Association Study of Serotonin Transporter Gene Polymorphisms with Obstructive Sleep Apnea Syndrome in Chinese Han Population

Weihua Yue, MD[1,2]*; Huiguo Liu, MD[3]*; Jishui Zhang, MD[4]; Xianghui Zhang, BS[2]; Xiaoping Wang, MD[2]; Tieqiao Liu, MD[2]; Pozi Liu, MD[2,5]; Wei Hao, MD, PhD[2]

*[1]Mental Health Institute, the Second Xiangya Hospital, Central South University, Changsha, P. R. China; [2]Key Laboratory for Mental Health, Ministry of Health, Institute of Mental Health, Peking University, Beijing, P.R. China; [3]Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China; [4]Beijing Children's Hospital Affiliated to Capital University of Medical Sciences, Beijing, P.R. China; [5]The Second Affiliated Hospital of Tsinghua University, Beijing, P.R. China; *Both authors contributed equally to this work*

Background: Since the serotonin (5-HT) is associated with circadian rhythm and breathing regulation, the serotonin transporter (5-HTT), which plays an important role in serotoninergic transmission, might be a strong candidate gene in the pathogenesis of obstructive sleep apnea syndrome (OSAS).

Objective: To investigate the association of *5-HTT* gene polymorphisms with OSAS and clinical characteristics.

Methods: We genotyped the *5-HTT* gene linked polymorphic region (5-HTTLPR) and a variable number of tandem repeats at intron 2 (STin2.VNTR) in 254 OSAS patients and 338 healthy controls in Chinese Han population.

Results: In total sample, the 10-repeat allele of STin2.VNTR was significantly associated with OSAS (P = 0.007, OR = 1.72, 95% CI = 1.15~2.58), but no association was found in 5-HTTLPR. In male subjects, both polymorphisms showed significant association with OSAS (Allele L: P = 0.005, OR = 1.44, 95% CI = 1.11 to 1.87; Allele 10: P = 0.002, OR = 1.94, 95% CI = 1.26 to 3.00). Two haplotypes, S-12 and L-10, constructed by the above polymorphisms also revealed significant associations with OSAS (global P-values were 0.020 for total sample and 0.0006 for male subjects, respectively). Male patients carrying the haplotype S-12 showed a significantly lower apnea / hypopnea index (AHI), depressive factor, plasma 5-HT level and 5-hydroxyindolacetic acid (5-HIAA) levels, but higher episodic memory, when compared with non-S-12 carriers (P < 0.05). However, no significant differences were found in excessive daytime sleepiness or other psychological function across haplotype carriers (P > 0.05).

Conclusions: These findings support that *5-HTT* gene may be involved in susceptibility to OSAS, especially with sex-dependent effect.

Keywords: Obstructive sleep apnea syndrome (OSAS), serotonin transporter (5-HTT), association study

Citation: Yue W; Liu H; Zhang J; Zhang X; Wang X; Liu T; Liu P; Hao W. Association study of serotonin transporter gene polymorphisms with obstructive sleep apnea syndrome in chinese han population. *SLEEP* 2008;31(11):1535-1541.

OBSTRUCTIVE SLEEP APNEA SYNDROME (OSAS) HAS BEEN SHOWN TO AGGREGATE SIGNIFICANTLY WITHIN FAMILIES.[1-3] FAMILY STUDIES HAVE SUGGESTED that the risk of OSAS may be from 2- to 4-fold as great in relatives of patients with OSAS than in controls,[4] and that nearly 40% of the variance in the apnea hypopnea index (AHI) of patients with OSAS may be explained by genetic factors.[1] It is likely that genetic factors accompanied with craniofacial structure, body fat distribution and neural control of the upper airway muscles interact to produce the OSAS phenotype.[5-7] However, the role of specific genes that influence the development of OSAS has not been identified.

Although several previous studies had presumed that several susceptible genes, such as angiotensin converting enzyme (*ACE*), *ApoE4*, and insulin receptor substrate (*IRS*) -1 might be involved in the pathogenesis of OSAS,[8-10] the genetic markers for this disorder remain elusive. Several lines of pharmacological, neurobehavioral, and therapeutic evidence have implicated that serotonin (5-HT) is involved in the pathogenesis of OSAS.[11-13] By regulating the magnitude and duration of serotonergic responses, the serotonin transporter (5-HTT) is central to the fine tuning of brain serotonergic neurotransmission and of the peripheral actions of 5-HT.[14] The 5-HTT protein is encoded by a single gene, the solute carrier family 6, member 4, SLC6A4 (i.e., 5-HTT), which locates on chromosome 17q11.1-17q12.[15,16] Two common polymorphisms have been described in the gene: a deletion /insertion of 44 bp in the promoter region approximately 1 kb upstream of the transcription site (5-HTTLPR) and a variable number of tandem repeats in intron 2 (STin2.VNTR) containing 9,10, or 12 copies of a 16-17 bp repeat element located in intron 2.[16-18] These polymorphisms make the *5-HTT* a strong candidate gene for study in sleep disorders. In previous studies, the rat with *5-HTT* gene knockout showed disturbed sleep.[19] Yılmaz et al. reported that the 12/10 and LL genotypes were overrepresented in 20 male patients with OSAS.[20] To further assess whether the *5-HTT* gene could be implicated in the vulnerability to OSAS, we performed an independent case-control association study in a Chinese Han population.

