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Relationship between zinc finger protein 36 *(ZFP36)* gene polymorphisms and obstructive sleep apnea

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ABSTRACT. Recent data have indicated that inflammation may have an important correlation with obstructive sleep apnea (OSA). Studies have indicated a relationship between OSA and *TNF-α* gene polymorphisms. Zinc finger protein 36 (ZFP36) regulates *TNF-α* mRNAs. However, *ZFP36* gene polymorphisms have not been investigated in OSA. Therefore, we conducted the present case-control study to assess whether variances in *ZFP36* gene polymorphisms account for differences in TNF-α levels in patients with moderate-to-severe OSA. This case-control study aims to investigate the relationship between genetic variations in the *ZFP36* gene and moderate-to-severe OSA. Three common single nucleotide polymorphisms of the *ZFP36* gene (rs251864, rs3746083, and rs17879933) were evaluated in a group of patients with moderate-to-severe OSA (N = 408) and in a control

group (N = 394) by using TaqMan polymerase chain reaction analysis. The moderate-to-severe OSA group and the control group exhibited significant differences in the distributions of rs251864 and rs17879933 genotypes and alleles ($P < 0.05$). TNF-α levels were significantly different not only among the three rs251864 genotypes but also between the II genotype and the DD + ID genotypes of rs17879933. However, no significant differences in sleep apnea parameters in the three *ZFP36* gene polymorphisms were observed. Logistic regression analyses demonstrated that TNF-α and the three *ZFP36* gene polymorphisms were not independently associated with OSA. *ZFP36* might be involved in *TNF-α* regulation. However, *ZFP36* gene variants were not independent risk factors for moderate-to-severe OSA.

Key words: Obstructive sleep apnea; Zinc finger protein 36; Tumor necrosis factor-α; Gene polymorphisms

INTRODUCTION

Obstructive sleep apnea (OSA) is a major cause of neurocognitive and behavioral dysfunctions, as well as of cardiovascular and metabolic morbidities (Capdevila et al., 2008). OSA is the most common sleep-disordered breathing abnormality (Weiss et al., 2007) and often results in apnea or hypopnea, which can lead to snoring. OSA is a common medical condition and a form of disordered sleep breathing. The disease is classified as mild, moderate, or severe based on the number of apneas and/or hypopneas per hour of sleep, and this classification is known as the apnea-hypopnea index (AHI). OSA is present in 2% of women and 4% of men living in Western communities. The prevalence of OSA has been reported to be 3.7% in a Japanese population (Asaoka et al., 2010) and 49.3% in a Chinese population with hypertension (He et al., 2010). Male gender, older age, higher body mass index (BMI), increased waist-to-hip ratio, greater neck circumference, arterial hypertension, smoking, and snoring are related to OSA. However, the mechanisms underlying these major risk factors are not well understood. Many potential mechanisms, such as inflammation, chronic sympathetic activation, endothelial dysfunction, increased endothelin levels, hypercoagulability, rennin-angiotensin system stimulation (Wolf et al., 2007), and genetics (Campana et al., 2010) have linked OSA to cardiovascular disease.

TNF-α is one of the most important inflammatory factors involved in sleep regulation (Krueger, 2008). The current study elucidates the specific contribution of *TNF-α*-308G gene polymorphism to increased plasma TNF-α levels when OSA is present, and links OSA symptoms of excessive daytime sleepiness to increased TNF-α levels and the *TNF-α*-308G gene polymorphism (Khalyfa et al., 2011).

Zinc finger protein 36 (*ZFP36*), which is one of the best-characterized adenylate and uridylate-rich element (ARE)-binding proteins, regulates inflammatory cytokine (*TNF-α*, *IL-6*) mRNA expression (Lai et al., 2006; Sanduja et al., 2012), particularly in inflammatory syndromes, such as rheumatoid arthritis, systemic lupus erythematosus, and ulcerative colitis. In addition, recent research has shown that *ZFP36* is a promising candidate gene for obesity-associated metabolic complications. Recent data have also suggested that OSA is highly associated with metabolic syndromes. Therefore, we investigated whether a relationship be-

tween OSA and *ZFP36* exists. We conducted the present case-control study to assess whether variances in *ZFP36* gene polymorphisms account for the differences in TNF-α levels with moderate-to-severe OSA. We also examined whether sleep apnea parameters correlate with *ZFP36* genotype and plasma TNF-α levels.

