Reviewer’s report

**Title:** The Estrogen Receptor 1 locus associated with migraine susceptibility is restricted to the 3' end of the gene

**Version:** 2  **Date:** 6 December 2005

**Reviewer:** Jane Worthington

**Reviewer’s report:**

**General**

**Review of article:**

The estrogen receptor 1 locus associated with migraine susceptibility is restricted to the 3’ end of the gene. NJ Colson et al.

1) **Background:**

In this paper the authors have attempted to further investigate the association of the estrogen receptor alpha locus and migraine. A previous publication from this group had identified association to a synonymous SNP in exon 8. They here explore the possibility that other SNPs in LD with the SNP initially investigated, are the responsible for the association.

**Major compulsory revisions**

2) **Study Design**

The authors state that the previously published association with the exon 8 SNP (rs2228480) may be due to linkage disequilibrium (LD) with a causative variant. However, the SNPs chosen for investigation by the authors, in an attempt to refine the region of association, reside 155 kb and 257 kb upstream, and are thus unlikely to be in LD with this SNP. In this case, the HapMap data in the CEU sample may have highlighted SNPs showing LD with the exon 8 polymorphism in this population, and would have better informed the researchers about the SNPs that are more likely to explain the previously identified association. The authors state in the Conclusions (Paragraph 2, line 5) that an absence of LD between the exon 4 and exon 8 SNPs has previously been reported, thus raising the question of why the exon 4 and intron 1 SNPs were chosen as candidate polymorphisms.

3) **Title:**

The statement made in the title is too specific. The authors have genotyped two polymorphisms within the 295.7kb of the ESR1 gene, one in intron 1 and another in intron 4, further to the exon 8 SNP investigated in the previous publication. It does not follow from the lack of association of these two SNPs with susceptibility to migraine that the association is restricted to the 3’ region of the gene. No more variants in the 3’ region have been genotyped, and many other SNPs in the upstream 5’ region have not been investigated.

The data reported in this article do not further our understanding of the role of polymorphism in ESR1 in susceptibility to migraine.

**Minor essential revisions**

1.) **Third paragraph, line 12:** The ‘rs’ number provided for the PvuII polymorphism is incorrect. The ‘rs’ number for this SNP is rs2234693 (Johansson et al. Ann Rheum Dis 2005;64:1611-1617).
Background:
2.) Third paragraph, first sentence: Some background regarding the role of hormones such as oestrogen in the biology of migraine should be given. Although this has been clarified in the Conclusion section, it should be provided in the Introduction also, as this information is essential to the understanding of why ESR1 was chosen as a candidate for a migraine susceptibility gene.

3) Third paragraph, final sentence: This sentence should read “The minor allele at each of these SNPs has been shown to have a frequency of >20%”, and the population in which this was demonstrated should be given.

Methods:
4.) Genotyping: RFLP analysis for SNP genotyping is a rather out-dated technique, recognised to be error prone. It is advisable to include samples of known genotype (determined by sequencing) on each gel and to check for Hardy Weinberg Equilibrium in control samples. The volume of DNA polymerase used in the PCR should be stated.

Discretionary revisions:
7) Background:
Second paragraph, last sentence: Examples of those loci showing association with susceptibility to migraine could be cited.

What next?: Reject because scientifically unsound

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
'I declare that I have no competing interests'