SUBJECTS AND METHODS

Subjects

The study sample consisted of 254 patients with OSAS (220 males and 34 females) diagnosed by using the overnight polysomnography (PSG). The patients who met the diagnostic criteria of OSAS[21] were recruited from the sleep laboratory of the Second Xiangya Hospital, Central South University (CSU) and Tongji Hospital, Tongji Medical College, Huazhong University

Submitted for publication December, 2007
Accepted for publication August, 2008
Address correspondence to: Prof. Wei Hao, MD, PhD, Mental Health Institute, the Second Xiangya Hospital, Central South University, 139, Middle Renmin Road, Changsha, Hunan, P.R. China 410011, Tel: +86-731-5292156, Fax: +86-731-5360160, E-mail: weihao57@china.com; or Prof. Pozi Liu, MD., liupozi@mail.tsinghua.edu.cn

of Science and Technology (HUST), China. In OSAS patients, the mean age was 45.2 ± 11.8 y, the mean duration of complaints was 6.5 ± 2.7 y; apnea / hypopnea index (AHI) was 53.9 ±16.4 events/h; body mass index (BMI) was 25.1 ± 6.5 kg/m^2; educational level was 9.3 ± 3.4 y, respectively.

Three hundred and thirty-eight healthy control subjects (291 males and 47 females) were screened for a personal or family history to exclude sleep disorders. The mean age was 43.2 ± 12.7 y, the BMI was 22.6 ± 5.7 kg/m^2, and the educational level was 10.9 ± 4.6 y, in healthy control group.

All subjects were unrelated Chinese Han individuals. Patients with OSAS and healthy control subjects were screened to exclude definite psychiatric disorders (Axis I disorders of the Diagnostic and Statistical Manual of Mental Disorders, DSM-IV) and taking psychotropic medication regularly. Those with illnesses in which a serotonergic mechanism has been implicated, such as migraine or irritable bowel syndrome, and those taking medications with known effects on the serotonin system (such as SSRIs drugs) were also excluded. The subjects with heart failure, chronic obstructive pulmonary disease, and/or recent cerebral apoplexy were also excluded. All subjects gave written informed consent and the protocol of our study had been approved by the Medical Ethics Committees of Central South University and Huazhong University of Science and Technology.

METHODS

Nocturnal PSG Measurements and Neuropsychological Examination

Each patient received overnight polysomnographic (PSG) examination. Nocturnal PSG measurement and analyses of sleep records were accomplished as we previously described.[22] The diagnosis of OSAS in the present study met the PSG diagnostic criterion of an AHI > 5 events/h. An apnea was defined as a complete cessation of airflow ≥ 10 sec. Hypopnea was defined as a decrease in airflow of at least 50% with a concomitant fall ≥ 4% in arterial oxygen saturation followed by an arousal response (as indicated by α waves on EEG, increased submental electromyogram levels, or increased body movements). The AHI was calculated by dividing the total number of apnea and hypopnea episodes by the hours in sleep.

A total of 108 patients also received neuropsychological status and cognitive examination, and a general questionnaire concerning demographic data. The excessive daytime sleepiness (EDS) and psychological symptoms of OSAS patients were evaluated with the score of Epworth Sleepiness Scale (ESS) and general severity index (GSI) of the Symptom Checklist-90 (SCL-90), respectively.[22-24] Memory and executive function in patients were primarily assessed by memory quotient (MQ) of Wechsler Memory Scale—Chinese Revision (WMS-CR, standardized by Gong Yaoxian), and performance intelligent quotient (PIQ) of Wechsler Adult Intelligent Scale—Chinese Revision (WAIS-CR, standardized by Gong Yaoxian).

Plasma and gDNA Preparation

Venous blood samples were obtained from all the subjects when they woke up in the morning. After the blood sample was centrifuged at 3000 rpm for 15 min, the supernatant plasma was extracted. Genomic DNA was isolated from the blood of experimental subjects as Smith et al. described.[25] Plasma and gDNA samples were either used immediately or stored at -70 °C until required.

Genotyping

The 2 polymorphisms (LPR and STin2.VNTR) of *5-HTT* gene were genotyped according to Collier's report.[26] The primers sequences were as follows: 5'-GGC GTT GCC GCT CTG AAT TGC-3' (sense) and 5'-GAG GGA CTG AGC TGG ACA ACC AC-3' (antisense) for 5-HTTLPR, 5'-GTC AGT ATC ACA GGC TGC GAG-3' (sense) and 5'-TGT TCC TAG TCT TAC GCC AGT G-3' (antisense) for STin2.VNTR. The PCR amplification was performed in a 25 μL volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.25 μM of each primer, 1U of Taq DNA polymerase and 40 ng genomic DNA. The conditions used for PCR amplification included an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, 60-61°C for 30 sec, 72°C for 40 sec, and a final elongation at 72°C for 7 min. PCR products of the 2 polymorphisms were separated on 6% non-denaturing polyacrylamide gels and bands visualized with silver nitrate ($AgNO_3$) staining. The fragments sizes were 484 base pairs (bp) (S allele) and 528 bp (L allele) for 5-HTTLPR, 267 bp (allele 10-repeats) and 300 bp (12 repeats) for STin2.VNTR, respectively.

