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Genetic analysis of candidate SNPs for metabolic syndrome in obstructive sleep apnea (OSA)

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ABSTRACT

Obstructive sleep apnea (OSA) is a common disorder characterized by the reduction or complete cessation in airflow resulting from an obstruction of the upper airway. Several studies have observed an increased risk for cardiovascular morbidity and mortality among OSA patients. Metabolic syndrome (MetS), a cluster of cardiovascular risk factors characterized by the presence of insulin resistance, is often found in patients with OSA, but the complex interplay between these two syndromes is not well understood. In this study, we present the results of a genetic association analysis of 373 candidate SNPs for MetS selected in a previous genome wide association analysis (GWAS). The 384 selected SNPs were genotyped using the Illumina VeraCode Technology in 387 subjects retrospectively assessed at the Internal Medicine Unit of the "Virgen de Valme" University Hospital (Seville, Spain). In order to increase the power of this study and to validate our findings in an independent population, we used data from the Framingham Sleep Study which comprises 368 individuals. Only the rs11211631 polymorphism was associated with OSA in both populations, with an estimated OR = 0.57 (0.42–0.79) in the joint analysis ($p = 7.21 \times 10^{-4}$). This SNP was selected in the previous GWAS for MetS components using a digenic approach, but was not significant in the monogenic study. We have also identified two SNPs (rs2687855 and rs4299396) with a protective effect from OSA only in the subpopulation with abdominal obesity. As a whole, our study does not support the idea that OSA and MetS share major genetic determinants, although both syndromes share common epidemiological and clinical features.

1. Introduction

Obstructive sleep apnea (OSA) is a common disorder characterized by the reduction or complete cessation in airflow resulting from an obstruction of the upper airway. This obstruction results in repetitive breathing pauses during sleep. As a consequence, the architecture of sleep is disrupted: there is a decrease in REM sleep as well as deeper stages of non-REM sleep (Berry et al., 1998).

Several studies have observed an increased risk for cardiovascular morbidity and mortality among OSA patients (Marin et al., 2005; Marshall et al., 2008; Peppard et al., 2000). Metabolic syndrome (MetS), a cluster of cardiovascular risk factors characterized by the presence of insulin resistance (Grundy et al., 2004), is often found in patients with OSA, but the exact nature of this relationship is still controversial (Coughlin et al., 2004; Gruber et al., 2006; Ip et al., 2002; Reichmuth et al., 2005). Repetitive hypoxias have been shown to cause insulin resistance (Braun et al., 2001) and the use of continuous positive airway pressure (CPAP) has been evaluated in OSA patients in relation with mortality (Campos-Rodriguez et al., 2012) and insulin sensitivity. Again the results are still conflicting: whereas some studies reported a better metabolic profile after CPAP treatment (Brooks et al., 1994; Lam et al., 2010; Sharma et al., 2011), other reports failed to identify improved insulin sensitivity (Smurra et al., 2001; West et al., 2007) or a reduction in blood pressure levels (Campos-Rodriguez et

Abbreviations: ABPM, ambulatory blood pressure monitoring; ACE, angiotensin converting enzyme; AHI, apnea hypopnea index; AntiHT, antihypertensive; APOE, apolipoprotein E; ATPIII, Adult Treatment Panel III; BMI, body mass index; CEBP, CCAAT/enhancer binding protein; CHOL, cholesterol; CPAP, continuous positive airway pressure; dbGAP, database of Genotypes and Phenotypes; DBP, diastolic blood pressures; DNA, deoxyribonucleic acid; ET-1, endothelin 1; FEV1, forced expiratory volume at 1 s; GWAS, genome wide association analysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LEP, leptin; LEPR, leptin receptor; AF, minor allele frequency; MetS, metabolic syndrome; NHLB, National Heart, Lung, and Blood Institute; OGTT, oral glucose tolerance test; OSA, obstructive sleep apnea; PLCB1, 1-phosphatidylinositol-4, 5-bisphosphate phosphodiesterase beta-1; PLCB4, 1-phosphatidylinositol-4, 5-bisphosphate phosphodiesterase beta-4; Post-BD, post-bronchodilator; Pre-DB, pre-bronchodilator; QTL, quantitative trait locus; REM, rapid sleep movement; SBP, systolic blood pressure; SNPs, single nucleotide polymorphisms; TGs, triglycerides; TNFα, tumor necrosis factor alpha; WC, waist circumference.

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al., 2007; Iellamo and Montano, 2006). Regarding the prevalence of the individual MetS components in OSA patients, it is particularly high for hypertension and obesity. Some authors have even reported a dose dependent effect between blood pressure and OSA severity (Barcelo et al., 2005; Nieto et al., 2000).

In the pathogenesis of OSA, genetic factors play also an important role, explaining up to 40% of the variance in the apnea hypopnea index (AHI), a quantitative measure of OSA (Palmer et al., 2004; Pillar and Lavie, 1995; Redline et al., 1995; Strohl et al., 1978) Nevertheless, there are few data regarding specific genes associated with OSA. The candidate gene approach has been the standard for identifying genes associated with most common diseases. In the case of OSA, it has been focused in genes affecting upper airway and ventilator control (such as serotonin-2 receptors of transcription or the transcription factor Phox2b) and, particularly in genes related to metabolic syndrome components (Kent et al., 2010; Riha et al., 2009) such as genes encoding the angiotensin converting enzyme (ACE), endothelin (ET-1), leptin and its receptor (LEP, LEPR), tumor necrosis factor alpha (TNFα) or apolipoprotein E (APOE). Unfortunately none of them have been consistently replicated. Recently published meta-analyses have investigated the role of APOE, TNFα and ACE gene polymorphisms in OSA pathogenesis, but they only found a statistical significant association for the TNFα gene (Huang et al., 2012; Lee et al., 2012; Varvarigou et al., 2011).

