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Association of *CHRNA4* gene rs1044396 and rs1044397 polymorphisms with Parkinson's disease symptoms and smoking

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ABSTRACT. We assessed the *CHRNA4* exon 5 rs1044396 and rs1044397 polymorphisms and investigated their relationship with Parkinson's disease (PD) severity and several non-motor symptoms. Ninety-seven patients with primary PD and 108 controls were recruited, and their smoking history identified. Patients with PD were assessed using the unified PD rating scale (UPDRS), Hoehn & Yahr (H&Y) grade, Hamilton depression rating scale (HAMD), visual analogue 10-points scale (VAS), and the Pittsburgh sleep quality index (PSQI). Polymerase chain reaction amplification and direct sequencing was performed on genomic DNA to identify polymorphic variants. Statistical analysis demonstrated that there were no gender differences in rs1044396(C→T) and rs1044397(G→A) frequencies. More smokers were identified among carriers of rs1044396 CT/TT genotypes. We also found no differences between PD and control groups in frequencies of either polymorphism. However, in women, PD onset was latest in rs1044397 GA/AA (P = 0.015). rs1044396 CT/TT genotype carriers and

rs1044397 GG genotype patients with PD had higher VAS scores. No differences were found on the course of PD, H&Y grade, or UPDRS-II or -III scores between various genotypes, nor were differences found on scores of HAMD, nocturia, or PSQI in PD patients. Our results suggested that the *CHRNA4* rs1044396 CT/TT genotype is related to cigarette smoking, that the rs1044397 polymorphism may associate with PD age of onset in women, and that rs1044396 and rs1044397 may relate to pain in PD patients, but not to the course or severity of disease, or to depression or nocturnal or sleeping disorders.

Key words: Parkinson's disease; Smoking; Motor symptoms; Non-motor symptoms; Nicotinic acetylcholine receptor

INTRODUCTION

Acetylcholine (Ach) is a major neurotransmitter of the basal ganglia. Ach levels show a relative increase in the striatum of patients with Parkinson's disease (PD) due to a reduction of dopamine levels. Ach acts through binding to either the muscarinic acetylcholine receptor (mAChR) or the nicotinic acetylcholine receptor (nAChR). Artane (benzhexol hydrochloride) exerts an inhibitory effect against binding of Ach to mAChR in the brain, which relieves static tremor and rigidity in patients with PD. Nicotine is the main component of tobacco with a high affinity for nAChR. The ultimate effect of nicotine is to increase dopamine in the reward system; thus, nicotine assists to ameliorate the symptoms of PD (Hernán et al., 2002; Allam et al., 2004). A recent study has also shown that nicotine may also relieve dyskinesia in a Parkinson's disease animal model (Bordia et al., 2013).

So far, there have been fewer studies on cerebral nAChR than on mAChR. The majority (90%) of the encephalic region contains nAChR-α4β2 subunits, and activation of α4β2 subunits may promote differentiation of undifferentiated neural progenitors (Takarada et al., 2012); patients with PD show decreased nAChR-α4β2 subunit density in the brain. It has been reported that α-synuclein inhibits α4β2-nAChR activation, and might influence the regulation of cholinergic signaling (Liu et al., 2013). These results suggest that α4β2-nAChR might mediate a cholinergic disorder in PD. α4β2-nAChR exhibits the highest affinity to nicotine, suggesting that α4β2-nAChR might be the common tie between PD symptoms relieved by nicotine.

The alpha4 subunit gene of the nAChR is *CHRNA4*. The main functional coding region of *CHRNA4* is located in exon 5. Studies have shown that cigarette smoking is related to the rs1044396 polymorphism in this exon (Zhang et al., 2005; Kamens et al., 2013). Therefore, the question arises of whether this polymorphism influences PD susceptibility, and/or subsequently mediates the therapeutic effect of nicotine on PD. There had been a report that the *CHRNA4* rs1044396 polymorphism was associated with visuospatial attention in healthy adults (Greenwood et al., 2012), but the relationship of this polymorphism with attention deficiency was not observed in patients with PD (Hudson et al., 2010). In addition to the consideration of the relationship of rs1044396 with behavioral and psychiatric processes, PD patients also commonly display complicated motor and non-motor symptoms, which may relate to disorders of the cholinergic system. Therefore, this study determined the rs1044396 polymorphism status and investigated the relationship of rs1044396 with PD severity along with several non-motor symptoms of PD, including depression, pain, and nocturia and sleep-

