Reviewer's report

Title: Paraoxonase 1 (PON1) polymorphisms are not related with the risk for brain astrocytoma and meningioma.

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Reviewer: Susan Searles Nielsen

Reviewer's report:

In “Paraoxonase 1 (PON1) polymorphisms are not related with the risk for brain astrocytoma and meningioma” Martinez et al. conducted a case-control study in which individuals with brain tumors were compared to healthy individuals with regard to two amino acid changing polymorphisms in the PON1 gene, Q192R and L55M. The authors appropriately build the case for examining this relationship. However, the methods section lacks important detail, and some results are not presented in accord with accepted practice (the two histologic types are combined in some analyses, and the odds ratios for heterozygotes use an inappropriate reference group). In addition, given the modest sample size and potential for biased odds ratios (described below), the authors overemphasize the lack of association. Nonetheless, these can be addressed, and (in as much as bias is not present) the authors’ conclusion remains meaningful, because the ORs are quite close to null for both tumor types, i.e. the lack of association is not simply a matter of statistical non-significance.

As to whether this study contributes to the body of literature, its impact is modest because of its small size and prior similar published results. It would add substantially more if the C-108T SNP in PON1 could also be addressed, because it is strongly indicative of PON1 levels, yet has not been considered previously in an adult brain tumor study (and would complement the study that observed differences in PON1 levels based on post-diagnosis blood). (PON1 activity or levels cannot be assessed directly here because blood was preserved by EDTA.)

Major compulsory revisions:

1. Are all cases Caucasian Spanish as were controls? Cases of any other race or ethnicity should be excluded from all analyses because PON1 genotype differs by race and ethnicity.

2. Controls are younger than cases, and by design controls are largely college-educated. It is possible that genotype proportions differ by age (change over time), or even by education (this has been observed in non-Spanish Caucasians), so both age and education should be considered as potential confounders. Adjust for these in the comparisons, or state that adjustment made no difference in odds ratios. Examination of statistical significance or use of backward elimination is not sufficient to address whether confounding affected odds ratios.
3. How comparable are controls to cases? Specifically: A) Were any exclusions made of “healthy” controls that may not have been made of cases, either outright (e.g. PON1-related conditions like heart disease) or built into the control selection method (healthy enough to be enrolled in college or employed as a professor)? B) Were cases from the same region(s) as controls? This would mainly be an issue if PON1 genotype likely differed between regions of Spain (e.g. between Madrid and Badajoz), or between Spain and Portugal.

4. Remove all results, including Table 1, in which the two very distinct tumor types are combined.

5. For the genotype ORs, a non-linear model is imposed even though based on their known function, these polymorphisms would likely act in a linear fashion. Dominant or recessive models (as is assumed for the homozygous ORs) might possibly be defensible. However, use of both homozygous wildtype and homozygous mutant individuals combined together as the reference group for the heterozygotes’ ORs is not. (Although the reference group is not shown or stated as is customary [please include], one can recreate the ORs presented and determine that the reference group for the 55 Leu/Met individuals is the Leu/Leu and Met/Met homozygotes combined together; and likewise for Q192R.)


Minor essential revisions:

1. Abstract (Background): The second sentence is “methods” but is very similar to what is already stated there.

2. Abstract (Methods): More detail is required. Are these adults only, or were some children included? Where were the cases identified? Where were the controls obtained? What is the race and ethnicity of cases and controls? When were cases diagnosed?

3. Abstract (Results): Because of the small sample size, it is uninformative to simply state there were no statistically significant differences. Therefore, also indicate that the genotype frequencies were similar when comparing cases and controls.

4. Abstract (Conclusions): Only two PON1 SNPs were investigated, so the authors cannot conclude that “PON1 polymorphisms” are not related with brain tumors. (The authors could indicate that “common amino acid changing polymorphisms in PON1” are not.)

5. Use the accepted format of the polymorphism names (Q192R and L55M).

6. Introduction (paragraph 3): A) it would be more accurate to state that “some insecticides” rather than “pesticides” (much broader) are metabolized by PON1; and B) “PON1” is incorrectly italicized when referring to the PON1 protein (italics only apply to the PON1 gene).
7. Introduction: The description of the functional effect of Q192R is not as relevant as a discussion of the effect of this amino acid change on chlorpyrifos and diazinon, and preferably in vivo not in vitro (PON1 is not important to the in vivo disposition of parathion [paraoxon]). Also, it would be helpful to indicate whether the L55M has any known functional effect due to the amino acid change. (See Table 1 in Searles Nielsen et al. 2010, cited below.)

8. Methods: Cases need to be described with much greater detail. Where were they identified? What does “unselected” mean – were there any exclusions due to not agreeing to participate, too sick to provide a blood sample, already deceased (survival may be associated with genotype), etc.? When were cases diagnosed? Where did they originate from – were they all living in the region of Badajoz as were the controls? What years were they diagnosed? How was the diagnosis verified? What was the age range (to indicate whether any children or elderly individuals were included)? What was their race and ethnicity (all should be Caucasian Spanish like controls)?

9. Methods: Controls and their recruitment need to be described in greater detail so the reader can determine whether they represent a reasonable comparison group. (See all comments in major comment #3).

10. Discussion (paragraph 2): “Primitive [not primary] neuroectodermal tumors”. Note also that the cited study of childhood brain tumors has been expanded to include more children and more PON1 polymorphisms (Searles Nielsen S, McKean-Cowdin R, Farin FM, Holly EA, Preston-Martin S, Mueller BA. Childhood brain tumors, residential insecticide exposure, and pesticide metabolism genes. Environ Health Perspect. 2010 Jan;118(1):144-9). Because these analyses were in children, they need not be described in detail. Comparison of the present work should mainly focus on prior studies in adults.

11. Discussion: The potential for bias based on the manner in which cases were identified/included, and controls were obtained, should be addressed, at least briefly. (See major comments 1-3, above.)

12. Discussion: In the absence of exposure to chlorpyrifos and diazinon, one would expect no association between PON1 genotype and brain tumors. Therefore it would be useful to mention the extent to which cases and controls were likely exposed to these agents, either occupationally, residentially or via diet. It is a limitation that the study was unable to consider this, as the above study in children observed an interaction despite there being (as in the present work) no difference in genotype overall.

13. Discussion (last paragraph): Removed reference to statistical power to observe an OR # 1.8 for an association with both tumor types combined. Presentation of the subtype-specific power calculations is a strength and nicely included here. Those in the Methods section could be deleted.

14. As noted earlier, it would be ideal if additional PON1 SNPs could be included; without this, an inability to include other functional PON1 SNPs and/or PON1 activity measurements should be stated as a limitation.

15. A few minor typographical errors are present.
Discretionary Revisions:

1. Given the small sample size, the title is rather strongly worded.
2. Generally the gene, not the enzyme product, is “polymorphic” (see abstract and paragraph 3 of the introduction).
3. The “odds ratios” for the alleles are not necessary. Further, they are essentially presented in duplicate (the OR for 55 Met is simply the inverse of the OR presented right above it for 55 Leu; and likewise for Q192R).
4. Bonferroni correction seems unnecessary since so few comparisons were made in these analyses, especially since few/none were likely statistically significant even without the correction.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.