Author's response to reviews

Title: Two common nonsynonymous paraoxonase 1 (PON1) gene polymorphisms do not show a strong association with the risk for brain astrocytoma and meningioma

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Author's response to reviews: see over
Sirs,

Thank you for your letter and for the opportunity to resubmit the manuscript MS: 2011529362335605 entitled “Two common nonsynonymous paraoxonase 1 (PON1) polymorphisms are not related with the risk for brain astrocytoma and meningioma”.

We are thankful to reviewers and we agree with their comments. Two reviewers considered the manuscript adequate for publication as it is, but another reviewer requested additional changes. The manuscript was extensively revised and all concerns raised by the reviewers are addressed in the revised version of the manuscript. Changes are in red font in the revised version of the manuscript.

Reviewer Comments:

Reviewer #1:

Reviewer's report:

The organized presentation of the revisions is much appreciated. Most, but not all, comments were addressed. In addition, some revision to the new text is recommended.

Major compulsory revisions:

1. It remains that it is not generally accepted to present results for astrocytoma and meningioma combined into one group. The authors note that none of the other reviewers requested this change, but all are experts in other fields (none appear to have published studies focused on brain tumors). If the authors must retain any presentation of both tumor types combined, then they must provide justification (i.e. an explanation and references as to why the etiology of these two distinct tumor types, arising from completely different types of cells, might be similar).

Done

In addition, discussion of the study being powered to detect an overall OR of 2.0 should be removed. Any reference to power in relation to the combined group of tumors
overemphasizes the study’s ability to detect any departure from null (the histology-specific power calculations are far more informative).

Done

2. The Abstract and Methods state that all participants were from “the central area of Spain,” but it appears that some of the cases may have been from Badajoz (on the border with Portugal) and all of the controls were from Extremadura in the same area, whereas the remainder of cases appear to have been recruited from Madrid (in central Spain). Please clarify, and indicate in the Discussion section that it is a limitation that controls were a “convenience sample” of younger, very healthy individuals from a different city than all/most cases.

About 80% of patients and 60% of control subjects were from Madrid and the rest of patients and controls were from Extremadura (Badajoz and Cáceres). Madrid and Extremadura are very close geographically, and no genetic differences have been observed in previous studies involving individuals from Madrid and Extremadura. This is now stated in the discussion.

3. Tables 1-2 still do not indicate the reference group for the ORs, and I reiterate that the “use of both homozygous wildtype and homozygous mutant individuals combined together as the reference group for the heterozygotes’ ORs is not” defensible/meaningful for these SNPs.

The ORs in Tables have been recalculated by using the dominant, recessive and allelic models. The risk for heterozygotes was not included in the tables.

4. The authors state that failure to include PON1 activity levels “does not invalidate the findings.” This is incorrect given that their findings are a lack of association: Use of genotype as a surrogate for measurement of actual activity levels introduces substantial measurement error which would likely bias results toward null. Inability to include PON1 activity is a major limitation here.

The text has been corrected.

Underscoring this, one of the studies mentioned (Kafadar et al.2006) observed no association between adult brain tumors and PON1 genotype, but did observe an association with PON1 activity. This citation seems more relevant here than the study on stroke. Another important reference to cite in this context is a recent article first authored by one of the world’s foremost experts on PON1, which states: “Previous studies have shown that the determination of PON1 status, which reveals both PON1(192) functional genotype and serum enzyme activity
level, is required for a meaningful evaluation of PON1's role in risk of disease or exposure” (Furlong CE et al. Human PON1, a biomarker of risk of disease and exposure. Chem Biol Interact. 2010 Mar 23.)

Both references were included and commented in the discussion.

5. The authors very appropriately note that their study was sufficiently powered to detect an OR # 2.1 for astrocytoma and OR # 2.5 for meningioma. It would be important for them to further acknowledge that sporadic disease-genotype associations this strong are unlikely to be biologically plausible (Wacholder et al. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst. 2004 Mar 17;96(6):434-42.) This should be briefly noted in the Discussion, and given this and the above limitations, the paper should be retitled to be more neutral.