Detection of Plasma 5-HT and 5-HIAA Levels

The plasma 5-HT and 5-hydroxyindolacetic acid (5-HIAA) levels were measured by using the high-performance liquid chromatography (HPLC) - electrochemical detecting (L-ECD-6A) in 108 OSAS patients. The mobile phase was 20 mM sodium citrate (adjusted pH 4.50) filtered by a 0.45 μM filter membrane, with 5% (v/v) columbian spirit. The filtered and degassed mobile phase was delivered by a dual piston pump at a flow-rate of 0.7 mL/min. The separation was performed with a 150-mm long, 6-mm internal diameter Shim-pak CLC-phenyl column (Shimadzu, Japan). The column temperature was retained at 35 °C and the voltage of the electrochemical detector was 0.75 V. The plasma 5-HT and 5-HIAA contents were identified by retention times (28 min) and measured by the ratio of peak areas for the samples *vs.* standard solutions. 5-HT and 5-HIAA were prepared as 1 mg/mL stock solution in 0.1 M perchloric acid with 100 mM EDTA.[27]

Statistical Analyses

The genetic power estimates were performed with the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/). Deviation of the genotype counts from the Hardy-Weinberg equilibrium was tested using a chi-square goodness-of-fit test. Statistical differences in genotypic, allelic, and haplotypic distribution between OSAS and control subjects were evaluated by the chi-square test. Odds ratio (OR) and their 95% confidence intervals (95% CI) were calculated to evaluate the effects of different alleles. The pairwise linkage disequilibrium (LD)

Table 1—Comparison of Demographic, Daytime Functions, and Biochemical Index in OSAS Patients and Healthy Control Subjects

	OSAS (n = 254)	Controls (n = 338)	*t*-value	χ^2	P-value
Sex: Male, n (%)	220 (86.6%)	291 (86.1%)	-	0.033	0.856
Female, n (%)	34 (13.4%)	47 (13.7%)			
Age (y)	45.20 ± 11.84	43.19 ± 12.65	1.093	-	0.317
BMI (kg/m^2)	25.12 ± 6.51	22.63 ± 5.73	1.304	-	0.178
Educational level (y)	9.32 ± 3.44	10.94 ± 4.61	0.911	-	0.347
ESS scores[a]	16.04 ± 5.52	4.35 ± 3.62	2.378*	-	0.014
GSI of SCL-90 [a]	0.91 ± 0.42	0.26 ± 0.11	2.653*	-	0.011
MQ of WMS [a]	86.46 ± 13.51	101.90 ± 14.78	3.452*	-	0.002
PIQ of WAIS [a]	93.27 ± 16.06	99.12 ± 18.85	1.815	-	0.056
Plasma 5-HT level (ng/ml) [a]	168.51 ± 42.68	138.94 ± 36.60	5.491**	-	<0.001
Plasma 5-HIAA level (ng/ml) [a]	184.52 ± 54.19	157.96 ± 60.30	4.360*	-	<0.001

ESS: Epworth Sleepiness Scale; GSI: General Severity Index; SCL-90: symptom checklist-90; MQ: memory quotient; WMS: Wechsler Memory Scale; PIQ: performance intelligent quotient; WAIS: Wechsler Adult Intelligent Scale; 5-HIAA: 5-hydroxyindolacetic acid. [a]N. case: 108, N. control: 150; *P < 0.05; **P < 0.001.

Table 2—Genotype and Allele Frequencies and Association of 5-HTT Gene Polymorphisms in OSAS and Control Groups

Marker	n	Genotype [a]			χ^2	P-value	HWE	Allele [a]		X^2	P-value	Empirical	OR
LPR		SS	SL	LL			P-value	S	L			P-value [b]	(95%CI)
Total Cases	254	114(44.9)	106(41.7)	34(13.4)	2.906	0.233	0.241	334(65.7)	174(34.3)	3.115	0.077	0.259	1.25(0.97-1.59)
Total Controls	338	173(51.2)	131(38.8)	34(10.1)			0.217	477(70.6)	199(29.4)				
Male Cases	220	84(38.2)	106(48.2)	30(13.6)	10.417	**0.005**	0.706	303(65.6)	159(34.4)	7.541	**0.006**	**0.007**	1.44(1.11-1.87)
Male Controls	296	155(52.4)	113(38.2)	28(9.5)			0.269	456(73.3)	166(26.7)				
Stin2.VNTR		10/10	10/12	12/12				10	12				
Total Cases	254	6(2.4)	46(18.1)	202(79.5)	6.565	**0.037**	0.095	58(11.4)	450(88.6)	7.154	**0.007**	**0.034**	1.72(1.15-2.58)
Total Controls	338	3(0.9)	41(12.1)	294(87.0)			0.250	47(7.0)	629(93.0)				
Male Cases	220	4(1.8)	457(20.5)	171(77.7)	9.377	**0.009**	0.607	53(12.0)	387(88.0)	9.258	**0.002**	**0.009**	1.94(1.26~3.00)
Male Controls	296	3(1.0)	33(11.1)	260(87.8)			0.105	39(6.6)	553(93.4)				

[a]Frequencies (%) are shown in parenthesis. Significant P values (<0.05) are in boldface; [b]Empirical P-values were generated after 10,000 Monte Carlo simulations.

analysis (using *D'* value) was applied to detect the inter-marker relationship in control group. These analyses were performed by SHEsis (Bio-X Life Science Research Center, Shanghai, China), a powerful software platform for analyses of LD, haplotype construction, and genetic association at polymorphism loci.[28] The HAP - Haplotype Resolution: Version 3.0 (http://research.calit2.net/hap/) was used for haplotype distribution in OSAS patients.[29] The comparisons of demographic data, sleep characteristics, psychological status, and cognitive function across various groups were determined by *t*-tests or chi-square tests using SPSS 11.5. All tests were 2-tailed, and significance level was set at P < 0.05.

