Coexistence of CACNA1A, ATP1A2, and KCNN3 gene mutation in migraine patients with human platelet polymorphism

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ABSTRACT

The prevalence rate of migraine in the Malaysian population is approximately 9%. Platelet dysfunction has often been linked to the pathophysiology of migraine. Increased platelet activation, hyperadhesion, and serotonin release are observed during migraine attacks and also sometimes in the headache-free periods of the migraine patients.

Recent studies have revealed that the human platelet HPA-1a/1b polymorphism in the glycoprotein IIb/IIa (GPIIb/IIa) gene causes platelet hyper-adhesiveness leading to an increased risk for

There is evidence for the role of genetic factors in migraine, and elucidating the genetic basis of this disabling condition remains the focus of much research. The prevalence rate of migraine in the Malaysian population is approximately 9%. Platelet dysfunction has often been linked to the pathophysiology of migraine. Increased platelet activation, hyperadhesion, and serotonin release are observed during migraine attacks and also sometimes in the headache-free periods of the migraine patients.

In this study, conducted at the Neurology Clinic, Hospital University Sains Malaysia, Kelantan, Malaysia between April 2004 and March 2005, the HPA1a/1b polymorphism were analyzed by polymerase chain reaction using the allele specific oligonucleotide technique to detect the presence of CACNA1A, ATP1A2, and KCNN3 genotypes.

Results: We found that the CACNA1A gene mutation alone was present in only one patient who presented with classical migraine with aura. The gene mutations on ATP1A2 and KCNN3 were seen in none of our 4 cases with migraine.

Conclusion: There is no coexistence between the platelet HPA-1a/1b polymorphism and the ATP1A2 and KCNN3 gene mutations, though one classical migraine patient with HPA-1a/1b polymorphism had the CACNA1A gene mutation. Larger studies are warranted to confirm these findings.

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thrombotic episodes, especially myocardial infarction.\textsuperscript{4} It has also been well documented that in migraineurs, the platelets get easily activated leading to hyper-adhesion and serotonin release.\textsuperscript{5} We did a preliminary study on 80 migraine patients and found that the HPA-1a/1b polymorphism was seen in 4 patients of which 3 had migraine with aura.\textsuperscript{6} Genetic studies have suggested the possible role of numerous candidate genes leading to migraine susceptibility.\textsuperscript{7} Mutations in the CACNA1A, ATP1A2, and KCNN3 genes have been reported in migraine patients, especially in the dominantly inherited hemiplegic migraine.\textsuperscript{8} The aim of this study was to look for any possible coexistence of the CACNA1A, ATP1A2, and KCNN3 genotypes in 4 of our migraine patients, who incidentally had yet another genetic aberration in the form of human platelet HPA-1a/1b polymorphism. This coexistence has not been studied so far.

**Methods.** Eighty consecutive patients, attending the Neurology Clinic at Hospital Universiti Sains Malaysia, Kelantan, Malaysia between January 2005 and December 2005, with headaches that fulfilled the International Headache Society criteria for migraine, and who had no other neurological diseases or overt systemic diseases were studied for the presence of platelet HPA-1a/1b polymorphism. Four of the 80 patients who were positive for the polymorphism were further taken up for the present study. The study was approved by the research and ethics committee, Universiti Sains Malaysia. The DNA of the 4 patients was extracted from fresh blood using commercial extraction kit (QIAGEN, Inc). Samples were stored at -20\textdegree C until required. Target DNA was amplified by polymerase chain reaction (PCR) using the specific oligonucleotide primers. The oligonucleotide primers used for PCR amplification were purchased from First Base Lab (Kuala Lumpur, Malaysia). The PCR reaction mixture consisted of 1x PCR buffer, 200 \(\mu\)M of dNTP, 2.0 mM MgCl\(_2\), 1 \(\mu\)M (50 pmol) of each primer, and 50 ng of genomic DNA in total 100 \(\mu\)l reaction volume. Amplification was carried out in Thermal Cycler (Eppendorf) for 35 cycles. The PCR products were purified using a GeneClean II kit (Bio 101 Corp., La Jolla, CA, USA) before proceeding with direct sequence analysis using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

**Results.** Of the 4 cases with the platelet HPA1a/1b polymorphism, we found that the CACNA1A gene mutation alone was present in only one (who presented with classical migraine with aura.) The gene mutations on ATP1A2 and KCNN3 were seen in none of our 4 cases (Table 1).

**Discussion.** Studies performed so far have not led to the ultimate identification of the gene(s) responsible for the various forms of migraine.\textsuperscript{9} Only for the familial hemiplegic migraine (FHM1) - a rare autosomal dominant form of migraine, CACNA1A gene mutation has been identified on chromosome 19p13.\textsuperscript{10} Some other studies have suggested that mutations in ATP1A2,\textsuperscript{11} and KCNN3\textsuperscript{12} on chromosome 1q23 may be involved in migraine although contradictory data have also been reported.\textsuperscript{13} We had reported earlier\textsuperscript{13} that 4 out of 80 migraine patients tested positive for the HPA1a/1b

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**Table 1** - The clinical and laboratory data of 4 patients with migraine studied. The CACNA1 gene is the \(\alpha_{1A}\) subunit of a neuronal voltage-gated P/Q-type calcium channel, the ATP1A2 is the gene encoding the ATP1A2 \(\text{Na}^+/\text{K}^+\) ATPase subunit and KCNN3 is the calcium-activated potassium channel gene.

<table>
<thead>
<tr>
<th>Description</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/gender</td>
<td>20/female</td>
<td>19/female</td>
<td>23/female</td>
<td>44/female</td>
</tr>
<tr>
<td>Total duration of headache</td>
<td>5 years</td>
<td>3 years</td>
<td>7 years</td>
<td>13 years</td>
</tr>
<tr>
<td>Maximum duration of each episode</td>
<td>12 hours</td>
<td>30 hours</td>
<td>8 hours</td>
<td>6 hours</td>
</tr>
<tr>
<td>Aura</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>MIDAS score</td>
<td>18</td>
<td>15</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Neurological examination</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>CT scan brain</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Gene mutation on CACNA1</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Gene mutation on ATP1A2</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Gene mutation on KCNN3</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>HPA1a/1b polymorphism</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>

MIDAS - Migraine disability assessment, Na - sodium, K - Potassium, ATPase - Adenosine triphosphatase
polymorphism, a genetic abnormality resulting in platelet hyper adhesiveness. This genetic abnormality has been shown to be the result of variations on 5 genes of which the GPIIIa gene located on the chromosome 17q21-22 seems important.14

The intention of the present study was to find out whether mutations in all or any of the CACNA1A, ATP1A2, or KCNN3 genes could also be coexistent in patients with migraine having the human platelet HPA-1a/1b polymorphism. Our study is a small and preliminary one. It suggests that there may not be any coexistence of the platelet polymorphism and the ATP1A2 and KCNN3 gene mutations, except perhaps the CACNA1A gene mutation. Larger studies are warranted to throw more light on this subject.

References