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Nutrigenetic genotyping study in relation to Sleep Apnea Clinical Score

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Abstract

Background Conflicting results regarding associations between single nucleotide polymorphisms (SNPs) in IRS1, ACE, APOE, PPARG, MTHFR, 5HT2AR, BDNF, and FTO genes and obstructive sleep apnea have been reported in previous studies.

Objective To assess pleiotropic associations between these gene polymorphisms that are commonly being studied in a nutrigenetic test and sleep apnea.

Methods One hundred and nine subjects of Caucasian origin who have performed a commercially available nutrigenetic test that includes the aforementioned polymorphisms were divided into two groups depending on the results of their Sleep Apnea Clinical Score (SACS ≤ 15 or > 15). Statistical significant differences in the prevalence of the polymorphisms under study between the groups were assessed with the Chi-squared test. Possible associations of the polymorphisms with SACS and BMI were further evaluated with logistic regression analyses.

Results From the polymorphisms studied, only variant rs9939609 in the FTO gene was more prevalent in people with high sleep apnea clinical score ($\chi^2 = 7.1$, $P = 0.029$). However, this association was attenuated after adjustment for body mass index (OR = 0.653, $P = 0.178$).

Conclusion We failed to confirm previously reported associations between the majority of the studied polymorphisms and sleep apnea. Body weight seems to be an important cofounding factor that needs to be accounted for, when genetic association studies are performed for sleep apnea.

Keywords Sleep apnea · Nutrigenetics · Gene polymorphisms

Introduction

Both sleep apnea and obesity are common interrelated diseases with an enormous public health burden. The exact pathogenic mechanisms of obstructive sleep apnea (OSA) are not yet fully elucidated. It is certainly a multifactorial disease and several genetic factors have been reported to be related with this syndrome. The study of these factors is interesting both for the elucidation of the pathophysiological mechanisms as well as the application of biomarkers for diagnosis of the disease or the estimation of the risk of its complications [1].

A Pubmed search, as well as a search in Public Health Genomics Knowledge Base (https://phgkb.cdc.gov/PHGKB/startPagePhenoPedia.action) reveals more than 100 genes that have been reported to be associated with obstructive sleep apnea syndrome. However, several candidate gene studies show conflicting results. Since these studies are known to have limited replication validity, further research is needed for the validation of the reported associations.

As may be expected, many genes that have been reported to be associated with OSA are also related to metabolic or inflammatory pathways. As a consequence, several of these genes can be found in commercially available nutrigenetic tests that are being applied with increased frequency lately for weight control and/or other personalized lifestyle changes. Thus, an examination of these genes is useful not only for validation reasons, but also because such studies offer

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valuable information on factors such as body weight that can bias the results of previous genotyping and OSA studies [2].

Methods

One hundred twenty-one normal weight, overweight, and obese subjects who have performed a commercially available nutrigenetic test (GENOSOPHY-Discover Yourself) and have agreed to provide their data for research purposes were asked to answer Sleep Apnea Clinical Score (SACS) by means of a telephone interview. Of them, 109 subjects completed the survey.

SACS is a validated screening tool for OSA based on neck circumference, hypertension, habitual snoring, and bed partner reports of nocturnal gasping or choking respiration [3]. A score greater than 15 is used for the prediction of OSA [4]. The Genosophy nutrigenetic test offers personalized recommendations based on the genotyping of 128 single nucleotide polymorphisms. Among others, this test examines polymorphisms in IRS1 (rs294364), ACE (insertion/deletion), APOE (rs429358, rs7412), PPARG (rs1801282), MTHFR (rs1801131, rs1801133), and 5HT2AR (that have been reported in the past to be associated with OSA). It also tests common BDNF (rs6265) and FTO (rs9939609) polymorphisms that have been examined previously [2, 5] in relation to obesity and sleep apnea. These polymorphisms were also included in current analysis.

SNPs were genotyped using the TaqMan assays, provided by Life Technologies (Database: https://www.lifetechnologies.com/es/en/home/life-science/pcr/real-time-pcr/real-time-pcrassays.html). We performed genotyping on the OpenArray™ SNP Genotyping System (Life Technologies, Carlsbad, CA, USA) using microscope slide-sized plates and the OpenArray Accufill autoloader (Life Technologies, Carlsbad, CA, USA), following the manufacturer's instructions. Participants were divided into two groups depending on the results of their Sleep Apnea Clinical Score (SACS ≤ 15 or > 15). Statistical significant differences for nominal and ordinal data were assessed with the *t* test and Chi-squared test respectively. Multiple logistic regression was utilized for further evaluation taking BMI status (≤ 25 or > 25) into account.