RESULTS

Daytime Functions and Biochemical Status of OSAS Patients

There were no differences in age, sex distribution, body mass index (BMI), or educational level between OSAS and healthy control groups (P > 0.05). Compared with healthy controls, OSAS patients suffered from striking daytime dysfunctions. The total ESS scores (16.04 ± 5.52 vs. 4.35 ± 3.62, P = 0.014), and the GSI of SCL-90 (0.91 ± 0.42 vs. 0.26 ± 0.11, P < 0.011) in OSAS group were significantly higher than those in healthy control group. However, the scores of memory quotient (MQ) (86.46 ± 13.51 vs. 101.90 ± 14.78, P = 0.002) in OSAS group was significantly lower than that in control group. No significant difference was found in performance intelligence quotient (PIQ) of WAIS-CR between OSAS and controls groups (P > 0.05). The plasma levels of 5-HT (168.51 ± 42.68 ng/mL *vs.* 138.94 ± 36.60 ng/mL) and 5-HIAA (184.52 ± 54.19 ng/mL *vs.* 157.96 ± 60.30 ng/mL) in OSAS patients were significantly higher than those in healthy controls (P < 0.05) (Table 1).

Genetic Association Analyses

The size of our sample (254 OSAS patients and 338 healthy control subjects) was sufficient to detect a difference with a genetic power of about 80.6% assuming an odds ratio (OR) of 2 with a minor allele frequency (MAF) of 0.1. Genotype frequencies above 2 polymorphisms (5-HTTLPR or STin2.VNTR) in either case or control groups were in Hardy-Weinberg equilibrium (P > 0.05), which suggested that the study sample came from a general population, without any effects of natural selection or migration.

No significant differences in allele distributions or genotype frequencies of 5-HTTLPR were found between patients and control subjects (P > 0.05). In contrast, significant differences

Table 3—Haplotype Frequencies Estimation and Comparison of 5-HTT Gene Polymorphisms in OSAS and Control Groups

Group	Haplotype	Haplotype frequency		χ^2	P-value	Empirical	Odds ratio	Global	
		Cases	Controls			P-value[b]	(95% CI)	χ^2	P-value
Total	S-10	27.90(5.5)	23.97(3.5)	2.766	0.096	0.267	1.57 (0.92 to 2.69)	9.798	**0.020**
	S-12	306.10(60.3)	456.03(67.5)	6.562	**0.010**	**0.034**	0.73 (0.58 to 0.93)		
	L-10	30.10(5.9)	23.03(3.4)	4.185	**0.040**	0.078	1.82 (1.02 to 3.24)		
	L-12	143.90(28.3)	172.97(25.7)	1.110	0.292	0.750	1.15 (0.89 to 1.49)		
Male	S-10	18.92(4.3)	20.83(3.5)	0.416	0.518	0.724	1.23 (0.65 to 2.33)	17.104	**0.0006**
	S-12	255.08(58.0)	402.17(67.9)	10.830	**0.001**	**0.002**	0.65(0.50 to 0.84)		
	L-10	34.08(7.7)	18.17(3.1)	11.484	**0.0007**	**0.0009**	2.65(1.48 to 4.75)		
	L-12	131.92(30.0)	150.83(25.5)	2.574	0.108	0.251	1.25 (0.95 to 1.65)		

[a]Frequencies are shown in parenthesis. [b]Empirical P-values were generated after 10,000 Monte Carlo simulations.
Significant P values (<0.05) are in boldface.

in frequency of genotypes and alleles were found in the STin2.VNTR (Allele: 10>12, χ^2 = 7.154, df = 1, P = 0.007, OR = 1.72, 95% CI = 1.15 to 2.58; Genotype: χ^2 = 6.565, df = 2, P = 0.037) (Table 2). Pairwise linkage disequilibrium (LD) analysis between 5-HTTLPR and STin2.VNTR showed that 2 markers were in modest LD (*D'* = 0.423). Two-locus haplotype analyses revealed that haplotype S-12 (P = 0.010, OR = 0.73, 95% CI = 0.58 to 0.93) and haplotype L-10 (P = 0.040, OR = 1.82, 95% CI = 1.02 to 3.24) were associated with OSAS in the total group (global P-value was 0.020) (Table 3).

After gender stratification, significant differences were found between male OSAS patients and male controls in genotype and allele frequencies of both 5-HTTLPR (Allele: L>S, χ^2 = 7.541, df = 1, P = 0.006, OR = 1.44, 95% CI = 1.11 to 1.87; Genotype: χ^2 = 10.417, df = 2, P = 0.005) and STin2.VNTR (Allele: 10>12, χ^2 = 9.258, df = 1, P = 0.002, OR = 1.94, 95% CI = 1.26 to 3.00; Genotype: χ^2 = 9.377, df = 2, P = 0.009) (Table 2). Furthermore, two haplotypes S-12 and L-10 also demonstrated significant evidence of association with OSAS in male group (S-12: P = 0.001, OR = 0.65, 95% CI = 0.50 to 0.84; L-10: P = 0.0007, OR = 2.65, 95% CI = 1.48 to 4.75, respectively) (Table 3). These results were still significant after 10,000 Monte Carlo simulations (Tables 2 and 3). Since the genotype frequencies of above 2 polymorphisms in female group showed significant deviations from Hardy-Weinberg equilibrium, we did not make analyses in female groups.

