Lack of Association of Serotonin 2A Receptor Gene in Japanese Patients with Obstructive Sleep Apnea Syndrome

Kayoko Ikegawa[1], Masao Ota[2], Nobumitsu Kobayashi[1], Yunden Droma[1]
Hironobu Yaegashi[3], Michiko Nishizawa[1], Masanori Yasuo[1], Kazuhisa Urushihata[1]
Hiroshi Yamamoto[1] and Masayuki Hanaoka[1]*

1) *The First Department of Internal Medicine, Shinshu University School of Medicine*
2) *Department of Legal Medicine, Shinshu University School of Medicine*
3) *Hiro Internal Medicine Clinic*

Background : The contraction of the genioglossus muscle is realized by the binding of serotonin with serotonin 2A receptor through modulating the hypoglossal motor output. When the genioglossus muscle relaxes, it causes glossoptosis and upper airway obstruction. Therefore, the variations of the serotonin 2A receptor gene (*HTR2A*) are hypothesized to be associated with obstructive sleep apnea syndrome (OSAS) according to the pathogenesis of OSAS. To investigate the association of the *HTR2A* gene with OSAS in the Japanese population, we conducted the current case-control association study.

Methods : The subjects included 145 male patients with OSAS who were diagnosed by overnight polysomnography (PSG) and 133 male controls who were normal in PSG. All the subjects were of Japanese origin with respect to ethnicity. Ten tag single nucleotide polymorphisms (SNPs) in the *HTR2A* gene were genotyped with TaqMan SNP genotyping. A multivariate logistic regression analysis was applied with adjustments of age and body mass index (BMI).

Results : There were no significant differences of allelic frequencies of the ten tag SNPs between patient and control groups. In addition, in sub-analyses among the patients with OSAS, we did not detect any associations of these SNPs with the severity of OSAS (apnea hypopnea index cutoff : 40 events/h) and with the degree of obesity (BMI cut off : 25 kg/m^2).

Conclusions : This study did not prove the hypothesis regarding the association of variations of the serotonin 2A receptor gene (*HTR2A*) with OSAS. The *HTR2A* gene variations were less likely to participate in the pathogenesis of OSAS in Japanese. *Shinshu Med J 65 : 153—162, 2017*

(Received for publication October 27, 2016 ; accepted in revised form December 27, 2016)

Key words : 5-hydroxytryptamine, gene, obstructive sleep apnea syndrome, polymorphism

I Introduction

Obstructive sleep apnea syndrome (OSAS) is characterized by repeated partial or complete collapse of the pharynx during sleep, which results in apnea or hypopnea, associated with oxygen desaturation and arousal from sleep[1]. OSAS is associated with metabolic syndrome, cardiovascular diseases, and neuropsychological sequelae[2]. In addition, traffic and work-related accidents are frequently attributed to OSAS, which leads substantial social and economic costs[2]. Clarifying the risk factors that confer susceptibility to OSAS would contribute not only to the identification of diagnostic and prognostic biomarkers but also to the promotion of therapeutic and preventive strategies for individuals with a high risk of OSAS. In addition to the risk factors of age,

* Corresponding author : Masayuki Hanaoka
The First Department of Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
E-mail : masayuki@shinshu-u.ac.jp

gender, and body mass index (BMI), recent studies have identified that genetic factors are closely associated with OSAS[3)-5)]. For example, a family study suggested that the risk of OSAS might be higher in relatives of patients with OSAS than in controls[3)]. In addition, Redline and Tishler reviewed data in relation to OSAS and suggested that nearly 40 % of the variance in the apnea hypopnea index (AHI) in patients with OSAS might be explained by genetic factors[4)]. Strong evidences suggested that genetic factors were interactively associated with craniofacial structure, body fat distribution, and neural control of the upper airway muscles to produce the OSAS phenotype[4)].

The neurotransmitter, 5-hydroxytryptamine (5-HT, or serotonin), works in the central nervous system to regulate various visceral and physiologic functions, including sleep, appetite, pain perception, hormone secretion, thermoregulation, and sexual behavior[6)]. In addition, several lines of pharmacological, neurobehavioral, and therapeutic evidences have implicated serotonin is involved in the pathogenesis of OSAS[6)-9)]. Serotonin controls genioglossus muscle activity by binding the serotonin 2A receptor (HTR2A), which modulates hypoglossal motor output. Contraction of the genioglossus muscle, which is innervated by 5-HT neurons, prevents collapse of the upper airway[7)8)]. Previous studies in obese rats demonstrated that increased expression of *HTR2A* could effectively maintain stable upper airways and normal breathing[9)]. Experiments *in vitro* showed that polymorphisms in the *HTR2A* gene could influence the level of receptor expression[10)].

