%) and mean body mass index (BMI) of 27.5 ± 4.3 kg/m^2. None of the genotyped markers showed a statistically significant association with OSA after adjusting for age and BMI. A predictive algorithm included the variables gender, age, snoring, hypertension, mouth breathing and number of T alleles of *PER3 (rs228729)* presenting 76.5% specificity and 71.6% sensitivity.

Conclusions. No genetic variant tested showed a statistically significant association with OSA phenotype. Logistic regression analysis resulted in a predictive model for diagnosing OSA that, if validated by larger prospective studies, could be applied clinically to allow risk stratification for OSA.

KEY WORDS: obstructive sleep apnea, genetic polymorphisms, phenotype, algorithms, case-control studies

RIASSUNTO

Scopo. *Valutare la correlazione tra alcuni geni potenzialmente coinvolti nella sindrome delle apnee notturne (OSA) ed i fenotipi clinici di OSA e proporre un modello predittivo generale per la diagnosi di OSA.*

Metodi. *Uno studio caso-controllo ha confrontato modelli di polisonnografia, dati clinici, comorbidità, parametri dentali, e dati genetici riguardanti i polimorfismi di PER3, BDNF, NRXN3, APOE, HCRTR2, MC4R tra soggetti con diagnosi di OSA e controlli non affetti etnicamente appaiati. Un modello logistico di regressione è stato sviluppato per predire l'OSA utilizzando i dati combinati.*

Risultati. *Lo studio caso-controllo ha incluso 161 pazienti OSA e 81 controlli. L'età media era di 53,5 ± 14,0 anni, per la maggior parte maschi (57%) e con indice di massa corporea (BMI) medio di 27,5 ± 4,3. Nessuno dei marcatori genetici studiati ha mostrato un'associazione statisticamente significativa con OSA dopo aver aggiustato il modello per età e BMI. L'algoritmo predittivo ha incluso le variabili genere, età, russamento, ipertensione, respirazione orale e il numero di alleli T di PER3 (rs228729), mostrando una specificità del 76,5% e una sensibilità del 71,6%.*

Conclusioni. *Nessuna delle varianti genetiche analizzate ha mostrato un'associazione sta-*

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tisticamente significativa con i fenotipi OSA. L'analisi di regressione logistica ha espresso un modello predittivo per la diagnosi di OSA che potrebbe essere applicato in clinica per la stratificazione del rischio per OSA, una volta validato in studi prospettici più ampi.

PAROLE CHIAVE: *apnea ostruttiva del sonno, polimorfismi genetici, fenotipo, algoritmo, studi caso-controllo*

Introduction

Obstructive sleep apnoea (OSA) is characterised by recurrent events of upper airway obstruction during sleep associated with clinical signs and symptoms such as excessive sleepiness, snoring, choking, and breathing interruptions during sleep [1]. OSA has a high prevalence [2] and is related to increased morbidity and mortality from all causes [3].

Although considerable progress has been made in understanding the pathophysiology of OSA [4,5], the precise mechanisms that lead to upper airway obstruction are not fully understood [6]. Despite the heterogeneity and complexity of OSA, diagnosis, assessment of severity and management of OSA remain linked to a single indicator, the apnoea hypopnea index (AHI) [6].

Several factors that increase the risk of OSA are well established, such as obesity, body fat distribution, craniofacial morphology, and neural control of the upper airway muscles [5,7]. These risk factors are, in part, genetically determined. Identification of genes associated with risk factors for OSA will undoubtedly help to understand the molecular mechanisms underlying the disorder and aid in the management of OSA [8-11].

The discovery of genotypic and clinical markers can help in the early detection of the disease, as well as in the prevention and personalised treatment of this pathology. Thus, the aim of this study was to evaluate the correlation between several presumed candidate genes, phenotypes and clinical data that may contribute to the development of OSA and to propose a comprehensive predictive model for its diagnosis.

