Association between Single Nucleotide Polymorphisms in Gamma-Aminobutyric Acid B Receptor, Insulin Receptor Substrate-1, and Hypocretin Neuropeptide Precursor Genes and Susceptibility to Obstructive Sleep Apnea Hypopnea Syndrome in a Chinese Han Population

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Significance of the Study

- To investigate genotype-phenotype changes associated with SNPs of rs1801278 *(GABBR1)*, rs29230 *(IRS-1)*, and rs9902709 *(HCRT)* genes in Chinese Han individuals. This study revealed that the SNPs of rs1801278 and rs29230 were susceptible markers for the increasing risk of obstructive sleep hypoapnea syndrome, but rs9902709 did not appear to be a susceptibility locus.

Abstract

Objective: To investigate genotype-phenotype changes between rs29230 in γ-aminobutyric acid B receptor *(GABBR1)*, rs1801278 in insulin receptor substrate-1 *(IRS-1)*, and rs9902709 in hypocretin neuropeptide precursor *(HCRT)* and obstructive sleep apnea hypopnea syndrome (OSAHS) in Chinese Han individuals. ***Materials and Methods:*** A total of 130 patients with OSAHS and 136 age- and gender-matched healthy controls were enrolled in this study. A brief description of DNA extraction and genotyping is given. Mul-tivariate unconditional logistic regression analysis adjusted for gender and age was used to estimate the associations of single nucleotide polymorphisms (SNPs) rs29230 *(GABBR1)*, rs1801278 *(IRS-1)*, and rs9902709 *(HCRT)* with OSAHS risk. Subgroup analysis was performed to evaluate differences in these SNPs among subgroups according to gender, body mass index (BMI), and severity of disease. ***Results:*** Genotype and allele frequencies of rs29230 were significantly different between cases and controls (p = 0.0205 and p = 0.0191, re-spectively; odds ratio = 0.493, 95% confidence interval = 0.271–0.896), especially for male patients (p = 0.0259 and p = 0.0202, respectively). Subgroup analysis according to BMI also revealed a significant allele difference for rs29230 between cases and controls in the overweight subgroup (p = 0.0333). Furthermore, allele and genotype frequencies of rs1801278 showed significant differences between cases and controls (p = 0.0488 and p = 0.0471, respectively). How-

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ever, no association was observed between rs9902709 and OSAHS risk (p = 0.2762), and no differences were identified in other subgroups. ***Conclusion:*** In this study, there was an association between variants of rs29230 and rs1801278 and OSAHS risk in the Chinese Han population but not for rs9902709.

Introduction

Obstructive sleep apnea hypopnea syndrome (OSAHS) is a common disorder characterized by the limitation of airflow resulting from repetitive episodes of partial or complete upper airway collapse during sleep [1]. These recurrent episodes usually lead to repetitive breathing pauses during sleep, accompanied by snoring, marked sleep fragmentation, transient hypoxia and hypercapnia, and chronic excessive daytime sleepiness [2]. While ventilation is normal in waking patients with OSAHS, sleep-related reductions in motoneuronal activity result in collapse of the upper airway. With changing lifestyles and dietary habits, the global prevalence of OSAHS has been determined to be fairly high, and is estimated to affect up to 17% of middle-aged men and 9% of middle-aged women [3].

The pathogenesis of OSAHS is not fully elucidated at the hereditary level. However, genetic and epidemiological studies indicate that OSAHS susceptibility is associated with multiple genetic, environmental, and developmental factors [4]. The pathophysiology of OSAHS may be investigated by detecting novel genetic susceptibility markers, which could lead to the development of new preventive and curative methods. Genetic variants in-crease the risk of OSAHS due to involvement in pathways of the upper airway anatomy, airway collapsibility, ventilation, and sleep-wake control [5].