The human *HTR2A* gene comprises 3 exons and locates in the q14-21 region of chromosome 13[11)]. Several important single nucleotide polymorphisms (SNPs) in the *HTR2A* gene were studied in order to detect associations with susceptibility to OSAS, however, diverse results were shown by various ethnic populations regarding the association between SNPs of the *HTR2A* gene with susceptibility to OSAS[12)-17)]. Indeed, racial and ethnic differences in OSAS have been evidenced by international studies[18)-20)] in which the emerging data suggested that certain ethnic groups may be at increased risk for OSAS. At present, it is unclear about the association of SNPs in the *HTR2A* gene with susceptibility to OSAS in the Japanese population because of insufficient genetic data about this issue[12)]. In order to understand the associations of the *HTR2A* gene with OSAS in the Japanese, we genotyped and analyzed a large number of tag SNPs in the *HTR2A* gene in a case-control association study with a relatively large sample size of Japanese male patients with OSAS.

Ⅱ Patients and Methods

A Patients

This study was approved by the Ethics Committee of Shinshu University (permission number : 298). Written informed consent was obtained from all patients and controls prior to their inclusion in the study. All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

This study enrolled 145 male Japanese patients with OSAS. All subjects were unrelated Japanese individuals with permanent residences in Japan. Of these, 125 patients were consecutive referrals to Shinshu University Hospital and Hiro Internal Medicine Clinic from April 2001 to March 2012 ; the other 20 patients were long-distance truck drivers diagnosed with OSAS through an OSAS screening check-up at Shinshu University from 2006 to 2007. The diagnosis of OSAS was based on criteria determined by the American Academy of Sleep Medicine (AASM)[21)]. These criteria were AHI ≥ 15 events/h or AHI ≥ 5 events/h plus a clinical presentation of OSAS symptoms. The AHI was monitored continuously during a night of sleep with polysomnography (PSG). The clinical OSAS symptoms were defined as a score ≥11 by the degrees of habitual snoring and daytime sleepiness on the Epworth Sleepiness Scale (ESS)[22)]. The patients were excluded when they had renal failure, hypothyroidism, acromegaly, central sleep apnea, or psychiatric disorders. For sub-analysis classified by BMI, WHO defines overweight as a BMI greater than or equal to 25. The patients were further classified into obese OSAS (BMI ≥ 25 kg/

m^2 ; n = 51) and non-obese OSAS (BMI <25 kg/m^2 ; n = 94) subgroups. For sub-analyses classified by AHI, we followed the criteria in previous studies[23]. The patients were further classified into severe OSAS (AHI ≥ 40 events/h ; n = 70) and mild or moderate OSAS (AHI <40 events/h ; n = 75) subgroups.

Control subjects consisted of 133 healthy, unrelated male Japanese through an OSAS screening check-up at Shinshu University from 2006 to 2007. To ensure the control subjects were free from sleep-related breathing disorders, they were selected with the following criteria : absence of sleep disturbances ; no symptoms related to any disordered breathing during sleep ; AHI <5 events/h ; and oxygen saturation by pulse oximetry (SpO2) >90 % in an overnight PSG.

B Polysomnography (PSG)

All patients with OSAS and control subjects underwent overnight PSG (Alice III ; Chest Ltd ; Tokyo, Japan). Polysomnography consisted of a continuous polygraphic recording from multiple surface leads, including leads for an electroencephalography (EEG, C3-A2, C4-A1, O2-A1, and O3-A2), for a bilateral electro-oculography, for chin and lower leg electromyography, and for electrocardiography (ECG). Recordings also tracked output from thermistors for nasal and oral airflows, thoracic and abdominal impedance belts for respiratory effort, a pulse oximeter for SpO2, a tracheal microphone for snoring, and sensors for detecting body position during sleep. An apnea episode was defined as the complete cessation of airflow for at least 10 seconds (s). Hypopnea was defined as at least a 50 % reduction in airflow for at least 10s, accompanied by a reduction in SpO2 of at least 4 %. AHI was the key indicator for OSAS diagnosis ; AHI was defined as the number of apnea or hypopnea events per hour during sleep time, based on results from the overnight PSG.