Materials and methods

Participant identification and evaluation

A case-control study was carried out in a medical-dental clinic in Belo Horizonte, Brazil, and recruitment period was August 2018 to January 2020. Study participants were divided into two groups: patients diagnosed with OSA (case group) and non-OSA participants (control group). Each participant underwent complete OSA-focused clinical evaluation and polysomnography. The following inclusion criteria were used for defining OSA cases: diagnosis of OSA according to the American Academy of Sleep Medicine criteria [12] and absence of craniofacial dysmorphism, genetic syndromes with OSA as part of the spectrum of manifestations, drug and alcohol abuse, psychiatric disorders, and dementia; age 18 to 85 years and body mass index (BMI) ≤ 35 kg/m^2. The control group consisted of individuals referred due to clinical suspicion of OSA, but who did not meet diagnostic criteria (AHI < 5 per hour). For stratification of cases and controls the following variables were used: socioeconomic status, sex, age, height, weight, and BMI (≤ 35 kg/m^2). BMI was limited to ≤ 35 kg/m^2 and age to < 85 years to avoid increased AHI levels. In addition, dental data such as the presence of self-reported bruxism, pain in masticatory muscles, noise upon movement of the temporomandibular joint, tongue size, floor of mouth (i.e., sublingual space), presence of open or crossed bite were also analysed.

Genetic analysis

Genomic DNA was extracted from peripheral blood [13] or saliva [14]. 20 ng of DNA of each genotyped individual was used for TaqMan SNP genotyping assays for *PER3* gene variants (*rs228697, rs228727, rs228729* and *rs10462020*), *BDNF* (*rs6265*), *NRXN3* (*rs10146997*), *APOE* (*rs7412* and *rs429358*), *HCRTR2* (*rs2653349*), *MC4R* (*rs17782313*) according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Genotyping was performed by real-time polymerase chain reaction using allelic discrimination using a Stratagene Mx3005 instrument (MxPro QPCR System, 2007 Software, La Jolla, CA). At least 10% of the samples were genotyped twice for quality control.

Data analysis

Data were analysed using the statistical program R (version 3.6.3). In univariate analysis, logistic regression models with a logarithmic link function were adjusted using the participant's diagnosis as a dependent variable (case-control) and as an independent variable all clinical-epidemiological variables using the generalised linear model function. For genetic variables, the analysis was carried out based on five genetic models: co-dominance, dominance, recessive, over dominant and log-additive with the SNPassoc package.

Predictive OSA model using logistic regression

A logistic regression model was developed to predict OSA. The variables that reached a level of significance less than 0.20 in the univariate analysis were considered

suitable for the final model. However, only variables with $p < 0.05$ remained in the multivariate logistic regression model.

Results

Overall, 161 OSA cases and 81 controls were included. The mean age of the participants was 51.5 ± 14.3 years with a male predominance (57.0%) and a mean BMI of 26.6 ± 4.3 kg/m^2. The mean age of cases was 53.5 ± 14.1 years and mean BMI was 27.5 ± 4.4 kg/m^2; mean age of the controls was 47.4 ± 13.9 years and the mean BMI was 24.8 ± 3.8 kg/m^2. The polysomnographic variables of the sample participants showed a mean of AHI 17.05 ev/h, AHI in REM 21.05 ev/h, minimum saturation 82.01%, desaturation index 16.13/h. Sociodemographic, clinical, and dental variables are presented in Table I. Dental data were not available in the medical records of 16% patients.

Polysomnographic data showed a significant difference between cases and controls for AHI, AHI REM, AHI NREM, AHI dorsal, AHI non-dorsal, mean time of apnoea/hypopnoea, score 3 of arousal threshold, minimum saturation, and desaturation index (Tab. II).

