Author’s response to reviews

Title: Prevalence of at-risk genotypes for genotoxic effects decreases with age in a randomly selected population in Flanders: a cross sectional study.

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Author’s response to reviews:

Oland, 21/7/2011

To Prof. Philippe Grandjean and Prof. David Ozonoff, Editors in Chief of Environmental Health.

Dear Colleagues,

Herewith I present, for publication in your journal, our revised manuscript "Prevalence of at-risk genotypes for genotoxic effects decreases with age in a randomly selected population in Flanders: a cross sectional study." by Hans B. Ketelslegers, Roger W.L. Godschalk, Ralph W.H. Gottschalk, Ad M. Knaapen, Gudrun Koppen, Greet Schoeters, Willy F. Baeyens, Vera Nelen, Joep P.M. Geraedts, Joost H.M. van Delft, Jos C.S. Kleinjans and Nicolas A. van Larebeke.

It is an original research article based on the Flemish human biomonitoring program, has not been previously published and is not under consideration for publication elsewhere.

Participation of human subjects did not occur until after informed consent was obtained.

The author(s) declare that they have no competing interests.

All authors have read the manuscript, agree that the work is ready for submission to a journal and accept responsibility for the manuscript's contents.

In my opinion the manuscript is well suited for publication in Environmental
Health because it brings some evidence suggesting that persons, carrying a higher number of at risk alleles in genes involved in the protection against genotoxic substances, are less likely to reach age 50 to 65, this in sufficient health to be able to participate in a biomonitoring study. Our study, performed in Flanders, a typical densely populated and highly industrialized West European region, might contribute to the discussion concerning the relevance of inter-individual differences in susceptibility with regard to human health risk assessment. This is certainly a topic of public health interest and of importance pertaining to the environment.

All changes made to the original manuscript are highlighted in red in the revised manuscript.

The editorial office wrote:

“Your MS would benefit from a native speaker proofreading it. Some sections are hard to understand so there are flow issues as well as some article and other grammatical ones too.”. A serious effort has been done to improve the English.

“On the title page the periods after the title and addresses should be removed. The email addresses should be formatted as author's initials:email address e.g. JS:joe.shmoe@university.edu. The heading “Corresponding author should be moved above the Email addresses and the colon and all other information removed. In the abstract section, removed the periods after the subheadings and the text below the subheadings, and Conclusions should be plural. Remove the key words section. In numbers 100,000 and higher, use a comma in the thousands place, not a period. On page 5, 41.23% should be together on one line. In the final text section, the heading should read Conclusions, and the first few words should be removed. Change the section before the Competing interests to read List of Abbreviations and the one after it to read Authors' contributions. In the References section, the references should be left justified and authors' names up to 30 listed before using the abbreviation et al.” All this has been done.

“It looks like the first two sentences in the Background need references.” The reference “Crimmins EM, Beltran-Sanchez H: Mortality and morbidity trends: is there compression of morbidity? J Gerontol B Psychol Sci Soc Sci 2011, 66:75-86.” has been added to substantiate the statement “Cancer and cardiovascular diseases are the main causes of severe morbidity and mortality in the developed world.” The statement: “In western populations, morbidity and mortality rates of cardiovascular diseases, cancer and some other potentially fatal diseases increase rapidly between 15 and 65 years of age.” is substantiated sufficiently by the rest of the paragraph, referring to two papers and to detailed Flemish data.

Response to the comments of reviewer Franco Merlo.

The reviewer wrote:

“The authors describe the study population (1583 adults and 1679 adolescents) as being selected at random within the Flemish Environment and Health Survey
(FLEHS). As stated there was some self-selection in participation of the adults in the study due to fact that traveling to local community centres was required and sick people may have been self excluded. However it is not clear from the manuscript how many of the randomly selected did participate in the study. In page 8 the study subjects are indicated as selected as well as participants. I suggest to include a simple table (or chart) showing (among adults and adolescents) the numbers of “randomly selected”, the number (and %) of participants and non participants and the numbers (and %) of available DNA. This will help the readers to understand the magnitude of self selection and the consequences on the results and their interpretation.”

A new table was added as asked by the reviewer (table 1 in the revised manuscript).

