Lack of association between CGRP-related gene polymorphisms and medication overuse headache in migraine patients

Masakazu Ishii¹*, Hirotaka Katoh¹, Tatsuya Kurihara¹, Ken-ichi Saguchi², Shunichi Shimizu¹,⁴ and Mitsuru Kawamura³

¹Department of Pharmacology, Toxicology and Therapeutics, Division of Physiology and Pathology, Showa University School of Pharmacy, Tokyo 142-8555, Japan
²Department of Neurology, Showa University School of Medicine, Tokyo 142-8666, Japan
³Department of Pharmacy Education, Showa University School of Pharmacy, Tokyo 142-8555, Japan
⁴Laboratory of Pharmacology, Department of Clinical Pharmacy, Yokohama College of Pharmacy, Yokohama 245-0066, Japan

Abstract

We investigated whether calcitonin gene-related peptide (CGRP)-related gene polymorphisms are involved in the aggravation of migraines due to medication overuse. In total, 47 migraine patients (6 males and 41 females; 36.4 ± 10.3 years) and 22 medication overuse headache (MOH) patients (1 male and 21 females; 39.6 ± 9.9 years) who had migraine participated in this study. Calcitonin gene-related polypeptide-alpha (CALCA, α-CGRP, Insertion/Deletion rs1553005, rs145837941) and CGRP receptor (receptor activity-modifying protein 1: RAMP1, rs3754701, rs7590387) were analyzed by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP) methods. No significant differences were observed in the genotype distributions of CALCA and RAMP1 between migraine patients and MOH patients. The results of this study showed no association between CGRP-related gene polymorphisms and the complication of MOH in migraine patients.

Introduction

Migraine patients are particularly prone to developing medication overuse headache (MOH) [1-3]. Moreover, it has been shown that 56.8% of migraine sufferers use over-the-counter medicine alone [4]. Although MOH is known to be caused by triptan, ergotamine, opioid, and/or analgesic overuse in patients with headache [1], 85.1% of MOH patients overuse combination analgesics according to research by Imai et al. [2]. Most patients return to an episodic migraine pattern following drug withdrawal. However, the complication of MOH markedly decreases the quality of life of these patients [1]. In addition, the incidence of comorbidity with depression is higher in MOH patients than in migraine patients [3,5]. Therefore, the aggravation of migraines due to medication overuse needs to be prevented.

Calcitonin gene-related polypeptide-alpha (CALCA, α-CGRP) is a potent vasodilator and one of the mediators of neurogenic inflammation. Plasma levels of calcitonin gene-related peptide (CGRP), later called α-CGRP, are elevated in migraine patients [6,7], and an infusion of CGRP can trigger a migraine attack [8]. In addition, CGRP antagonists have good efficacy in the treatment of acute migraine attacks [9, 10]. Thus, CGRP is a key molecule in migraine pathogenesis. Interestingly, in MOH model animals, triptan increased CGRP levels [11]. In addition, exposure to µ opioids such as morphine also increased CGRP in cultured dorsal root ganglion cells [12,13]. Therefore, increasing CGRP through medication overuse seems to aggravate migraines. On the other hand, Menson et al. [14] showed no significant association between the intronic 16 bp deletion in the CALCA gene and migraine. Sutherland et al. [15] also reported that CALCA polymorphisms (rs3781719, rs145837941) and CGRP receptor (receptor activity-modifying protein 1: RAMP1, rs3754701, rs7590387) are not involved in the pathogenesis of migraine. However, to the best of our knowledge, there have been no studies on the relationship between CGRP-related gene polymorphisms and MOH.

In present study, we focused on CGRP-related gene polymorphisms such as CALCA and RAMP1 and investigated the relationship between CGRP-related gene polymorphisms and the complication of MOH in migraine patients.

Methods

Subjects

We enrolled 47 migraine patients [6 males and 41 females: 5 with migraines with aura (MA), 36 with migraines without aura (MO), 6 with both MA and MO at different times; 36.4 ± 10.3 years of age] and 22 MOH patients who had migraine (1 male and 21 females: 1 with MA and 21 with MO; 39.6 ± 9.9 years of age) who were seen in an outpatient clinic of the Department of Neurology, Showa University...
East Hospital, Tokyo, Japan, between May 2010 and January 2011. These subjects were the same as those included in a previous study [16]. The incidence of depression was significantly higher in MOH patients than in migraine patients (p<0.001) [16]. The medications that were overused 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), 2004 [17]. We also confirmed by interview that migraine patients did not overuse headache medications. The revised ICHD-II criteria were used to diagnose MOH [1]. MOH patients were questioned about their primary headaches by headache specialists. In addition, these headache specialists confirmed the primary headache according to the ICHD-II criteria after treating MOH. Although the subjects of the present study included not only patients with migraines but also patients with migraines and tension-type headaches, patients with tension-type headaches were excluded. We used the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [18] to diagnose major depressive disorder.