ing disorders. Until now, there have been no studies on these aspects of PD. rs1044397 is an adjacent polymorphism to rs1044396, 30 bp downstream and also in *CHRNA4* exon 5; this polymorphism was also reported to be associated with cigarette smoking (Chu et al., 2011; Chen et al., 2013); therefore, rs1044397 polymorphisms were examined in this study as well.

MATERIAL AND METHODS

Patients and controls

Ninety-seven patients with primary PD were included in this study. All met the criteria of the United Kingdom PD Society Brain Bank for PD. Of these patients, 50 were men and 47 were women, with ages ranging from 50 to 84 years old (mean age: 65.86 ± 10.01 years). The course of PD was 1-16 years. Patients were assessed with the unified PD rating scale (UPDRS), Hoehn & Yahr (H&Y) grade of the PD stage, the Hamilton depression rating scale (HAMD), and the Pittsburgh sleep quality index (PSQI); pain was quantified by the visual analogue 10-points scale (VAS); nocturia times were recorded as numbers of micturition in the duration of sleeping at night; nocturia was identified when micturition at night occurred more than twice.

The control group consisted of 108 healthy volunteers, including 64 men and 44 women, between 45 and 76 years old (mean age: 60.65 ± 10.62 years); PD, Parkinsonism, schizophrenia, and depression were precluded. Both patient and control groups were of Chinese Han ethnicity. There was no significant difference in gender between these two groups. Smokers were identified as those who smoked one or more cigarettes per day for a duration of over 1 year.

Genotyping

Genomic DNA was extracted from blood cells by the phenol chloroform method. The whole DNA sequence of *CHRNA4* was based on GenBank AL12182. The DNA fragment in exon 5 was amplified with primers F: 5'-GGG CCC GGC TCC TGG ATT ACA CA-3', and R: 5'-GGT CCC TCA GCG TCC AGC ACA T-3' (PAGE purified). Polymerase chain reaction (PCR) was performed including 0.5 µL DNA template, 0.5 µL primers, 2 µL dNTPs, 2.5 µL buffer, 0.25 U Taq DNA Polymerase, and double-distilled water to 25 µL. The denaturation temperature was 95°C, annealing temperature was 60°C, and the extension temperature was 72°C, in a total of 35 cycles. PCR products were PAGE purified and sequenced with capillary sequencer ABI3730xl; the sequences were recorded with the Chromas 2.23 software (SinoGenoMax, Chinese National Human Genome Center, Beijing, China).

Statistical analysis

Statistical analyses were performed with the SPSS 18.0 software (SPSS, Chicago, IL, USA). Genotypes of rs1044396 and rs1044397 were inspected using the Hardy-Weinberg equilibrium test. The chi-square test (χ^2 test) was used to evaluate the genotype distribution between men and women. The association of the genotypes with PD susceptibility and smoking behavior was analyzed with binary logistic regression (age and gender as covariate factors); P values were adjusted with false-discovery rate (FDR) (aF = PiN / i). Analysis of variance of factorial design was used for testing the associations of rs1044396 and rs1044397 polymorphisms with PD severity and non-motor symptoms; P values were adjusted with Bonferroni's correction.

The study was approved by the local Ethics Committee, and all patients signed written informed consent.

RESULTS

Sequence of rs1044396 and rs1044397 polymorphisms

rs1044396(C→T) and rs1044397(G→A) polymorphic variants were detected by direct sequencing (Figure 1). Both polymorphisms did not lead to amino acid change and were not linked. rs1044396 and rs1044397 polymorphism allele and genotype frequencies were analyzed by the Hardy-Weinberg equilibrium test. Frequencies of rs1044396 and rs1044397 genotypes fulfilled Hardy-Weinberg equilibrium both in control (Table 1) and PD groups (Table 2) .

