Reviewer's report

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

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Reviewer: Birsen Can Demirdogen

Reviewer's report:

Major Compulsory Revisions:
The authors have determined PON1 192Q/R and 55L/M genotypes of patients with brain tumors and respective controls.

1) The question posed by the authors is not well defined. The authors presumably try to associate slow metabolism of organophosphorous compounds by polymorphic forms of PON1 with brain tumors. However, this link is rather weak and cannot be generalized for all of the currently used organophosphates (OPs).

There are several OPs such as chlorpyrifos, diazinon, fenitrothion and primiphos-methyl. These compounds are substrates for PON1 and there are polymorphisms in PON1 gene that affect enzymatic activity. Although not clearly put forward, the hypothesis implied by the authors makes sense until here. However, the problem is, rate of metabolism of organophosphorous pesticides by a given PON1 isoform, thus the extent of protection against a given OP changes with the specific compound.

In a study carried out by Li et al. 2000 (Li et al. Pharmacogenetics 2000;10:1-13), either human PON1 192Q or 192R isoform was injected intravenously into PON1 knockout mice and the effects of OPs on brain and diaphragm AChE were determined. In the case of chlorpyrifos oxon, both isoforms were protective, and PON1 192R offered about 50% better protection than PON1 192Q. Both PON1 192R and 192Q offered equal protection against diazoxon, and neither human PON1 isoform protected against the toxicity of paraoxon, extending the surprising findings described above. The results from the kinetic analysis of substrate hydrolysis by purified human PON1192 isoforms provide an explanation for such findings. Although the PON1R192 isoform is eight times more efficient than the PON1Q192 isoform in hydrolyzing paraoxon (Km/Vm D 6.27 versus Km/Vm D 0.71), neither isoform hydrolyzes paraoxon as efficiently as diazoxon or chlorpyrifos oxon (Li et al. 2000).

The hypothesis would be allright if a given PON1 isoform protected against all of the currently used OPs to the same extent or only one OP compound was in use. Thus, it is difficult to determine which PON1 isoform represents a risk factor for the development of brain tumors. The authors did not mention which isoform is expected to be the risky one and why. They did not discuss the differential
activity of PON1 genotypes towards different OP substrates.

2) Authors have determined only the genetic polymorphisms of the subjects and did not measure PON1 activities and/or concentrations. However, genetic polymorphisms are not the sole determinants of PON1 activity; it was reported that paraoxonase activities can vary by 13 fold within a given genotype (Jarvik et al. Arterioscler. Thromb. Vasc. Biol. 2000;20;2441-2447; Richter R, Furlong CE. Pharmacogenetics. 1999;9:745–753; Brophy et al. Pharmacogenetics. 2000;10:453–460). Several factors affect PON1 activity, such as physiological and pathological states, dietary and lifestyle factors and environmental chemicals. In the study of Jarvik et al 2000, no genotype effect was detectable unless activity phenotype was also considered. Therefore, it was strongly suggested that PON1 activities and/or concentration should be determined together with PON1 genotypes for correlation with disease susceptibility (Mackness et al. Arterioscler Thromb Vasc Biol 2001;21(9):1451–7; Draganov et al. Naunyn-Schmiedeberg's Arch Pharmacol 2004;369(1):78–88).

The authors state in the discussion that: “PON1 should hypothetically act as a detoxifying enzyme at this level, causing the hydrolysis of the acetylcholinesterase-inhibiting oxons (activated intermediates) of some organophosphorus compounds [29], decreasing the possible arrive of these compounds to the brain”. Studying only the genetic polymorphisms of PON1 is not enough to reject this hypothesis.

Even the authors themselves touched this point in the discussion, when they cited the study by Kafadar et al. who found decreased PON1 activity in brain tumor patients compared to controls, but couldn’t find an association between PON1 192Q/R genotypes and brain tumors.

Measuring PON1 activity of patients and controls towards several OP substrates would give valuable tools to determine subjects susceptible to OP poisoning. Then, an association between brain tumors and susceptibility to OP compounds could be sought.

Authors should have discussed the reasons for not employing PON1 activity and/or concentration determinations and make it clear that they are already aware of the limitations of such a genetic association study.

3) No information is given on any incidence of exposure of the patients and controls and/or their parents to organophosphorous compounds. As discussed by the authors, in the study by Searles-Nielsen et al. there was a significant association between PON1 -108T allele and exposure to pesticides.

Recruiting brain tumor patients from agricultural workers or people who claim to be exposed to pesticides would have been more informative as to the role of PON1 in formation of brain tumors.

4) In the abstract, it is not put forward clearly why the authors decided to study PON1 polymorphisms in brain tumors. In discussion section, the background information supplying the link between xenobiotics (including OPs, environmental pollutants and drugs) and brain tumors is missing.
5) I think a table stating the demographic characteristics and some laboratory data of patients and controls should have been given.

Minor Essential Revisions:

There are several errors in the language of the manuscript. Some of the mistakes are listed below:

• At page 5, the word “of” (given in red) should be added to the following sentence:

For every polymorphism tested, genomic DNA of twenty individuals carrying no mutations, twenty heterozygotes and twenty homozygotes for rs1050450 were analyzed...

• At page 5 the word “text” should be corrected as “test” in the following sentence:

The intergroup comparison values and the significance of the gene-dose effect were calculated by using the chi-square test or the Fisher’s exact test text.

• At page 6, the word “on” should be removed from the following sentence:

To our knowledge, only two previous reports addressed on the possible role of PON1 polymorphisms in the risk for brain tumors...

• At page 6, the following sentence should be corrected for grammar:

In the present study, we found not significant differences neither in the frequencies of PON1-55 and PON1-192 genotypes, nor in the frequencies of the allelic variants of these polymorphisms in patients with meningioma and grade II/III astrocytoma when compared with healthy controls

Level of interest: An article of limited interest

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

Statistical review: No, the manuscript does not need to be seen by a statistician.