Comparisons of Clinical and Biochemical Index among Different Haplotype Carriers

In 108 OSAS patients with daytime function data, there were 91 male patients with OSAS, consisting of only 6 carriers and 85 non-carriers of the haplotype L-10, 51 carriers and 40 non-carriers for the haplotype S-12. Then we compared the difference in sleep characteristics, psychological status, cognitive function, and plasma 5-HT level between haplotype S-12 carriers and non-carriers in male OSAS patients.

Compared with non-carriers, patients carrying haplotype S-12 showed a significantly lower plasma 5-HT (143.64 ± 21.33 ng/mL *vs.* 185.47 ± 29.10 ng/mL, P < 0.001) and 5-HIAA (161.07 ± 32.16 ng/mL *vs.* 197.57 ± 39.07 ng/mL, *P* < 0.001) levels, and apnea / hypopnea index (AHI) (48.56 ± 19.43 events/h *vs.* 63.23 ± 20.30 events/h, P < 0.05), depressive factor of the SCL-90 (0.25 ± 0.10 *vs.* 0.88 ± 0.24, P < 0.05), and higher episodic memory score of the WMS (10.39 ± 2.62 *vs.*3.09 ± 1.70, P < 0.05). However, no significant differences were found in excessive daytime sleepiness (EDS), psychological status and other cognitive functions across different haplotype carriers (P > 0.05) (Table 4).

As mentioned above,[16,18,21] previous literatures have suggested that the *5-HTT* polymorphisms might play a potential role in mood disorders in particular and the psychiatric symptoms might be common comorbidities in OSAS. To exclude the effects of *5-HTT* polymorphisms on psychiatric comorbidities, we compared the 5-HTTLPR and STin2.VNTR allele frequencies between patients with GSI > 2 (n = 63) and ones with GSI ≤ 2 (n = 45) groups according to the SCL-90 scores. There were no significant difference in allele frequencies of above polymorphisms between GSI of SCL-90 > 2 and GSI ≤ 2 groups in OSAS patients (LPR: χ^2 = 2.692, df = 1, P = 0.101; STin2.VNTR: χ^2 = 1.648, df = 1, P = 0.199). These results suggested that the effect of *5-HTT* polymorphisms on OSAS might be independent of the psychiatric comorbidities.

DISCUSSION

Daytime Functions and Biochemical Status of OSAS

Previous literatures have reported that the OSAS might be associated with daytime dysfunctions.[30,31] As we reported previously, the OSAS patients showed significantly more psychological symptoms, such as somatization, obsession-compulsion, depression, anxiety.[22] In our sample, OSAS patients also showed significantly higher ESS and SCL-90 scores but lower memory quotients when compared with the healthy control subjects. These findings further support previous hypothesis that neuropsychological deficits in patients with OSAS.[30,31]

The neurobiochemical mechanism of OSAS has been long concerned for exploring the pathogenesis of OSAS. Serotonin, acting in the peripheral nervous system, can exacerbate sleep-related apnea, and systemically administered serotonin antagonists reduce sleep disordered respiration in rats, bulldogs, and even in human beings.[32-34] Our findings that the enhancement of plasma levels of serotonin (5-HT) and its metabolite, 5-hydroxyindolacetic acid (5HIAA), further suggested there might be hyperserotonergic neurotransmission in OSAS.

Table 4—Comparisons of Nocturnal PSG, Daytime Functions and Biochemical Index in Haplotype S-12 Carriers and Non-Carriers (mean ± SD)

	S-12 carriers (n = 51)	Non-S-12 carriers (n = 40)	*t*-value	P-value
(S_1+S_2) %[#]	67.87 ± 29.13	70.02 ± 28.32	0.968	0.340
SWS %[#]	12.47 ± 4.21	16.59 ± 5.03	1.087	0.234
REM %[#]	19.23 ± 7.21	13.42 ± 7.24	1.292	0.172
Arousals	8.93 ± 3.28	11.57 ± 6.46	0.875	0.482
AHI (events/h)	48.56 ± 19.43	63.23 ± 20.30	2.437*	0.020
Min SaO_2 (%)	72.22 ± 12.54	69.07 ± 14.89	0.926	0.356
ESS scores	10.01 ± 3.47	13.04 ± 5.52	1.378	0.170
GSI of SCL-90	0.54 ± 0.19	0.97 ± 0.41	0.499	0.629
Depressive factor of SCL-90	0.25 ± 0.10	0.88 ± 0.24	2.960*	0.007
MQ of WMS	81.16 ± 16.98	89.90 ± 15.66	1.426	0.155
Episodic memory of WMS	10.39 ± 2.62	3.09 ± 1.70	2.685*	0.008
PIQ of WAIS	86.13 ± 15.24	91.85 ± 17.62	1.815	0.212
Plasma 5-HT level (ng/mL)	143.64 ± 21.33	185.47 ± 29.10	8.019**	<0.001
Plasma 5-HIAA level (ng/mL)	161.07 ± 32.16	197.57 ± 39.07	7.543**	<0.001

[#]Values for sleep stages are expressed as a percentage of sleep period time; SWS: slow wave sleep; Arousals: frequencies of brief arousals from sleep; AHI: apnea / hypopnea index; Min SaO_2: minimum oxygen saturation; ESS: Epworth Sleepiness Scale; GSI: General Severity Index; SCL-90: symptom checklist-90; MQ: memory quotient; WMS: Wechsler Memory Scale; PIQ: performance intelligent quotient; WAIS: Wechsler Adult Intelligent Scale; 5-HIAA: 5-hydroxyindolacetic acid. *P < 0.05; **P < 0.001.