As the techniques of genome wide analysis have become available, they have been also applied to the study of the genetic causes of OSA. To date, three whole-genome analyses have been published (Palmer et al., 2003a, 2004; Relf et al., 2010). As a result, new candidate chromosomal regions for OSA have been identified, some of them including genes associated with inflammatory responses.

In a previous study, we performed a genome wide analysis in the general population aimed at identifying genes associated with metabolic syndrome and its endophenotypes (Gayan et al., 2010). We selected the 384 markers with the best score for association using both a monogenic and a digenic approach. In this paper, we present the results of the monogenic association analysis of these selected polymorphisms with OSA in a cohort of 387 hypertensive patients with polysomnographic data available. We also explore the role of several demographic, anthropometric and biochemical values in the development of OSA.

2. Material and methods

2.1. Patients

The genetic association study of OSA includes 387 subjects retrospectively assessed at the Internal Medicine Unit of the "Virgen de Valme" University Hospital (Seville, Spain). All of them are hypertensive patients. All participants gave their written consent to participate in the study. The study protocol was approved by the Ethics Committee of the "Virgen de Valme" Hospital (Seville, Spain).

In order to increase the power of this study and to validate our findings in an independent population, we used data from the Framingham Sleep Study. This subpopulation of the population-based Framingham study comprises 368 individuals with recorded AHI values.

2.2. Interventions

For the OSA study, AHI was measured by overnight unattended limited channel polysomnography. OSA was defined as an AHI of 5 events per hour or more.

Other phenotype measurements determined are body mass index (BMI), waist circumference (WC), systolic and diastolic blood pressures (SBP, DBP) and fasting glucose, 2-hour glucose (OGTT, oral glucose tolerance test), fasting insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglyceride (TG) levels.

SBP and DBP were measured by ABMP (using the noninvasive portable validated recorder Spacelab 90207; Spacelabs Medical; Redmond, WA).

After an overnight fasting period, 20 ml of blood was obtained from an antecubital vein without compression. Plasma glucose was determined in duplicate by a glucose-oxidase method adapted to an autoanalyzer (Hitachi 704, Boehringer Mannheim, Germany). Total cholesterol, triglyceride and HDL cholesterol levels were determined by enzymatic methods using commercial kits (Boehringer Mannheim, Germany). LDL cholesterol level was calculated by the Friedewald formula.

The metabolic syndrome status was established according to ATPIII definition as it was recently modified: the presence of at least three components between abdominal obesity (WC $\geq$ 102 cm in men, 88 cm in women), hypertriglyceridemia (TGs $\geq$ 150 mg/dl), hypertension ($\geq$85/130 mm Hg), HDL-c (<40 mg/dl men, <50 mg/dl women) and fasting glucose $\geq$ 100 mg/dl (Grundy et al., 2004).

2.3. Genotyping and quality control

The 384 SNPs genotyped in this study were selected after performing a monogenic and a digenic genome wide association study (GWAS) on metabolic syndrome components. For this study, we used Affymetrix 250k data from 801 Caucasian subjects derived from a cross-sectional population-based epidemiological survey in Spain aimed at investigating the prevalence of metabolic syndrome and related components (Gayan et al., 2010; Lorenzo et al., 2001; Martinez-Larrad et al., 2005).

We obtained 5 ml of peripheral blood from all patients and controls to isolate germline DNA from leukocytes. DNA extraction was performed in a MagNa Pure LC Instrument (Roche Diagnostics) according to the manufacturer's instructions.

The 384 selected SNPs were genotyped using the Illumina VeraCode Technology and the BeadXpress Reader according to the manufacturer's instructions.

All SNPs were subjected to quality control filters. Specifically we only selected SNPs successfully genotyped in at least 95% of individuals and with a p-value for Hardy–Weinberg equilibrium (HWE) larger than 10^{-4}. Finally, 373 SNPs were used in the genetic association study of OSA.

2.4. Statistical analyses

In order to analyze the associations between OSA and demographic/clinical data, we used regression procedures. To select the independent determinants of OSA we used a stepwise linear regression model using AHI as the dependent variable; those traits associated with $p < 0.05$ in this model were used as covariables for the genetic association study of OSA. These analyses were performed using SPSS software.

We used unconditional logistic regression models for the statistical genetic analysis. We performed independent analyses in both populations (i.e. Valme and Framingham) and a joint analysis controlling by population effect (included as a covariate in the model). Joint analysis has been described as more powerful than meta-analysis (Skol et al., 2006). We also performed a meta-analysis in order to compare the consistency of both approaches. For these genetic association analyses, we used PLINK software (Purcell et al., 2007).

We carried out a Breslow–Day test to investigate the independence of these associations with metabolic syndrome (ATPIII definition) and abdominal obesity. In instances when evidence of heterogeneity between-clusters was found, we run stratified analysis. We also tested the effect of hypertension in the Framingham population.