An association between Ala20Val polymorphism and C/C variant of the *GABBR1* Phe658Phe polymorphism and OSAHS had been reported in Turkish individuals [6], but not for the genotype and allele frequencies of *IRS-1* rs1801278 [7]. In addition, also, an association between the C allele of the *HCRT* rs9902709 variation and OSAHS was reported in a Japanese population [8]. Hence based on these findings the objective of this study was to investigate genotype-phenotype changes between rs29230 in γ-aminobutyric acid B receptor *(GABBR1)*, rs1801278 in insulin receptor substrate-1 *(IRS-1)*, rs9902709 in hypocretin neuropeptide precursor *(HCRT)* and OSAHS in Chinese Han individuals.

Subjects and Methods

Subjects

A total of 130 Han patients with OSAHS (20 females and 110 males) and 136 controls (24 females and 112 males) who were examined at the Sleep Disorders Center of Zhejiang Hospital (Hangzhou, China) were enrolled in this study in southeast China (fig. 1). The subjects were divided into two subgroups based on body mass index (BMI). The patient cohort consisted of 116 overweight (BMI ≥25) and 14 normal-weight (BMI <25) individuals, while the control group comprised 92 overweight and 44 normal-weight subjects. Each of them provided a written informed consent prior to being enrolled in the study. The research was conducted in accordance with the World Medical Association Declaration of Helsinki and approved by the Ethics Committee of Zhejiang Hospital.

OSAHS Diagnosis and Exclusion Criteria

Study participants underwent diagnostic overnight polysomnography (Alice LE; Philips Respironics Inc., Murrysville, Pa.,

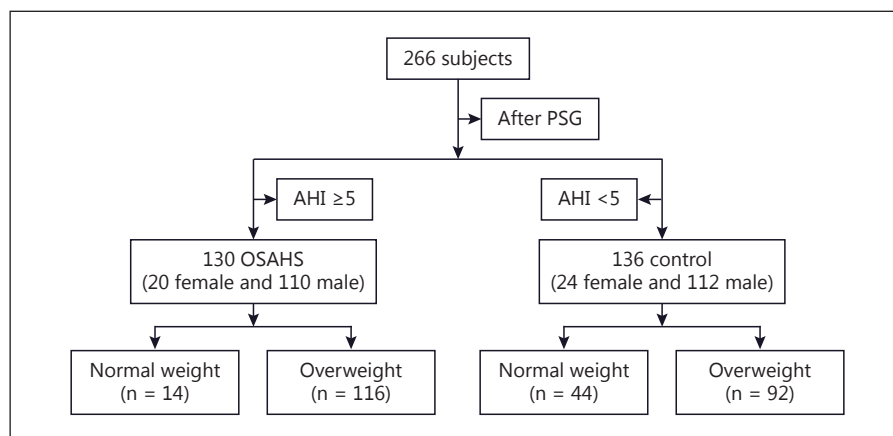**Fig. 1.** Flowchart for subject recruitment. PSG = Polysomnography.

Table 1. Oligonucleotide sequences used for genotyping

Gene	SNP	Allele/Primers	Sequences
IRS-1 (*Homo sapiens*)	rs1801278	Allele	ATGGGCAGACTGGGCCCTGCACCTCCC[G/A]GGG-CTGCTAGCATTTGCAGGCCTA
		First primer	5′-ACGTTGGATGATGGTCATGTAGTCACCCCG-3′
		Second primer	5′-ACGTTGGATGGTCGAGATGGGCAGACTGG-3′
		Extension primer	5′-AAATGCTAGCAGCCC-3′
GABBR1 (*Homo sapiens*)	rs29230	Allele	ATGGTTACCACATTGGGAGGAACCAG[T/C]CCTTTCGTCTGCCAGGTGAGGAGG
		First primer	5′-ACGTTGGATGTCGATGGTTACCACATTGGG-3′
		Second primer	5′-ACGTTGGATGCCTGTAAGGAATTTGCCCAC-3′
		Extension primer	5′-TGGGAGGAACCAGTT-3′
HCRT (*Homo sapiens*)	rs9902709	Allele	CTCTGGGATGGTGAGTCACCCCTCC[A/G]TC-CCTGGATCTTTACCTTTGTGGAA
		First primer	5′-ACGTTGGATGGCCAGACACCATGAACCTTC-3′
		Second primer	5′-ACGTTGGATGTTTGCTTCTCTGGGATGGTG-3′
		Extension primer	5′-GGAAAGGTAAAGATCCAGGGA-3′