C Genotyping

DNA was extracted from whole blood with a QuickGene 800 (Fuji Film, Tokyo, Japan). Genomic DNA was prepared at 10-15 ng/μL for the TaqMan SNP genotyping assay. We genotyped ten SNPs that spanned the region between the 3'-untranslated region (UTR) and the 5'-UTR of the *HTR2A* gene. These SNPs were : rs3803189 (in the 3'-UTR), rs977003, rs9567737, rs9316232, rs2224721, rs2770296, rs731779, rs9567746, rs2070036, and rs6311 (in the 5'-UTR). The ten SNPs were selected based on the following information from the NCBI dbSNP database : (a) located within the *HTR2A* gene ; (b) minor allele frequency over 10 % in Japanese populations ; (c) average heterozygosity of 30 % ; (d) density of at least one SNP per 5 kb ; and (e) availability for validation assays. Furthermore, these ten SNPs could tag another 38 SNPs in the *HTR2A* genes in a Japanese population by producing a coefficient of determination (r^2) >0.8, when evaluated with tagger software from the International HapMap project[24] (**Table 1**).

The SNP Genotyping Assay Mix contained forward and reverse primers and FAM™ and VIC™ dye-minor groove binder-labeled probes (Applied Biosystems Inc., Tokyo, Japan). Allelic discrimination of the ten SNPs was performed according to the manufacturer's instructions for the TaqMan® SNP Genotyping Assay with an Applied Biosystems 7500 Fast Real-time PCR System (Applied Biosystems Inc., Foster City, CA, USA). After thermal cycling, genotype data were acquired automatically and analyzed with sequence detection software (SDS v1.3.1, Applied Biosystems Inc.).

D Statistical analysis

Quantitative data were expressed as the mean ± standard deviation (SD). The Mann-Whitney U test was used to evaluate significant differences between cases and controls in age, BMI, and AHI. Frequencies of genotypes and alleles were expressed in decimals. The Hardy-Weinberg equilibrium (HWE) for each SNP was confirmed with the Chi-square test. Significant differences in allele frequencies between two groups were evaluated with the Chi-square test (2 × 2 contingency table). The effects of ancestral alleles on inheritance of OSAS were evaluated with multivariate logistic regression analyses, assuming a dominant mode and a recessive mode. The values of pair-wise linkage disequilibrium (LD) of the ten SNPs were measured with Haploview software[25]. Results are expressed with odds ratios (OR) with 95 % confidence interval (CI) values, after adjusting for

Table 1 Tagging efficiency of the ten SNPs of *HTR2A* for a Japanese population

Test SNPs	Alleles captured	Number of SNPs
rs3803189	rs977003, rs1923882, rs7322347, rs3125	4
rs977003	rs3803189, rs1923882, rs977003, rs7322347, rs3125	5
rs9567737	rs6561333, rs6561333	2
rs9316232	rs1923888, rs1923888, rs2296972, rs9567739, rs655888, rs3742279, rs1745837, rs622337, rs655854	9
rs2224721	rs2224721	1
rs2770296	rs2770297, rs2770298, rs1928040	3
rs731779	rs9567746, rs2770293, rs582854, rs9567746, rs9316235, rs9526245	6
rs9567746	rs731779, rs2770293, rs582854, rs9316235, rs9526245	5
rs2070036	rs2070036	1
rs6311	rs6311, 6313	2
	Total	38

Evaluated at coefficient of determination (r^2) >0.8 by tagger software through International HapMap project (http : //hapmap.ncbi.nlm.nih.gov/)

Table 2 Characteristics of subjects with OSAS and controls

	Patients with OSAS	Controls
Number of subjects	145	133
Age (years)	56.9 ± 13.6*	43.3 ± 12.7
BMI (kg/m^2)	27.2 ± 5.0*	23.4 ± 3.2
AHI (events/h)	42.2 ± 19.2*	3.5 ± 3.7

All subjects were male. Data are expressed as mean ± SD.
* p <0.001 versus controls by Mann-Whitney U test.

age and BMI[26]. P values <0.05 indicated statistical significance. Corrected P values (Pc) were calculated by multiplying the number of alleles in a given locus.

Ⅲ Results

A Characteristics of subjects with OSAS and controls

The final analyses were based on genetic data from 145 male patients with OSAS and 133 male controls. The average AHI was significantly higher in the OSAS group than in the control group (42.2 ± 19.2 vs. 3.5 ± 3.7 events/h, P <0.001, **Table 2**). The average age and BMI were significantly greater in the patients with OSAS than in the controls (**Table 2**).

