

Author's response to reviews

Title: Influence of Genetic factors on Toluene diisocyanate-related symptoms

Authors:

Karin Broberg (karin.broberg@med.lu.se)
Hakan Tinnerberg (hakan.tinnerberg@med.lu.se)
Anna Axmon (anna.axmon@med.lu.se)
Margareta Warholm (margareta.warholm@kemi.se)
Agneta Rannug (agneta.rannug@ki.se)
Margareta Littorin (margareta.littorin@med.lu.se)

Version: 3 **Date:** 15 April 2008

Author's response to reviews: see over

Title: Influence of Genetic factors on Toluene diisocyanate-related symptoms

Thank you for the opportunity to revise our manuscript according to the reviewer's and editorial comments.

Please, find our comments below.

Sincerely yours,

Anna Axmon, Karin Broberg, Margareta Littorin, Margareta Warholm, Agneta Rannug, and Håkan Tinnerberg

Reviewer: Piero Maestrelli

Reviewer's report:

The authors clarified most of the issues raised in the review and modified the manuscript accordingly. There are few minor points to consider.

Minor Essential Revisions

1. Statistical methods, pag 11, 2nd sentence: It should probably clearly state that exposed and non exposed groups were analysed separately, avoiding to mention stratification.

1. We have now altered the sentence to: "The genetic influence on the symptoms was evaluated by comparing different genotypes among exposed and non-exposed separately in logistic regression analysis."

2. Pag 8-last para, Legend to table 1: It is still not clear to me the meaning of the sentence. All the genetic analyses were performed in far more than 7 subjects.

2. We have now altered this sentence on page 8 as well as in the legend to table 1: "Moreover, all 246 individuals approved analysis of metabolizing genes, whereas 239 individuals approved analysis of immune-related genes."

Reviewer: Ari Hirvonen

Reviewer's report:

Major Compulsory Revisions

1) I still believe that the adjusted data should be presented rather than the unadjusted and suggest that this correction is made and the text modified accordingly.

1. We are now presenting the adjusted data in table 2 and refer to the unadjusted data when they affect the significance level in the text. We adjusted for sex, age, smoking and atopy and due to missing information, 112 non-exposed and 128 exposed workers could be included in the adjusted analysis compared to 114 non-exposed and 132 exposed workers in the unadjusted analyses (statistical section page 11).

2) The justification given for not studying any gene-gene interactions is quite confusing and partly hard to understand. For instance, how can the authors know beforehand that there will not be any strong gene-gene interactions between the studied genes? Undeniably the numbers are rather small for these interaction analyses but on the other hand the authors report results on NAT1 with only 0 and 3 slow acetylators in symptomatic and non-symptomatic subjects among exposed workers, and 2 and 1 slow acetylators in symptomatic and non-symptomatic subjects among unexposed workers, respectively. These numbers certainly are far too small for any reliable interpretations, as discussed further below. In any case, I still think that the potential gene-gene interaction analyses should be performed and the outcomes reported, whether or not anything significant was found. In case of no meaningful findings there of course is no need to show the data but a note that these analyses were done with no significant findings is sufficient.

2. It could be argued that for performance of gene-gene interaction analysis, all possible gene-gene combinations should be evaluated. However, we believe that this type of analysis is not justified for this size of study. We have therefore performed gene-gene interaction analysis limited to the analysis to genotypes that were significantly associated with symptoms in the single gene analysis. For most combinations, there were statistically significant associations between gene-gene combinations and symptoms. However, the results did not differ much from the results in the single gene analysis and there was no evidence for departure from an additive effect. The exception was the results from the combination of *GSTM1* and *GSTP1* for cough with mucus among non-exposed workers. The results are presented on page 15 and are discussed in the Discussion on page 19.

3) As requested, the results of NAT1 putative slow acetylator genotypes vs. dry cough have now been included in Table 2. However, as discussed above, this unfortunately revealed that these results based on far too small numbers of the slow acetylators for any meaningful interpretations. This should be properly discussed. The authors may also consider removing these data from the results.

It is true that there are very few individuals with the NAT1 slow acetylator genotype, and we therefore excluded those results in the first version, but added them to the second version due to the comments of the reviewer. However, we believe that the results for *NAT1* slow are very uncertain and we have now removed them from the analysis. We have also added a comment on this in the discussion (page 17): “For the lower airways, an association between *NAT1* slow genotype and increased risk for dry cough was found (data not shown), but it was only among non-exposed and there were no symptomatic exposed individuals with slow genotype, making it very difficult to evaluate the effect of this genotype. “

4. The fact that different NAT1 genotypes were compared to each other in the case of eye symptoms (IM vs R) and dry cough (IM+R vs S) is also very confusing. Further confusion causes that in the statistical methods section the IM and S genotypes were said to be combined in the case of comparison with R but based on the above this was not done in the case of eye symptoms. These discrepancies need to be properly explained.

