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

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ABSTRACT

الأهداف: للنظر في أية احتمالية لتحولات الجين (CACNA1A)، والجين (ATP1A2) والجين (KCNN3)، لدى المرضى المصابين بالشقيقة، والذين أصيبوا بتشوهات متعددة في الصفائح البشرية (HPA-1a/1b) والتي تعرف أيضاً بالتشوهات الجينية المتعددة (P1A1/A2).

الطريقة: أجريت هذه الدراسة بعيادة الأعصاب – مستشفى سينس الجامعي – ماليزيا، خلال الفترة ما بين أبريل 2004م وحتى مارس 2005م. تم تحليل الحمض النووي (DNA) لأربعة مرضى مصابين بالشقيقة مع التشوه المتعدد للجين (HPA1a/1b) بواسطة تفاعل سلسلة الخمائر الناقلة (PCR)، وباستعمال تقنية (ASO) لتحديد وجود أنواع الجين (CACNA1A) والجين (ATP1A2) والجين (KCNN3).

النتائج: وجدنا أن تحول الجين (CACNA1A) لوحده كان حاضراً لدى مريض واحد، والذي حضر وهو يعاني من شقيقة كلاسيكية مع نسمة. لم يتم ملاحظة وجود تحول الجين (ATP1A2) والجين (KCNN3) في أي مريض من الحالات الأربعة المصابة بالشقيقة.

خاتمة: لم يكن هنالك ظهور مشترك بين التشوه المتعدد للصفائح (HPA-1a/1b) وتحولات الجين (ATP1A2) والجين (KCNN3)، على الرغم من وجود مريض واحد يعاني من الشقيقة الكلاسيكية لديه تشوه متعدد في الجين (HPA-1a/1b) ولديه تحول في الجين (CACNA1A). يجب القيام بالمزيد من الدراسات الكبيرة للتأكد من هذه النتائج.

Objectives: To look for any possible coexistence of CACNA1A, ATP1A2, and KCNN3 gene mutations in migraine patients who had human platelet HPA-1a/1b polymorphism, which is also known as PlA1/A2 polymorphism.

Methods: The study was carried out at the Neurology Clinic, Hospital University Sains Malaysia, Kelantan, Malaysia between April 2004 and March 2005. The DNA from 4 patients who had migraine with 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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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.[1] The prevalence rate of migraine in the Malaysian population is approximately 9%.[2] 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.[3] 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

thrombotic episodes, especially myocardial infarction.[4] It has also been well documented that in migraineurs, the platelets get easily activated leading to hyper-adhesion and serotonin release.[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.[6] Genetic studies have suggested the possible role of numerous candidate genes leading to migraine susceptibility.[7] Mutations in the CACNA1A, ATP1A2, and KCNN3 genes have been reported in migraine patients, especially in the dominantly inherited hemiplegic migraine.[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-Ia/Ib 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^{0}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 µM of dNTP, 2.0 mM $MgCl_2$, 1 µM (50 pmol) of each primer, and 50 ng of genomic DNA in total 100 µl 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.[9] Only for the familial hemiplegic migraine (FHM1) - a rare autosomal dominant form of migraine, CACNA1A gene mutation has been identified on chromosome 19p13.[10] Some other studies have suggested that mutations in ATP1A2,[11] and KCNN3[12] on chromosome 1q23 may be involved in migraine although contradictory data have also been reported.[13] We had reported earlier[13] that 4 out of 80 migraine patients tested positive for the HPA1a/1b

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

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

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.

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