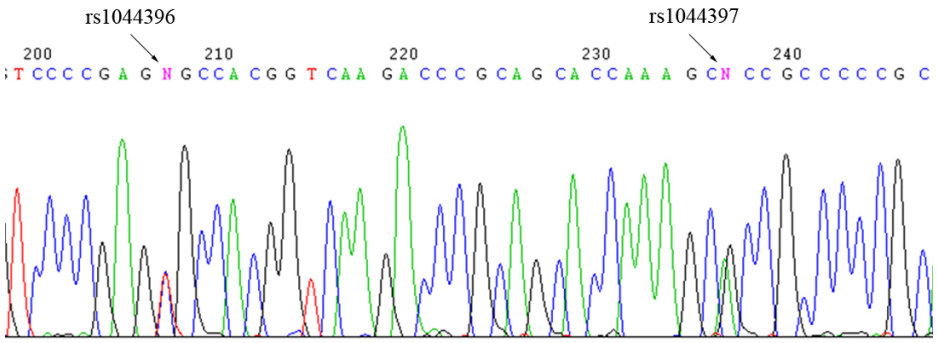

Figure 1. Sequence of rs1044396 and rs1044397 polymorphisms in the *CHRNA4* gene (heterozygote).

Table 1. Hardy-Weinberg equilibrium test in the control group.

Polymorphisms		Control [N (%)]		χ^2	P	95%CI
		Actual values	Theoretical values			
rs1044396	CC	55 (0.509)	53 (0.491)	0.405	0.843	0.794-0.891
	CT	41 (0.380)	45 (0.417)			
	TT	12 (0.111)	10 (0.093)			
rs1044397	GG	36 (0.333)	37 (0.343)	0.078	0.935	0.902-0.968
	GA	54 (0.500)	52 (0.481)			
	AA	18 (0.167)	19 (0.176)			

Data were analyzed with the chi-square test. CI = confidence interval.

Table 2. Hardy-Weinberg equilibrium test in the PD group.

Polymorphisms		PD [N (%)]		χ^2	P	95%CI
		Actual values	Theoretical values			
rs1044396	CC	57 (0.588)	58 (0.598)	0.177	0.944	0.914-0.975
	CT	36 (0.371)	34 (0.351)			
	TT	4 (0.041)	5 (0.052)			
rs1044397	GG	43 (0.443)	38 (0.392)	2.316	0.259	0.201-0.318
	GA	35 (0.361)	45 (0.464)			
	AA	19 (0.196)	14 (0.144)			

Data were analyzed with the chi-square test. PD = Parkinson's disease; CI = confidence interval.

There was no difference in rs1044396 and rs1044397 genotype frequencies between male and female subjects (Table 3).

Table 3. Genotype distributions in male and female subjects.

Polymorphisms		Total subjects [N (%)]		χ^2	P	95%CI
		Male (N = 114)	Female (N = 91)			
rs1044396	CC	58 (0.509)	54 (0.593)	2.279	0.320	0.275-0.401
	CT	48 (0.421)	29 (0.319)			
	TT	8 (0.070)	8 (0.088)			
rs1044397	GG	38 (0.333)	41 (0.451)	2.951	0.199	0.146-0.252
	GA	54 (0.474)	35 (0.385)			
	AA	22 (0.193)	15 (0.165)			

Data were analyzed with the chi-square test. CI = confidence interval.

Relationship of rs1044396 and rs1044397 polymorphisms with smoking

Ten patients with PD (0.3%) had a smoking history; 47 patients (43.5%) among the control subjects had a smoking history; this difference was significant (Pearson's $\chi^2 = 28.076$, $P < 0.001$). For genotype analysis, non-smokers (61.8%) were more likely than smokers (34.0%) to be represented in subjects carrying the rs1044396 CC genotype, regardless of the model of inheritance of the variant allele (dominant or recessive trait) ($P < 0.05$). There was no difference in smoking behavior between subjects carrying various rs1044397 genotypes ($P > 0.05$; Table 4).

Table 4. Genotype distributions in smokers and non-smokers.

