

Lack of Association of Insulin Receptor Substrate Gene Polymorphisms with Obstructive Sleep Apnea Syndrome

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Abstract

Sleep apnea syndrome is associated with increased prevalence of diabetes and has recently shown to be associated with insulin resistance. The aim of the present study was to investigate the relationships between insulin resistance, insulin receptor substrate-1 (IRS-1), insulin receptor substrate-2 (IRS-2) gene polymorphisms and obstructive sleep apnea syndrome (OSAS). The study population consisted of 56 consecutive patients with OSAS and 26 subjects without OSAS were enrolled in the study. Genotyping of IRS-1 and IRS-2 were amplified by polymerase chain reaction (PCR). Insulin resistance was estimated using the homeostasis model assessment (HOMA). In OSAS patients, 2 (3.6%) had G972R gene polymorphism and 54 (96.4%) had no nucleotide substitution in IRS-1 gene whereas in the control group, there was no nucleotide substitution in IRS-1 gene ($p>0.05$). Besides, 47 OSAS patients (84.0%) had no nucleotide substitution, 3 (5.3%) had G1057D heterozygous, 1 (1.8%) had P1033P heterozygous, 3 (5.3%) had P1033P homozygous and 2 (3.6%) had P1033P heterozygous/G1057D heterozygous polymorphisms in IRS-2 gene. In the control subjects, 21 (80.8%) had no nucleotide substitution, 3 (11.5%) had P1033P homozygous and 2 (7.7%) had P1033P heterozygous polymorphisms in IRS-2 gene ($p>0.05$). There was no significant difference between two groups in terms of fasting glucose and HOMA-IR. It was observed that IRS-1 and IRS-2 gene polymorphisms didn't increase risk for OSAS. Besides, there was no association between IRS-1 and IRS-2 polymorphisms and HOMA in OSAS.

Key Words: Obstructive sleep apnea syndrome, insulin receptor substrate-1 gene polymorphism, insulin receptor substrate-2 gene polymorphism

(Rec.Date: Apr 12, 2013)

Accept Date: May 14, 2013)

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Introduction

Excessive daytime sleepiness is an important public health concern associated with increased morbidity and mortality.[1, 2]. Multiple factors likely underlie the pathophysiology of this condition with considerable inter-individual variation. As with other common disorders, including obesity and diabetes mellitus, obstructive sleep apnea syndrome (OSAS) is likely to result from multiple gene-gene interactions occurring in a suitable environment .[3, 4].

Sleep apnea syndrome is associated with increased prevalence of type 2 diabetes [5] and has recently been shown to be a risk factor for incident diabetes [6]. Besides, in non-diabetics, OSA is associated with insulin resistance [7]. In previous studies [8- 10] the relationships between insulin receptor substrate (IRS) genes and glucose metabolism disorders have been shown. IRS molecules are key mediators in insulin signaling and play a central role in maintaining basic cellular functions such as growth, survival, and metabolism [9]. In fact, insulin receptor substrate-1 (IRS-1) appears to have its major role in skeletal muscle for glucose metabolism, whereas insulin receptor substrate-2 (IRS-2) appears to regulate hepatic insulin action as well as pancreatic beta cell development and survival [10]. The most prevalent IRS-1 variant, a Gly-->Arg change at the codon 972, has been reported to be increased in prevalence among patients with type 2 diabetes [11]. Moreover, the G1057D polymorphism in the IRS-2 gene has been reported to be associated with insulin resistance, obesity and type 2 diabetes [12]. However, little is known about its possible association with OSAS. The aim of the present study was to investigate the relationships between IRS-1 and IRS-2 gene polymorphisms and sleep apnea syndrome.

Subjects and Methods

The study population consisted of 56 consecutive patients with OSAS (28 men, mean age 50.1 ± 9.6 yrs, BMI 34.8 ± 4.9 kg/m²). Twenty six subjects without OSAS (15 men, mean age 49.5 ± 6.3 yrs, BMI 34.3 ± 5.5 kg/m²) were enrolled in the study as a control group. The study and control groups were matched for age, gender, and BMI. The subjects of the control group were selected among patients who were admitted to the Internal Medicine Outpatient Clinic for various reasons and matched randomly to OSAS patients using a computed technique. They were all questioned in details by the same doctor who was experienced in sleep

medicine (OB), and excluded if they had any symptoms related to OSAS. The demographic information (age, gender, history of smoking), anthropometric measurements (height, weight, body mass index and neck circumference) and medical history were evaluated in two groups. Besides, full overnight in-laboratory polysomnography were performed in OSAS patients. The control group did not undergo polysomnographic evaluation since none of them had symptoms related to OSAS. Subjective daytime sleepiness was assessed by using the Epworth Sleepiness Scale and >10 was considered as sleepiness [13]. A two point bioelectrical impedance apparatus calibrated for adults (Tanita TBF 300, TANITA Corp.) was used to measure the percentage body fat (%BF) and fat mass in all groups. The local ethics committee approved the study and all subjects gave informed consent.