USA). The baseline examination comprised pulsoxymetry, electrophysiological signals (electroencephalography, electrooculography, and electromyography), electrocardiography, observation of thoracic movement and the analysis of sleep stages, snoring phases, and awakening reactions. Recordings were reviewed independently by two sleep specialists (J.D. and T.T.). Apnea was defined as the continuous cessation of airflow for >10 s, and hypopnea as a ≥50% reduction in airflow for >10 s with oxygen desaturation of ≥4%. The apnea-hypopnea index (AHI) was calculated as the total number of apnea and hypopnea events per hour of sleep. Patients were grouped according to OSAHS severity as follows: mild OSAHS (AHI 5–15 events/h), moderate OSAHS (AHI 16–30 events/h), and severe OSAHS (AHI >30 events/h). The exclusion criteria were: hypothyroidism, acromegaly, micrognathia, central sleep apnea, rhinitis, nasal polyposis, tonsil hypertrophy, or cardiac insufficiency; chronic obstructive pulmonary disease or interstitial lung disease; a history of drug or alcohol abuse, and depression, and any kind of treatment for OSAHS. 129 subjects were excluded accordingly.

DNA Extraction and Genotyping

Peripheral blood samples were obtained from each participant and stored at −70°C. The DNA was isolated from human peripheral leukocytes of whole-blood samples using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, Calif., USA). The single nucleotide polymorphisms (SNPs) *HCRT* rs9902709, *GABBR1* rs29230, and *IRS-1* rs1801278 were amplified using the GeneAmp PCR System 9700 (ABI Co. Ltd., New York, N.Y., USA). Primers for polymerase chain reaction (PCR) and single-base extension were designed using Assay Designers software version 3.0 (Sequenom Inc., San Diego, Calif., USA) and were processed following standard protocols for iPLEX chemistry. Primers were synthesized by Sangon Biotech Shanghai Co. Ltd. (Shanghai, China; table 1).

Multiplex PCR was performed in 5-μl volumes containing 0.1 units of HotStar Taq polymerase (Qiagen, Hilden, Germany),

Table 2. Clinical and polysomnographic characteristics of the study subjects

Variables	Patients	Controls	p value
Demographics			
Gender, n			0.817
Male	110 (84.6%)	112 (82.4%)	
Female	20 (15.4%)	24 (17.6%)	
Mean age ± SD, years	51.78±12.24	50.88±8.21	0.617
Mean BMI ± SD	28.17±3.62	25.38±3.17	
Normal weight, n	14 (10.77%)	44 (32.35%)	0.005
Overweight, n	116 (89.23%)	92 (67.65%)	
Severity of disease, n			<0.001
Normal	0	136	
Mild	26 (20%)	UN	
Moderate	22 (16.92%)	UN	
Severe	82 (63.08%)	UN	
Polysomnography (means ± SD)			
AHI, events/h	35.66±19.52	3.43±1.12	<0.001
Male	38.78±18.16	3.32±2.05	
Female	25.76±15.13	3.12±1.07	
Overweight	39.12±22.32	4.09±1.87	
Normal weight	28.34±13.32	2.89±0.97	
MAI, events/h	26.28±16.09	4.87±1.56	<0.001
LSaO₂, %	74.27±11.15	96.85±3.8	<0.001
ODI4, events/h	43.42±25.17	4.16±1.08	
Sleep stages, % of TST			
NREM1	54.21±18.79	47.9±20.04	<0.001
NREM2	99.73±32.31	92.1±36.21	<0.001
NREM3/4	60.51±19.95	46.23±15.72	<0.001
REM	53.23±18.31	39.74±16.36	<0.001

LSaO₂ = Lowest oxygen saturation; MAI = microarousal index; NREM = non-rapid eye movement sleep; ODI4 = oxygen desaturation index; REM = rapid eye movement sleep; SD = standard deviation; TST = total sleep time; UN = unknown.

