Neurology Asia 2014; 19(3) : 277 – 281

Polymorphisms of nitric oxide synthase and GTP cyclohydrolase I genes in Japanese patients with medication overuse headaches

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Abstract

We investigated whether polymorphisms of the endothelial nitric oxide synthase (eNOS), neuronal NOS (nNOS), and GTP cyclohydrolase I (GTPCH) genes are involved in the aggravation of migraine induced by overuse of medications. We studied 47 patients with migraine (six males and 41 females; 36.4 ± 10.3 years of age) and 22 patients with migraine exhibiting medication overuse headache (MOH, one male and 21 females; 39.6 ± 9.9 years of age). The genotypes of polymorphisms of the eNOS (rs1799983), nNOS (rs2682826), and GTPCH (rs841) genes were analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The genotypic distributions of rs2682826 (T/T plus T/C vs. C/C, $P = 0.254$), rs1799983 (G/G vs. G/T plus T/T, $P = 1.000$), and rs841 (T/T plus T/C vs. C/C, $P = 0.149$) were not significantly different between patients with migraine and patients with MOH. The results of this study showed an absence of association between the polymorphisms of eNOS, nNOS, and GTPCH genes and the complication of MOH in patients with migraine.

INTRODUCTION

Nitric oxide (NO) is produced from L-Arg by the constitutive NO synthase (cNOS) enzyme that is activated by Ca^{2+}, and is an important signaling molecule for the regulation of vital functions such as vascular tone and neurotransmission. NO generated by endothelial NOS (eNOS), one of the cNOS, in the endothelial cells of cerebral arteries and/or by neuronal NOS (nNOS), which is another type of cNOS, in trigeminal neurons contributes to the pathophysiology of migraine.[1] NO donors such as glyceryl trinitrate cause headaches.[1-3] Moreover, NO levels during a migraine attack are higher in patients with migraine compared with those in controls.[4] Thus, NO may play a crucial role in migraine. Patients with migraine are particularly prone to the complication of medication overuse headache (MOH).[5-7] In addition, the frequency of comorbidity with depression is higher in patients with MOH than that in patients with migraine.[7,8] Because a reduction in NO synthesis is associated with the pathogenesis of depression[9-11], NO synthesis may be reduced in migraine patients with MOH.

Polymorphism of the eNOS (rs1799983) gene is a risk factor for migraine with aura.[12] In contrast, other groups have reported that the polymorphism of rs1799983 does not contribute to migraine.[13,14] Furthermore, polymorphism of the nNOS gene (rs2682826) was not involved in the pathophysiology of migraine.[15] However, the involvement of polymorphisms of the eNOS and nNOS genes in the aggravation of migraines by the overuse of medications has not been reported. In addition, tetrahydrobiopterin (BH4), an essential cofactor of NOS, is a regulator of NOS function that controls the release of NO and/or reactive oxygen species,[16-18] but we found no reports of a relationship between MOH and polymorphism of the gene that encodes GTP cyclohydrolase I (GTPCH), which is a rate-limiting enzyme involved in BH4 synthesis.

Therefore, we conducted the present study to investigate the association between polymorphisms of eNOS (rs1799983), nNOS (rs2682826), and GTPCH (rs841, a rate-limiting enzyme for BH4 synthesis) genes and the complication of MOH in patients with migraine.

METHODS

Subjects

We enrolled 47 patients with migraine [six males and 41 females: five with migraine with aura

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(MA), 36 with migraine without aura (MO), and six with MA + MO; 36.4 ± 10.3 years of age] and 22 patients with MOH (one male and 21 females: one with MA and 21 with MO; 39.6 ± 9.9 years of age) who were admitted to the Department of Neurology in an outpatient clinic of the Showa University East Hospital, Tokyo, Japan between May, 2010 and January, 2011. These subjects were the same as those included in previous studies.[19] Depression was significantly more frequent in patients with MOH than that in patients with migraine ($P < 0.001$).[19] The medications that were overused were combination analgesics in 14 patients (64%), analgesics in nine patients (41%), and triptan in two patients (9%).[19]

Migraine was diagnosed according to the International Classification of Headache Disorders, 2nd Edition (ICHD-II), 2004.[20] Moreover, we confirmed, via interviews, that patients with migraine did not overuse medications. The revised ICHD-II criteria were used for the diagnosis of MOH.[5] Patients with MOH were questioned regarding their primary headache by headache specialists. Moreover, headache specialists confirmed the primary headache after treating MOH, according to the ICHD-II criteria. Although the subjects of this study included not only patients with migraine but also patients with migraine and tension-type headache, patients with tension-type headache were excluded from this study. We used the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV)[21] for the diagnosis of major depressive disorder.

All patients were Japanese. In addition, all patients who gave their informed consent, including those with migraine and patients with MOH, were enrolled in this study. The clinical study was approved by the Ethics Committee for Genome Research of Showa University.

Genotyping

Genomic DNA was extracted from whole blood using the NucleoSpin® Blood QuickPure kit (NIPPON Genetics Co., Ltd., Tokyo, Japan). Polymorphisms of GTPCH (rs841), eNOS (rs1799983), and nNOS (rs2682826) genes were studied. Polymorphisms of each gene were determined as described in previous reports.[12,22,23] These genotyping assays were performed on a maximum of 30 samples, plus a positive control. The primer sequences and restriction enzymes used for the detection of polymorphisms of three genes and their expected fragment sizes are shown in Table 1.

PCR products or restriction-enzyme-treated PCR fragments were run on 3% agarose gels and stained with ethidium bromide.

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). Significance was set at $P < 0.05$.

