

Analysis of Specific Migraine Candidate Genes Mapping to Human Chromosome 1

By

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ABSTRACT

Migraine, comprised of migraine with aura (MA) and migraine without aura (MO), is a painful neurovascular disease, affecting approximately 16% of the general population. It is characterised by a wide variety of symptoms including headache, nausea and vomiting, and photo- and phonophobia. The disorder is complex involving not only multiple genes, but also specific environmental factors, which can induce attacks in genetically predisposed individuals. Hyperhomocysteinaemia is a known risk factor for cerebrovascular, peripheral vascular and coronary heart disease. The Methylene tetrahydrofolate Reductase (*MTHFR*) enzyme is involved in homocysteine metabolism. Furthermore, it has been reported that a homozygous mutation (677C to T; Ala to Val) in the 5,10-*MTHFR* gene is associated with an elevation in plasma homocysteine levels (Frosst *et al.*, 1995). This common mutation in the *MTHFR* gene has recently been associated with migraine *with aura* in a Japanese cohort (Kowa *et al.*, 2000). The present study was designed to determine the prevalence of the *MTHFR* C677T mutation in Australian patients with migraine and to determine whether this mutation is associated with the disease in Caucasians. A large case-control study, consisting of 270 patients with migraine (167 *with aura* and 103 *without aura*), and 270 normal matched controls was investigated. Genotypic results indicated that the prevalence of the homozygous (T/T) genotype in migraine sufferers (15%) was higher than that of controls (9%) ($P = 0.084$). Furthermore, the frequency of the mutant (T/T) genotype in individuals with MA (19%) was significantly higher than in controls (9%) ($P = 0.006$). Interestingly, the risk of MA was ~2.5-fold higher in sufferers possessing the

homozygous variant (OR = 2.52, CI: 1.42 – 4.47, $P = 0.0012$). To confirm the *MTHFR* allelic association with MA, family-based tests were performed in an independent pedigrees group, where only those with MA were considered affected. Results from both the Pedigree Disequilibrium Test (PDT) and Family-Based Association Test (FBAT) analysis revealed slight, although not significant (PDT test, $P = 132$; and FBAT test, $P = 0.390$), over-transmission of the mutant allele (T) from parents to affected offspring. However, despite the *MTHFR* variant having a high heterozygosity (0.48), there were a limited number of informative transmissions for the *MTHFR* variant in the pedigree group resulting in reduced power for these tests. In conclusion, our results support the trends reported in the Japanese migraine study and suggest that the homozygous 677T gene variant causing mild hyperhomocysteinaemia, is a genetic risk factor for migraine, and indicate that further studies investigating the role of this gene are warranted.

Mutations in various ion channel genes are responsible for neurovascular and other neurological disorders. Inherited ion channel mutations or “channelopathies” are increasingly found to be the cause of various neurological disorders in humans. Wittekindt and colleagues (1998) reported that the calcium-activated potassium channel (*hKCa3*) gene is a good candidate for schizophrenia and bipolar disorder (BD), as well as for other neurological disorders such as migraine. The *hKCa3* gene is a neuronal small conductance calcium-activated potassium channel, which contains a polyglutamine tract, encoded by a polymorphic CAG repeat in the gene. The *hKCa3* gene encodes a protein of 731 amino acids containing two adjacent polyglutamine sequences in its N-terminal domain separated by 25 amino acids. The C-terminal polyglutamine sequence is highly

polymorphic in length (Austin *et al.*, 1999). *hKCa3* plays a critical role in determining the firing pattern of neurons via the generation of slow after-polarization pulses and the regulation of intracellular calcium channels (Kohler *et al.*, 1996). Three distinct mutations in the $\alpha 1$ calcium channel gene have been shown to cause SCA-6, episodic ataxia-2 and familial hemiplegic migraine (FHM) (Ophoff *et al.*, 1996). The *hKCa3* gene contains a highly polymorphic CAG repeat that was initially mapped (Chandy *et al.*, 1997) to a schizophrenia locus on chromosome 22 (Pulver *et al.*, 1994). Recently Austin *et al.* (1999) re-mapped *hKCa3* and found it to reside on chromosome 1q21, a region that has been linked to FHM (Austin *et al.*, 1999), a rare subtype of MA (Ducros *et al.*, 1997; Gardner *et al.*, 1998), and a region recently showing genetic linkage to typical migraine (Lea *et al.*, 2002).