Site		Non-smokers (N = 148) [N (%)]	Smokers (N = 57) [N (%)]	AD OR (95%CI)	P value	AR OR (95%CI)	P value
rs1044396	CC	94 (0.618)	18 (0.340)	0.275 (0.116-0.655)	0.004[a]	0.033 (0.006-0.175)	0.000[b]
	CT	55 (0.362)	22 (0.415)				
	TT	3 (0.020)	13 (0.245)				
rs1044397	GG	64 (0.421)	15 (0.283)	1.242 (0.496-3.109)	0.643	1.404 (0.497-3.963)	0.522
	GA	64 (0.421)	25 (0.472)				
	AA	24 (0.158)	13 (0.245)				

Data were analyzed with binary logistic regression; adjusted age and gender were covariates. [a]False-discovery rate (FDR) correction was performed (aF = PiN / i), aF = 0.004 x 4 / 1 = 0.016. [b]FDR correction was performed (aF = PiN / i), aF = 0.000 x 4 / 1 = 0.000. AD = autosomal dominant inheritance; AR = autosomal recessive inheritance; OR = odds ratio; CI = confidence interval.

Analysis of the PD group

Association of rs1044396 and rs1044397 with PD

The distribution of rs1044396 and rs1044397 genotypes is listed between PD and control groups in Table 5. No difference was found in genotype frequencies between these two groups.

Table 5. Genotype distributions in PD and control groups.

Site		PD (N = 97) [N (%)]	Control (N = 108) [N (%)]	AD OR (95%CI)	P value	AR OR (95%CI)	P value
rs1044396	CC	57 (0.588)	55 (0.509)	0.918 (0.448-1.884)	0.816	0.305 (0.083-1.123)	0.074
	CT	36 (0.371)	41 (0.380)				
	TT	4 (0.041)	12 (0.111)				
rs1044397	GG	43 (0.443)	36 (0.333)	0.650 (0.311-1.357)	0.251	1.525 (0.678-3.428)	0.308
	GA	35 (0.361)	54 (0.500)				
	AA	19 (0.196)	18 (0.167)				

Data were analyzed with binary logistic regression; adjusted age and gender were used as covariates. False-discovery rate (FDR) correction was not performed for P > 0.05. PD = Parkinson's disease; AD = autosomal dominant inheritance; AR = autosomal recessive inheritance; OR = odds ratio; CI = confidence interval.

rs1044396 and rs1044397 polymorphisms and PD severity

The course of PD was similar between subjects carrying various rs1044396 and rs1044397 genotypes. H&Y stage and scores of UPDRS-II and -III showed no differences between common and variant genotypes (Table 6).

Table 6. CHRNA4 gene polymorphisms and severity of PD (means ± SD).

Site		Course of PD	H&Y	UPDRS-II	UPDRS-III
rs1044396	CC	5.583 ± 0.533	2.131 ± 0.116	10.471 ± 1.024	22.903 ± 1.633
	CT/TT	4.281 ± 0.718	2.274 ± 0.157	11.116 ± 1.379	24.909 ± 2.200
	F value	1.436	1.127	0.060	0.001
	P	0.235	0.292	0.808	0.979
rs1044397	GG	5.164 ± 0.692	2.312 ± 0.151	11.941 ± 1.328	24.249 ± 2.119
	GA/AA	4.971 ± 0.550	2.105 ± 0.120	9.900 ± 1.056	23.360 ± 1.684
	F value	0.004	1.246	0.817	0.114
	P	0.949	0.268	0.369	0.737

According to autosomal dominant pattern of inheritance, analysis of variance of factorial design was used. Age and gender were also fixed factors; PD patients were divided into three groups (<60, 60-70, and >70 years old); Bonferroni's corrections were performed. PD = Parkinson's disease; H&Y = Hoehn and Yahr staging scale; UPDRS = unified PD rating scale.

CHRNA4 polymorphisms and age of onset of PD

According to the autosomal dominant pattern of inheritance, PD onset was earliest in female carriers of the rs1044397 GG genotype (56.817 ± 1.729 years); the next was male carriers of rs1044397 GA/AA; the third was male carriers of rs1044397 GG genotype; the latest onset was female carriers of rs1044397 GA/AA genotypes (61.343 ± 1.342 years) (P = 0.015; Table 7). There was no difference in PD onset age across the various rs1044396 genotypes.