RESULTS

The genotypic distributions of polymorphisms of eNOS (rs1799983; G/G vs. G/T plus T/T, $P = 1.000$), nNOS (rs2682826; T/T plus T/C vs. C/C, $P = 0.254$), and GTPCH (rs841; T/T plus T/C vs. C/C, $P = 0.149$) genes were not significantly different between patients with migraine and patients with MOH (Table 2).

DISCUSSION

In the present study, no association was observed between polymorphisms of eNOS (rs1799983), nNOS (rs2682826), and GTPCH (rs841) genes and the complication of MOH in patients with migraine.

The frequency of comorbidity with depression is higher in patients with MOH than that in patients with migraine.[7,8] Moreover, in this study, we confirmed that the incidence of depression

Table 1 Primers and restriction enzymes used for genotyping

Polymorphism	Primer	Restriction enzyme	Product size (bp)	Reference
eNOS (rs1799983)	5'-CAT GAG GCT CAG CCC CAG AAC-3' 5'-AGT CAA TCC CTT TGG TGC TCA-3'	MboI	T: 119 and 87 G: 206	[12]
nNOS (rs2682826)	5'-ACT CCT TGA GTT TTC CTG CTG CGA-3' 5'-CCA TGT TCC AGT GGT TTC ATG CAC AC-3'	Eco72I	T: 128 C: 100 and 28	[22]
GTPCH (rs841)	5'-GTT GGT TGC CGA TCG TAC TG-3' 5'-CAG TAT ACT GGG CAC AGT TC-3'	TaiI	T: 361 C: 225 and 136	[23]

Table 2. Genotype distributions of GTPCH, nNOS and eNOS genes polymorphisms

		Total		Migraines		MOH		
		n=69	%	n=47	%	n=22	%	*P* value
eNOS (rs1799983)	G/G	57	82.6	39	83.0	18	81.8	
	G/T	12	17.4	8	17.0	4	18.2	
	T/T	0	0.0	0	0	0	0	
	G/G	57	82.6	39	83.0	18	81.8	1.000
	G/T,T/T	12	17.4	8	17.0	4	18.2	
nNOS (rs2682826)	T/T	11	15.9	7	14.9	4	18.2	
	T/C	26	37.7	16	34.0	10	45.5	
	C/C	32	46.4	24	51.1	8	36.4	
	T/T,T/C	37	53.6	23	48.9	14	63.6	0.254
	C/C	32	46.4	24	51.1	8	36.4	
GTPCH (rs841)	T/T	15	21.7	11	23.4	4	18.2	
	T/C	28	40.6	21	44.7	7	31.8	
	C/C	26	37.7	15	31.9	11	50.0	
	T/T,T/C	43	62.3	32	68.1	11	50.0	0.149
	C/C	26	37.7	15	31.9	11	50.0	

was significantly higher in patients with MOH than that in patients with migraine.[19] In addition, NO synthesis is attenuated in patients with depression,[9,11] and platelet NOS activity and plasma NOx levels are decreased in patients with major depression.[10] Thus, the reduction in NO synthesis may be associated with the pathogenesis of depression. Polymorphisms of rs1799983 lead to a substitution at nucleotide 894 (G to T), resulting in the conversion of glutamate (Glu) to aspartate (Asp) at codon 298, and are determinants of decreased eNOS activity[24]; moreover, their importance in the onset of chronic artery disease has been established.[25] In addition, a recent study showed that the rs2682826 T allele increased the vulnerability to recurrent depressive disorder.[26] However, we showed that rs1799983 and rs2682826 did not affect the pathophysiology of MOH.

BH4 is an essential cofactor for NOS and aromatic L-amino acid hydroxylases, including tryptophan hydroxylase, which is associated with serotonin (5-HT) biosynthesis.[16-18] 5-HT plays a pivotal role in the pathogenesis of migraine[27-29] and depression.[30,31] The depletion of tryptophan, which is a precursor of 5-HT, increases nausea, headaches, and photophobia in patients with migraine.[32] Moreover, decreases in 5-HT levels in platelets have been observed in migraine patients with MOH.[33,34] However, in our previous study, polymorphisms of the tryptophan hydroxylase 2 gene (rs4565946, rs4570625, and rs4341581) were not associated with the complication of MOH in patients with migraine.[35] In addition, in the present study, we did not find an association between MOH and polymorphism of the GTPCH gene (rs841).

Chronic exposure to antimigraine drugs, such as triptans, alters 5-HT receptors in the brain.[36,37] Calabresi and Cupini (2005) showed that the balance between 5-HT and dopamine systems may play a crucial role in MOH sensitization and in the action of various forms of drugs.[38] However, polymorphisms of 5-HT transporter (5-HTT) gene-linked polymorphic region (5-HTTLPR, NG_011747), 5-HT_{2A} (rs6313), 5-HT_{1B} (rs6296), and monoamine oxidase A (MAOA) (rs6323), and MAOA variable number of tandem repeats (MAOA VNTRs, NG_008957) genes were not associated with the complication of MOH in patients with migraine.[19] Other polymorphisms of 5-HT-related genes may contribute to the aggravation of migraine by the overuse of medication. Further genetic studies are needed to identify not only other 5-HT-related, but also other NO-related, polymorphisms of genes associated with the complication of MOH in patients with migraine. Because the sample size was the primary limitation of this study, future studies using larger samples must be undertaken to elucidate the relationship between these polymorphisms of genes and MOH.

ACKNOWLEDGEMENT

This study was supported in part by a grant from the Private University High Technology Research Center Project that matched a fund subsidy from the Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT). The authors would like to thank Enago (www.enago.jp) for the English language review.

DISCLOSURE

Conflicts of Interest: None

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