All patients were Japanese. All patients who provided informed consent, including those with migraines and the subset with MOH, were enrolled in the study. 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 gene polymorphisms of CGRP (Insertion/Deletion (I/D) [14], rs1553005, rs145837941) and RAMP1 (rs3754701, rs7590387) [15] were studied. The polymorphism of each gene was determined by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP) methods. Primer sequences, restriction enzymes, and reaction (PCR) and PCR-restriction fragment length polymorphism (PCR-RFLP) methods. Primer sequences, restriction enzymes, and expected fragment sizes of the gene polymorphisms are shown in Table 1. The 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 χ² test or Fisher’s exact test using Excel Statistics 2008 for Windows (Excel Toukei, Social Survey Research Information Co., Tokyo, Japan). P values ≤ 0.05 were considered significant.

**Table 1:** Primers and restriction enzymes used for genotyping.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer</th>
<th>Restriction enzyme</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCA I/D</td>
<td>5’TGG GGG AGA AGG GTA GGA CT-T</td>
<td>I: 303</td>
<td>D: 287</td>
</tr>
<tr>
<td>rs1553005</td>
<td>5’TGG AGA GCA GCC CAT GA-3’</td>
<td>I/D</td>
<td>I/D</td>
</tr>
<tr>
<td>rs145837941</td>
<td>5’TGA ACA CAC CAG CCT GTT GG3’</td>
<td>AluI: 167</td>
<td>C: 142 and 25</td>
</tr>
<tr>
<td>rs7590387</td>
<td>5’TGG ACT CTG GTT GAT AGC CAT GG-3’</td>
<td>AclI: 373</td>
<td>T: 215 and 158</td>
</tr>
<tr>
<td>RAMP1 rs3754701</td>
<td>5’TGC ACA GCA GCC CAT GA-3’</td>
<td>XcmI: 284</td>
<td>C: 194 and 90</td>
</tr>
</tbody>
</table>

**Results**

The genotype distributions of polymorphisms of the CALCA (Insertion/Deletion, I/I vs. D/D plus D/D, p=1.000; rs1553005, G/G vs. G/C plus C/C, p=0.646; rs145837941, A/A vs. A/G plus G/G, p=1.000) and RAMP1 (rs3754701, A/A vs. A/T plus T/T, p=0.573; rs7590387, G/G vs. G/C plus C/C, p=0.342) genes were not significantly different between migraine patients and MOH patients (Table 2).

**Discussion**

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], in addition to the tumor necrosis factor (TNF)-β gene polymorphism [20]. Thus, gene polymorphisms that are unrelated to 5-hydroxytryptamine seem to be associated with the aggravation of migraines by medication overuse.

In the present study, we focused on the relationship between CGRP-related gene polymorphisms and the complication of MOH in migraine patients. However, no association was observed between CGRP (I/D, rs1553005, rs145837941) and RAMP1 (rs3754701, rs7590387) gene polymorphisms and the aggravation of migraines by medication overuse. Recently, Munksgaard et al. [21] reported that no change in CGRP was detected despite dramatic reduction in headache frequency after the detoxification of MOH patients, suggesting that...
CGRP is not involved in MOH. Although Cernuda-Morollon et al. [22] reported that CGRP levels in peripheral blood were a biomarker for chronic migraine outside migraine attack times, analgesic overuse did not significantly influence CGRP levels. Therefore, CGRP may be unrelated to MOH in migraine patients. However, because the small sample size was a limiting factor in the present study, larger genetic studies are required to identify CGRP-related gene fragments that may be associated with MOH in migraine patients.

Acknowledgments

We thank Mika Yatagawa, a student at Showa University School of Pharmacy, for her technical assistance. This study was supported in part by a grant from the Private University High Technology Research Center Project with a matching fund subsidy from the Ministry of Education, Culture, Sport, Science, and Technology (MEXT), Japan.

References

1. Headache Classification Committee, Olesen J, Bousser MG, Diener HC, Dodick D, et al. (2006) New appendix criteria open for a broader concept of chronic migraine. Cephalalgia 26: 742-746. [Crossref]

Copyright: ©2015 Ishii M. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.