Table 3. Allele and genotype distribution in the patients and controls

Gene	Cases	Controls	χ^2	p value	OR (95% CI)
HCRT rs9902709					
Allele			1.1857	0.2762	
A	255 (0.981)	260 (0.942)			
G	5 (0.019)	16 (0.058)			2.443 (0.465–12.820)
Genotype			1.1408	0.5653	
AG	5 (0.038)	6 (0.044)			
AA	125 (0.962)	128 (0.941)			
GG	0 (0.000)	2 (0.015)			
GABBR1 rs29230					
Allele			5.4914	0.0191	
T	185 (0.717)	228 (0.838)			
C	73 (0.283)	44 (0.162)			0.493 (0.271–0.896)
Genotype			7.7729	0.0205	
CC	12 (0.093)	0 (0.000)			
TT	65 (0.504)	95 (0.699)			
CT	52 (0.403)	41 (0.301)			
IRS-1 rs1801278					
Allele			3.8819	0.0488	
A	0 (0.000)	9 (0.033)			
G	260 (1.000)	263 (0.967)			
Genotype			3.9421	0.0471	
AG	0 (0.000)	9 (0.067)			
GG	130 (1.000)	127 (0.933)			

10 ng of whole genome-amplified genomic DNA, 2.5 pmol of each primer, and 2.5 μmol of dNTP. Thermocycling was carried out at 94°C for 15 min followed by 45 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min, with a final incubation at 72°C for 3 min. Unincorporated dNTPs were deactivated using 0.3 units of shrimp alkaline phosphatase (Sequenom Inc.). Primer extension was performed using 5.4 pmol of each primer extension probe, 50 μmol of the appropriate ddNTP combination, and 0.5 units of iPLEX enzyme (Sequenom Inc.). Extension reactions were carried out at 94°C for 15 min followed by 45 cycles at 94°C for 20 s, then 56°C for 30 s, and 72°C for 1 min, and a final incubation at 72°C for 3 min.

Thermocycling A cation exchange resin was used to remove residual salt from the reactions. Purified primer extension reaction products were spotted into a 384-well SpectroCHIP using the MassARRAY Nanodispenser (Sequenom Co.) and determined by a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer. Genotype calling was performed in real time with MassARRAY RT software version 3.0.0.4 and analyzed using MassARRAY Typer software version 3.4.

Statistical Analysis

Continuous values were expressed as means ± standard deviation. Hardy-Weinberg equilibrium testing was carried out for all three SNPs. Pearson's χ^2 tests were used to compare the alleles and genotypes between cases and controls, and stratified analyses were performed to evaluate the difference of these SNPs among subgroups based on gender, BMI, and severity of disease. Odds ratios

(ORs) and 95% confidence intervals (CIs) were obtained with Plink software (OpenLink Software Inc., Burlington, Mass., USA). p values <0.05 were considered statistically significant.

Results

Characteristics of Study Subjects

Clinical and polysomnographic characteristics of study subjects are shown in table 2. The mean age was 51.78 ± 12.24 years (range = 31–67) in cases and 50.88 ± 8.21 years (range = 31–60) in controls, Following BMI classification, 116 OSAHS patients (89.23%) had a higher likelihood of being overweight, while, by contrast, 44 healthy subjects (32.35%) with normal weight were more prevalent in the control group; the difference between the two groups was significant (p = 0.005). We examined Hardy-Weinberg equilibrium in the controls and cases with observed p values of 0.00212 for rs9902709, 0.86024 for rs1801278, and 0.85051 for rs29230.