Polysomnography

All the patients in the study population underwent full overnight in-laboratory polysomnography with a 44-channel recording system (Compumedics E series, Melbourne, Australia). The electroencephalography electrodes were positioned according to the international 10–20 system. PSG consisted of monitoring of sleep by electroencephalography, electrooculography, electromyography, airflow, and respiratory muscle effort, and included measures of electrocardiographic rhythm and blood oxygen saturation. Thoracoabdominal plethysmograph, oro-nasal temperature thermistor and nasal-cannula-pressure transducer system were used to identify apneas and hypopneas. Transcutaneous finger pulse oximeter was used to measure oxygen saturation. Sleep and respiratory events were recorded and scored according to the standard methods [14, 15]). Apnea-hypopnea index (AHI) was the sum of the number of apneas and hypopneas per hour of sleep. OSAS was defined as an AHI of 5 events/h-1 and the presence of clinical symptoms e.g. excessive daytime sleepiness, loud snoring, witnessed apneas and nocturnal choking [16].

Molecular Analysis

IRS1 gene G972R polymorphism genotyping

Two ml of whole blood samples was collected into EDTA–anticoagulated tubes by standard vein puncture method. Genomic DNA was extracted from EDTA– anticoagulated whole blood samples employing the QIAmp Blood DNA mini-kit (Qiagen, Hilden, Germany)

following manufacturer's instructions. DNA concentration was determined by the Nano Drop digital spectroscopy according to the manufacturer's instructions and diluted as 100ng/μl.

Polymerase chain reaction (PCR) and enzyme digest

IRS-1 gene polymorphism was genotyped by method of Baroni et al. [17] They designed primers spanning a region of 198 bp using the following primers: forward 5'-GCTTTCCACAGCTCACCTTC-3' and reverse 5'-GGTAGGCCTGCAAATGCTA-3'.

PCR conditions: Amplification was carried out on a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA) in a 25 μl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific, Inc., CA) containing 1μl genomic DNA solution, Platinum Enhancer Buffer, 2.0 mmol MgCl₂, 50 μmol/l each of the dGTP, dATP, dTTP and dCTP (Promega, Madison, WI), 5 pmol each forward and reverse primers and 1.0 U Platinum Taq polymerase (Invitrogen, Carlsbad, UK). The cycling conditions comprised a hot start at 95°C for 10 min, followed by 35 amplification cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 25 s, followed by one elongation step at 72°C for 5 min.

Digestion conditions: The amplified IRS-1 products were digested by SmaI (5.0 units) in a total volume of 20 μl including NEBuffer 4 (New England Biolabs, Beverly, MA, USA) for at least 2 h at 25°C. The IRS-1 fragments (wild-type GG-171 and 27 bp; heterozygous GA-198, 171 and 27 bp and homozygous AA-198 bp) were run on a 2% agarose gel containing Etidium Bromide and visualized under ultraviolet illumination.

IRS-2 gene G1057D polymorphism genotyping

Two ml of whole blood samples was collected into EDTA-anticoagulated tubes by standard venipuncture method. Genomic DNA was extracted from EDTA-anticoagulated whole blood samples employing the QIAamp Blood DNA mini-kit (Qiagen, Hilden, Germany) following manufacturer's instructions. DNA concentration was determined by the NanoDrop digital spectroscopy according to the manufacturer's instructions and diluted as 100ng/μl.

PCR and enzyme digest

IRS-2 gene polymorphism was genotyped by method of .Lautier et al. [18]. They designed primers spanning a region of 198 bp using the following primers: forward 5'-TCCTTGACGGCCTCCTGT-3' and 5'-AAGGCCTCGACTCCCGACA-3'.

Reverse primers

PCR conditions: Amplification was carried out on a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA) in a 25 µl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific Inc., CA) containing 1 µl genomic DNA solution, Platinum Enhancer Buffer, 2.5 mmol MgCl₂, 50 µmol/l each of the dGTP, dATP, dTTP and dCTP (Promega, Madison, WI), 5 pmol each forward and reverse primers and 1.0 U Platinum Taq polymerase (Invitrogen, Carlsbad, UK). The cycling conditions comprised a hot start at 950C for 10 min, followed by 35 amplification cycles at 950C for 30 s, 580C for 30 s, and 720C for 25 s, followed by one elongation step at 72°C for 5 min.

