Author's response to reviews

Title: Two-stage case-control association study of dopamine-related genes and migraine

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

Thank you for the review of our manuscript entitled “Two-stage case-control association study of dopamine-related genes and migraine”. We also thank the referees and the associate editor for their comments; we appreciate the fact that, despite their different degree of criticism, they concurred in the importance of our results for those working in the field of migraine genetics.

Here follows our point-by-point response to the concerns raised by the reviewers.

1) Referee 2

• The authors have undertaken a number of SNP association analyses and after applying a false discovery rate of 10% found only 2 SNPs in the DRD2 and TH genes that showed association. Haplotype analyses of the tested SNPs in these genes were then undertaken, as was sub-group analyses for the DRD2 risk haplotype. It would be of interest to see if the original SNP associations in these two genes relate more to MA or MO sub-types. Results of analysis of these sub-types for the associated SNPs should be presented, as well as for the risk haplotypes for both markers.

We performed these subgroup analyses and found that in population 1, DRD2 rs2283265 was associated with both MO and MA, while TH rs2070762 was only associated with MO. However, as it happened when both phenotypes were analyzed together, none of these associations were replicated in population 2. This is reflected in the text of Results and in the newly included Table 1b. Tellingly, splitting the analysis trades in lower statistical power: for DRD2 rs2283265 decreased from 86.6% (MO+MA) to 75.7% (MO) and 69% (MA); for TH rs2070762 decreased from 66% (MO+MA) to 43.8% (MO) and 42.8% (MA), considering only population 1, a disease prevalence of 0.12, a significance level (α) of 0.05 and an odds ratio (OR) of 1.7. Therefore, caution should be used when interpreting the results of the migraine subgroups analyses, particularly the association of rs2070762 with MO in the first cohort of our study.

• The authors have discussed other published results for dopamine-related genes in the Discussion section of the paper. In relation to the DBH results, they note that polymorphisms in the DBH promoter have shown association in some studies but “other DBH polymorphisms were not associated with migraine [9, 26, 32, 35]”. This is a confusing statement and does not clarify or accurately present the results from other studies – in particular in Todt et al’s recently published study on dopamine-related genes in migraine with aura, the most positively associated marker for their study was a DBH non-promoter polymorphism. It is clear that the authors understand this and even discuss these results in more detail later in their discussion, but this initial inaccurate statement needs
correction.

We deleted the general statement referring to non-promoter DBH polymorphisms since we agree that is was misleading. In the new version of the manuscript, while still giving the references of previous negative studies, we emphasize Todt et al results regarding DBH rs2097629.

- Finally there are a few minor grammatical corrections needed in the paper including:
  - “It is to mention, however, that a …” correct to “It is worth mentioning however,...”
  - “Conversely, many evidences point to …” correct to “Conversely, much evidence points to…”

We appreciate the style corrections. We reworded the sentences as proposed by the reviewer.

2) Referee 1

Major Compulsory Revisions

- The manuscript of Corominas et al. report an association study for eight genes involved in the dopamine system in a migraine sample series from Spain. Their first cohort indicated a possible significant association; however replication in an independent cohort was not supportive of these findings. The SNPs associated in the first cohort were not significantly associated in a German study indicating a likely false positive nature for the association. Therefore this is in essence a negative association study, and as such over 3,500 words to describe the study are excessive.
  The authors should consider rewriting the study as a short report.

While we agree that our results can be considered altogether negative, this genetic association study was carried out following a relatively novel design and, in our view, this should suffice to claim its publication as an original, standard-length article. First, we examined a group of genes that pertain to a single transmitter pathway that has been considered relevant to migraine pathogenesis. This stands in contrast with many previous studies exploring association with a single (or few) markers in one (or few) susceptibility gene(s). Second, we followed a genetic coverage approach, thus covering both exonic and intronic regions. And third, we recruited a population which granted enough power as to analyze a range of SNPs with MAF>0.15 and included a follow-up sample. In the field of migraine genetics such a design
was rather unprecedented at the outset of the study; admittedly, a similar study in the German population (see ref. by Todt et al) was published while our work was in preparation, and a comparison between the two studies is given in the Discussion section.

- Just to highlight this excess, the statistical descriptions are an overkill, power analysis in the methods are unnecessary, and the equipment used to extract and measure the concentration of their DNA is irrelevant.

Following the referee indications we have substantially reduced or deleted most of these descriptions (see Methods). We left some abridged statistical power calculations in, since we feel that they are required to understand the rationale behind the specific step-wise analysis we elected (i.e., whole population vs. clinical subgroup analysis)

- Also the long and convoluted description of positive findings in the results and discussion is inappropriate since the association is not replicated in the second cohort.