B Associations of the ten tag SNPs with OSAS

All the ten SNPs were in HWE for both the OSAS and control groups. There were no significant differences in the allelic frequencies of the ten tag SNPs between the two groups (**Table 3**). In addition, after adjusting for age and BMI, the multivariate logistic regression analysis did not show any effects of the ancestral SNP alleles on OSAS inheritance, assuming either the dominant mode or the recessive mode (**Table 3**). Moreover, there were no significant differences in frequencies of the observed haplotypes between the controls and OSAS patients.

C Associations of the ten tag SNPs with obesity and with severity of OSAS

In the sub-analysis concerning obese and non-obese OSAS subgroups classified by BMI (cut-off value : 25 kg/m^2), significant associations were not detected regarding the ten tag SNPs of the *HTR2A* with obese-OSAS (**Table 4**).

In the sub-analysis concerning severe and mild or moderate OSAS subgroups classified by AHI (cut-off value : 40 events/h), rs2770296 and rs731779 seemed to be associated with severe OSAS (P = 0.040, 0.047, respectively, **Table 5**) ; however, such significant

Table 3 A allele frequencies and genotype distributions of tag SNPs of *HTR2A* gene in patients with OSAS (N = 145) and controls (N = 133)

dbSNPs Location	Alleles (1/2)*	Allele 1 Frequency		P†	Genotype distributions						P‡ 11/12 + 22	P‡ 11 + 12/22
					11*		12*		22*			
		OSAS	Controls		OSAS	Controls	OSAS	Controls	OSAS	Controls	OR (95 % CI)	OR (95 % CI)
rs3803189 3'-UTR	T/G	0.769	0.756	0.71	0.593	0.564	0.352	0.383	0.055	0.053	0.28 0.47 (0.12–1.88)	0.45 0.79 (0.44–1.44)
rs977003 Intron	A/C	0.748	0.741	0.83	0.559	0.534	0.379	0.413	0.062	0.053	0.51 1.23 (0.67–2.25)	0.91 1.07 (0.31–3.69)
rs9567737 Intron	T/C	0.62	0.621	0.99	0.361	0.379	0.519	0.483	0.120	0.138	0.76 0.90 (0.45–1.80)	0.44 0.76 (0.38–1.52)
rs9316232 Intron	A/G	0.548	0.485	0.14	0.290	0.241	0.517	0.408	0.193	0.271	0.66 1.14 (0.63–2.07)	0.71 1.25 (0.40–3.91)
rs2224721 Intron	G/T	0.603	0.526	0.07	0.386	0.286	0.435	0.481	0.179	0.233	0.57 0.84 (0.46–1.54)	0.84 0.91 (0.36–2.31)
rs2770296 Intron	T/C	0.700	0.673	0.49	0.490	0.443	0.421	0.459	0.089	0.098	0.40 1.29 (0.71–2.33)	0.99 1.06 (0.37–2.76)
rs731779 Intron	A/C	0.766	0.759	0.87	0.600	0.579	0.331	0.361	0.069	0.06	0.81 1.14 (0.40–3.25)	0.43 0.67 (0.25–1.80)
rs9567746 Intron	A/G	0.741	0.726	0.67	0.565	0.519	0.352	0.413	0.083	0.068	0.33 0.74 (0.41–1.35)	0.76 1.21 (0.36–4.07)
rs2070036 Intron	T/G	0.686	0.68	0.88	0.455	0.466	0.462	0.421	0.083	0.105	0.15 1.72 (0.81–3.64)	0.19 1.52 (0.82–2.83)
rs6311 5'-UTR	C/T	0.5	0.534	0.43	0.241	0.278	0.518	0.511	0.241	0.211	0.72 1.13 (0.57–2.26)	0.82 0.93 (0.47–1.82)

Allelic frequencies and genotypic distributions are expressed as decimals.

＊1/2 indicates ancestral allele/derived allele according to the NCBI dbSNP database.

† *P* values obtained by Chi-square test (2 × 2 contingency table).

‡ *P* values of the dominant mode (11/12 + 22) and the recessive mode (11 + 12/22) as well as their corresponding OR (95 % CI) values are obtained by multivariate logistic regression analysis after adjustment for age and body mass index (http : //statpages.org/logistic.html).

associations vanished after correcting with the P values (Pc = 0.40, 0.47, respectively, **Table 5**). No other significant associations were detected regarding the ten tag SNPs of the *HTR2A* with severe OSAS (**Table 5**).

