Author's response to reviews

Title: Epigenetic mechanisms in migraine: a promising avenue?

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Author's response to reviews: see over
Dear Dr Lee,

We were very pleased with the positive feedback we received from the peer review of our manuscript titled: "Epigenetic mechanisms in migraine: a promising avenue?" by E. Eising et al. We have revised the manuscript according to the recommendations of both reviewers. The changes we made are highlighted in yellow in the revised manuscript and addressed point-by-point below.

I have one additional remark: Arn van den Maagdenberg will be replacing me as corresponding author in all future correspondence, since I will be working elsewhere from January 1st 2013.

Yours sincerely,

Nicole Datson
We thank the Reviewers for their helpful comments and provide a point-by-point rebuttal below. Textual changes based on comments of the Reviewers are indicated by page and line number and are highlighted in yellow in the revised Manuscript.

**Reviewer's report Reviewer 1:**
This is a well written concise review of the potential role of epigenetic mechanisms in the pathogenesis and treatment of migraine. It covers an important topic in a complicated, evolving field of research.

**Minor essential revisions**
1. There should be consistency in citing references. For some reviews it is cited [reviewed in 18], for other reviews (eg references 20, 21) it is not.

We have adapted the citations throughout the Manuscript, so that all reviews are now cited as references 20 and 21.

2.1 In the section of the epigenetic effects of estrogen, it is probably known to most readers that women have much higher prevalence of migraine than men, and that female reproductive hormones likely play a role, but this should be mentioned.

2.1: We had already mentioned the increased frequency of migraine in females in the Background section of the manuscript, but have now moved this information to the section on the epigenetic effects of estrogen to make the role of estrogens more clear. This section now reads:

“Modulators of migraine attack frequency include female sex hormones, given that migraine affects 2-3 times more females than men, and the occurrence is influenced by the menstrual cycle…” [page 3, line 20-22].

2.2 Some may know that estrogen lowers the threshold for CSD, and that drops in estrogen (with menses and postpartum) precipitate attacks. Given this, the second paragraph of the section on environmental factors is unclear with regards to the example it gives in FHM mice. It is my understanding that the investigators found that, as with humans, female mice were more susceptible to spreading depression than male mice. This difference was reversed (ie less susceptible, ie increased threshold for CSD induction) if the female mice had their ovaries removed, and then partially restored by replacement of the hormone estrogen (ie. decreased threshold).

2.2: We agree with the Reviewer that besides the effects of the ovariectomy and the estrogen replacement it was also shown that female FHM mice have an increased susceptibility to CSD induction under basal conditions compared to male FHM mice. We have now added this to our manuscript [page 4, line 3-6]:

“For instance, female transgenic migraine mice carrying a pathogenic gene mutation that causes familial hemiplegic migraine (FHM) in humans [7] have an increased susceptibility for CSD induction compared to male transgenic migraine mice [8, 9]. Ovariectomy of these fema-
le migraine mice reduced the susceptibility for CSD induction, which was partially abrogated by estrogen replacement [9]. ”

2.3 In the second sentence of the second paragraph under environment factors, eliminate the word “patients” or change to humans.

2.3: We have changed the word “patients” to “humans” [page 4, line 3].

Discretionary revisions
1. In the first sentence I suggest changing from “1 to 3 day” to “4 to 72 hours”, since many attacks last less than 1 day

We have adapted this accordingly [page 2, line 13].

2. In the paragraph of early life stress, it suggest mentioning that early life stress also increases risk of developing important migraine comorbidities, such as depression, and of developing alterations in the immune system, such as inflammation, since these are discussed as separate entities in the following sections.

We have added the links between early life stress and risk of developing depression and between early life stress and alterations in the immune system in the section [page 5, line 7-10]:
“Interestingly, early life stress is a risk factor for developing depression [22], a disorder with increased comorbidity with migraine (see below), and first evidence has also associated it with alterations of the immune system [23, 24], a condition that is also associated with migraine (see below).”

3. Following the section on Genetic risk factors for migraine affecting epigenetic modifications, the concept that heritability of epigenetic changes may potentially extend transgenerationally should be mentioned, especially since genes for the common forms of migraine have been elusive, despite the fact that it is appears in most cases to be an inherited condition.