We trimmed down many of these paragraphs. Positive findings in the first cohort are mentioned, but discussion has been shortened.
As a result of this trimming, made to meet the criticisms in this and the previous point, the word count in the revised version of the Methods and Discussion sections has diminished by 15%.

- In addition, we feel that the study is not comprehensive. The authors have attempted to cover a wide range of dopamine genes but have traded in genetic coverage for this increased number of genes. The authors have used a minor allele frequency of either 0.15 or 0.25; far over the standard 0.05 used in most studies.

As part of a parsimonious SNP-based association study design, we adjusted the MAF cut-offs to the statistical power provided by the sample. Indeed, this was already emphasized in our original manuscript (Discussion, 1st paragraph: "...SNPs with low frequencies, which would require very large sample sizes to produce significant results, were not selected"). We realize that, in the best possible scenario, with a huge number of samples, no cut-off would be necessary. However, practical issues such as sample size and budget limitations impose some constraints. Thereby, for genes with a large quantity of SNPs we even used a MAF of 0.25.
In our case and control population 1, using a MAF cut-off of 0.15 yielded a reasonable statistical power at 87.3%; using a 0.05 “standard” cut-off would have provided only a 62% power (considering, as described in the Materials & Methods section, a disease prevalence of 0.12, a significance level (α) of 0.05 and an odds ratio (OR) of 1.7). Further, some of the SNPs with MAF<0.15 are in linkage disequilibrium with each other, thus limiting the benefit of lowering the cut-off.

In addition, our design was based on the hypothesis of common disease – common variant (CDCV), classically invoked in complex diseases. We notwithstanding acknowledge that the alternative hypothesis of complex disorders arising as the result of interaction of multiple rare genetic variants (RVCD) is also plausible in migraine.

All in all, we got suboptimal coverage of three of eleven genes involved in dopaminergic transmission, but in any case our study represents one of the most complete analyses of this neurotransmission system in migraine to date.

- On top of these decreased capture of genetic information they have not attempted to re-genotyped or identify alternative SNPs for any of those that failed genotyping. In the extreme case, they did not even have a single tagging SNP in DRD4 while still presenting data on the tables and mentioning it in the text. Others like SLC6A3 had 5 tagging SNPs ungenotyped out of the 9 SNPs originally selected; this reduced the gene coverage to 0.45 according to the authors’ standards, but will be much reduced assuming a MAF of 0.05 rather than the 0.25 selected for this gene.

Rather than reporting only the results of the genes that we genotyped successfully, we have elected to openly present our design (see Supplementary Table 1, in additional file 1), including any shortcoming associated with our kick-off strategy. We agree that one downside of it was a certain reduction in the capture of genetic information in the case of a few genes. Nevertheless, in very few migraine studies has been such a high number of genes and SNPs analyzed at one time (Todt et al. 2009, Nyholt et al. 2009 and our report on serotonine-related genes, Corominas et al. 2009). The referee is right when pointing out that it is not appropriate to present data on a gene for which genotyping did fail. Other than a brief mention in Methods (to specify that DRD4 was part of the original design) and in Discussion (to make clear that DRD4 was not included in the analysis because genotyping failed), we have removed any comment related to DRD4 from the Results section.
Current association studies in complex diseases are still ranging from the classical association studies focused in a specific candidate gene, often involving a single marker, to the hypothesis-free, genome-wide association studies (none still published in the field of migraine). Our strategy of using criteria of genetic coverage and pathway-based gene analysis stands at a midpoint between them. Of course, medium or high-throughput technologies, such as the one used here, have some drawbacks, which include losing genetic coverage in some cases. Some of the “holes” in our study may deserve further scrutiny, but this will only become feasible when a bigger cohort will allow us to interrogate those SNPs with lower MAF.

Discretionary Revisions

- We suggest to the authors to reconsider this study, maybe it would be more appropriate to take the focus of out the high number or genes and analyze one or two genes comprehensively.

Further studies, focused on genes involved in monoaminergic neurotransmission, whether comprised in the present study or not, are clearly needed in migraine research. This will include genes for which we found a positive signal in our first cohort, genes insufficiently covered in our study and, of course, DRD4. But we consider this future work. Our view, anyhow, is that in the absence of definitive genome-wide association results, pathway-oriented and reasonably comprehensive analyses should provide better clues to migraine pathophysiology than examination of one or two isolated genes.

3) Associate Editor:

When providing the additional analyses regarding the migraine subtypes, as requested by the second reviewer: if a positive association with either subtype is found in the first cohort, then also analyze the subtypes in the second cohort.”

We did analyze the subtypes in the second cohort whenever a subtype-specific association was identified in the first cohort. Again, none of the significant results could be replicated. This is shown in Table 1B and stated in Results.