Table 7. *CHRNA4* polymorphisms and age of onset of PD in men and women.

Site		Age of onset in men (means ± SD, years)	Age of onset in women (means ± SD, years)	F value	P
rs1044396	CC	56.487 ± 1.339	59.569 ± 1.283	3.781	0.056
	CT/TT	59.830 ± 1.657	59.300 ± 1.815		
rs1044397	GG	57.981 ± 1.628	56.817 ± 1.729	6.184	0.015
	GA/AA	57.720 ± 1.355	61.343 ± 1.342		

According to the autosomal dominant pattern of inheritance, analysis of variance of factorial design was used. Age, gender, and the two polymorphisms were fixed factors; PD patients were divided into three group (<60; 60-70, and >70 years old); Bonferroni's corrections were performed. PD = Parkinson's disease.

CHRNA4 polymorphisms and non-motor symptoms of PD

In 97 patients with PD, 31 (32%) showed HAMD scores <8, 45 (46.4%) had HAMD scores of 8-20, 20 (20.6%) had HAMD scores of 20-35; the HAMD score was over 35 in one patient (1%). There was no difference on HAMD scores between groups carrying different rs1044396 or rs1044397 genotypes.

Fifty-five patients (56.7%) suffered from pain. rs1044396 CT/TT genotype carriers had higher VAS scores (6.037 ± 0.528) than CC genotype carriers; rs1044397 GG genotype carriers demonstrated high VAS scores (6.059 ± 0.518) as well, but these did not differ significantly from other genotype carriers after FDR correction (Table 8).

Fifty-one patients (52.6%) showed nocturia, there were no differences in frequencies of nocturia between various rs1044396 or rs1044397 genotype carriers. Fourteen patients (14.4%) received PSQI scores of 0-5; 24 (24.7%) had PSQI scores of 6-10; 29 (29.9%) received PSQI scores of 11-15; and 30 had PSQI scores of 16-21 (30.9%). There was no difference in PSQI scores between groups carrying different genotypes.

Table 8. *CHRNA4* gene polymorphisms and non-motor symptoms of PD (means ± SD).

Site		Depression (N = 97)	Pain (N = 55)	Frequency of nocturia (N = 97)	PSQI score (N = 97)
rs1044396	CC	12.687 ± 1.366	4.605 ± 0.367	2.447 ± 0.257	11.102 ± 1.809
	CT/TT	16.352 ± 2.886	6.037 ± 0.528	2.239 ± 0.544	13.980 ± 1.711
	F value	0.330	4.877	0.062	0.313
	P	0.569	0.032[a]	0.330	0.579
rs1044397	GG	16.928 ± 2.879	6.059 ± 0.518	2.113 ± 0.542	14.250 ± 1.707
	GA/AA	12.111 ± 2.432	4.583 ± 0.376	2.573 ± 0.256	10.831 ± 0.805
	F value	1.019	5.293	0.855	0.407
	P	0.318	0.026[b]	0.360	0.527

According to an autosomal dominant pattern of inheritance, analysis of variance of factorial design was used. Gender was also a fixed factor; age and UPDRS-III scores were covariates, and the results were assessed as the scores of UPDRS-III = 25.4433, age = 65.8660, and course of disease = 5.4227 years. The results for pain were assessed as UPDRS-III scores = 26.5091, age = 65.4364, and course of disease = 5.8545 years. [a]FDR correction (aF = PiN / i), aF = 0.032 x 5 / 1 = 0.160; [b]FDR correction (aF = PiN / i), aF = 0.026 x 5 / 1 = 0.130; PD = Parkinson's disease; PSQI = Pittsburgh sleep quality index; FDR = false-discovery rate.

