Author's response to reviews

Title: Polymorphisms in xenobiotic metabolizing genes (EPHX1, NQO1 and PON1) in lymphoma susceptibility: a case control study

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Version: 3 Date: 19 March 2013

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Version: 2 Date: 19 March 2013

Author's response to reviews: see over

Reviewer: Kathryn Barry
Reviewer's report:

Major Compulsory Revisions
1. The authors state that an aim of the paper was to compare the genotype distribution in non-exposed and exposed subjects and they mention the
possibility of genotype selection in the exposed population (see 5th paragraph of
the Discussion section). However, it is unclear why the genotype distribution
would be related to exposure. Please elaborate.

AR: This aim concerning the exposure has been deleted from the manuscript
following reviewer 3 suggestion and therefore now there is no need for such
description.

2. Please add a description of the results for all genetic models evaluated. For
example, there should be some mention of the results with the dominant genetic
model, even if non-significant. The methods section should also mention the
various genetic models considered.

AR: Dominant and co-dominant models have now been commented in Methods
and Results.

3. The study conclusion in the abstract that “this study demonstrates that the
PON1 GG genotype is a risk factor for B-cell lymphomas…” is overly strong
given the relative small size of the study compared to genome-wide association
studies and the need for replication. The authors mention the need for replication
in relation to the finding of a possible association with differential carcinogen
exposure, but should also emphasize this for the overall association of the PON1
GG genotype with lymphoma outcomes.

AR: The conclusion has been modified accordingly by saying “This study
indicates that the PON1 GG genotype could be a risk factor for B-cell lymphomas,
although confirmation in larger and independent series is needed”

Discretionary Revisions

1. Recommend removing discussion/references for cancer outcomes other than
lymphoma because the association for a particular SNP with cancer risk could
potentially vary by the type of cancer and therefore findings for other cancers
than lymphoma may not be relevant to the current paper.

AR: We have deleted some references on non-lymphoid tumors. However, in
order to accomplish with previous reviewer’s requirements we have kept some
references needed to justify that the SNPs studied have also reported no
association with cancer.

2. In the Discussion section (paragraph 5), the authors state the limited sample
size did not allow testing of gene-environment interactions and that “in order to
take into account the possible environmental influence we stratified both cases
and controls according to exposure,” which is essentially the same idea. Instead
of stating that testing of gene-environment interactions was not possible, suggest
re-wording along the lines that the authors attempted to account for the possible
influence of environmental exposures by stratifying according to exposure, but
the analysis was limited by small numbers.
AR: This part of the study has been deleted from the manuscript following reviewer 3 suggestion.

3. Did participation rates or any other factors differ between the cases included from the previous study and the cases diagnosed in 2011? Also, it would be helpful if the Methods section could include the specific numbers of participants drawn from different sources.
AR: The part of the questionnaire has been deleted from the manuscript following reviewer 3 suggestion. The only differences in cases from 2011 compared to prior ones is that all belonged to the HUSMR institution, which had previously provided most of the cases included in the study. This information is now included in the Methods.

Minor Revisions
1. There are some grammatical issues, for example a fragment in paragraph 2 of the Background section: "And, in particular...".
AR: This paragraph has been deleted from the manuscript. English language in the manuscript has now been revised by a native English.

2. In the Methods section, suggest writing out the title for the WHO document and defining WHO.
AR: The reference to the WHO book is now detailed

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: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer: Pierluigi Cocco
Reviewer’s report:
The revised version of this paper matches some points this reviewer raised on the original submission, but not the major point, which was the inadequacy of the study design to pursue the aim of assessing the interaction between polymorphisms in metabolic genes and occupational and environmental exposures in affecting risk of lymphoma. As stressed in the previous review, selection bias from the source of the controls (all were blood donors, and
therefore not an unbiased sample of the general population) makes results based on the questionnaire information invalid. Another non amendable problem is the extremely generic type of information that was collected and analyzed. The conclusion is therefore unwarranted that subjects carrying the PON1 GG genotype would be at greater risk of lymphoma if residing in proximity of an industrial area, where, based on the list of the plants reported in the Methods section, there is no indication of a specific pollution from organophosphate insecticides, the major substratum of that gene.