Association Analyses of OSAS with 5-HTT Gene Polymorphisms

Dysfunction of the serotoninergic system has long been suspected to be involved in sleep disorders and respiration diseases.[13,20,35-37] Serotonergic fibers broadly innervate the thalamus and may influence the sleep wake cycle, attention, and other processes through modulation of neurons in this structure.[38] New findings in past few years also have brought significant insight into the neural mechanisms governing upper airway dilator muscle function and provided on neurobiochemical mechanisms, emphasizing a role for serotonergic mechanism for OSAS.

Synaptic serotonin is inactivated by presynaptic reuptake, which is mediated by the serotonin transporter (5-HTT). Two polymorphisms, 5-HTTLPR (*5-HTT* gene-linked polymorphic region) and VNTR (variable number tandem repeats of 17 bp sequence in the second intron and has several alleles), have been frequently described in several disease models. The function of VNTR is thought to affect enhancer function and thus transcription of the gene.[39] 5-HTTLPR is a deletion insertion polymorphism located at the 5'-flanking regulatory region of the *5-HTT* and creates short (S) and long (L) alleles. The reuptake of serotonin in cells homozygous for the L form (or L/L) of the promoter polymorphism was found to be 1.9 to 2.2 times as great as that in cells carrying one or two endogenous copies of the S (or S/L, or S/S) allele. That is, the presence of S allele is associated with decreased 5-HT reuptake, which, in turn, results in longer serotonergic activity and corresponds to low serotonin uptake activity.[40] Based on above evidence, we hypothesized that the *5-HTT* gene variations may implicate in determining the susceptibility to OSAS and then investigate the association of *5-HTT* gene with OSAS.

In the present study, we found that the allele 10-repeat of the STin2.VNTR was associated with OSAS in both total (P = 0.007, OR = 1.72, 95% CI = 1.15 to 2.58) and male subjects (P = 0.002, OR = 1.94, 95% CI = 1.26 to 3.00). Although the 5-HTTLPR did not show susceptibility to OSAS in total subjects, the L allele showed significant differences between male patients and male controls (P = 0.006, OR = 1.44, 95% CI = 1.11 to 1.87). Haplotype analysis is a powerful strategy that markedly increased data significance compared to single-locus analysis. In this study, 2 haplotypes (S-12 and L-10) constructed of 5-HTTLPR and STin2.VNTR provided convergent evidence for a significant role of the *5-HTT* gene in the genetic predisposition to OSAS, especially in a subgroup of male subjects (global P-values were 0.020 for total sample and 0.0006 for male subjects, respectively). Our data further provided evidence implicating the *5-HTT* gene itself might play a role in the pathogenesis of OSAS, especially with gender effect. As we all known, the prevalence of OSAS is much higher in male than in female (2-4:1). Therefore the stratification of sex in our sample might further help to investigate the association of OSAS with *5-HTT* gene polymorphisms. On the other hand, our findings just support suggestive association between *5-HTT* polymorphisms and OSAS, considering the limited power of our sample size (254 OSAS patients and 338 healthy controls) with a MAF<0.10 for Stin2.10 in controls and a much lower OR of 1.44 for LPR in male subjects. These preliminary results should be further verified or replicated in another samples to exclude the possibility of false positive association.

Yilmaz et al. ever reported that the 12/10 and LL genotypes were over-represented in the male patients compared with male controls.[20] Interestingly, there are significant ethnic differences between European-American and Chinese Han populations in genotype or allele frequencies of 5-HTTLPR and STin2.VNTR in the *5-HTT* gene.[41] Weese-Mayer et al. also have reported the racial differences in their research of association of *5-HTT* and sudden infant death syndrome (SIDS).[42] And the genotype and allele distributions in the sample of Yilmaz were similar to those of European-American population. However, the potential risk allele susceptibility to OSAS in our Chinese Han sample were the same as those in the sample of Yılmaz. These convergent findings further support the potential implications of 5-HTT in the pathogenesis of OSAS.

Previous studies have indicated that 5-HTTLPR might be a potential susceptible factor in behavioral or mental disorders, as the long allele had a higher transcriptional activity than the short one.[16,25] The VNTR in intron 2 of *5-HTT* gene is thought to affect enhancer transcription of the gene. In the present study, STin2.10 allele of the *5-HTT* gene may confer susceptibility to OSAS in about 21.3% of our patients with OSAS. This might be explained with several possible mechanisms. Firstly, the repeat is followed by an activating protein (AP-1) motif, a putative binding site for a transcription factor comprising the heterodimer c-fos / c-jun, which may play a role in the regulation of 5-HTT expression.[15] Alternatively, a small number of VNTR repeats may influence the stability of transcription of messenger RNA. Similarly, the VNTR at the insulin gene locus IDDM2 might influence the expression of insulin messenger RNA in pancreatic cell lines.[43] To date, some hypotheses have suggested that micro- and mini-satellite polymorphisms play a role in complex multigenic disorders and behavioral phenotypes.[44] Further efforts are required to determine whether the 5-HTT-VNTR is of direct functional significance or whether it is closely linked to another functional variant.