MATERIAL AND METHODS

Moderate-to-severe OSA subjects

A total of 408 subjects with moderate-to-severe OSA based on polysomnography (PSG) were referred to The Center of Diagnosis, Treatment, and Research of Hypertension in Xinjiang between January 2010 and December 2010. Subjects with confounding conditions, such as diabetes, congestive heart failure, asthma, chronic obstructive pulmonary disease, cirrhosis, or renal impairment, were excluded from the study. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of the People's Hospital of Xinjiang Uygur Autonomous Region. Written informed consent was obtained from all participants.

PSG recordings

Polysomnograms were recorded using an E-series Compumedics system (Compumedics Ltd., Abbotsford, Victoria, Australia) and the recording array comprised and investigated: a C3/M2 electroencephalogram; left and right electrooculograms; an electrocardiogram; a submental electromyogram; airflow from a nasal pressure transducer and an oronasal thermocouple (Compumedics Ltd.); body position; thoracic and abdominal excursions (inductance plethysmography); finger pulse oximetry (Masimo SET Radical, Masimo, Irvine, CA, USA), set to an average time of 8 s and sampled at 1 Hz; left and right leg movements (piezoelectric sensors); and a sound pressure meter (RION Co. Ltd., Tokyo, Japan).

Recordings were made as part of a routine diagnostic service based on best clinical and laboratory practices. Overnight staff members were instructed to correct respiratory sensors that were not functioning correctly when the patient awoke, but they made no other effort to maximize signal integrity.

PSG scoring

PSGs were scored by one of three scorers using the Compumedics Profusion PSG 2 software. All scorers participated in intra- and inter-laboratory scoring concordance programs. Scoring was based on the Tsara rules for sleep (Tsara et al., 2009). The AHI was defined as the number of apneas and hypopneas per hour of sleep and was confirmed using an electroencephalogram. OSA severity was determined based on a combination of the severity of daytime sleepiness and the AHI value. Mild OSA was scored as 5 to 15 events per hour, moderate OSA was scored as 15 to 30 events per hour, and severe OSA was scored as greater than 30 events per hour.

Sleep apnea parameters

Hypopnea was defined as follows: 1) ≥50% reduction in airflow compared with the

baseline value, recorded by nasal pressure cannula, or by induction plethysmography or oronasal thermistors; 2) duration ≥10 s; and 3) occurrence of ≥50% reduction in airflow during at least 90% of the event.

Apnea was defined as follows: 1) ≥90% reduction in airflow compared with the baseline value, recorded by oronasal thermistors or nasal pressure cannulas; 2) duration ≥10 s; and 3) occurrence of aforementioned reduction in airflow during at least 90% of the event.

The oxygen desaturation index (ODI 4) was defined as events of decreased blood O_2 saturation ≥4% for 1 h. $TRTSAO_2$ <90% was defined as the time with arterial oxygen saturation (SaO_2) <90% and the proportion of sleep time with SaO_2 below 90%.

Control subjects

Control subjects (N = 394) without OSA (AHI ≤ 5 events per hour based on PSG) were recruited from the physical examination center within the same period.

TNF-α evaluation and biochemical criteria

Blood samples from patients who fasted overnight were taken in the morning from the antecubital vein. The samples were divided into aliquots, separated within 30 min, and stored at -80°C until they were transported to the People's Hospital of XinJiang Uygur Autonomous Region-certified laboratories for analyses.

We used radioimmunoassays to measure TNF-α levels (North Biotechnology Research Institute, Beijing, China). In addition to routine blood examinations, data such as lipid profiles, glucose levels, and uric acid levels, and anthropometric measurements were obtained (neck and abdomen circumferences). The patients completed a set of questionnaires, which included questions about demographic information, personal history, detailed previous history, family history of diseases, and lifestyle.

Genotyping of *ZFP36* single nucleotide polymorphisms (SNPs)

Genomic DNA was prepared from blood samples using the PAX gene blood DNA kit (QIAGEN/BD Company, Hombrechtikon, Switzerland) based on manufacturer instructions.