Biochemical Analysis

Serum concentration of glucose was determined by enzymatic procedures and serum insulin was measured by chemilumminance.

Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) from fasting glucose and insulin concentrations using the following formula [19]:

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows (13.0) software package. Numerical variables were expressed as mean ± standard deviation. The groups were compared using the Mann Whitney test. The relationship between OSAS risk and gene polymorphism was evaluated using Fisher's Exact test.

Results

The characteristics of the study and control groups are shown in Table 1. The patients with OSAS had higher mean systolic blood pressure, waist, hip and neck circumferences compared with the control group (p=0.01). Of 56 OSAS patients, 25 (44.6%) had dyslipidemia, 24

(42.9%) had hypertension, 7 (12.5%) had cardiovascular disease and 7 (12.5%) had type 2 diabetes mellitus. In the control group, 9 (34.6%) had hypertension, 5 (19.2%) had dyslipidemia and 1 (3.8%) had type 2 diabetes mellitus. The mean Epworth sleepiness score and AHI were found to be 12.4 ± 6.6 and 25.6 ± 2.1 events/h, respectively in the patients with OSAS.

Table 1. Characteristics of the study and control groups*.

	OSAS Group (n=56)	Control Group (n=26)	P
Age (years)	50.1±9.6	49.5±6.3	NS
Men, n (%)	28 (50.0%)	15 (57.7%)	NS
Smokers, n (%)	16 (28.6%)	6 (23.1%)	NS
Body weight (kg)	93.3±15.0	92.2±7.9	NS
Body mass index (kg/m ²)	34.8±4.9	34.3±5.5	NS
Waist circumference (cm)	110.4±11.3	95.5±9.9	0.01
Hip circumference (cm)	118.6±7.9	101.5±9.6	0.01
Neck circumference (cm)	40.6±3.1	35.8±2.3	0.01
Fat mass (kg)	42.1±9.1	40.0±7.3	NS
% Fat	43.9±10.0	42.9±5.8	NS
Systolic blood pressure (mmHg)	126.2±14.0	113.9±19.3	0.01
Diastolic blood pressure (mmHg)	69.2±15.8	61.3±13.8	NS

* Data are expressed as mean ± SD, unless otherwise stated.

There was no significant difference between two groups in terms of mean plasma levels of fasting glucose and HOMA-IR, whereas mean fasting insulin levels of the OSAS patients were higher than the control group (8.5 ± 2.6 vs. 7.3 ± 2.5 , $p=0.05$) (Table 2).

Table 2. Biochemical analysis of the study and control groups*

	OSAS Group (n= 56)	Control Group (n= 26)	<i>p</i>
Fasting glucose (mg/dl)	91.3±10.3	91.3±6.9	NS
Fasting insulin (μU/ml)	8.5±2.6	7.3±2.5	0.05
HOMA-IR	1.9±0.7	1.6±0.6	NS

* Data are expressed as mean ± SD

Abbreviation: HOMA-IR= Homeostasis model assessment-insulin resistance

In OSAS, 2 (3.6%) patients had G972R gene polymorphism and 54 (96.4%) had no nucleotide substitution in IRS-1 gene and in the control group, there was no nucleotide substitution in IRS-1 gene ($p>0.05$). Besides, 47 OSAS patients (84.0%) had no nucleotide substitution, 3 (5.3%) had G1057D heterozygous, 1 (1.8%) had P1033P heterozygous, 3 (5.3%) had P1033P homozygous and 2 (3.6%) had P1033P heterozygous/G1057D heterozygous polymorphisms in IRS-2 gene. In the control subjects, 21 (80.8%) had no nucleotide substitution, 3 (11.5%) had P1033P homozygous and 2 (7.7%) had P1033P heterozygous polymorphisms in IRS- 2 gene ($p>0.05$). IRS gene polymorphisms of the OSAS and control groups are shown in Table 3.

Table 3. Insulin receptor substrate (IRS) gene polymorphisms in the study and control groups*

	OSAS Group (n= 56)	Control Group (n= 26)	<i>p</i>
IRS-1 gene polymorphism (+)	2 (3.6%)	-	NS
IRS-2 gene polymorphism (+)	9 (16.1%)	5 (19.2%)	NS

* Data are expressed as the number of patients (%).