Polymorphisms in Case and Control Cohorts

For SNP rs29230, allele and genotype frequencies were 71.7% (T), 28.3% (C), 9.3% (CC), 50.4% (TT), and

Table 4. Allele and genotype distribution in the patients and controls both according to gender

Gene	Male		p value	OR (95% CI)	Female		p value	OR (95% CI)
	cases	controls			cases	controls		
HCRT rs9902709								
Allele								
A	215 (0.977)	215 (0.960)			40 (1.000)	45 (0.938)		
G	5 (0.023)	9 (0.040)	0.4206	2 (0.359–11.149)	0 (0.000)	3 (0.062)	0.3558	
Genotype								
AG	5 (0.045)	5 (0.044)			0 (0.000)	2 (0.083)		
AA	105 (0.955)	105 (0.938)			20 (1.000)	22 (0.917)	0.3501	
GG	0 (0.000)	2 (0.018)	0.6092					
GABBR1 rs29230								
Allele								
T	155 (0.705)	188 (0.839)			27 (0.750)	40 (0.833)		
C	65 (0.295)	36 (0.161)	0.0202	0.467 (0.243–0.895)	9 (0.250)	8 (0.167)	0.650	0.7 (0.149–3.282)
Genotype								
CC	13 (0.118)	0 (0.000)						
TT	57 (0.518)	76 (0.679)			10 (0.556)	15 (0.625)		
CT	40 (0.364)	36 (0.321)	0.0259		8 (0.444)	9 (0.375)	0.6039	0.625 (0.105–3.707)
IRS-1 rs1801278								
Allele								
A	0 (0.000)	5 (0.022)			0 (0.000)	3 (0.063)		
G	220 (1.000)	219 (0.978)	0.0839		40 (1.000)	45 (0.937)	0.3558	
Genotype								
AG	0 (0.000)	5 (0.045)			0 (0.000)	2 (0.083)		
GG	110 (1.000)	107 (0.955)	0.0818		20 (1.000)	22 (0.917)	0.3501	

40.3% (CT) in patients, and 83.8% (T), 16.2% (C), 69.9% (TT), and 30.1% (CT) in controls; differences were significant between the two groups ($\chi^2 = 5.4914$, p = 0.0191, OR = 0.493, 95% CI = 0.271–0.896, and $\chi^2 = 7.7729$, p = 0.0205), indicating that possession of the C allele is associated with susceptibility to OSAHS (table 3). To further explore allele and genotype frequency differences, the subgroup analysis demonstrated that the allele and genotype distributions of rs29230 were 70.5% (T), 29.5% (C), 11.8% (CC), 51.8% (TT), and 36.4% (CT) in male patients, and 83.9% (T), 16.1% (C), 67.9% (TT), 32.1% (CT), and 0% (CC) in male controls, indicating that possession of at least one C allele is significantly more frequent in patients than controls (p = 0.0202, OR = 0.467, 95% CI = 0.243–0.895, and p = 0.0259; table 4). However, no differences were identified between female patients and controls (table 4). Likewise, the C allele of rs29230 was more prevalent in cases than controls in the overweight subgroup (p = 0.0333, OR = 0.487, 95% CI = 0.250–0.951; table 5).

For rs1801278 variants, only the G allele was detected in cases, with A and G alleles present in controls; a significant difference was observed between cases and controls ($\chi^2 = 3.8819$, p = 0.0488; table 3). Moreover, the GG genotype was significantly more common in patients than controls ($\chi^2 = 3.9421$, p = 0.0471; table 3). However, subgroup analysis according to gender, BMI and severity of disease indicated that no significant associations with OSAHS risk were identified (tables 4–6).

Allele and genotype frequencies of rs9902709 were 98.5% (A), 1.5% (G), 3.1% (AG), and 96.9% (AA) in cases, and 96.3% (A), 3.7% (G), 4.4% (AG), 94.1% (AA), and 1.5% (GG) in controls; the lack of significance between the two groups indicates that rs9902709 variants are not involved in the etiology of OSAHS ($\chi^2 = 1.1857$, p = 0.2762, OR = 2.443, 95% CI = 0.465–12.82, and $\chi^2 = 1.1408$, p = 0.5653; table 3). Furthermore, stratified analysis according to gender, BMI, and severity of disease also revealed no associations with OSAHS (tables 4–6).

