Tryptophan Hydroxylase 2 Gene Polymorphisms in Japanese Patients with Medication Overuse Headaches

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Abstract-

Purpose: We investigated whether tryptophan hydroxylase 2 (TPH2) gene polymorphisms were involved in the aggravation of migraines due to the overuse of medication.

Methods: Forty-seven migraine patients (6 males and 41 females; 36.4 ± 10.3 years) and 22 MOH patients (1 male and 21 females; 39.6 ± 9.9 years) who had migraines participated in this study. The genotypes for the TPH2 gene polymorphisms (rs4565946, rs4570625, and rs4341581) were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods.

Results: The rs4565946, rs4570625, and rs4341581 genotypes were similarly distributed between migraine patients and MOH patients.

Conclusion: The results of this study showed no association between tryptophan TPH2 gene polymorphisms and the complication of MOH in patients with migraines.

Key Words: medication overuse headache, migraine, tryptophan hydroxylase 2

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INTRODUCTION

Serotonin (5-HT) plays a pivotal role in the pathogenesis of migraines [1-3]. The depletion of tryptophan, a precursor of 5-HT, increases nausea, headaches, and photophobia in migraine patients [4]. Interestingly, migraine patients are particularly prone to the complication of medication overuse headaches (MOH), in contrast to tension-type headaches [5-7]. Moreover, decreases in the levels of 5-HT in platelets have been observed in migraine patients with MOH [8,9]. Therefore, 5-HT may play a crucial role in the complication of MOH in migraine patients.

5-HT is also known to be involved in the pathogenesis of psychiatric disorders such as major depression [10-12]. Tryptophan hydroxylase 2 (TPH2) is a rate-limiting enzyme in the synthetic pathway for brain 5-HT. Recently, Marziniak et al. [13] showed that TPH2 polymorphisms (rs4570625, rs11178997, rs4341581, and rs4565946) did not play a major role in the pathogenesis

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of migraines. On the other hand, the TPH2 gene polymorphism, rs4570625 was shown to be associated with the pathogenesis of depression [14]. Previous studies also revealed a higher percentage of comorbidity with depression in MOH patients than in migraine patients [7,15]. We also confirmed the higher incidence of depression in MOH patients than in migraine patients [16]. However, the TPH2 gene polymorphisms involved in the aggravation of migraines by the overuse of medication have not yet been identified.

Therefore, we carried out the present study to investigate the association of THP2 gene polymorphisms with the complication of MOH in migraine patients.

METHODS

Subjects

We enrolled 47 migraine (6 males and 41 females; 5 with migraines with an aura (MA), 36 with migraines without an aura (MO), and 6 with MA + MO; 36.4 ± 10.3 years) and 22 MOH (1 male and 21 females; 1 with MA and 21 with MO; 39.6 ± 9.9 years) patients who were admitted to the Department of Neurology in the outpatient clinic of Showa University East Hospital, Tokyo, Japan, between May 2010 and January 2011. These patients had participated in a previous study, in which the incidence of depression was shown to be significantly higher in MOH patients than in migraine patients ($p<0.001$) [16]. The overused medications were combination analgesics in 14 patients (64%), analgesics in 9 patients (41%), and triptans in 2 patients (9%) [16].

Migraines were diagnosed according to the International Classification of Headache Disorders, 2nd Edition (ICHD-II) in 2004 [17]. We also confirmed with an interview that the migraine patients in the present study had not previously overused medication. The revised ICHD-II criteria were used for the diagnosis of MOH [5]. MOH patients were asked about primary headaches by headache specialists. Moreover, headache specialists confirmed primary headaches after the recovery of patients from MOH, according to the ICHD-II criteria. Although the subjects included in the present study were not only patients with migraines, but also patients with migraines and tension-type headaches, patients with tension-type headaches only were excluded from this study. We used the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) to diagnose major depressive disorder [18].

All patients were Japanese. We enrolled all patients with migraines and patients with MOH who provided informed consent for this study, and did not select patients. This clinical study was approved by the Ethics Committee for Genome Research of Showa University.

Genotyping

Genomic DNA was extracted from whole blood using NucleoSpin® Blood QuickPure (NIPPON Genetics Co., Ltd., Tokyo, Japan). The determination of SNP rs4570625 was performed by a polymerase chain reaction (PCR)-based method in accordance with Kim et al [19]. The sense oligonucleotide primer for rs4570625 was 5'-TTT TAT GAA AGC CAT TAC ACA T-3', and the antisense primer was 5'-TTC CAC TCT TCC AGT TAT TTT-3'. PCR products were digested for 10-12 h at 37˚C with *Psi* I (New England Biolabs). The 204 bp fragment indicated the presence of the *G* allele (no *Psi* I restriction site) and the 55 bp and 149 bp fragments indicated the presence of the *T* allele (the presence of the *Psi* I restriction site).