Results

Demographic characteristics and genotyping results of the two groups (SACS ≤ 15, SACS > 15) are presented in Tables 1 and 2 respectively. In relation to genotyping study, a statistically significant difference between the two groups was found only for the rs9939609 variant of the FTO ($P = 0.029$). However, this association was attenuated after adjustment for body mass index (Table 3).

Discussion

In several circumstances, genotyping enables more personalized choices and is increasingly applied either for healthcare or lifestyle purposes. By leveraging this torrent of data in combination with relevant phenotypic characteristics, additional observational studies can be performed in a relatively cost-effective way. Herein, we utilize genetic data provided by a nutrigenetic exam in order to test previously reported associations of specific polymorphisms with sleep apnea. More importantly, in contrast to many previous studies, single nucleotide polymorphisms in IRS1, ACE, APOE, PPARG, IL6, MTHFR, 5HT2AR, BDNF, and FTO genes are studied in relation to sleep apnea score as well as to obesity status which can be regarded is a significant confounding factor.

Angiotensin converting enzyme is an important factor of blood pressure homeostasis and a key target of hypertension therapy. ACE insertion/deletion (I/D) polymorphisms are commonly studied in lifestyle genetic tests since these are strongly related to endurance vs strength phenotype in athletes respectively. Several studies and at least five meta-analyses have been published on the possible association of I/D polymorphisms with sleep apnea. The majority of them conclude that there is no statistically significant association except for a subgroup of Asian origin people with hypertension. In this particular group, the insertion-insertion (II) polymorphism is related to OSA syndrome. In our study, we failed to show any associations of this polymorphism with the sleep apnea clinical score. We did not perform a subgroup analysis since the participants were of Caucasian origin and the great majority of them had no known hypertension.

APOE gene has three possible isoforms (ε2, ε3, and ε4) determined by two SNPs (rs429358, rs7412). APOEε4 status is the strongest genetic risk factor for age-related cognitive decline and has been related with OSA in several studies, although two meta-analyses of these studies have shown conflicting results [6, 7]. A probable explanation is that ethnicity and most importantly age-related bias were not corrected for in the majority of these studies. Another important issue is the very low prevalence of the ε2 and ε4 isoforms that does not permit meaningful comparisons in small groups of people. In our group of young Caucasian people, we could not find any association between APOE alleles and sleep apnea clinical score.

PPARγ2 is studied in various nutrigenetic tests since it is implicated in lipid metabolism and it has been shown to influence weight regain upon weight loss diets [8]. A study on obese Asian Indians with OSA had revealed a significantly higher frequency of PPARγ2 (Ala12) allele when compared

Table 1 Basic characteristics of the participants as stratified into two groups based on their Sleep Apnea Clinical Score (SACS)

		SACS ≤ 15	SACS > 15	*P* values
Sex	Men	12	19	$\chi^2 = 0.016$, $P = 0.898$
	Women	33	45	
Mean value of age (SD and range)		38.6 (7.96, 27–59)	39.7 (8.59, 26–61)	$t = -0.655$, $P = 0.514$
BMI	≤ 25	29	9	$\chi^2 = 27.357$, $P < 0.001$
	> 25	16	55	

to obese Asian Indians without OSA [9]. Our study, as well as a previous study on a Chinese population [10] did not show this kind of association. A possible explanation is that the Ala12 allele (CC polymorphism) is highly frequent in the general population and especially in obese patients.

Methylene tetrahydrofolate reductase (MTHFR) gene is implicated in homocysteine metabolism a significant cardiovascular risk factor especially in patients with OSA. Two common polymorphisms of the MTHFR gene that affect homocysteine levels have been studied revealing no statistically significant associations with the OSA status [11].

The IRS is the main substrate of insulin receptor tyrosine kinase and represents a key element in the actions of insulin and insulin-like growth factor. IRS-1 polymorphisms and especially rs2943641 has been strongly associated with the possibility of diabetes mellitus development. This SNP is in linkage disequilibrium with rs1801278 which was found to be associated with occurrence of OSAS only in male patients [12] but this was not replicated in a later candidate gene study [13]. In contrast to our findings and to the abovementioned papers, a recent study on Chinese Han population reported an association between rs1801278 and sleep apnea attributing this to the fact that the association between OSAHS and type 2 diabetes mellitus and insulin resistance is independent of obesity [14]. We did not perform a subgroup analysis in relation to sex or diabetes occurrence since our groups consist mainly from young women without DM.