Table 5. Allele and genotype distribution in the patients and controls according to BMI

Gene	Normal weight		p value	OR (95% CI)	Overweight		p value	OR (95% CI)
	cases	controls			cases	controls		
HCRT rs9902709								
Allele								
A	28 (1.000)	82 (0.976)			227 (0.978)	175 (0.951)		
G	0 (0.000)	2 (0.024)	0.5602	3.029 (0.737–12.455)	5 (0.022)	9 (0.049)	0.2615	2.591 (0.464–14.47)
Genotype								
AG	0 (0.000)	2 (0.048)			5 (0.043)	5 (0.054)		
AA	14 (1.000)	40 (0.952)	0.5566		111 (0.957)	85 (0.924)		
GG					0 (0.000)	2 (0.022)	0.5116	
GABBR1 rs29230								
Allele								
T	23 (0.958)	72 (0.857)			165 (0.711)	155 (0.842)		
C	1 (0.042)	12 (0.143)	0.5882	1.833 (0.199–16.916)	67 (0.289)	29 (0.158)	0.0333	0.487 (0.250–0.951)
Genotype								
TT	10 (0.833)	30 (0.714)			58 (0.500)	60 (0.652)		
CT	0	0			45 (0.388)	32 (0.348)		
CC	2 (0.167)	12 (0.286)	0.5573	2.000 (0.191–20.899)	13 (0.112)	0 (0.000)	0.0506	
IRS-1 rs1801278								
Allele								
A	0 (0.000)	4 (0.048)			0 (0.000)	4 (0.022)		
G	28 (1.000)	80 (0.952)	0.4057		232 (1.000)	180 (0.978)	0.1106	
Genotype								
AG	0 (0.000)	5 (0.119)			0 (0.000)	4 (0.043)		
GG	14 (1.000)	37 (0.881)	0.3968		116 (1.000)	88 (0.957)	0.1088	

Discussion

The present study showed that the SNPs *GABBR1* rs29230 and *IRS-1* rs1801278 were susceptibility candidates for OSAHS in Chinese Han individuals. However, no association between *HCRT* rs9902709 and OSAHS was revealed.

The patency of the upper airway during respiration is controlled mostly by muscle activity, which also prevents collapse due to negative airway pressure [9]. Hypoglossal motoneurons are cholinergic elements that innervate tongue muscles and play important roles during respiration. Dysfunction of the hypoglossal motoneurons appears to be implicated in OSAHS [10]. The hypoglossal motor nucleus releases GABA, which has an important physiological function through its association with a large family of receptors, including the metabotropic GABA$_B$ receptor [11]. This is encoded by *GABBR1*, which maps to chromosome 6, contains 22 exons, and spans at least 18 kb [12, 13]. It is therefore conceivable that *GABBR1* variations correlate with the etiology of OSAHS.

Our present study showed significant differences in *GABBR1* SNP rs29230 between OSAHS cases and controls (p = 0.0205), especially in male and overweight patients, which was concordant with those reports. The previous research by Bayazit et al. [6] showed that *GABBR1* polymorphisms are associated with the occurrence of OSAHS in the Turkish population. However, the overweight individuals in OSAHS patients were more prevalent than healthy controls, which may increase the possibility of obtaining spurious findings because an increased body weight can cause the development of OSAHS. Nevertheless, our findings and previous studies indicate that *GABBR1* is a susceptibility locus for OSAHS, independently of ethnic background and environmental agents.