Three SNPs (rs251864, rs3746083, and rs17879933) were selected from the National Center for Biotechnology Information SNPdb and the International HapMap Project databases (http://www.ncbi.nlm.nih.gov and http://www.hapmap.org, respectively), which served as templates for PCR-based genotyping by using a previously described Taq amplification method and the 7900 HT Fast Real-Time PCR System (Applied Biosystems Inc., USA) (Li et al., 2010). TaqMan SNP genotyping assay primers and probes were chosen based on specific sequences for the genes of interest available on the Applied Biosystems website (http://www.appliedbiosystems.com). PCRs (5 μL) were performed using 1 μL 25 ng/μL DNA or H_2O for negative control samples, 0.125 μL 20 μM probe, and 2.25 μL 20 μM master mix (Applied Biosystems Inc.). The reaction was cycled 43 times with a denaturation step at 95°C for 10 s, an annealing step at 60°C for 1 min, and a final elongation step at 72°C for 7 min.

The following three SNPs in the *ZFP36* gene were genotyped: rs251864 (39897293A > G) in the promoter exon; rs3746083 (39898667C > T) in the second exon; and rs17879933 (39899917_39899918insTT) in the 3' untranslated region. The three SNPs selected were suc-

cessfully genotyped in 802 subjects. For genotyping quality control, case and control subjects were randomly distributed across the PCR plates. Sequenced samples were also genotyped to detect genotyping errors. The success rate for genotyping was >99%, and the minor allele frequencies for all subjects were >5%.

Statistical analysis

All statistical analyses were performed using the SPSS statistical software version 16.0 (Chicago, IL, USA). Data are reported as means ± standard deviation or median (interquartile range). Inter-group comparisons were made using unpaired *t*-tests, Mann-Whitney U-tests, or chi-square (χ^2) tests. Hardy-Weinberg equilibrium tests were performed using the SNP Alyze software version 2.1 (Dynacom Co. Ltd., Japan). Inter-group differences of SNP genotype or allele frequency were analyzed using χ^2 tests. Logistic regression analyses were performed to calculate odds ratios with 95% confidence intervals. A P value less than 0.05 was considered to be statistically significant.

RESULTS

Characteristics of moderate-to-severe OSA and control subjects

The baseline characteristics of 408 moderate-to-severe OSA subjects and 394 control subjects are summarized in Table 1.

Table 1 Characteristics of moderate-to-severe obstructive sleep apnea (OSA) patients and control subjects.

	Moderate-to-severe OSA group	Control group	t/χ^2	P value
Number of subjects	408	394		
Gender (male/female)	342/66	323/71	0.48	0.48
Age (years)	48.52 ± 9.59	48.76 ± 8.87	0.93	0.34
Neck circumference (cm)	41.54 ± 3.74	38.2 ± 4.41	6.34	<0.00
Abdomen circumference (cm)	102.39 ± 9.58	88.56 ± 7.93	21.71	<0.00
BMI (kg/m^2)	28.81 ± 3.70	23.46 ± 2.17	21.05	<0.00
SBP (mmHg)	140.44 ± 18.86	120.89 ± 17.43	10.81	<0.00
DBP (mmHg)	95.07 ± 14.02	73.78 ± 12.54	21.56	<0.00
FPG (mM)	5.54 ± 1.56	5.07 ± 3.13	2.21	0.03
Uric acid (mM)	373.18 ± 88.51	300.89 ± 85.67	9.65	<0.00
Total cholesterol (mM)	4.63 ± 0.94	3.43 ± 1.34	10.09	<0.00
Triglycerides (mM)	2.45 ± 1.57	2.20 ± 1.80	1.93	0.05
HDL (mM)	1.09 ± 0.28	1.30 ± 0.39	9.87	<0.00
LDL (mM)	2.59 ± 0.70	2.63 ± 0.92	0.96	0.33
TNF-α (fM)	64.72 ± 8.32	30.56 ± 13.34	7.02	<0.00

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting blood glucose; HDL: high density lipoprotein; LDL: low density lipoprotein; TNF-α: tumor necrosis factor-alpha.

The gender, age, fasting blood glucose (FPG) levels, triglyceride (TG) levels, and low-density lipoprotein (LDL) levels of the patients showed no significant differences. Compared with the control group, the subjects in the moderate-to-severe OSA group had larger neck and abdomen circumferences as well as higher systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), uric acid (UA), total cholesterol (TC), and TNF-α levels. However, the OSA subjects had lower high-density lipoprotein (HDL) levels.

Genotype and allele frequencies

Table 2 presents the genotype and allele frequencies of the *ZFP36* gene in patients with moderate-to-severe OSA and the control subjects.

Table 2. Genotype and allele frequencies in moderate-to-severe obstructive sleep apnea (OSA) and control subjects.