DISCUSSION

nAChR is a ligand-gated ion channel, consisting of eight α and three β subunits; the various subunits comprise a pentamer of nAChR subtypes. α4β2-nAChR contains the major nicotine-binding site. Close attention has been paid to this subtype because of its complex function. α4β2-nAChR may be of either $(\alpha4)_2(\beta2)_3$ or $(\alpha4)_3(\beta2)_2$ composition, each of which shows different receptor sensitivity (Mazzaferro et al., 2011). $(\alpha4)_2(\beta2)_3$-nAChR displays high sensitivity to nicotine, and $(\alpha4)_3(\beta2)_2$ displays low sensitivity (Moroni et al., 2006). The configuration may be decided by the ratio of *α4* and *β2* mRNA levels, roughly corresponding to the concentration of available subunits during receptor assembly.

The α4 subunit gene (*CHRNA4)* includes six exons. Exon 5 comprises the main functional coding region, encoding the four trans-membrane determinants (TM1-4) and the intracellular cyclic structure of the nAChR α4 subunit. In this study, rs1044396(C→T) and rs1044397(G→A) polymorphic variants in exon 5 of *CHRNA4* were detected in 97 patients with PD, and in 108 control subjects. These are nonsense mutations with a high ratio of varia-

tion. The amino acids encoded by rs1044396 and rs1044397 are located within the intracellular cyclic structure, close to the protein kinase C phosphorylation site. The allele frequencies of rs1044396(C→T) and rs1044397(G→A) in this study fulfilled Hardy-Weinberg equilibrium in both PD and control groups.

Long-term nicotine exposure might increase the nAChR density in the brain (Chu et al., 2011). The α4 subunit plays an important role in drug addiction (McGranahan et al., 2011). Deletion of the α4 subunit increases sensitivity to nicotinic-induced depression (Markett et al., 2011). The results of this study showed that rs1044396 CT/TT genotype carriers were more likely to be smokers ($P < 0.01$). This result was similar to that from a previous report that found a relationship between rs1044396 CT/TT genotypes and cigarette smoking (Chu et al., 2011). rs1044396 does not cause amino acid change, the function of this mutation is still unclear. Does it affect the DNA spatial configuration, and then affect the efficiency of transcription? Therefore, the ratio of $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ might be changed, so that the individuals carrying differing genotypes might exhibit different sensitivities to nicotine through the reward system, leading to dissimilar behaviors of cigarette smoking. Male smokers were more prevalent than female smokers in most countries, but rs1044396 and rs1044397 genotypes showed no differences between men and women in this study, suggesting that the different smoking prevalence between the two genders might be caused by social factors aside from genetic reasons.

The nAChR density decreases in the neocortex and basal ganglia of patients with PD; the reduction in the substantia nigra reaches 70%. It has been reported that the α4 subunit plays an important role in movement control (McGranahan et al., 2011). Presynaptic α4β2-nAChR promotes the release of glutamic acid and activates serotonergic neurons (Garduño et al., 2012), and thus might affect PD susceptibility and levodopa complications. In this study, frequencies of rs1044396 and rs1044397 polymorphisms showed no differences between PD and control groups, suggesting that these two polymorphisms appeared to have no relationship with PD susceptibility. Smokers were significantly less frequent in PD than in control groups, as seen both in previous studies and in this investigation; there also has been a report that cigarette smoking interacts with *MAO-B* and *GSTP1* genes to influence PD susceptibility (Deng et al., 2004). It could be considered that the negative relation of cigarette smoking and PD might be due to pharmacologic actions of nicotine rather than to a genetic relationship.

The statistical analysis showed that PD onset was latest in women carrying rs1044397 GA/AA polymorphisms, and the age of onset of PD was the earliest in women carrying the rs1044397 GG genotype. This result evokes to thinking about the role of rs1044397 to *CHRNA4* transcription efficiency, if rs1044397 polymorphism affects the ratio of α4 and β2 subunits; therefore, the sensitivity of α4β2-nAChR to α-synuclein changed correspondingly. Anyway, it was supposed that rs1044397 might influence PD to some extent, although the *CHRNA4* rs1044396 and rs1044397 polymorphisms did not correlate with the course of PD, H&Y grade, or UPDRS-II or -III score, meaning that they did not relate to PD severity.