Done

Minor essential revisions:

1. Italicize “PON1” when referring to the gene (abstract and few locations in the text).

Done

2. Abstract (Methods): State the age range and year of diagnosis of cases. It also should be clear that this was not a population-based sample of cases. For example, something as simple as describing patients as “consecutive patients” would imply this, while conveying that they were not specially selected, a strength of this study.

Done

3. Introduction (paragraph 1): A) The statistic provided in the first sentence should only include primary brain tumors (and state it as such), as well as state that this is globally.

Done

B) I would not characterize brain tumors as being “common at all ages.”

Corrected

C) Clarify that it is “in adults” that gliomas and meningiomas are the most common histologic types; these types are very rare in children, in whom several other tumor types clearly predominate.

Done

4. Introduction: It is incomplete to say that the Q allele results “in a reduced metabolic activity.” Note that this is for some substrates only. Given the discussion of the possible
relationship between pesticides and brain tumors, perhaps mention that rodents' brains are less protected from chlorpyrifos if they have the Q isoform instead of the R isoform.

Corrected

5. Methods: Cases’ age ranges and the hospitals where they were recruited should be specified. I presume they were the same two hospitals listed in the ethics section, but please confirm/clarify.

Corrected

6. Methods (genotyping): rs1050450 is located on a different chromosome than PON1.

Thank you for the observation the text was corrected,

7. Methods (statistical analysis): “Backward logistic regression was performed for multiple comparison analysis.” It is not clear what this means, and this “method” does not seem to match the reporting of results (for example, confirming that the ORs were unchanged by inclusion of potentially confounding factors does not happen when one is eliminating variables based on p-value [backward logistic regression]).

Thank you for the observation the text was corrected,

8. Discussion: The introduction (emphasis on pesticides) and the discussion (focus on anti-oxidant properties of PON1) are now incongruous.

We do not agree. The study was based on the putative association of PON1 with brain tumors. We believe that pesticides and the anti-oxidant properties of PON1 may play a role in such putative association, as it has been proposed by several authors.

9. Discussion: It remains a limitation that the study was unable to consider PON1 C-108T genotype. This SNP has been repeatedly shown to be highly functional, it is the only PON1 SNP ever to have been associated with brain tumors, and is not in strong linkage disequilibrium with the SNPs included here.

The study focused on nonsynonymous SNPs. The C-108T genotype has been studied by other authors.

10. Minor typographical errors are present.

Discretionary Revisions:

1. Abstract (Results): Thank you for indicating that the genotype and allele frequencies were similar when comparing cases and controls. The prior sentence is somewhat redundant and
could be easily be eliminated and just provide non-significant range of p-values in parenthesis at the end of the sentence. (Please make it clear whether these are for all cases combined, or for the two histologic types separately.)

Corrected

2. Results: The long list of p-values is tedious. A suggestion (since there was little indication of association regardless) is to summarize with only one-three sentences and provide the associated range of p-values in parentheses.

We believe that it is better to give the data as detailed as possible and therefore we did not modify the text.

3. Methods and/or Discussion: Is it surprising that an unselected, consecutive group of brain tumor patients was exclusively Hispanic, given the amount of immigration to Madrid?

All patients were Caucasian, not Hispanic. We only included Caucasian Spanish subjects as stated in the manuscript. This was intended to avoid confounder factors related to ethnicity.

4. Discussion (CYP P450s): This family of enzymes is an important means of detoxification of chlorpyrifos and diazinon (not just activation to oxon forms) (“would activate” implies that that is all that they do).

Corrected

5. Discussion (paragraph 2): It is specifically (and only) PON1 Q192R that has a differential effect by substrate, not PON1 genotype in general.

Corrected

We hope that you consider the revised version of the manuscript adequate for publication in BMC Neurol.

We are looking forward to hear from you.

Yours sincerely,

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