Ⅳ Discussion

In the present study, we densely genotyped ten SNPs of the *HTR2A* gene (rs3803189 in the 3'-UTR, rs977003, rs9567737, rs9316232, rs2224721, rs2770296, rs731779, rs9567746, rs2070036, and rs6311 in the 5'-UTR), those that could tag another 38 SNPs along the *HTR2A* gene (**Table 1**), in 145 male patients with OSAS and 133 male controls. The results demonstrated that there was no association between the ten tag SNPs of the *HTR2A* gene and the susceptibility to OSAS in a Japanese population. In addition, no genetic associations were detected between these SNPs and the severity of OSAS (AHI ≥40 events/h) or overweight in OSAS (BMI ≥25 kg/m^2). The reliability of these results were convinced by the facts that all patients and controls were strictly diagnosed with standard PSG examinations and that multivariate logistic regression analyses were adjusted for significant differences in age and BMI.

The biological pathways underlying OSAS are mediated by genes involved in serotonergic receptor transmission ; thus, these genes attract interest as candidate genes that might confer susceptibility to OSAS. Serotonin plays important roles in sleep-wake behavior and appetite regulation ; it is also in-

Table 4 A allelic frequencies of the ten tag SNPs of the *HTR2A* gene between subgroups classified by BMI (cuto-ff : 25 kg/m^2) among the patients with OSAS

dbSNPs	Alleles (1/2)*	Allele 1 Frequency		$P^{\dagger}$	$Pc^{\ddagger}$
		Obese OSAS (N = 94)	Non-obese OSAS (N = 51)		
rs3803189	T/G	0.777	0.755	0.676	6.756
rs977003	A/C	0.761	0.725	0.510	5.102
rs9567737	T/C	0.628	0.608	0.740	7.398
rs9316232	A/G	0.580	0.490	0.143	1.432
rs2224721	G/T	0.601	0.608	0.910	9.103
rs2770296	T/C	0.691	0.716	0.668	6.677
rs731779	A/C	0.724	0.833	0.062	0.616
rs9567746	A/G	0.745	0.735	0.862	8.616
rs2070036	T/G	0.686	0.686	0.999	9.985
rs6311	C/T	0.521	0.461	0.325	3.252

Allelic frequencies and genotypic distributions are expressed as decimals.
＊1/2 indicates ancestral allele/derived allele according to the NCBI dbSNP database.
† *P* values obtained by Chi-square test (2×2 contingency table).
‡ Corrected P value calculated by multiplying the number of alleles in a given locus.

Table 5 A allelic frequencies of the tag SNPs of the *HTR2A* gene between subgroups classified by AHI (cut-off : 40 events/hour) among the patients with OSAS

dbSNPs	Alleles (1/2)*	Allele 1 Frequency		$P^{\dagger}$	$Pc^{\ddagger}$
		Severe OSAS (N = 70)	Mild & Moderate OSAS (N = 75)		
rs3803189	T/G	0.800	0.736	0.194	1.943
rs977003	A/C	0.753	0.743	0.837	8.373
rs9567737	T/C	0.647	0.593	0.345	3.453
rs9316232	A/G	0.567	0.529	0.515	5.148
rs2224721	G/T	0.640	0.564	0.188	1.878
rs2770296	T/C	0.753	0.643	0.040	0.402
rs731779	A/C	0.813	0.714	0.047	0.467
rs9567746	A/G	0.787	0.693	0.068	0.683
rs2070036	T/G	0.713	0.657	0.303	3.028
rs6311	C/T	0.513	0.486	0.638	6.383

Allelic frequencies and genotypic distributions are expressed as decimals.
＊1/2 indicates ancestral allele/derived allele according to the NCBI dbSNP database.
† *P* values obtained by Chi-square test (2×2 contingency table).
‡ Corrected P calculated by multiplying the number of alleles in a given locus.

volved in upper airway dilator muscle activity through its modulation of hypoglossal motor output[8)9)]. In particular, the serotonin 2A receptor was found to be the predominant excitatory serotonin receptor subtype in hypoglossal motor neurons[27)] ; indeed, administration of a serotonin 2A receptor agonist improved upper airway stability in an animal model[28)]. A significant association of the rs9526240 SNP in the *HTR2A* gene with OSAS was detected in an African-American population, however, which was greatly attenuated after adjusting for BMI (the P value was attenuated from 0.0000523 to 0.0126 after adjustment)[29)]. The rs9526240 SNP is located in the intron of the *HTR2A* gene, and its function is currently unknown. This association attenuation suggested that *HTR2A* may influence OSAS through pleiotropic pathways