In this study, the following non-motor symptoms of PD were included: depression, pain, frequency of nocturia, and PSQI. It has been reported that *CHRNA4* rs1044396 polymorphisms were related to negative emotion (Markett et al., 2011; Tsai et al., 2012); the hypothalamic-pituitary-adrenal axis was proposed to interact with *CHRNA4*, which then became a risk factor for depression (Reuter et al., 2012). In this study, 68% of patients exhibited HAMD scores >8, but rs1044396 and rs1044397 genotypes had no relation to bad mood or depression. The pathogenesis of depression in PD might be more complicated than in the normal population, so the relation of depression in PD to *CHRNA4* might be masked.

Patients with PD carrying rs1044396 CT/TT genotypes had higher VAS scores in this study, as well as patients with rs1044397 GG genotypes. These results suggested that *CHRNA4* gene mutation might be related to pain in PD, although this finding was observed before correction for multiple analyses. It has been reported that α4β2-nAChR was related to pain in a previous study (Rode et al., 2012), and that selective α4β2-nAChR agonists might enhance the analgesic effect (Zhu et al., 2011), but these were not studied on PD. The caudate nucleus contains pain response neurons and participates in pain perception, integration, and convection; AChR in the caudate nucleus may participate in this process.

The increase in frequencies of nocturia in patients with PD might be due to a dopamine disorder in the basal-frontal circuit, and a reduction of central inhibition to micturition (Sakakibara et al., 2012), or it might be caused by urinary retention. In this study, patients with nocturia accounted for 52.6% of all patients with PD, but nocturia was not shown to be related to *CHRNA4* rs1044396 and rs1044397 polymorphic variation. It is still unknown whether the central cholinergic system is related to nocturia in PD. Artane, which is an antagonist of mAChR, had an effect in the treatment of nocturia, but has been seldom used because of its side effects. Sleep disorders are a common symptom of PD. AChR is a type of neurotransmitter that is related to sleep and wakefulness. It maintains alertness and cortex activity at a time of wakefulness. It can enhance alertness and prolong the cortex activity during wakefulness and paradoxical sleep. Rapid eye movement sleep is a marker of highly effective and wakeful activity of the AChR. Gamma aminobutyric acid (GABA) neurons in the ventrolateral preoptic area are sleep-promoting cells. Ach inhibits GABA cells through affecting mAChRs in the postsynaptic membrane, and influences the sleep cycle through affecting nAChRs in the presynaptic membrane of noradrenaline terminals (Saint-Mleux et al., 2004). There has been a report that a hypersensitive alpha4 receptor of mice mediates sleep-wake cycle alteration (Fonck et al., 2005). In this study, rs1044396 and rs1044397 polymorphisms had no relation to sleep disorders. Further investigation is needed to discuss the relationship of alpha4 subunits and the cholinergic system with sleep disorders in PD.

In summary, the nAChR alpha4 subunit gene *CHRNA4*, and the polymorphic variants rs1044396(C→T) and rs1044397(G→A) in exon 5 of *CHRNA4* were detected in 97 patients with PD and 108 controls in this study. There was no difference in the frequency of these two polymorphisms between men and women. Carriers of rs1044396 CT/TT genotypes had more smokers.

Frequencies of rs1044396 and rs1044397 polymorphisms showed no differences between PD and control groups. PD onset was latest in women carrying rs1044397 GA/AA polymorphisms and earliest in women carrying the rs1044397 GG genotype. rs1044396 CT/TT genotype carriers and rs1044397 GG genotype patients with PD showed higher VAS scores.

No differences were found in the course of PD, H&Y grades, and UPDRS-II or -III scores between the various genotypes. rs1044396 and rs1044397 polymorphisms showed no relation to depression, nocturia, or PSQI.

CHRNA4 rs1044396 CT/TT genotypes show a relationship with cigarette smoking. rs1044396 and rs1044397 polymorphisms had no relation to PD susceptibility, but rs1044397 might associate with onset age of PD, and rs1044396 and rs1044397 polymorphisms might relate to pain in PD. rs1044396 and rs1044397 polymorphisms did not relate to the course or severity of disease, depression, nocturia, or sleep disorders.

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