Patients with OSA reported significantly more persistent nocturnal snoring than controls, with rates of 34.3% and 17.4% ($p < 0.001$), respectively. In addition, there was a significant difference between cases and controls for the presence of hypertension (23.1% *vs* 6.2%, $p = 0.015$), lung disease self-reported (asthma, bronchitis, chronic obstructive pulmonary disease) (3.3% *vs* 0.4%, $p = 0.038$) and presence of mouth breathing (28.5% *vs* 4.9%, $p < 0.001$) in patients with OSA compared with controls. Regarding dental data, there was a significant difference between cases and controls for enlarged tongue (54.5% *vs* 16.5%, $p = 0.000$), high palate (54.5% *vs* 18.2%, $p = 0.003$) and Angle class II malocclusion (27.3% *vs* 6.2%, $p = 0.007$) in patients with OSA (Tab. III).

Univariate analysis results revealed a significant difference for gender, age, weight and BMI between cases and controls (Tab. III).

Table I. Sociodemographic, clinical, dental variables of patients in the sample.

Variable	Category	%
Sociodemographic		
Gender male/female	57/43	
Age (mean/SD)	51.5 ± 14.3	
BMI	26.6 ± 4.3	
Clinical		
Snoring every night		66
Daytime sleepiness		59
Wake up feeling refreshed		50
Alcohol consumption		47
Mouth breathing		34
Hypertension		29
Decreased libido		28
Dental		
Ogival palate		73
Increased tongue		71
Pain on palpation TMJ/muscles		45
Bruxism		43
TMJ noise		33
Floor of mouth		12
Crossbite		13
Mallampati	Grade 1	2.43
	Grade 2	8.53
	Grade 3	25.60
	Grade 4	53.65
Malocclusion (%)	Class II	27.3

SD: standard deviation; BMI: body mass index; TMJ: temporomandibular joint.

Analysis of genetic factors

BDNF (*rs6265*) and *PER3* (*rs228729*) were the only variants that showed a significant association with OSA ($p = 0.013$; $p < 0.001$, respectively). However, after adjusting for age and BMI, *BDNF* (*rs6265*) and *PER3* (*rs228729*) no longer exhibited a significant association with OSA phenotype ($p = 0.062$; $p = 0.066$, respectively) (Tab. IV). None of the other genotypes had an association between the tested genotype and OSA phenotype (Tab. IV).

Prediction of the presence of OSA using logistic regression model

The values obtained in the multivariate logistic regression model (Tab. V) indicated that the probability of a patient having OSA can be best predicted in the equation derived from the final model:

$$D_x = \frac{1}{[1 + exp\ (-z)]}$$

$z = -3.705 - 0.855 \times (X1) + 0.031 \times (X2) + 1.643 \times (X3) + 1.105 \times (X4) + 1.093 \times (X5) + 1.209 \times (X6) + 0.528 \times (X7)$

Where: Dx = diagnosis; exp = exposure; X1 = 1, if the gender is female, otherwise 0; X2 = age of the patient in years; X3 = 1, if subjective snoring is present, otherwise 0; X4 = 1, if hypertension is present, otherwise 0; X5 = 1, if not breathing well through the nose, otherwise 0; X6 = number of T alleles of *PER3 (rs228729)*.

Table II. Comparison between cases and controls for polysomnographic variables.