Variants	Genotype/allele	Moderate-to-severe OSA group (N/%)	Control group (N/%)	χ^2	P value
rs251864	AA	142 (35.1)	184 (46.9)	13.18	<0.00
	AG	189 (46.8)	164 (41.8)		
	GG	73 (18.1)	46 (11.3)		
	A	473 (58.5)	532 (67.5)	13.77	<0.00
	G	335 (41.5)	256 (32.5)		
rs3746083	CC	376 (92.4)	370 (93.9)	1.50	0.47
	CT	30 (7.4)	24 (6.1)		
	TT	1 (0.2)	0 (0.0)		
	C	782 (96.1)	764 (97.0)	0.93	0.34
	T	32 (3.9)	24 (3.0)		
rs17879933	DD	8 (2.0)	4 (1.0)	22.40	<0.00
	ID	50 (12.3)	99 (25.3)		
	II	349 (85.7)	291 (73.7)		
	D	66 (8.1)	107 (13.6)	12.43	<0.00
	I	748 (91.9)	681 (86.8)		

In this study, the three *ZFP36* gene SNPs (rs251864, rs3746083, and rs17879933) were successfully genotyped and showed a Hardy-Weinberg equilibrium behavior ($P > 0.05$). The control and moderate-to-severe OSA groups showed significant differences in the genotype and allele distributions of rs251864 and rs17879933 ($P < 0.05$). No significant differences were observed between the moderate-to-severe OSA subjects and the control samples in the genotype and allele distribution of rs3746083.

Comparison of intermediate phenotypes and TNF-α in *ZFP36* rs251864, rs3746083, and rs17879933 genotypes

We combined the rs3746083 CT and TT genotypes as one group and the rs17879933 DD and ID as another group because the rs3746083 TT and rs17879933 DD genotypes were few. We then compared the intermediate phenotypes and TNF-α levels in the *ZFP36* rs251864, rs3746083, and rs17879933 genotypes. Differences in the intermediate phenotypes and in TNF-α levels in the *ZFP36* rs251864, rs3746083, and rs17879933 genotypes are shown in Table 3.

No significant differences in the neck circumference, abdomen circumference, BMI, SBP, DBP, UA, FPG, TC, TG, HLD, LDL, and TNF-α between the two-rs3746083 genotypes were observed. Among the three rs251864 genotypes, abdomen circumference (AA, AG, and GG genotypes were 97.05 ± 9.32, 99.57 ± 9.28, and 101.06 ± 11.37 cm, respectively), BMI (AA, AG, and GG genotypes were 26.91 ± 4.34, 27.02 ± 4.57, and 28.83 ± 3.71 kg/m^2, respectively), and TG levels (AA, AG, and GG genotypes were 2.29 ± 0.96, 2.47 ± 1.71, and 2.51 ± 1.74 mM, respectively) were significantly different, and the rs251864 GG genotype had the highest values.

Table 3. Association of ZFP36 single nucleotide polymorphisms with clinicopathological data.