We then evaluated the association of the haplotype S-12 consisted of 5-HTTLPR and STin2.VNTR with sleep structure, daytime function, and plasma 5-HT / 5-HIAA levels among male OSAS patients. Individuals carrying the haplotype S-12 showed a significantly lower plasma 5-HT and 5-HIAA levels and apnea / hypopnea index (AHI represents the severity of OSAS) ($P < 0.05$). This also indirectly supports our findings that presence of haplotype S-12 allele might against OSAS. However, no significant differences were found in EDS, psychological status, and cognitive function across different haplotype carriers ($P > 0.05$). Several studies focused on the association of the *5-HTT* gene polymorphisms and behavioral diseases. A recent literature has proposed that the S allele of *5-HTT* gene might be associated with reduced hippocampal volume and lower memory in the older adults.[45] Another study reported that the STin2.12 may be associated with weaker working memory in major depression.[46] Yu et al. have reported that the major depressive patients carrying the L/L genotype had a significantly better response to fluoxetine when compared to the S allele carriers on the basis of total ($P = 0.013$), core ($P = 0.011$), psychic anxiety ($P = 0.005$), and somatic anxiety ($P = 0.002$) symptoms of the Hamilton Depression Rating Scale-score percentage change.[47] In our study, we did not compare the therapeutic effect in OSAS patients. However, we did find the association of the 5-HTT haplotype with the depressive symptoms by using the SCL-90. On the other hand, the episodic memory scores of WMS also might be associated with the 5-HTT polymorphisms. Egan et al. also reported that the brain-derived neurotrophic factor (*BDNF*) val/met polymorphism might affect the human episodic memory and hippocampal function in schizophrenia.[48] These results further suggested that the daytime sequences of OSAS may due to much more complex mechanism.

CONCLUSIONS

This study provides some support for the hypothesis that allele L of 5-HTTLPR and allele 10 of the STin2.VNTR in the *5-HTT* gene might be a susceptible factors to OSAS, especially within the male patients. Compared with non-S-12 carriers, OSAS patients with haplotype S-12 constructed by two above polymorphisms might play a potential role for OSAS and clinical characteristics. Due to the potential limitations of sample size and population stratification, the case-control study may lead to false-positive findings. Therefore, genotype analysis of more markers in *5-HTT* and replication of this finding in large independent samples and family-based association studies will be required to further strengthen that *5-HTT* gene plays a role in genetic susceptibility to OSAS.

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

REFERENCES

1. Redline S, Tishler PV, Tosteson TD, et al. The familial aggregation of obstructive sleep apnea. Am J Respir Crit Care Med 1995;151:682-7.
2. Schwab RJ, Pasirstein M, Kaplan L, et al. Family aggregation of upper airway soft tissue structures in normal subjects and patients with sleep apnea. Am J Respir Crit Care Med 2006;173:453-63.
3. Pillar G, Lavie P. Assessment of the role of inheritance in sleep apnea syndrome. Am J Respir Crit Care Med 1995;151:688-91.
4. Young T, Peppard PE, Gottlieb DJ. Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med 2002;165:1217-39.
5. Partinen M, Telakivi T. Epidemiology of obstructive sleep apnea syndrome. Sleep 1992;15:S1-4.
6. Buxbaum SG, Elston RC, Tishler PV, Redline S. Genetics of the apnea hypopnea index in Caucasians and African Americans: I. Segregation analysis. Genet Epidemiol 2002;22:243-53.
7. Taheri S, Mignot E. The genetics of sleep disorders. Lancet Neurol 2002;1:242-50.
8. Barceló A, Elorza MA, Barbé F, Santos C, Mayoralas LR, Agusti AG. Angiotensin converting enzyme in patients with sleep apnoea syndrome: plasma activity and gene polymorphisms. Eur Respir J 2001;17:728-32.
9. Bliwise DL. Sleep apnea, ApoE4 and Alzheimer's disease 20 years and counting? J Psychosom Res 2002;53:539-46.
10. Bayazit YA, Erdal ME, Yilmaz M, et al. Insulin receptor substrate gene polymorphism is associated with obstructive sleep apnea syndrome in men. Laryngoscope 2006;116:1962-5.
11. Sood S, Morrison JL, Liu H, Horner RL. Role of endogenous serotonin in modulating genioglossus muscle activity in awake and sleeping rats. Am J Respir Crit Care Med 2005;172:1338-47.
12. Kraiczi H, Hedner J, Dahlöf P, Ejnell H, Carlson J. Effect of serotonin uptake inhibition on breathing during sleep and daytime symptoms in obstructive sleep apnea. Sleep 1999;22:61-7.
13. Carley DW, Olopade C, Ruigt GS, Radulovacki M. Efficacy of mirtazapine in obstructive sleep apnea syndrome. Sleep 2007;30:35-41.
14. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527-31.
15. Lesch KP, Balling U, Strauss K, et al. Organisation of the human serotonin transporter gene. J Neural Transm Gen Sec 1994;95:157-62.
16. Ramamoorthy S, Bauman AL, Moore KR, et al. Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. Proc Natl Acad Sci U S A 1993;90:2542-6.

17. Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter expression. J Neurochem 1996;66:2621-4.
18. Battersby S, Ogilvie AD, Smith CA, et al. Structure of a variable number tandem repeat of the serotonin transporter gene and association with affective disorder. Psychiat Genet 1996;6:177-81.
19. Fabre V, Boutrel B, Hanoun N, et al. Homeostatic regulation of serotonergic function by the serotonin transporter as revealed by nonviral gene transfer. J Neurosci 2000;20:5065-75.
20. Yılmaz M, Bayazit YA, Ciftci TU, et al. Association of serotonin transporter gene polymorphism with obstructive sleep apnea syndrome. Laryngoscope 2005;115:832-6.
21. Rechtschaffen A, Kales A, eds. A manual of standardized techniques and scoring system for sleep stages of human subjects. Los Angeles (CA): Brain Information Service and Brain Research Institute; 1968.
22. Yue W, Hao W, Liu P, Liu T, Ni M, Guo Q. A case-control study on psychological symptoms in sleep apnea-hypopnea syndrome. Can J Psychiatry 2003;48:318-23.
23. Furuta H, Kaneda R, Kosaka K, Arai H, Sano J, Koshino Y. Epworth sleepiness scale and sleep studies in patients with obstructive sleep apnea syndrome. Psychiatry Clin Neurosci 1999;53:301-2.
24. Derogatis L, Lopman R, Covil L. SCL-90: an outpatient psychiatric rating scale preliminary report. Psychopharmacology 1973;9:13-28.
25. Smith CA, Gough AC, Leigh PN, et al. Debrisoquine hydroxylase gene polymorphism and susceptibility to Parkinson's disease. Lancet 1992;339:1375-7.
26. Collier DA, Stöber G, Li T, et al. A novel functional polymorphism within the promoter of the serotonin transporter gene: Possible role in susceptibility to affective disorders. Mol Psychiatry 1996;1:453-60.
27. Auerbach SB, Minzenberg MJ, Wilkinson LO. Extracellular serotonin and 5- hydroxyindoleacetic acid in hypothalamus of the unanesthetized rat measured by in vivo dialysis coupled to high-performance liquid chromatography with electrochemical detection: dialysate serotonin reflects neuronal release. Brain Res 1989;499:281-90.
28. Shi Y, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15:97-8.
29. Halperin E, Eskin E. Haplotype Reconstruction from genotype data using imperfect phylogeny. Bioinformatics 2004;20:1842-9.
30. Naegele B, Thouvard V, Pepin J, et al. Deficits of executive cognitive functions in patients with sleep apnea syndrome. Sleep 1995;18:43-52.
31. Cheshire KH, Engleman I, Deary C, et al. Factors impairing daytime performance in patients with sleep apnea / hypopnea syndrome. Arch Intern Med 1992;152:538-41.
32. Veasey SC, Chachkes J, Fenik P, Hendricks JC. The effects of ondansetron on sleep-disordered breathing in the English bulldog. Sleep 2001;24:155-60.
33. Fenik P, Ogawa H, Veasey SC. Hypoglossal nerve response to 5-HT3 drugs injected into the XII nucleus and vena cava in the rat. Sleep 2001;24:871-8.
34. Carley DW, Olopade C, Ruigt GS, Radulovacki M.Efficacy of mirtazapine in obstructive sleep apnea syndrome. Sleep 2007;30:35-41.
35. Python A, Steimer T, de Saint Hilaire Z, Mikolajewski R, Nicolaidis S. Extracellular serotonin variations during vigilance states in the preoptic area of rats: a microdialysis study. Brain Res 2001;910:49-54.
36. Sakai K, Takada T, Nakayama H, et al. Serotonin-2A and 2C receptor gene polymorphisms in Japanese patients with obstructive sleep apnea. Int Med 2005;44:928-33.
37. Fenik P, Veasey SC. Pharmacological characterization of serotonergic receptor activity in the hypoglossal nucleus. Am J Respir Crit Care Med 2003;167:563-9.
38. Monckton JE, McCormick DA. Neuromodulatory role of serotonin in the ferret thalamus. J Neurophysiol 2002;87:2124-36.
39. MacKenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. PNAS 1999;96:15251-5.
40. Serretti A, Catalano M, Smeraldi E. Serotonin transporter gene is not associated with symptomatology of schizophrenia. Schizophr Res 1999;35:33-9.
41. Kunugi H, Hattori M, Kato T, et al. Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. Mol Psychiatry 1997;2:457-62.
42. Weese-Mayer DE, Zhou L, Berry-Kravis EM, et al. Association of the serotonin transporter gene with sudden infant death syndrome: a haplotype analysis. Am J Med Genet 2003;122A:238-45.
43. Kennedy GC, German MS, Rutter WJ. The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. Nat Genet 1995;9:293-8.
44. Comings DE. Polygenic inheritance and micro/mini-satellites. Mol Psychiatry 1998;3:21-31.
45. O'Hara R, Schröder CM, Mahadevan R, et al. Serotonin transporter polymorphism, memory and hippocampal volume in the elderly: association and interaction with cortisol. Mol Psychiatry 2007;12:544-55.
46. Dömötör E, Sárosi A, Balogh G, et al. Association of neurocognitive endophenotype and STin2 polymorphism in major depressive disorder. Neuropsychopharmacol Hung 2007;9:53-62.
47. Yu Y, Tsai S, Chen T, et al. Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. Mol Psychiatry 2002;7:1115-9.
48. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 2003;112:257-69.