Variable	Category	Cases	Controls	Coefficient	Standard error	Z value	*P* value
Polysonographic							
AHI (mean/SD)		24.3 ± 16.8	2.6 ±1.5	1.069	0.209	5.113	**0.000**
Apnoea ratio (mean/SD)		1.8 ± 7.9	0.3 ± 0.4	0.217	0.344	0.631	0.528
Hypopnoea ratio (mean/SD)		3.1 ± 12.2	4.4 ± 19.3	-0.006	0.010	-0.550	0.576
REM AHI (mean/SD)		25.8 ± 21.0	3.5 ± 4.7	0.188	0.030	4.745	**0.000**
NREM AHI (mean/SD)		22.6 ± 18.1	1.8 ± 1.7	1.041	0.232	4.492	**0.000**
AHI dorsal (mean/SD)		20.1 ± 23.7	3.0 ± 4.7	0.134	0.039	3.435	**0.001**
Non-dorsal AHI (mean/SD)		16.8 ± 20.9	3.6 ± 10.3	0.108	0.033	3.243	**0.001**
Average time of apnoea/hypopnoea (mean/SD)		29.2 ± 15.8	20.3 ± 15.2	0.059	0.024	2.483	**0.013**
Arousal threshold (n/%)	2	64/26.4	23/9.5	-0.965	0.535	-1.805	0.071
	3	37/15.3	23/9.5	-1.444	0.546	-2.643	**0.008**
Minimum saturation (mean/SD)		79.2 ± 11.5	87.9 ± 8.1	-0.181	0.031	-5.899	**0.000**
Time below 90% (mean/SD)		14.2 ± 27.9	6.1 ± 21.3	0.020	0.012	1.733	0.083
Oxygen desaturation index (mean/SD)		20.4 ± 20.2	2.8 ± 6.8	0.206	0.044	4.734	**0.000**

SD: standard deviation, AHI: apnea-hypopnea index, REM: rapid eye movement, NREM: non rapid eye movement.

The area under the ROC curve was 79.4% (CI 95% 72.2%-86.6%) suggesting that the model achieves 76.5% specificity and 71.6% sensitivity assuming a 0.763 cutoff bridge (Fig. 1).

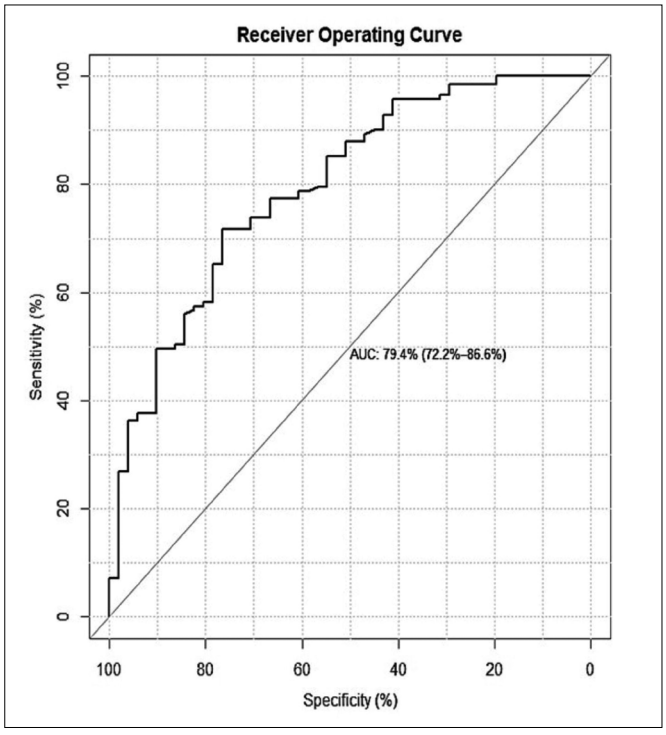

Figure 1. The ROC curve for the logistic regression model was based on the definition of the chance of having OSA. The area under the ROC curve was 79.40%.

Discussion

In this report, the contribution and association of sequence variants of several OSA candidate genes [15-20] was tested in Brazilian OSA cases. None of these variants was significantly associated with OSA phenotype.