that influence both airway stability and obesity. Moreover, the positive association of the rs6311 (−1438G/A) in the *HTR2A* gene with OSAS were reported in Chinese[13)14)], Turkish[15)], and Brazilian[16)17)] populations, yet those significances were uncertain because of the absence of adjustment for BMI in these studies[13)−17)]. It is well known that obesity is the most common characteristic of adults with OSAS. There are probably both shared and unshared genetic factors that underlie the susceptibilities to OSAS and obesity[4)]. Thus, the association between the *HTR2A* polymorphisms and OSAS might be partially explained by a common causal pathway involving both AHI and BMI pathogeneses[30)]. Nevertheless, it is absolutely necessary to adjust BMI in statistical analyses to minimize the possibility of false-positive or conflicting results in genetic association studies on OSAS.

The rs6311 and rs6313 SNPs of the *HTR2A* gene were the most attractive candidates, based on previous studies on genetic variants associated with OSAS[12-17)]. One meta-analysis revealed that rs6311 was significantly associated with susceptibility to OSAS, but not rs6313[31)]. The rs6311 is a polymorphism in the promoter of *HTR2A*, with functional significance in serotonergic neurotransmission. A structure-function equation model suggested that this promoter polymorphism might affect both transcription factor binding and promoter methylation, and thus, it might alter the rate of *HTR2A* transcription in a methylation-dependent manner[32)]. The rs6311 is in complete linkage disequilibrium ($r^2 = 1.0$) with rs6313 in the Japanese population on the genetic dataset of HapMap. Regarding the rs6313, it is a synonymous variant, with no resulting change in the amino acid sequence, though it may affect the mRNA stability, quantity, and/or translation, which could affect protein expression[33)]. Additionally, the rs6313 SNP may also affect methylation of the *HTR2A* promoter[34)]. Pollesskaya and Sokolov observed that the T allele of rs6313 was associated with an elevated number of *HTR2A* receptors in the central nervous system[33)]. Although true, we did not find any associations of these two SNPs with the susceptibility to OSAS in the present Japanese patients.

The HTR2A receptor is located primarily in the neurocortex, caudate nucleus, nucleus accumbens, olfactory tubercle, and the hippocampus, in the central nervous system, while being marginally distributed in the hypoglossal motor nucleus in the peripheral nervous system[35)]. Thus, the HTR2A receptor mainly targets biological molecules in serotonergic-rich areas of the central nervous system involved in neuronal excitation, behavioral effects, learning, and anxiety, but has a minor function in excitatory transmission at the serotonergic-poor area of the hypoglossal motor nucleus in the peripheral nervous system[35)]. We interpreted this to mean that the scant density and minor function of the HTR2A receptor in the hypoglossal motor nucleus might partly explain the negative results found in the present study. At present, the relations of the hypoglossal nerve and serotonin receptor have been demonstrated in animals by animal experiments; however, the distribution of the serotonin receptor in the medulla oblongata (where the nucleus of the hypoglossal nerve exists) has not yet been evidenced in humans. Additional mechanisms other than the HTR2A receptor might be involved in OSAS pathophysiology as well. For example, craniofacial morphologic abnormalities are more severe in Asian populations than in Caucasians with the same range of BMI or the degree of obesity[36)37)]. Endothelin-receptor-A[38)] and transforming growth factor-beta 2[39)] are concerned with craniofacial morphologic abnormalities, and it is suggested that these genes be analyzed regarding the genetic background of OSAS pathophysiology.

The obvious limitation of the present study was that the age and BMI of the patient group did not match those of the control group, although adjustments were applied to the statistical analyses for theoretical correlations. We did not restrict age or BMI in the process of selecting subjects because we aimed to include a relatively large sample size to achieve adequate statistical power. In practice, it is difficult to recruit large sample sizes of an OSAS group and control group matched in the age and

BMI, these being the two major risk factors for developing OSAS.

Ⅴ Conclusion

This study showed that ten SNPs in the *HTR2A* gene (rs3803189, rs977003, rs9567737, rs9316232, rs2224721, rs2770296, rs731779, rs9567746, rs2070036, and rs6311) and their tagged 38 SNPs were not associated with susceptibility to OSAS in a Japanese population. Further studies on different genes that might be associated with OSAS, such as genes involved with craniofacial morphology[38], transforming growth factor-beta 2[39], endothelin-receptor-A, or a whole genome scan, might elucidate the role of genetics in the pathogenesis of OSAS in the Japanese population.