	ZFP36 rs251864			ZFP36 rs3746083		ZFP36 rs17879933	
	AA	AG	GG	CC	CT + TT	DD + ID	II
Neck circumference (cm)	40.36 ± 4.01	40.78 ± 4.48	40.36 ± 4.37	40.58 ± 3.73	40.25 ± 4.10	40.50 ± 3.27	40.42 ± 3.87
Abdomen circumference (cm)	97.05 ± 9.32	99.57 ± 9.28	101.06 ± 11.37[a]	95.89 ± 11.72	97.16 ± 10.03	94.12 ± 12.48	96.71 ± 11.45
BMI (kg/m^2)	26.91 ± 4.34	27.02 ± 4.57	28.83 ± 3.71[a]	26.91 ± 4.34	26.74 ± 3.71	26.14 ± 4.51	27.14 ± 4.26[a]
SBP (mmHg)	140.56 ± 20.45	139.96 ± 20.73	140.45 ± 19.73	132.36 ± 21.01	131.10 ± 19.17	128.97 ± 20.25	133.79 ± 21.08[a]
DBP (mmHg)	87.21 ± 17.21	91.54 ± 16.27	90.00 ± 15.89	87.21 ± 17.21	88.45 ± 16.06	82.87 ± 6.93	88.66 ± 17.09[a]
UA (mM)	327.96 ± 110.08	337.51 ± 91.34	329.22 ± 109.01	327.96 ± 110.09	327.51 ± 91.34	302.89 ± 109.27	335.27 ± 108.16[a]
FPG (mM)	5.31 ± 1.49	5.42 ± 1.21	5.31 ± 1.47	5.31 ± 1.49	5.42 ± 1.21	5.09 ± 1.03	5.36 ± 1.55
TC (mM)	4.20 ± 1.41	4.52 ± 0.96	4.22 ± 1.39	4.20 ± 1.41	4.52 ± 0.96	4.04 ± 1.53	4.26 ± 1.35
TG (mM)	2.29 ± 0.96	2.47 ± 1.71	2.51 ± 1.74[a]	2.50 ± 1.74	2.45 ± 0.90	2.38 ± 1.90	2.49 ± 1.67
HLD (mM)	1.17 ± 0.37	1.11 ± 0.33	1.17 ± 0.37	1.17 ± 0.37	1.11 ± 0.33	1.20 ± 0.42	1.16 ± 0.35
LDL (mM)	2.57 ± 0.80	2.58 ± 0.91	2.57 ± 0.81	2.57 ± 0.80	2.58 ± 0.90	2.56 ± 0.83	2.57 ± 0.80
TNF-α (fM)	56.07 ± 55.64	60.04 ± 58.69	62.03 ± 59.49[a]	59.13 ± 57.52	60.98 ± 59.49	59.16 ± 58.79	65.37 ± 58.40[a]
AHI (events/h)	32.20 (22.15-47.05)	31.40 (20.10-46.55)	27.50 (22.40-45.00)	31.20 (21.35-46.75)	32.80 (24.65-43.15)	27.15 (20.00-41.40)	32.30 (21.70-46.90)
Average heart rate at night (n)	67.55 ± 8.23	67.66 ± 9.50	65.47 ± 7.55	67.49 ± 8.63	65.87 ± 9.49	67.40 ± 9.21	67.37 ± 8.62
Average SaO_2 (%)	91 (89-93)	91 (89-92)	92 (90-93)	91 (89-93)	91 (89.5-93)	91 (89-92)	91 (89-93)
Lowest SaO_2 (%)	74 (68-80)	74 (68-79)	72 (64-80)	74 (67-80)	74 (64-77.50)	71 (64-80)	74 (68-80)
ODI 4 (events/h)	273 (188-415)	280 (201-424)	247 (196-394)	275.50 (194.50-420.00)	297.00 (212.00-417.50)	265.50 (186.00-395.00)	279.00 (197.00-421.00)
Time with SaO_2 < 90% (min)	58.00 (25.00-140.00)	87.00 (34.50-178.50)	58.00 (26.00-154.00)	66.50 (27.50-162.50)	67.00 (19.00-152.00)	69.50 (41.00-181.00)	67.00 (26.00-155.00)
TRTSAO_2 <90%	16.17 (7.08-38.48)	24.07 (8.46-45.68)	14.61 (5.94-35.64)	18.88 (7.81-43.62)	17.86 (4.94-38.30)	20.26 (8.70-58.98)	18.24 (7.42-42.56)

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; UA: uric acid; FPG: fasting blood glucose; TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; TNF-α: tumor necrosis factor-alpha; AHI: apnea-hypopnea index; SaO_2: arterial oxygen saturation; ODI: oxygen desaturation index; TRTSAO_2 <90%: percentage of sleep time with O_2 saturation below 90%; [a]P < 0.05.

Between the two genotypes groups (DD + ID and II genotypes) of rs17879933, BMI (26.14 ± 4.51 and 27.14 ± 4.26 kg/m^2, respectively), SBP (128.97 ± 20.25 and 133.79 ± 21.08 mmHg, respectively), DBP (82.87 ± 6.93 and 88.66 ± 17.09 mmHg, respectively), and UA (302.89 ± 109.27 and 335.27 ± 108.16 μM, respectively) were significantly different, and the rs17879933 II genotype had higher values than the rs17879933 DD + ID genotype. Finally, TNF-α levels were different in *ZFP36* rs251864 (AA, AG, and GG genotypes were 56.07 ± 55.64, 60.04 ± 58.69, and 62.03 ± 59.49 fM, respectively) as well as in *ZFP36* rs17879933 (DD + ID genotype 59.16 ± 58.79 fM and II genotype 65.37 ± 58.40 fM).