The choice of variants for the present study was based on previous studies. For example, Canales et al. [23] found an association between OSA and decreased morning expression of clock genes, such as PER3. Yuksekkaya et al. [21] reported that the BDNF *rs6265* (196G/G) genotype may be useful to assess OSA in non-obese patients. Furthermore, findings showed that BDNF, but not NGF, was significantly increased in a subpopulation of muscle fibres in patients with snoring and OSA [20]. The MC4R gene is largely expressed in the hypothalamus and is intimately involved in appetite regulation, autonomic and endocrine functions, and insulin resistance. OSA is one of the deleterious consequences of obesity. A case report of a child with obesity at 23 months of age showed homozygous mutations of the *MC4R* gene (Pro299his) through DNA sequencing of the genes involved in early-onset obesity. Overnight polysomnography was significant for severe OSA (AHI 36 events/h and oxygen saturation 50%). The authors suggested that future research may provide more information on possible associations between MC4R and OSA [17]. Central abdominal fat is a strong risk factor for diabetes, cardiovascular disease and OSA. Common variants in NRXN3 are associated with waist circumference, BMI and obesity [24]. A case-control study investigated whether variations in the *APOE-ε* gene were associated with craniofacial changes, AHI and BMI

Table III. Comparison between cases and controls for all clinical-epidemiological and dental variables.

Variable	Category	Cases	Controls	Coefficient	Standard error	Z value	*P* value
Sociodemographic							
Gender male/female		104/57	33/47	-0.964	0.279	-3.458	**0.001**
Age (mean/SD)		53.6 ± 14.0	47.3 ± 14.0	0.030	0.010	3.031	**0.002**
Weight		79.1 ± 15.0	69.4 ± 14.3	0.050	0.012	4.393	**0.000**
Height		1.7 ± 0.1	1.7 ± 0.1	0.016	0.025	0.647	0.518
BMI		27.5 ± 4.4	24.9 ± 3.8	0.179	0.042	4.233	**0.000**
Clinical							
Daytime sleepiness (n/%)		97/40.1	46/19.0	0.094	0.305	0.308	0.758
Snoring every night (n/%)		83/34.3	42/17.4	1.151	0.324	3.548	**0.000**
Wake up feeling refreshed (n/%)		57/23.6	37/15.3	0.320	0.291	1.090	0.272
Hypertension (n/%)		56/23.1	15/6.2	0.825	0.337	2.445	**0.015**
Cancer (n/%)		5/2.1	3/1.2	-0.211	0.745	-0.284	0.777
Diabetes (n/%)		16/6.6	4/1.7	0.731	0.579	1.263	0.207
Fibromyalgia (n/%)		3/1.2	4/1.7	-1.039	0.778	-1.335	0.182
Decreased libido (n/%)		43/17.8	24/9.91	-0.192	0.300	-0.619	0.536
Heart disorders (n/%)		10/4.1	3/1.2	0.103	0.619	0.166	0.868
Emotional problems (n/%)		38/15.7	16/6.6	0.190	0.341	0.585	0.559
Gastroesophageal reflux (n/%)		35/14.5	12/5.0	0.448	0.371	1.205	0.228
Thyroid disorders (n/%)		19/7.9	10/4.1	-0.084	0.421	-0.199	0.843
Pulmonary disorders (n/%)		8/3.3	1/0.4	2.161	1.041	2.075	0.038
Mouth breathing (n/%)		69/28.5	12/5.0	1.371	0.349	3.928	**0.000**
Smoking (n/%)		8/3.3	3/1.2	0.280	0.693	0.405	0.686
Alcohol consumption (n/%)		78/32.2	35/14.5	0.211	0.291	0.724	0.469
Dental							
Bruxism (n/%)		67/27.7	36/14.9	-0.252	0.291	-0.866	0.387
Pain on palpation TMJ / muscles (n/%)		77/31.8	32/13.2	-0.038	0.313	-0.122	0.903
TMJ noise (n/%)		59/24.4	20/8.3	0.225	0.322	0.699	0.484
Increased tongue (n/%)		132/54.5	40/16.5	1.690	0.437	3.892	**0.000**
Floor of mouth (n/%)		19/7.9	9/3.7	-0.299	0.426	-0.702	0.483
Ogival palate (n/%)		132/54.5	44/18.2	1.339	0.455	2.944	**0.003**
Mallampati (n/%)	Grade 2	1/0.4	7/2.9	13.620	1029.122	0.013	0.989
	Grade 3	27/11.2	20/8.3	15.817	1029.122	0.015	0.988
	Grade 4	114/47.1	27/11.2	17.006	1029.121	0.017	0.987
Malocclusion (n/%)	Class II	66/27.3	15/6.2	0.954	0.355	2.688	**0.007**
	Class III	15/6.2	6/2.5	0.235	0.504	0.466	0.641
Open bite (n/%)		2/0.8	1/0.4	-0.205	1.235	-0.166	0.868
Crossbite (n/%)		25/10.3	6/2.5	0.616	0.484	1.273	0.203