Ⅵ Funding Source

This research was financially supported by the Respiratory Failure Research Group in the Ministry of Health, Labour and Welfare, Japan. The funding source had no roles in study design ; in the collection, analysis and interpretation of data ; in the writing of the report ; and in the decision to submit the article for publication.

Ⅶ Conflict of Interest Disclosure Statement

No potential conflicts of interest were disclosed.

Ⅷ Acknowledgement

We are very grateful to Dr. Yoshimichi Komatsu (Suwatoyoda Clinic, Suwa) and Dr. Keisaku Fujimoto (Shinshu University School of Medicine, Matsumoto) for their contributions to the collection of samples for this study.

References

1) Douglas NJ, Polo O : Pathogenesis of obstructive sleep apnoea/hypopnoea syndrome. Lancet 344 : 653-655, 1994
2) Redline S, Young T : Epidemiology and natural history of obstructive sleep apnea. Ear Nose Throat J 72 : 20-21, 24-26, 1993
3) Redline S, Tishler PV, Tosteson TD, Williamson J, Kump K, Browner I, Ferrette V, Krejci P : The familial aggregation of obstructive sleep apnea. Am J Respir Crit Care Med 151 : 682-687, 1995
4) Redline S, Tishler PV : The genetics of sleep apnea. Sleep Med Rev 4 : 583-602, 2003
5) Casale M, Pappacena M, Rinaldi V, Bressi F, Baptista P, Salvinelli F : Obstructive sleep apnea syndrome : from phenotype to genetic basis. Curr Genomics 10 : 119-126, 2009
6) Richter DW, Manzke T, Wilken B, Ponimaskin E : Serotonin receptors : guardians of stable breathing. Trends Mol Med 9 : 542-548, 2003
7) Sood S : Role of endogenous serotonin in modulating genioglossus muscle activity in awake and sleeping rats. Am J Respir Crit Care Med 172 : 1338-1347, 2005
8) Kraiczi H : Effect of serotonin uptake inhibition on breathing during sleep and daytime symptoms in obstructive sleep apnea. Sleep 22 : 61-67, 1999
9) Sood S, Liu X, Liu H, Horner RL : Genioglossus muscle activity and serotonergic modulation of hypoglossal motor output in obese Zuker rats. J Appl Physiol 102 : 2240-2250, 2007
10) Myers RL, Airey DC, Manier DH, Shelton RC, Sanders-Bush E : Polymorphisms in the regulatory region of the human serotonin 5-HT2A receptor gene (HTR2A) influence gene expression. Biol Psychiatry 61 : 167-173, 2007
11) Chen K, Yang W, Grimsby J, Shih JC : The human 5-HT2 receptor is encoded by a multiple intron-exon gene. Molec Brain Res 14 : 20-26, 1992
12) Sakai K, Takada T, Nakayama H, Kubota Y, Nakamata M, Satoh M, Suzuki E, Akazawa K, Gejyo F : Serotonin-2A and 2C receptor gene polymorphisms in Japanese patients with obstructive sleep apnea. Intern Med 44 : 928-933, 2005