Comparison of sleep apnea parameters in the *ZFP36* rs251864, rs3746083, and rs17879933 genotypes

No significant differences in AHI, average heart rate at night, average SaO_2, lowest SaO_2, ODI, time with SaO_2 <90%, or percentage of sleep time with O_2 saturation below 90% ($TRTSAO_2$ <90%) among the *ZFP36* rs251864, rs3746083, and rs17879933 genotypes were observed (Table 3).

Multivariate analyses for *ZFP36* polymorphisms according to the logistic regression model

Table 4 shows the multivariate analyses of risk factors related to OSA and *ZFP36* rs251864, rs3746083, and rs17879933 using a conditional logistic regression model.

Table 4. Multivariate analyses of *ZFP36* polymorphisms according to the logistic regression model.

	B	S.E.	χ^2	Sig.	OR	95%CI	
Gender	0.13	1.71	0.01	0.94	1.14	0.04	32.53
BMI	0.33	0.14	5.69	0.02	1.39	1.06	1.82
Neck circumference	-0.04	0.33	0.01	0.91	0.97	0.50	1.85
Abdomen circumference	0.37	0.17	4.69	0.03	1.45	1.04	2.04
FPG	3.27	1.27	6.66	0.01	26.30	2.20	314.99
TC	-3.54	3.32	1.14	0.29	0.03	0.00	19.51
TG	0.60	1.00	0.37	0.55	1.83	0.26	12.98
TNF-α	-0.01	0.01	0.67	0.41	0.99	0.98	1.01
rs251864	-0.39	1.19	0.11	0.74	0.68	0.07	7.03
rs3746083	-1.37	2.06	0.45	0.53	0.25	0.00	14.28
rs17879933	-0.14	2.29	0.00	0.95	0.86	0.10	78.33

OR: odds ratio; CI: confidence interval; BMI: body mass index; FPG: fasting blood glucose; TC: total cholesterol; TG: triglycerides; TNF-α: tumor necrosis factor-alpha.

BMI, abdomen circumference, and FPG were independently associated with a significant predisposition to OSA. However, gender, neck circumference, TC, TG, TNF-α, and *ZFP36* rs251864, rs3746083, and rs17879933 variants were not independently associated with OSA.

DISCUSSION

In this study, the relationship between three *ZFP36* polymorphisms (rs251864, rs3746083, and rs17879933) with TNF-α levels and OSA was investigated in patients with moderate-to-severe OSA. The results of our study confirm that moderate-to-severe OSA sig-

nificantly increases the circulation of morning TNF-α plasma concentrations. Among the three *ZFP36* polymorphisms, significant differences were observed in the distributions of genotypes and alleles and TNF-α levels in subjects with rs251864 and rs17879933. However, no significant differences in sleep apnea parameters among the three *ZFP36* polymorphisms were found, and the *ZFP36* polymorphisms were not independently associated with OSA.

TNF-α is an inflammatory cytokine that has been reported to be elevated in patients with sleep apnea (Ciftci et al., 2004; Minoguchi et al., 2004; Ryan et al., 2006). Steiropoulos et al. (2010) reported increased TNF-α levels in subjects with OSA compared with those matched for BMI, waist-to-hip ratio, and waist circumference. However, the neck circumference, waist circumference, SBP, DBP, and BMI of the OSA subjects were significantly higher compared with those of the control subjects. Many studies have shown that obesity is a strong risk factor for OSA, which is associated with an increased risk of hypertension (Peppard et al., 2000; Angelico et al., 2010). Current evidence shows that OSA has specific correlations with metabolic syndrome. The potential mechanisms of the OSA-obesity-metabolic syndrome interaction involve sympathetic activation, oxidative stress, inflammation, and neurohumoral changes (Lam et al., 2012).

Current data indicate that OSA has a strong genetic basis, with 35-40% of variance attributed to genetic factors. Evidence has shown that genetic determinants of upper-airway muscle activity, craniofacial structure, obesity, fat distribution, and respiratory control may interact and cause OSA (Casale et al., 2009). Most genetic association studies have focused on the *TNF-α*, *ACE I/D*, and *APOE* genes (Cosentino et al., 2008; Popko et al., 2008; Bhushan et al., 2009; Yakut et al., 2010). Bhushan et al. (2009) reported that the frequency of the *TNF-α* (-308A) allele and serum TNF-α levels were significantly higher in obese Asian Indians with OSA.