SD: standard deviation; BMI: body mass index; TMJ: temporomandibular joint.

in patients with OSA. The polymorphisms that define the APOE-ε1-4 allele *rs429358* and *rs7412* were genotyped. There was no association of the APOE-ε4 allele with facial profile among these patients with OSA. However, the authors cautioned that in relation to genetic research, this study was underpowered due to the small sample size [18]. The orexin receptor type 2 (Ox2R or OX2), also known as hypocretin receptor type 2 (HcrtR2), is a protein that in humans is encoded by the *HCRTR2* gene. Peever et al. [15] demonstrated that genioglossus activity was increased by

Table IV. Association between polymorphisms and the presence of OSA.

Polymorphism	Genotyped controls	%	Genotyped cases	%	Non-adjusted				Adjusted (age and BMI)			
					OR	Lower	Upper	*P* value	OR	Lower	Upper	*P* value
BDNF rs6265	82	33.90	160	66.10	1.66	1.10	2.51	**0.013**	1.52	0.97	2.38	0.062
HCRTR2 rs2653349	78	33.10	158	66.90	1.08	0.64	1.82	0.768	1.02	0.58	1.79	0.936
MC4R rs17782313	82	34.30	157	65.70	1.18	0.78	1.8	0.428	0.75	0.45	1.24	0.258
NRXN3 rs10146997	81	34.00	157	66.00	0.96	0.61	1.53	0.874	0.91	0.55	1.51	0.718
APOE rs7412	81	34.00	157	66.00	0.82	0.40	1.69	0.622	0.46	0.2	1.08	0.079
PER3 rs228697	81	34.30	155	65.70	1.05	0.56	1.97	0.763	1.12	0.57	2.2	0.747
PER3 rs228727	82	34.30	157	65.70	1.29	0.87	1.93	0.204	1.35	0.87	2.11	0.184
PER3 rs228729	81	33.60	160	66.40	2.16	1.44	3.23	**< 0.001**	1.59	0.97	2.63	0.066
APOE rs429358	81	33.90	158	66.10	1.46	0.85	2.53	0.159	1.41	0.79	2.51	0.239
PER3 rs10462020	82	34.30	157	65.70	0.92	0.56	1.52	0.738	0.89	0.51	1.53	0.660

Table V. Logistic regression model for obstructive sleep apnoea.

Variable	Estimate	Standard error	Z value	*P* value	OR	Inf. Lim.	Sup. Lim.
(Intercept)	-3.71	1.052	-3.521	**0.000**	0.02	0.003	0.177
Gender female	-0.85	0.417	-2.048	**0.041**	0.43	0.184	0.954
Age	0.03	0.015	2.013	**0.044**	1.03	1.001	1.063
Subjective snoring	1.64	0.561	2.929	**0.003**	5.17	1.761	16.267
Hypertension	1.10	0.489	2.261	**0.024**	3.02	1.208	8.360
Mouth breathing	1.09	0.420	2.599	**0.009**	2.98	1.340	7.048
Ogival palate	1.21	0.574	2.107	**0.035**	3.35	1.082	10.532
T alelle PER3 rs228729	0.53	0.263	2.005	**0.045**	1.60	1.023	2.886

intracerebroventricular injection of orexin, therefore reductions in orexin may contribute to the suppression of upper airway dilator activity, which may facilitate OSA.