Discussion

Genetic factors are thought to play an important role in human development. Recent data indicate that obstructive sleep apnea may have a genetic basis. The pathogenesis of sleep apnea is related to a reduction in the size of the upper airway. The reduction in airway size is secondary to increased adipose tissue, alterations in craniofacial structure and enlargement of the surrounding soft tissue structures. Genetic factors are one of the factors that have been proposed to mediate the size of each of these anatomic risk factors for sleep apnea. Recent evidence is accumulating about the genetic loci for these structural risk factors that predispose to the development of obstructive sleep apnea [20]. In the present study, we found that IRS-1 and IRS-2 gene polymorphisms were not increased in OSAS. Of 56 OSAS patients, 3.6% had G972R gene polymorphism in IRS-1 gene, whereas 5.3% had G1057D heterozygous, 5.3% had P1033P homozygous, 3.6% had P1033P heterozygous/G1057D heterozygous and 1.8% had P1033P heterozygous polymorphism in IRS-2 gene.

IRS family acts as docking proteins between the insulin receptor and a complex network of intracellular signaling molecules [21- 25]. Results from targeted disruption of the IRS genes in mice have provided important clues to the functional differences among these related molecules, suggesting they play different and specific roles in vivo. The available data are consistent with the notion that IRS-1 and IRS-2 are not functionally interchangeable in tissues that are responsible for glucose production (liver), glucose uptake (skeletal muscle and adipose tissue), and insulin production (pancreatic beta cells). By contrast, IRS-3 and IRS-4 genes appear to play a redundant role in the IRS signaling system [20].

There are studies [24, 25] related angiotensin-converting enzyme (ACE) and IRS-1 gene polymorphisms [26], in related to sleep apne risk in Turkish patients with OSAS. Besides, IRS gene polymorphisms were investigated in glucose metabolism disorders [27, 28]. In a study, Bayazit et al. [28] suggested that the polymorphism of the IRS-1 gene at codon 972, especially Gly/Arg variant or the presence of the allele for Arg appears to be associated with occurrence of OSAS in male patients, whereas this polymorphism is not related to severity of OSAS. In this study, IRS-1 gene polymorphism was found to be 3.6% in OSAS patients but not in control group. IRS-2 gene polymorphism was measured 16.1% in OSAS and 19.2% in

control group. There was no statistically significant difference between two groups in terms of IRS-1 and IRS-2 polymorphism.

The relationship between OSA and daytime hypersomnolence is not completely understood. Most subjects with verified OSA do not report daytime sleepiness and there is increasing evidence that snoring without apneas or hypopneas might also relate to sleepiness [29]. Previously, no correlation was found between the genotypes of IRS-1 and polysomnography parameters on correlation analyses in the patients with OSAS [28]. In the present study, it was observed that BMI, hip circumference, fat mass and %fat were lower in OSAS patients with IRS-1 polymorphism but the number of patients with polymorphism was quite small. There was no significant difference between OSAS patients with and without IRS-2 polymorphism in terms of these parameters. Besides, subjective daytime sleepiness assessed by using the Epworth Sleepiness Scale was similar in patients with and without IRS gene polymorphism. Laukkanen et al. [29] reported that the common polymorphisms of the IGF-1R, IRS-1 and IRS-2 genes might be modified the weight change response to a lifestyle intervention. Similarly, IRS-1 gene polymorphism might be showed a protective effect from obesity in OSAS.

Obstructive sleep apnea is independently associated with insulin resistance, and its role in the atherogenic potential of sleep disordered breathing is worthy of further exploration [30]. In many studies [31- 36], there are several potential mechanisms of insulin resistance and impaired insulin secretion during intermittent hypoxia. However, there were no many studies related the relationship insulin resistance and IRS genotype in OSAS. Defects in muscle IRS-1 expression and function have been reported in insulin-resistant states such as obesity and type 2 diabetes. In contrast, polymorphisms of the other IRS genes do not appear to contribute to type 2 diabetes [20, 37]. In our findings, we did not find any association between IRS-1 gene polymorphisms and HOMA levels in OSAS. Likewise, no relationships between IRS-2 gene polymorphisms and HOMA levels were found in OSAS and control. However, our study involved small number of subjects so that its statistical power may be insufficient to detect small associations. Large based population studies are needed.

The limitations of this study must be taken into account. First, the numbers of subjects in the study and control groups were somewhat small. IRS gene polymorphism was higher in OSAS patients, but the difference was not significant probably because of sample size. Second, the

subjects in the control group did not undergo polysomnography as the waiting list of our sleep laboratory was quite long and it was very difficult to perform polysomnography even to the patients with obvious OSAS symptoms. However, they were all questioned in details and none of them had symptoms related to OSAS.

In conclusion, the results of the current study suggest that IRS-1 and IRS-2 gene polymorphisms didn't increase risk for OSAS. Besides, there were no associations between IRS-1 and IRS-2 polymorphisms and HOMA in OSAS patients. However, further studies with larger number of patients are needed to show the relationship between IRS gene polymorphism and sleep apnea.

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