ZFP36, or tristetraprolin, which is a zinc-finger protein, modulates *TNF-α* mRNA stability by first binding to an AU-rich element in its 3'-untranslated region, thereby promoting the deadenylation and destruction of the *TNF-α* mRNA (Lai et al., 2006). Carballo et al. (1998) showed that *ZFP36* inactivation in mice resulted in a complex inflammatory syndrome caused by increased TNF-α production. Bouchard et al. (2007) suggested that *ZFP36* was a promising candidate gene for obesity-associated metabolic complications.

ZFP36 is known as the gene encoding human tristetraprolin and is located on chromosome 19q13.1 (Taylor et al., 1991). *ZFP36* consists of two exons and one intron. In the present study, we chose three SNPs (rs251864, rs3746083, and rs17879933). The genotyping results are shown in Table 2. Significant differences were observed in the distributions of the rs251864 and rs17879933 genotypes and alleles. First, the frequencies of the AA, AG, and GG genotypes in rs251864 were 35.1, 46.8, and 18.1%, respectively, in the moderate-to-severe OSA subjects but 46.9, 41.8, and 11.3%, respectively, in the control subjects. The frequency of the G allele genotype in the moderate-to-severe OSA subjects (41.5%) also differed from that of the control subjects (32.5%). A similar result was observed in rs17879933, where the frequencies of the II genotype and I allele in the moderate-to-severe OSA subjects were higher than in the control subjects. Moreover, based on the comparison of the clinical characteristics among the different genotypes of SNPs rs251864 and rs17879933, significant associations were observed for the genotypes of rs251864 as well as the genotypes of rs17879933. Among the three rs251864 genotypes, abdomen circumference, BMI, TG levels, and TNF-α levels were significantly different, and the rs251864 GG genotype had the highest values. Between the two-genotype groups of rs17879933, BMI, SBP, DBP, UA, and TNF-α levels were significantly different, and the rs17879933 II genotype had higher values than the rs17879933 DD +

ID genotype. Suzuki et al. (2008) identified an SNP, namely SNP359 A/G (*ZFP36* rs251864), within the *ZFP36* promoter region that impacted promoter activity. Although no differences were observed in the allele frequencies of SNP359 A/G between healthy individuals and rheumatoid arthritis patients, the presence of the minor G allele inhibited *ZFP36* promoter activity by approximately twofold compared with the A allele. These results suggest that this SNP can modulate disease activity by negatively influencing *ZNF36*. These findings also indicate that genetic polymorphisms in the *ZFP36* gene can regulate TNF-α. Carrick et al. (2006) investigated the function of *ZFP36* in the development of autoimmune disorders by examining polymorphisms in human *ZFP36* across a large cohort of healthy individuals and patients with autoimmune diseases. In their study, 28 polymorphisms were identified, of which SNP 36*8 (*ZFP36* rs3746083), a C to T transition in exon 2, was associated with a higher incidence of rheumatoid arthritis in African-American individuals. However, in the present study, no significant differences were observed between the moderate-to-severe OSA subjects and control subjects in the distribution of rs3746083 genotypes and alleles and other clinical intermediate phenotypes, including TNF-α levels. These data suggest that the regulation of TNF-α through *ZFP36* is complicated.

In this study, no significant differences were observed in sleep apnea parameters, such as AHI, average heart rate at night, average SaO_2, lowest SaO_2, ODI, and $TRTSAO_2$ <90% in patients with *ZFP36* rs251864, rs3746083, and rs17879933 genotypes. However, multiple logistic analyses showed that BMI, abdomen circumference, and FPG, but not *ZFP36* rs251864, rs3746083, and rs17879933, were independent risk factors related to OSA, which suggests that *ZFP36* might only be involved in TNF-α regulation. OSA itself is a complex syndrome and obesity and metabolic syndromes are only several of its characteristics. Inflammation and genetics are other possible mechanisms of OSA with equally complex regulation.

In conclusion, significant differences were observed in the distribution of genotypes and alleles and TNF-α levels of *ZFP36* rs251864 and rs17879933. However, our study has several limitations. First, we only evaluated patients with moderate-to-severe OSA. Second, although studies have shown that *TNF-α* gene polymorphisms and TNF-α levels are related to OSA, *ZFP36* is not the only regulator of TNF-α. Moreover, the effect of a single gene is limited, particularly in OSA, which is a complex polygenic disease. Large-scale investigations are necessary for future joint analyses of multiple OSA candidate genes.

Conflicts of interest

The authors declare no conflict of interest.

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