A previous study evaluated the association of the *BDNF* variant *rs6265* (G > A) with OSA [21]. To avoid the confounding effect of obesity, the authors divided participants into four groups based on the presence/absence of OSA and/or obesity. No significant differences were observed regarding the *BDNF (rs6265)* polymorphism between patients with and without OSA, and there was also no significant association with the *BDNF* gene and OSA polymorphism in a regression analysis. In our study, unlike the study by Yüksekkaya et al. [21], age and BMI did affect the results, since *BDNF* 270C/T was significantly associated with OSA in a codominant, dominant, recessive, and log-additive model, although after adjusting for age and BMI there was no longer a significant association with OSA.

Several studies in humans have suggested that *PER3* plays a key role in maintaining circadian rhythm. Although there are still few studies on the relationships between circadian clock genes and OSA, it is important to note that the sleep-wake cycle can modulate circadian clock genes and, in turn, the sleep cycle circadian rhythm can affect the occurrence and duration of apnoea [22]. A study conducted in a cohort of elderly and obese veterans compared the relative gene expression of clock genes between those with and without OSA or related nocturnal hypoxaemia. The results showed that *PER3* expression was significantly decreased by 35% among those with OSA compared to those without. In addition, a trend of downregulation of *PER3* was observed with increasing severity of OSA [23]. The present study is the first to examine the association of *PER3 (rs228729)* with OSA. We showed that this variant was significantly associated with OSA in a codominant, dominant, recessive, and log-additive model, although after adjusting for age and BMI there was a significant association for dominant and heterozygosity, but there was no further significant association for OSA when analysing the log-additive.

Although the evidence points to the possibility of changes in the *BDNF* and *PER3* genes being relevant for the development of OSA [21-23], the type of analysis carried out in the present study did not support the notion of such an as-

sociation. These results may be due to the fact that there is indeed no relationship between the specific *BDNF* and *PER3* gene polymorphisms and OSA. Alternatively, the small sample size of the present cohort prevented any subtle association from being detected.

Using clinical and phenotypic data collected from patients with OSA, an algorithm was constructed that allows the prediction of OSA cases with reasonable specificity and sensitivity, which, if validated, may at least be applicable in South American populations. Although the *PER3 (rs228729)* variant, after adjusting for age and BMI, resulted in a significant association for dominant and heterozygosity, but no additional significant association for OSA when analysing the log-additive, it showed a $p < 0.05$ and remained in the model of multivariate logistic regression. Therefore, the results and the investigation provide a reference for investigations focusing on the identification of OSA in Brazilian and similar populations using the equation:

$$D_x = \frac{1}{[1 + exp\ (-z)]}$$

A limitation of this study is the fact that some polysomnographic examinations were performed in different clinics. Differences in the performance and analysis of polysomnographic examinations are known [25], which may lead to the need for validation of algorithms in different contexts. The results of this study should be replicated in larger and more independent Brazilian samples, preferably involving multicentre studies to minimise the study bias.

Conclusions

In conclusion, no genetic variant tested showed a significant association with OSA phenotype. Notwithstanding, logistic regression analysis enabled risk stratification for OSA diagnosis in Brazilian cases. These results should be replicated in a larger ethnically diverse cohort of cases in a prospective manner.

Conflict of interest statement

The authors declare no conflict of interest.

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Author contributions

LB-R, EF, LDM: study conception and design; MLRG, PGA, BG-F: acquisition, analysis and interpretation of data; RPS: statistical analysis; MLRG: drafting of the manuscript; LB-R, EF, LDM: critical revision of the manuscript; LB-R: study supervision.

All authors read and approved the final manuscript.

Ethical consideration

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Ethics Committee of the Universidade Federal de Minas Gerais #2.980.453) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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