The *hKCa3* polymorphism results in small variations in polyglutamine number, similar to those that occur in the calcium channel $\alpha 1a$ subunit gene (*CACNA1A*), which is encoded by CAG expansions and thought to cause Spinocerebellar Ataxia Type 6 via loss of channel function (Austin *et al.*, 1999). Given the recent linkage of FHM to the region of chromosome 1q21, to which *hKCa3* resides, and also linkage of typical migraine to this region, a large case-control study investigating this *hKCa3* CAG marker and consisting of 270 migraine and 270 stringently matched healthy controls was undertaken. Our results indicated that there was no statistically significant difference in allele distributions for this marker between migraine and non-migraine patients ($P > 0.05$). No significant difference in the allelic distribution was observed in the MA or MO groups when compared to controls ($P > 0.05$) and there was no significant difference in CAG repeat

length distribution between the migraine group and controls ($P = 0.92$), or between the MA and MO groups ($P = 0.72$) collectively. Hence, the CAG repeat in this gene does not show expansion in migraine. Overall, our results provide no genetic evidence to suggest that the *hKCa3* CAG repeat polymorphism is involved in migraine aetiology in Australian Caucasians. Thus the involvement of the *hKCa3* gene in migraine is not likely, although the *hKCa3* gene remains an important candidate for other neurological disorders that may be linked to the 1q21.3 chromosomal region.

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STATEMENT OF ORIGINALITY

The material presented in this Thesis has not been previously submitted for a degree or diploma in any university, and to the best of my knowledge contains no material previously published or written by another person except where due acknowledgment is made in the Thesis itself.

James Sundholm

PUBLICATIONS AND ABSTRACT PRESENTATIONS

Lea, R.A., Ovcaric, M., Sundholm, J., Solyom, L., MacMillan, J. and Griffiths, L.R. (2003). The methylenetetrahydrofolate reductase (MTHFR) gene variant (C677T) influences susceptibility to migraine with aura in caucasians. *Journal of Medical Genetics* (submitted 2/6/03).

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LIST OF ABBREVIATIONS

ABI 310	Applied Biosystems 310 Genetic Analyser
ACE	Angiotensin Converting Enzyme
AGE	Agarose Gel Electrophoresis
AT	Adenosine Thymine
bp	Base Pair
CACNA1A	Calcium Channel Alpha-1 Subunit Gene
CNS	Central Nervous System
(χ^2) test	Chi-Square Statistic
DNA	Deoxyribonucleic Acid
DNTP	Deoxynucleotide Triphosphates
dsDNA	Double Stranded DNA
EDTA	Ethylene Diamine Tetraacetic Acid
EtBr	Ethidium Bromide
FAM	Phosphoramidite Reagent
FHM	Familial Hemiplegic Migraine
GC	Guanine Cytosine
H ₂ O	Water
Hcy	Homocysteine
HCl	Hydrochloric Acid
<i>Hinf</i> I	Haemophilus Influenzae Rf
<i>hKCa3</i>	Calcium-activated Potassium Channel Gene
HWE	Hardy-Weinberg Equilibrium
IHS	International Headache Society
K ⁺	Potassium
KCl	Potassium Chloride
Me-Cbl	Methyl-Cobalamin
Me-THF	5-Methylenetetrahydrofolate
MS	Methionine Synthase
MTHFR	Methylenetetrahydrofolate Reductase Gene

LIST OF ABBREVIATIONS

MA	Migraine with Aura
MO	Migraine without Aura
MgCl ₂	Magnesium Chloride
SK	Small Conductance
NaCl	Sodium Chloride
NE	Norepinephrine
NINDB	National Institute of Neurological Diseases
NKM	0.14M NaCl-30mM KCl-3mM MgCl ₂
N ₂	Nitrogen
OD	Optical Density
OD ₂₆₀	Optical Density at 260nm
OR	Odds Ratio
P	Probability
PCR	Polymerase Chain Reaction
RE	Restriction Enzyme
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rpm	Revolutions Per Minute
RSB	10mM Tris-HCl, pH7.5-10m MNaCl-3mM
5-HT	Serotonin
SDS	Sodium Dodecyl Sulfate
ssDNA	Single Stranded DNA
TAE	40mM Tris-acetate, pH8-2mM EDTA
TAMRA	Labelled Size Standard
TDT	The Transmission/Disequilibrium Test
TE	10mM Tris-Cl-pH8-EDTA
TH	Tension-type Headache
U	Unit
v	Volume

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