5-HT2A receptor gene encodes for an important mediator of serotonin action. Serotonin (5-HT) is a neurotransmitter involved in several physiologic functions, such as sleep,

Table 2 Distribution of the genotypes in the groups with high (> 15) and low (≤ 15) Sleep Apnea Clinical Score (SACS)

Single nucleotide polymorphisms		SACS ≤ 15	SACS > 15	
ACE	II	12	16	$P = 0.785$
	ID	23	30	
	DD	10	18	
APOE	ε2	0	1	$P = 0.471$
	ε3	43	62	
	ε4	2	1	
PPARG rs1801282	GG	1	0	$P = 0.401$
	GC	5	5	
	CC	39	59	
MTHFR rs1801131	AA	26	38	$P = 0.956$
	AC	14	20	
	CC	5	6	
MTHFR rs1801133	CC	34	41	$P = 0.342$
	CT	9	16	
	TT	2	7	
IRS1 rs2943641	CC	23	30	$P = 0.904$
	CT	19	29	
	TT	3	5	
5HT2AR rs6311	CC	13	22	$P = 0.833$
	CT	19	25	
	TT	13	17	
FTO rs9939609	AA	8	26	$P = 0.029$ ($\chi^2 = 7.1$)
	AT	22	26	
	TT	15	12	
BDNF rs6265	CC	34	43	$P = 0.286$
	CT	11	18	
	TT	0	3	

Table 3 Multiple logistic regression with SACS (Sleep Apnea Clinical Score), BMI, and FTO variants. Odds ratio (OR) for SACS > 15 was not significant for FTO polymorphisms. On the contrary, overweight or obesity (BMI > 25) increases significantly the risk for SACS > 15

	Coefficient	OR	*P* value
FTO rs9939609 (T allele)	0.426	0.653	0.178
BMI > 25	2274	9.719	< 0.001

appetite, pain perception, and hormone secretion. In addition, the serotonergic system seems to have a relevant role in upper airway patency, as it excites airway motor neuron. Various serotonin receptors exist and 5-Ht2AR is among the best studied. Several meta-analyses that have been published on the association of 1438G/A and T102C polymorphisms with OSA converge to the fact that individuals carrying the 5-HTR 2A -1438G/A AA genotype had an increased risk of OSA syndrome [15–18]. However, in accordance with our findings, a significant association was not supported by the meta-analyses for T102C polymorphism.

BDNF and FTO variants have been correlated with obesity. FTO region harbors the strongest genetic association with obesity due to various mechanisms such as the repression of mitochondrial thermogenesis in adipocyte precursor [19], whereas BDNF is being studied in current nutrigenetic test because it has shown to affect emotional eating behaviors [20, 21]. In addition, a marked decrease of serum and plasma levels of BDNF has been recorded both in adults and children upon OSAS treatment. In accordance with previous studies, BDNF polymorphisms are not correlated to sleep apnea [2] whereas an association between FTO variants and sleep apnea is attenuated after adjustment for body mass index [5].

Pleiotropy, the phenomenon in which a genetic variant is associated with multiple phenotypes has been well known since many years. Current technological developments permit the application of a great number of genetic tests in a relatively low cost. Genome-wide studies, multiplex PCR, or DNA sequencing are examples of genetic data that are being accumulated for various normal or abnormal conditions. By leveraging this data, as in our case, additional genetic epidemiology studies can be performed in a cost effective way.

However, several limitations can be found. As in every observational study, verification of the findings is needed, whereas possible inaccurate clinical data or limited number of patients in certain groups of the study can result in missed associations. Specifically in our study, sleep apnea classification was based on a clinical score (SACS) rather than polysomnography (PSG) which is considered the gold standard in diagnosis of sleep apnea, although SACS is a validated score with good correlations with PSG results. A sleep apnea clinical score greater than 15 has been consistently found in a number of studies as one of the most reliable clinical scores based on validated questionnaires [22]. Nevertheless, despite its limitations, this study takes body weight into account as a possible confounding factor. In contrast, the majority of the previous publications on the correlation of specific SNPs with sleep apnea have failed to account for body weight, even though body weight appears to be an important cofounding factor that needs to be accounted for when genetic association studies are performed for sleep apnea.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study. All procedures performed in the study were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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