Our present study also identified a strong association between the *IRS-1* rs1801278 G allele or GG genotype and an increased risk of developing OSAHS in the Chinese Han population. To the best of our knowledge, the association between OSAHS and type 2 diabetes mellitus and insulin resistance is independent of obesity [14]. The IRS is the main substrate of insulin receptor tyrosine kinase,

Table 6. Allele and genotype distribution in OSAHS according to severity of disease

Gene	OSAHS mild	moderate	p value	OR (95% CI)	OSAHS mild	severe	p value	OR (95% CI)	OSAHS moderate	severe	p value	OR (95% CI)
HCRT rs9902709												
Allele												
A	52 (1.000)	44 (1.000)			52 (1.000)	159 (0.970)			44 (1.000)	159 (0.970)		
G	0 (0.000)	0 (0.000)			0 (0.000)	5 (0.030)	0.4215		0 (0.000)	5 (0.030)	0.4595	
Genotype												
AG	0 (0.000)	0 (0.000)			0 (0.000)	5 (0.061)			0 (0.000)	5 (0.061)		
AA	26 (1.000)	22 (1.000)			26 (1.000)	77 (0.939)	0.4171		22 (1.000)	77 (0.939)	0.4551	
GABBR1 rs29230												
Allele												
T	45 (0.865)	31 (0.705)			45 (0.865)	105 (0.675)			31 (0.705)	105 (0.675)		
C	7 (0.135)	13 (0.295)	0.3123	2.063 (0.499–8.530)	7 (0.135)	55 (0.325)	0.0924	2.648 (0.827–8.478)	13 (0.295)	55 (0.325)	0.6398	1.284 (0.450–3.663)
Genotype												
CC	2 (0.077)	2 (0.091)			2 (0.077)	6 (0.075)			2 (0.091)	6 (0.075)		
TT	20 (0.769)	15 (0.682)			20 (0.769)	35 (0.438)			15 (0.682)	35 (0.438)		
CT	4 (0.154)	5 (0.227)	0.7044		4 (0.154)	39 (0.487)	0.0775		5 (0.227)	39 (0.487)	0.146	
IRS-1 rs1801278												
Allele												
G	52 (1.000)	44 (1.000)			52 (1.000)	164 (1.000)			44 (1.000)	164 (1.000)		
Genotype												
GG	26 (1.000)	22 (1.000)			26 (1.000)	82 (1.000)			22 (1.000)	82 (1.000)		

and represents a key element in the actions of insulin and insulin-like growth factor. The IRS family consists of 4 closely related members, and *IRS-1* on chromosome 2q has been regarded as a plausible susceptibility locus for insulin resistance in type 2 diabetes mellitus. Among the known *IRS-1* polymorphisms, associations were previously observed with diabetes mellitus, cancer, multiple myeloma, and Alzheimer's disease [15–18]. However, Bayazit et al. [6] reported no significant differences in the genotype and allele frequencies of *IRS-1* rs1801278 between OSAHS patients and controls overall, but *IRS-1* variants in male Turkish patients appeared to be susceptible to OSAHS development via a gender-specific comparison [7], which was inconsistent with our finding. The discrepancy between our results and the previous study could be attributed to ethnic or environmental factors, so further studies are needed to verify the findings.

Additionally, the current research demonstrated that no association between rs9902709 variants and OSAHS was identified in Chinese Han individuals. Based on our results, it seems reasonable to speculate that *HCRT* variants are not involved in the development of OSAHS. The previous study reported that circulating hypocretin levels were significantly lower in OSAHS patients than controls,

and inversely correlated with the severity of OSAHS [19, 20]; however, a clinical trial by Ahmed et al. [8] had previously found that the frequency of the *HCRT* rs9902709 variation increased *HCRT* expression, and was associated with a decreased risk for OSAHS in the Japanese population. These differences between the two studies may be primarily attributed to environmental factors which have the potential to interact with the genetic background.

The present study had two limitations: the sample size was relatively small and OSAHS patients were more likely to be overweight than controls, which might have increased the chance of obtaining spurious findings. Future analysis should investigate a larger sample size, and include these risk factors.

Conclusion

The present study showed that SNPs rs1801278 and rs29230 were associated with an increased risk of OSAHS, and that *HCRT* did not appear to be a susceptibility gene for OSAHS.

Disclosure Statement

There is no conflict of interest.

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