Author's response to reviews

Title: Association analysis of a highly polymorphic CAG Repeat in the human potassium channel gene KCNN3 and migraine susceptibility

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Author's response to reviews: see over
29th July 2005

BioMed Central
Editorial Team

Re: Response to Reviewers - manuscript submission in BMC Medical Genetics.

Dear Sir/Madam

Please find enclosed the revised manuscript entitled “Association analysis of a highly polymorphic CAG Repeat in the human potassium channel gene \( KCN\text{N}3 \) and migraine susceptibility” by Robert Curtain, James Sundholm, Rod Lea, Mick Ovcaric, John MacMillan and Lyn Griffiths.

We have now addressed the major concerns of reviewers for this manuscript, as required, please find listed changes made in an accompanying rebuttal letter (below). We look forward to your future correspondence and thank you in advance for your consideration.

Yours Sincerely,

[Signature]

Professor Lyn R. Griffiths,
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Changes to Manuscript in Response to Reviewers Comments and Suggestions

Please find below our responses to the issues raised by reviewer 1, 2 and 3 for the manuscript entitled “Association analysis of a highly polymorphic CAG Repeat in the human potassium channel gene KCNN3 and migraine susceptibility” by Curtain et al.

RESPONSE TO REVIEWERS

Reviewer comments

Reviewer 1

1. The authors should present power calculations to demonstrate whether the sample size is sufficient to study the association between multiallelic markers and migraine.

The minimum allele frequency for migraine was 0.25% for 24 CAG repeats. In a sample size of n=404 alleles for migraine, utilizing a marker with a distance of 40kb from the possible trait-influencing locus gives an estimate of power of >75% to detect association at the 0.05 significance level. At distances closer to the possible locus (≤20kb) a power estimate of >84% was obtained, where 80% power is the conventional threshold (see Schork NJ, 2001).

Reviewer 2

1. The authors state in the introduction: ‘Given that FHM2 maps to C1q23 and KCNN3 localizes nearby at C1q21.3, it may be important to examine the prevalence of the second (highly polymorphic) KCNN3 CAG polymorphism in populations affected and unaffected with migraine’. Moreover, in the conclusions, they state: 'However, other migraine candidate genes near this cytogenetic region on chromosome 1 are currently being tested'. In fact, the gene for FHM2 has been found in 2003 (FHM2 was found to be an alpha subunit of the sodium-potassium pump (De Fusco et al., Nature Genetics 33, 192-196, 2003). Therefore, with the gene for FHM2 known, this rationale for investigating KCNN3 is not valid.

FHM2 and common migraine (MA/MO) are two distinct phenotypes of migraine disorder (see 1.1, 1.2, 1.2.4, International Headache Society, 2004). FHM2 is a rare form of common migraine and both the FHM1 and FHM2 genes do not cause the common forms of migraine (Lea et al, 2001, Jen et al, 2004, Curtain et al, 2005). Though a C1 locus for common migraine maps to C1q31 (Lea et al, 2002) and C1q23 (Curtain et al, 2005), with KCNN3 localised nearby (C1q21.3).
2. A study on KCNN3 in migraine appeared in Headache in February 2005 (A highly polymorphic poly-glutamine stretch in the potassium channel KCNN3 in migraine).

This paper has now been mentioned and extrapolated on in the Discussion section (page 13).

Reviewer 3

1. The authors should describe both CAG repeats in the gene in slightly more detail (the other repeat is much less polymorphic).

More detail of the second CAG repeat, that was analysed, and a description of the first CAG repeat has now been amended to page four of the introduction.

2. The authors should cite more studies that have examined ion channels in migraine, or perhaps just provide a reference to a review of such studies. Most such studies have been negative. This will provide a better context for their own work.

An explanation of ion channels in migraine has now been added and cited in the introduction (page 4).

3. On p4, second paragraph: this is highly confusing. The authors have tried to cram too much information into a single sentence. They should provide more information on the CACNA1A gene in familial migraine.

The sentence has now been revised with more information provided on CACNA1A and migraine.

4. The authors have not mentioned that FHM2 is now known to be caused by mutations in the ATP1A2 gene (although the reference is in their reference list, ref 11). This is important for two reasons: It shows that two familial forms of migraine have now been shown to be caused by mutations in ion channels. Secondly, their argument that the KCNN3 gene is close to the locus for FHM2 becomes invalid, as the gene for this condition is identified.

The mentioning that FHM2 is now known to be caused by mutations in the ATP1A2 gene is now stated on page four of the introduction.

5. The prevalence of migraine is given as 33% in women and 13.3% in men on page 3, but as 12% on page 12. These are two different studies, which not surprisingly, found different prevalence rates. They can be cited together, or the authors could choose one of them, but the way they are cited can confuse the reader.
The prevalence of migraine has now been given as 33% in women and 13.3% in men on page 3 and 12.

6. On page 12 the authors state that other studies have shown that the long CAG repeats are more common in patients with schizophrenia and bipolar disorder. They cite two of the early studies. There have been numerous studies since then and overall the conclusion is that this repeat is not involved in the pathogenesis of either of these psychiatric disorders. The authors could instead cite a meta-analysis (Glatt et al, 2003) that draws that conclusion.

The meta-analysis study has now been cited on page 13, with the drawn conclusion that this repeat is not involved in the pathogenesis of either of these psychiatric disorders.

7. The authors probably submitted their paper before they became aware of the work by Moessner et al, (Headache, Feb 2005, 45:132-136). These authors examined this repeat in a similar number of migraine patients and controls from Germany. Obviously this work needs to be cited and discussed in the revised manuscript.

This work by Moessner et al has now been cited and discussed on pages 13 and 14.

8. It appears to me that the p-values in Table 1 (and text) are not correct. I estimate that when the number of alleles longer or shorter than 19 repeats in patients and controls are compared, the p-value is 0.15, and when migraine patients with and without aura are compared, it is 0.4. Although these are not significant either, they differ from what the authors have stated.

The P values for all groups have now been updated in Table.1.

References


Jen JC, Kim GW, Dudding KA, Baloh RW. No mutations in CACNA1A and ATP1A2 in probands with common types of migraine. Arch Neurol. 2004 Jun;61(6):926-8

Lea RA, Shepherd AG, Curtain R P, Nyholt DR, Quinlan S, Brimage PJ, Griffiths LR. A typical migraine susceptibility region localizes to chromosome 1q31. Neurogenetics 2002;4:17-22