The determination of SNP rs4565946 was performed by a PCR-based method in accordance with Marziniak et al [13]. The sense oligonucleotide primer for rs4565946 was 5'-CAT CCA AGG CTG TGT CCA TA-3', and the antisense primer was 5'-TGT GTC ACG TTG GGC TTT TA-3'. PCR products were digested for 10-12 h at 37˚C with *Bpu* 10I (New England Biolabs). The 225 bp fragment indicated the presence of the *T* allele (no *Bpu* 10I restriction site) and the 93 bp and 132 bp fragments indicated the presence of the *C* allele (the presence of the *Bpu* 10I restriction site).

The determination of SNP rs4341581 was performed by a PCR-based method in accordance with Marziniak et al [13]. The sense oligonucleotide primer for rs4341581 was 5'-AGG ATT CAA CGA GGC TAA GAG-3', and the antisense primer was 5'-GTG AAG TTG CCG TGT CAC TC-3'. PCR products were digested for 10-12 h at

37˚C with *Hpy*CH4 V (New England Biolabs). The 247 bp fragment indicated the presence of the *T* allele (no *Hpy*CH4 V restriction site) and the 81 bp and 166 bp fragments indicated the presence of the *C* allele (the presence of the *Hpy*CH4 V restriction site).

Statistical analysis

Categorical variables were analyzed by χ^2 test or Fisher's exact test using Excel Statistics (Excel Toukei) 2008 for Windows (Social Survey Research Information Co., Tokyo, Japan). Values of $P<0.05$ were considered significant.

RESULTS

The genotypic distributions of rs4570625 (T/T vs T/G plus G/G, P = 0.728) and rs4565946 (T/T plus T/C vs C/C, P = 0.693) were not significantly different between migraine patients and MOH patients (Table 1). In addition, all patients had the T/T genotype in rs4341581 of the SNPs (Table 1).

DISCUSSION

In the present study, no association between TPH2 gene polymorphisms (rs4565946, rs4570625, and rs4341581) and the complication of MOH was observed in migraine patients.

The frequency of comorbidity with depression was higher in MOH patients than in migraine patients [7,15]. In addition, we also confirmed that the incidence of depression was significantly higher in MOH patients than in migraine patients [16]. Although the TPH2 gene polymorphism, rs4570625 has been shown to be related to the pathogenesis of depression using meta-analysis [14], THP2 gene polymorphisms including rs4570625 were not related to the complication of MOH in migraine patients.

We previously reported that gene polymorphisms such as methylenetetrahydrofolate reductase (rs1801133) and dopamine D2 receptor (rs6275) were associated with the complication of MOH in migraine patients [16], as well as the tumor necrosis factor (TNF)-β gene polymorphism [20]. Thus, 5-HT-unrelated gene polymorphisms seem to be related to the aggravation of migraines by the overuse of medication. On the other hand, 5-HT-related gene polymorphisms including the 5-HT transporter (5-HTT) gene-linked polymorphic region (5-HTTLPR, NG_011747), 5-HT2A (rs6313), 5-HT1B (rs6296), monoamine oxidase A (MAOA) (rs6323), and MAOA

Table 1. Genotype distributions of the TPH2 gene polymorphisms

		Migraines		MOH		
		n=47	%	n=22	%	P value
rs4570625	T/T	11	23.4	6	27.3	
	T/G	30	63.8	12	54.5	
	G/G	6	12.8	4	18.2	
	T/T	11	23.4	6	27.3	0.728
	T/G, G/G	36	76.6	16	72.7	
rs4565946	T/T	5	10.6	1	4.5	
	T/C	23	48.9	11	50.0	
	C/C	19	40.4	10	45.5	
	T/T, T/C	28	59.6	12	54.5	0.693
	C/C	19	40.4	10	45.5	
rs4341581	T/T	47	100.0	22	100.0	
	T/G	0		0		
	G/G	0		0		
	T/T	47	100.0	22	100.0	1.000
	T/G, G/G	0		0		

variable number of tandem repeats (MAOA VNTR, NG_008957) were not associated with the complication of MOH in migraine patients [16]. However, chronic exposure to anti-migraine drugs such as triptans was shown to alter the 5-HT receptors in the brain [21,22]. Moreover, Calabresi and Cupini (2005) showed that the balance between 5-HT and dopamine systems may play a crucial role in MOH sensitization and in various forms of drugs [23]. Although this study did not find an association between TPH2 gene polymorphisms and the complication of MOH in migraine patients, it is possible that other 5-HT-related gene polymorphisms may contribute to the aggravation of migraines by the overuse of medication. Further genetic studies are needed to identify susceptible 5-HT-related genes associated with the complication of MOH in migraine patients.

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