The Reviewer states that heritability of epigenetic modifications may extend transgenerationally and might explain a part of the missing heritability for migraine. This is a very interesting concept that we indeed should mention. We therefore added the following text on the subject:
“Despite large efforts GWA studies have until now explained only a fraction of the total heritability of migraine. One explanation for this so-called ‘missing heritability’ is the fact that GWA approaches are unsuited for capturing disease susceptibility DNA variants with a low allele frequency, but which are expected to have a larger effect size [55]; next generation sequencing is currently used to identify such variants. Another possible explanation could be that DNA is not the only carrier of heritable information; also epigenetic information can be transmitted across cell divisions and possibly even transgenerationally.” [Page 8, line 2-8]
Reviewer's report Reviewer 2:
This is a short review article which aims to justify the importance for future studies of epigenetic mechanisms in migraine. This topic is timely given the recent advances in the technologies available for epigenetic screen and the findings emerging in terms of complex diseases. Overall, the manuscript is interesting, well written and covers most of the relevant points in terms migraine

Major Compulsory Revision
1. In terms of the section "Epigenetics in Co-morbidity of Migraine". This section is limited to depression, which is certainly not the only recognized co-morbid condition for migraine. This section needs to be expanded to include pertinent information on other co-morbidities especially "ischaemic stroke".

We agree with the Reviewer that depression is not the only recognized co-morbid condition of migraine. We have therefore now also included information on epilepsy as disorder with bidirectional comorbidity and cardiovascular diseases including stroke and myocardial infarction. The section "Epigenetics in co-morbidities of migraine" now reads:

“Depression and epilepsy are two disorders that display bidirectional co-morbidity with migraine. Moreover, migraine is associated with an increased risk of cardiovascular disease including stroke and myocardial infarction [25]. Interestingly, depression also shares modulatory factors with migraine, such as female hormones and chronic stress, the latter of which is an established risk factor for depression [26]. A role for epigenetics has been suggested for all these co-morbid disorders of migraine, and has already been extensively reviewed [27-29]. In summary, the main proof for a role of epigenetic mechanisms in depression is evident from animal models for major depressive disorder that show large changes in epigenetic programming of stress related genes (e.g. Bdnf) that could be reversed by antidepressant treatment [30, 31]. Moreover, a recent study reported differential expression of DNMTs in peripheral white blood cells of patients with major depressive disorder and bipolar disorder, suggesting that aberrant epigenetic gene regulation may be associated with the pathophysiology of mood disorders [32]. The contribution of epigenetics in epilepsy is illustrated by the high occurrence of this disorder in Rett syndrome and alpha thalassemia mental retardation, two disorders caused by mutations in the epigenetic effector proteins MeCP2 and ATRX, respectively [33, 34]. In addition, in the brain of temporal lobe epilepsy patients increased DNA methylation was found at the promoter of Reelin [35], a gene involved in brain plasticity whose reduced expression contributes to epilepsy pathogenesis [36]. Also in blood and tissue of cardiovascular disease patients, as well as in cardiovascular disease models, aberrant DNA methylation levels were found, both globally and at cardiovascular disease associated genes [27]. Therefore, causal pathways shared between migraine and its co-morbid disorders may be modulated by epigenetic mechanisms.”

Minor Essential Comments
2. There should be at least some mention of the state of the art in molecular technologies currently available for surveying the epigenome and perhaps mention that many resear-
chers currently have large DNA-banks of migraineurs which would be amenable to epigenomic screening.

To add information on current techniques to study the epigenome, and the possibility to use these techniques to identify epigenetic factors involved in migraine using large DNA-banks of migraineurs, we have added the following text [page 8, line 8-19]:

“Recent techniques that couple array-based analysis or next generation sequencing to methods to study epigenetic marks enable genome-wide and high-throughput analysis of epigenetic marks. These techniques can analyze histone modifications (i.e. by chromatin immunoprecipitation (ChIP)) as well as DNA methylation (i.e. by bisulfite conversion of unmethylated cytosines or by immunoprecipitation of methylated DNA using antibodies (MeDIP) or methyl binding domains (MBD)) [56]. It therefore seems likely that the recently proposed epigenome-wide association studies, that can associate epigenetic marks to a trait (in addition to genetic variations found by GWAS) [57], will soon be put to use to further discover factors involved in migraine heritability. Because brain-tissue of migraine patients is hardly available, it may be feasible to use DNA banks consisting of large collections of stored DNA samples of migraineurs as a resource for identification of heritable DNA methylation marks that predispose to migraine.”