Author's response to reviews

Title: Association Analysis of Chromosome 1 Migraine Candidate Genes

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Author’s response to reviews: see over
Thank you for allowing us the opportunity to provide an opinion/point of view on the reviewer’s comments for our manuscript entitled “Association Analysis of Chromosome 1 Migraine Candidate Genes”.

Please see our attached response to reviewer’s comments

Referee's Comments to Author:

**REVIEWER’s REPORT 1:**

Reviewer: Michael Bjørn Russell
Reviewer's report:

**General**
The original International Classification of Headache Disorders should be on the reference list rather than a commented version.

_The correct reference has now been included as suggested._

Migraine without aura and migraine with aura should be analysed separately. Results related to combined data should be less emphasized.

_The results for the analysis of subtypes of migraine have now been added in Table 3 for all SNP markers and in Table 5 for the Fas Ligand (CA) repeat. The results have also been discussed in The Results section._

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**Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)**

Material and methods should be described in a separate section

_The Material and Methods sections have now been separated._

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**Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)**

Suggest that the discussion is also related to the FHM-2 mutation on chromosome 1, which was originally reported to be 1q31 (miscalculation), but actually is located to 1q23. Only about 10% of those with FHM have mutation in the ATP1A2 gene. Relate the results of the association.
In response to this concern, the section below has been added to page 8:

“Family linkage studies conducted by Gardner et al. originally implicated an additional FHM susceptibility locus within a broad region (44 cM) on chromosome 1q31 [1]. Independent research carried out by Ducros et al.[2] indicated a second FHM locus at 1q21–23, approximately 30 cM centromeric to the region reported by Gardner et al [1]. Further investigation of these FHM susceptibility regions has subsequently implicated a specific ATPase gene ATP1A2 on chromosome 1q23. The gene has been identified as having causal mutations in some FHM pedigrees with the locus now defined as FHM2 [3]. This region as well as the 1q31 region may also be involved typical migraine with studies suggesting that 1q31 may also be implicated in MO or MA susceptibility in a 82 independent pedigrees [4]. The present study investigated markers within both 1q23 and 1q31 regions for involvement in typical migraine. We tested polymorphisms from the KCNJ9, KCNJ10 and FasL genes (markers that had previously been tested in a number of other genetic disorders, including Multiple Sclerosis (MS)).”

REVIEWER ’s REPORT 2:

Reviewer: Pasquale Montagna
Reviewer’s report:
General
This paper details the findings of a genetic association study done in a sample of migraine patients with and without aura (243 patients versus 243 controls matched for age, sex and ethnicity). Several genes were investigated, all located on chromosome 1 regions found associated with FHM type 2 and actually containing the gene ATP1A2 responsible for this disease. The results of the association study were in the end negative for all markers, even though some significance was obtained for a marker in the KCNJ10 gene at least for the overall migraine sample (p0.02). The conclusion was reached that ATP1A2 does not contribute to the typical migraines and that indeed the genetic basis of migraine with aura is different from that of FHM, a maybe disappointing conclusion but one on which several other lines of evidence converge.

The paper is well written (except for the occasional typographical error), the rationale sound and well defined, methods are well described and appropriate.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Arguably, the numbers may be few, some authors believing that at least 500 patients are required for an association study of this kind. I think that the authors should acknowledge this difficulty, since no power calculation was done.
Also this seems to be a Hospital population, and as such it may be not representative of the general population.
The authors should also mention that genotyping failures occurred, and what kind of quality control over the genotyping procedures was performed.

In response to this query, we have now included more details on the population. The population studied in this report contains initially 275 cases vs 275 controls. To minimize potential bias from population stratification, the control group was matched for sex, age (+/- 5 years) and ethnicity. All genotypes for each of tested markers were determined by 2 independent researchers. Genotypes were only called when results were unambiguous. This meant that around 10% of analyzed DNA samples did not lead to a definite genotype.

Also in response to this reviewer, the section below discussing power issues has been added to page 11 to outline this:

“Furthermore, as the power of an association study is affected not only by sample size, but other unknown factors such the strength of the association or degree of difference between the case and control subjects, as well as the genetic effect (ie. penetrance), the possibility of false negative results should not be ruled out.”
We have probably not been very clear in our manuscript but the studied population has been generated from a general Australian community, not from a hospital environment. All individuals were of Caucasian origin and gave informed consent before participating in the research, organized by our Centre. Migraineurs were diagnosed as having either MA or MO, based strictly on criteria specified by the International Headache Society. Several articles have been published with results generated with this population [5] (Colson).

The subject section has now been modified page 13.


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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

I take exception with the starting line in the abstract, that MA is a subtype of common migraine (“common” migraine was the old term for migraine without aura, typical is better); and with the opening sentence of the introduction (common again, and also there are no "normal" symptoms of migraine; maybe the authors mean usual or common symptoms).

The word “common” has been replaced by “typical” in page 1 and page 2 as suggested and also the term “normal” has been removed and replaced by “usual” in page 3.

I am also puzzled by the description of ATP1A4 as a subunit of ATP1A2 (maybe of the Na/K ATPase? pages 3 and 10) and of CACNA1E as a subunit of CACNA1A (pages 5 and 10);)

The reviewer is quite correct in picking these inadvertent errors.

Section “ATP1A4 as a subunit of ATP1A2” has now been replaced by “ATP1A4 subunit of the sodium/potassium ATPase” pages 3 and 10.

Section “CACNA1E as a subunit of CACNA1A” have been replaced by “subunit of R-type voltage-dependent calcium channels” pages 5 and 11.

Zayas et al (page 7) is not referenced properly;

The reference has now been added correctly page 7.

and the authors reported no association rather than they “did not report an association” (page 9, line 13);

The sentence “did not report an association” has been replaced by “reported no association” in page 9.
KCNJ9 and KCNJ10 are KCN 9 and 10 in table 3;

*KCN 9 and 10 in Table 3 have been replaced by KCNJ9 and KCNJ10 respectively.*

reference 1 should be replaced by the original one;

*This reference has been replaced by the original one, which is “HCCIHS: Headache Classification Committee for the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain 2nd edition. Cephalgia 2004, 24 (Suppl 1):1-60.”*

and is reference 7 the correct one?

*This reference has been replaced by the correct one, which is “Shin DW, Pan Z, Kim EK, Lee JM, Bhat MB, Parness J, Kim DH, Ma J: A retrograde signal from calsequestrin for the regulation of store-operated Ca2+ entry in skeletal muscle. J Biol Chem 2003, 278(5):3286-3292.”*