“19 genes and 28 low penetrance polymorphisms were selected a priori based on commonly studied “susceptibility” genes involved in xenobiotic metabolism, oxidative stress defense and DNA repair. These genes have been reported in the literature as being associated with probability of being sick (from a variety of diseases, mainly chronic diseases such as cancer). The authors state that for these genes a clear ‘increased risk’ or a ‘protective effect’ was reported on the available literature. This is the correct approach to identify the study hypotheses. An index was generated for each gene polymorphism/alleles and a sum of risk alleles was computed for all polymorphisms to generate indexes that were used in the statistical analyses to compare the proportions of adolescents and adults carrying the “sum of gene/alleles” and the “gene-grouped” into 4 “biological processes” (i.e., biotransformation phase I, biotransformation phase II, oxidative stress, and DNA repair) separately.

This type of analysis, has the advantage of showing the study findings collapsed into 4 groups (+ the sum of gene/alleles) but has the disadvantage of hiding the differences in proportions of each of the 19 genes between adolescents and adults. I suggest the authors to report a table for each of the 19 genes/alleles (as reported in Table 1) with the n and % for adolescents and adults. This table will have the advantage of showing the frequency distribution of each of 19 genes/alleles in the study populations and allow readers to inspect the data. There is no need to perform statistical comparisons in such table.”

A new table was added as asked by the reviewer (table 3 in the revised
“The summary of the findings (Table 2) should include the number of subjects and the %, not only the latter and the mean number of risk alleles. As it is now it is not fully informative and there is no corresponding data for mean number of risk alleles to which the authors refer in the results section.”

The numbers of subjects were added to table 4 in the revised manuscript. The respective mean numbers of risk alleles are presented in the text and do, in my view, not belong in the table.

“As stated, the hypothesis that higher sensitivity to genotoxic agents decreases with age among persons capable and willing to participate in a biomonitoring study, seems reductive. The real hypothesis is tested among those capable and willing to participate in an a study but concern the general population (isn’t the FLEHS a Stratified Clustered Multi-Stage Design?). Please just say “….decreases with age”.”

The sentence “among persons capable and willing to participate in a biomonitoring study” was removed as asked by the reviewer in both the abstract and the discussion. As we are of the opinion that chronic diseases are probably at stake we wrote: “suggesting that persons carrying a higher number of at risk alleles (especially in phase II xenobiotic-metabolizing or DNA repair genes) are at a higher risk of morbidity and mortality from chronic diseases.”

“do not think it is correct to state that the results “..suggest that Flemish residents carrying more unfavorable genetic traits related to genotoxic effects were more likely to be severely ill or to have died before age 50 to 65”. The correct conclusion is that more unfavorable genes are detected in adults aged 50 to 65 than in adolescents. This may be interpreted as resulting from a higher mortality among subjects with unfavorable genetic traits. Then the discussion that follows is reasonable.”

As we are of the opinion that severe disease might have played a role in self selection by elderly subjects leading to a decreased participation of people carrying unfavourable genetic traits (see methods section), we prefer to keep the sentence “ Our results suggest that Flemish residents carrying more unfavorable genetic traits related to genotoxic effects were more likely to be severely ill (so as to avoid participation in a biomonitoring study, participation for which travelling some distance was required) or to have died before age 50 to 65.”

“Conclusion:
According to the previous comment, replace the statement “…in a randomly selected population of persons able and willing to participate in a biomonitoring
study” with “in a randomly selected Flemish population”.

The sentence “among persons capable and willing to participate in a biomonitoring study” was removed as asked by the reviewer. As we are of the opinion that chronic diseases are probably at stake we added: “suggesting that persons carrying a higher number of at risk alleles (especially in phase II xenobiotic-metabolizing or DNA repair genes) are at a higher risk of morbidity and mortality from chronic diseases.”

“Discretionary

It would interesting if the authors could attempt to quantify the proportion of deaths attributable to the differences in % between adolescents and adults detected. Indeed the leading causes of death do differ in adolescent and adults and it is not necessary true that the main causes in the age group not included in the study (17-49 years old) are related to the genotypes considered.”

At present we have no data to quantify the proportion of deaths attributable to the differences in % between adolescents and adults detected.

Response to the comments of reviewer Radim Sram.

The reviewer wrote:

“Remark - references:
# 20 name is De Coster CS
# 24 + volume+pages”

The corrections asked were made

I hope that your journal will be able to publish our paper and send you my best wishes.

Nik van Larebeke

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