From this revised version, we learn that the source of cases was heterogeneous, which might also have lead to changes in the geographical distribution of their residences in comparison to the blood donors, possibly resulting in an artifactual concentration of the observed excess of the PON1 GG genotype among the residents in proximity to the industrial area. Besides, source heterogeneity, in space and time, was apparently the reason for the low proportion of questionnaires available for the cases, which would make it difficult to generalize the results to all lymphomas (the old follicular lymphoma cases were all presumably excluded, although the authors are not explicit about this). Major compulsory revision

I would suggest the authors to concentrate their report on the metabolic gene polymorphisms, which are of interest per se, and to drop the questionnaire part of their paper, which would have required a specific study design instead of a post hoc assembly of generic data from heterogeneous sources. Following this reviewer’s suggestion, they might make full use of the whole study population, presenting the analysis by gender; a subgroup analysis by smoking might also still be acceptable, and the conclusion of the association of polymorphisms of genes implicated in xenobiotic metabolism on lymphoma risk still relevant, as the explored genes would not affect risk if not through the metabolic changes they induce in their substrata.

AR: Following the reviewer suggestion all data related to exposure assessment as well as the questionnaire part have been removed from the manuscript.

Minor essential revisions
1. Refer to the 2008 WHO classification of Lymphoma, and not to the “WHO book”.

AR: This issue has now been corrected.

2. The Bonferroni correction would not be necessary; the sentence “This association was restricted to males \( p = 0.008 \), but not present in females \( p = 0.05 \) “ is unjustified. Risk is elevated in both genders, and a formal test of heterogeneity would most likely suggest their consistency.

AR: Following the reviewer’s suggestion Bonferroni correction have been deleted from the manuscript and, accordingly, the sentence has now been rephrased as
follows: “This association was more evident in males (p= 0.008), than in females (p= 0.05)”

3. In the discussion, please describe honestly the source of cases and controls as a limitation.

AR: The following sentence has been introduced in order to deal with this issue “Although the study subjects and controls share the same geographical origin, they present some differentiating factors that are worthy of consideration. First, since the controls are blood donors, they are not exactly representative of the general population. Second, a significant degree of heterogeneity was present in the cases, since they presented various types of lymphoma that may be caused by different etiological factors.”

4. Please, do not tediously repeat the percentage frequency of each genotype in the text; briefly refer to what reported in the tables.

AR: Some percentage frequencies have been removed from the results.

5. This reviewer understands that another peer suggested to explore the gene-gene interaction, but what was the rationale for the “haplotype analysis” needs to be explained in the discussion. Was there any published report suggesting LD between the polymorphisms investigated in this paper? Also, a haplotype is defined is a combination of alleles at adjacent locations on the same chromosome that are transmitted together, or are statistically associated. The SNPS herein investigated are located in chromosome 1, 7, and 16; therefore, the authors should use the term “gene-gene interaction” to define the analysis they conducted.

AR: There are no references to LD for the chromosomal locations where the SNPs are. In fact, we did not detect LD amongst the studied SNPs. The rationale of performing the genotype combined analysis is that, under a mixture of different pollutants, the contribution of polymorphisms in different metabolizing enzyme could exert an additive effect in disease susceptibility than can only be identified considering them in combination. We agree with the reviewer that the use of haplotype for this purpose is confusing and thus it has been deleted and substituted by “genetic profile”. The following sentence has been added in the discussion:

“The rationale for performing the genetic profile analysis of the studied SNPs is that, under a mixture of different pollutants, the contribution of polymorphisms in different metabolizing enzymes could exert an additive effect in disease susceptibility which can only be identified considering the SNPs in combination”

6. Have the text revised by a native English expert in scientific language.

AR: English language in the manuscript has now been revised by a native English.

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.
Declaration of competing interests:
I declare that I have no competing interests