13) Chen H, Hu K, Zhu J, Xianyu Y, Cao X, Kang J, He J, Zhao P, Mei Y : Polymorphisms of the 5-hydroxytryptamine 2A/2C receptor genes and 5-hydroxytryptamine transporter gene in Chinese patients with OSAHS. Sleep Breath 17 : 1241-1248, 2013
14) Yin G, Ye J, Han D, Zhang Y, Zeng W, Liang C : Association of the 5-HT2A receptor gene polymorphisms with obstructive sleep apnea syndrome in Chinese Han population. Acta Otolaryngol 132 : 203-209, 2012
15) Bayazit YA, Yilmaz M, Ciftci T, Erdal E, Kokturk O, Gokdogan T, Kemaloglu YK, Inal E : Association of the -1438G/A polymorphism of the 5-HT2A receptor gene with obstructive sleep apnea syndrome. ORL J Otorhinolaryngol Relat Spec 68 : 123-128, 2006
16) de Carvalho TB, Suman M, Molina FD, Piatto VB, Maniglia JV : Relationship of obstructive sleep apnea syndrome with the 5-HT2A receptor gene in Brazilian patients. Sleep Breath 17 : 57-62, 2012
17) Piatto VB, Carvalho TB, De Marchi NS, Piatto VB, Maniglia JV : Polymorphisms in the 5-HTR2A gene related to obstructive sleep apnea syndrome. Braz J Otorhinolaryngol 77 : 348-355, 2011
18) Redline S, Tishler PV, Hans MG, Tosteson TD, Strohl KP, Spry K : Racial differences in sleep-disordered breathing in African-Americans and Caucasians. Am J Respir Care Med 155 : 186-192, 1977
19) Baldwin M, Kolbe J, Troy K, Belcher J, Gibbs H, Frankel A, Eaton T, Christmas T, Veale A : Racial differences in severity of sleep apnea between Maori, Pacific Islands and Europeans. Am J Respir Crit Care Med 153 : A357 (abstr), 1996
20) Ng TP, Seow A, Tan WC : Prevelence of snoring and sleep breathing-related disorders in Chinese, Malay and Indian adults in Singapore. Eur Respir J 12 : 198-203, 1998
21) The AASM manual for the scoring of sleep and associated events : Rules, terminology, and technical specification. American Academy of Sleep Medicine, Westchester, 2007
22) Johns MW : A new method for measuring daytime sleepiness : the Epworth sleepiness scale. Sleep 14 : 540-545, 1991
23) Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S : The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 328 : 1230-1235, 1993
24) International HapMap project (http : //hapmap.ncbi.nlm.nih.gov/), Accessed 26 June 2016
25) Barret JC, Fry B Maller J, Daly MJ : Haploview : analysis and visualization of LD and haplotype maps. Bioinfomatics 21 : 263-265, 2005
26) Pezzullo JC (2015) : Logistic Regression. http : //statpages.org/logistic.html Accessed 26 June 2016
27) Fenik P, Veasey SC : Pharmacological characterization of serotonergic receptor activity in the hypoglossal nucleus. Am J Respir Crit Care Med 167 : 563-569, 2003
28) Ogasa T, Ray AD, Michlin CP, Farkas GA, Grant BJ, Magalang UJ : Systemic administration of serotonin 2A/2C agonist improves upper airway stability in Zucker rats. Am J Respir Crit Care Med 170 : 804-810, 2004
29) Larkin E, Patel S, Goodloe R, Li Y, Zhu X, Gray-McGuire C, Adams MD, Redline S : A candidate gene study of obstructive sleep apnea in European Americans and African Americans. Am J Respir Crit Care Med 182 : 947-953, 2010
30) Palmer LJ, Buxbaum SG, Larkin E, Patel SR, Elston RC, Tishler PV, Redline S : A whole-genome scan for obstructive sleep apnea and obesity. Am J Hum Genet 72 : 340-350, 2002
31) Zhao Y, Tao L, Nie P, Lu X, Xu X, Chen J, Zhu M : Association between 5-HT2A receptor polymorphisms and risk of obstructive sleep apnea and hypopnea syndrome : A systematic review and meta-analysis. Gene 530 : 287-294, 2013
32) Falkenberg VR, Gurbaxani BM, Unger ER, Rajeevan MS : Functional genomics of *serotonin receptor 2A (HTR2A)* : Interaction of polymorphism, methylation, expression and disease association. Neuromolecular Med 13 : 66-76, 2011
33) Polesskaya OO, Sokolov BP : Differential expression of the 'C' and 'T' alleles of the 5-HT2A receptor gene in the temporal cortex of normal individuals and schizophrenics. J Neurosci Res 67 : 812-822, 2002

34) Polesskaya OO, Aston C, Sokolov BP : Allele C-specific methylation of the 5-HT2A receptor gene : Evidence for correlation with its expression and expression of DNA methylase DNMT1. J Neurosci Res 83 : 362-373, 2006
35) Hoyer D, Hannon JP, Martin GR : Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71 : 533-554, 2002
36) Li KK, Kushida C, Powell NB, Riley RW, Guilleminault C : Obstructive sleep apnea syndrome : a comparison between Far-East Asian and white men. Laryngoscope 110 : 1689-1693, 2000
37) Liu Y, Lowe AA, Zeng X, Fu M, Fleetham JA : Cephalometric comparisons between Chinese and Caucasian patients with obstructive sleep apnea. Am J Orthod Dentofacial Orthop 117 : 479-485, 2000
38) Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, Oda H, Kuwaki T, Cao WH, Kamada N : Elevated blood pressure and craniofacial abnormalities in mice deficient in endotheline-1. Nature 368 : 703-710, 1994
39) Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R, Boivin GP, Cardell EL, Doetschman T : TGF Beta-2 knockout mice have multiple development defects that are non-overlapping with other TGF Beta knockout phenotypes. Development 124 : 2659-2670, 1997

(2016. 10. 27 received ; 2016. 12. 27 accepted)