4. We made a mistake in table 2. For eye symptoms there should be IM+S vs R as stated in the statistical methods. This has now been corrected. We have now removed the results for *NAT1* slow (as explained above) from the table and the result section, but we make a comment about *NAT1* slow in the discussion (page 17).

Minor Essential Revisions

1) The p-values should be given for the significant differences in Table 1, both in the footnote of the table and in the text.

1). The p-values have now been added to the footnote of the table and the result section.

2) The corrections made in the abstract are somewhat slopily made and the following linguistic revisions are needed: i) give the missing p-value for interaction for the dry cough in the last line of abstract in page 2; ii) correct to read "statistical" in line 2 in page 3; iii) correct to read "symptoms than NAT1" in line 4 in page 3.

2) i) The p-value for interaction for dry cough is included; ii) this has now been corrected; iii) since we now have removed the results for NAT1, we have altered the sentence to: Other polymorphisms, showed associations to certain symptoms: among TDI-exposed, *NAT1**10 carriers had a higher risk of eye symptoms and *CCL5* -403 AG+AA as well as *HLA-DQB1* *05 carriers displayed increased risk of symptoms of the lower airways.

3) The numerous misspellings in page 19 concerning the citations on reference 12 have not been corrected although the authors claim to have done so. Once more, correct the citations in lines 2, 6, 7 and 9 in page 19 to read "Piirilä et al."

3) The misspellings have been corrected.

.

Editorial comments.

1. In addition, we have some editorial comments that we ask you to respond to. The abstract and the main text frequently uses the term 'statistically significant'. We would argue that, in a study like yours, attention must be paid to meaningful associations, even if they are not significant, while at the same time being aware of multiple comparison problems. We believe that confidence intervals are preferable to significance categories, although they may be substituted by p values for the sake of brevity. Please update the statistics section on p. 11 and the main text in this regard.

1. We agree with the editor that only focusing on the p-value is not very meaningful, in particular, when a large number of comparisons are being performed. We have in this study tried to consider patterns of associations between genetic markers and different symptoms and present those data although some are not statistically significant. For genetic markers with no obvious pattern of associations, we have due to space limitations chosen only to present the significant values and tried to be modest in the interpretation of the findings.

On page 11, we have tried to explain our approach as follows: "The data were analysed for patterns of associations between genetic markers and different symptoms and such patterns were

presented, although some effect estimates were not statistically significant. For genetic markers with no obvious pattern of associations, we have due to space limitations chosen only to present the significant effect estimates.” We also explain that the statistical significance refers to “ $P < 0.05$ (two-tailed) or, equivalently, to 95% CI for an OR that excludes 1.0” (statistical section page 11).

We hope that this information will be sufficient for the reader. However, if necessary, we will include the p-values for all significant findings.

2. In particular, the title should indicate study design (cross-sectional study). We suggest the following title: Influence of genetic factors on toluene diisocyanate-related symptoms: evidence from a cross-sectional study

3. Pluralize “Conclusion” (Conclusions) in the abstract. We have followed the suggestion by the editor.

4. Pg 3, first sentence, remove period. Period has been removed.

5. Rename “Introduction” as Background. We have followed the suggestion by the editor.

6. Under “subjects”, the second sentence requires modification (unclear), suggestion: “Out of a total 198 employees from five different facilities in southern Sweden, a group consisting of 114 workers, none/not occupationally exposed to isocyanates or performing any plastic or rubber work, was included as well?”. The suggested sentence has now been included in the manuscript.

7. On p. 7, second sentence, the verb should be 'has'. The verb has been changed.

8. Include “Conclusions” as section title in the text (currently only in abstract). We prefer not to include a separate “conclusion” section in the discussion.

9. In references, volume numbers should be bolded. The volume numbers have been changed.

10. Reference 20 is missing information (also check format-unclear which is being used). Use of “et al” is not appropriate (Ref 25). Reference 20 has been updated Reference 25 has now been corrected.

11. Rename “Figure Labels” as “Figure Legends”. We have followed the suggestion by the editor.