Author's response to reviews

Title: RAS mutations in early age leukaemia modulated by NQO1 rs1800566 (C609T) are associated with second-hand smoking exposures

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Major Compulsory Revisions

Q) The outcome of ALL patients <13 months has not be presented. The authors should add these data or explain why they cannot be added.

A- The data are added to the Figure 1.supl and results discussed in the text.

2) The frequency association between RAS mutations and NQ01 genotype should be presented. This is especially important given the data presented in Table 5 regarding the effect of smoking on the development of leukaemia in cases with NQ01 1609 CT/TT and a RAS mutation.

A- The results are presented in Supplementary Table 5 and described in the text.

3) In tables 4, 5 and 6 and in the text the authors need to clarify exactly how the NQ01 genotype status has been analyzed. I assume it is always CC v CT/TT. However, this is not clear.

A- The EAL potential risk association with NQO1C609T genotypes were previously investigate in a case-control study (ref 21), in which, a group of selected children without malignancies, from the same regions as the cases were age-matched. The NQO1 status was considered as at least one T allele variant for statistical analysis shown in the Tables notes (2,3,4) and supplemental table 6.

4) The authors should show the data referred to in the following sentence: “… and no association between maternal smoking and ALL or AML was found data not shown).”

A- The percentage of mothers who related smoking three months previous or during the index pregnancy (29.8% and 21.0%, respectively) were not
significantly significant associated with the risk of having offspring with EAL (OR, 1.84, 95%CI, 0.78-4.36 and OR, 1.53, 95% CI, 0.59-4.01, respectively).

5) In addition, the authors should show the association of smoking with MLL-r, RAS mutations and NQ01 genotype. The latter two are presented in Table 5 in combination, they should also be presented separately.

A- ok. The additional results are presented in table 2 and discussed in the text.

6) The final multivariate models should be presented in full.

A-OK. Following previous studies exploring the association of different variables with the occurrence of paediatric leukaemia (as in ref 17), a multivariable log-linear model was built including the variables RAS mutation, NQ01 status, presence of MLL-r and presence of smoker within the household (SMOKER) and two-by-two interaction terms between them. The Variables included in the model and the Full log-linear model descriptions are included in the table 4.

Full log-linear model: Log(count) = a + b1(Var1) + ... + b4(Var4) + c1 (Var1 * Var2) + c2 (Var1 * Var3) + c3(Var1 * Var4) + d1(Var2 * Var3) + d2(Var2 * Var4) + e1(Var3 * Var4).

7) I find the results perplexing. Does the variable “someone in the house ever smoked” include the mother themselves? Or is it “other” smokers. Either way I find it puzzling how these “other smokers” can be having such a large effect; given that there is no discernible effect at all for the mother herself smoking and the risk of NQ01609CT/TT and/or RAS mutated leukaemia. In fact there is almost a negative correlation. It is counterintuitive that “first” hand smoke has no effect whereas second hand smoke does. This apparent paradox needs to be acknowledged and discussed.

A- The variable "someone in the house ever smoked", but mothers, is characterized by relatives such as husband, grandparents and/or nanny "smokers" living in the house and taking care of the child. Yes, it is puzzling. However, the interpretation for this counter intuitiveness [first" hand smoke has no effect whereas second hand smoke does] could be also considered (or implied) that some mothers might had denied being a smokers due to guilty. This is one pitfall of epidemiological studies based on questionnaire responses. Censured topics and variables such drug and/or tobacco users values are depending upon the commitment of different actors under social pressures. However, the biologic plausibility for this findings is that cigar metabolites compounds carcinogens’ substances such phenols, toluene and others that are spread in the air by the smokers, and the secon.

8) Given the above paradox, further details of the “smoking” questions in the questionnaire should be provided; along with details of exactly how the variables are constructed.

A- Added in the methodology...

Data collection was gathered through face-to-face interview with case and control mothers, after signing a written informed consent. The questionnaire contain
inquires about, parental educational levels and family's socioeconomic background, mother and child health antecedents, medicines use, smoking, alcohol consumption, and maternal occupational history during pregnancy. Regarding the affirmativeness of maternal smoking antecedents and/or other person living at home, additional information were included, such as, the usual amount of daily smoked cigarettes during preconception, pregnancy and breastfeeding. Usual smoking frequency at these time windows were also collected as: no primary hand smokers; moderate smokers (less than 20 smoked cigarettes per day); and heavy smokers (20 or more smoked cigarettes per day).

9) The conclusion states that “The present data suggest that second-hand tobacco smoking exposures are associated with increased risk of EAL with MLL-r and RASmut modulated by NQO1 rs1800566 (C609T).” However, the authors do not present smoking data with respect to MLL-r status.
A-Yes, in the reviewed analysis the conclusion statement has been changed to: The present data demonstrated the increased risk association between maternal smoking and EAL with MLL-r. Additionally, suggests that second-hand tobacco smoking exposures are associated with increased risk of EAL with RASmut modulated by NQO1 rs1800566 (C609T).

Minor Essential Revisions
1) There is a typo “the?” in the second sentence of the second paragraph in the introduction.
A- ok

2) In the last paragraph of the results the authors refer to the “…risk of developing leukaemia…”. This is incorrect as all the cases have leukaemia. They should rephrase emphasizing that they are measuring an association between smoking and the presence of selected genetic characteristics.
A-OK

Discretionary Revisions
1) Table 2, 3 and 4 should be in the supplementary information.
A-OK

2) There are five major variables considered in this paper: ALL/AML, MLL-R, RAS mutation, NQ01 genotype and smoking. Hence the results are complicated and can be difficult to follow. I would suggest that the authors consider if all these variables are required. For example, do they need to separate ALL and AML in this context? The key results seem to be very similar. In addition, the division of cases by MLL status does not appear to add to the central message. The paper would be clearer if it were more focused.
A- We agreed and we have gathered ALL and AML in the NQO1 genotype and RAS analysis.

3) As FLT3 and BRAF mutations also result in deregulation of the MAPK
pathway, one could argue that these cases could be combined with the RAS mutations to form a “RAS pathway mutated” group.

A-OK.

To Reviewer 2-Wenlei Zhuo

Q-Why didn’t the authors adopt case-control as the research design?
Were the controls unavailable in their district?

A- Because RAS mutations and MLL rearrangements are of acquired mutations, case-control design is not appropriated. Actually the case-control design was applied previously in these cases, to test the smoker effect in ref 22. As well as, the same approach (case-control study) was done for NQO1 genotyping and described in ref 21 NQO1 rs1800566 (C609T), PON1 rs662 (Q192R), and PON1 rs854560 (L55M) polymorphisms segregate the risk of childhood acute leukemias according to age range distribution. Cancer Causes Control 2012, 23(11):1811-1819).

Q- the parental occupational exposures were not considered and assessed in the study. Could the authors obtain relevant information from the questionnaire survey?

A- We are agreed. This information is under considerations, although the final results not available for this manuscript.