Author’s response to reviews

Title: Influence of maternal and own genotype at tanning dependence-related SNPs on sun exposure in childhood

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Author's response to reviews:

Dear Milica Keckarevic Markovic,

We thank you for the time you have invested in considering our submission and we thank the reviewers for their comments and suggestions. We have carefully considered and addressed these issues, where possible.

Below we include a point by point description of the changes we have made to address the points raised during peer review.

Please also accept my apologies as there was an error in the version of the manuscript that I uploaded. One additional finding was omitted from my submission that has been added back into the revised manuscript. This finding relates to the association between the rs2073478 SNP and the star rating of sun block/cream used.
Many thanks,

Miss Jasmine Khouja

Influence of maternal and own genotype at tanning dependence-related SNPs on sun exposure in childhood

Jasmine Khouja, Sarah Lewis, Carolina Bonilla

Reviewer comments and response to reviewers

Xin Li (Reviewer 1): In the current study, the authors studied influence of maternal and own genotype at tanning dependence (TD)-related SNPs on sun exposure pattern in childhood. This is a relatively novel topic, considering the current knowledge on genetic variants that are associated with tanning dependency is still very limited. The study design and statistical analysis are straightforward, and the manuscript is well-prepared. However, I have several comments:

1. Selection of tanning dependency-related SNPs: The SNPs were selected based on two previous publications--ref 13 and 14. I understand that few papers have been published on TD-related SNPs, but I would suggest the authors to acknowledge the limitations of the selected SNPs in Discussion. For example, in Ref 14, the outcome of interest is ever used indoor tanning, which is not a good reflect of tanning dependency; Also noticed are the p-values of SNPs identified by those two papers, I would expect non-significant results after adjusting for multiple comparisons. Moreover, neither of them performed replication analysis to validate their findings.

RESPONSE

Thank you for your comment. This is true, the SNPs were not obtained from a GWAS of tanning dependence. Cartmel et al. (2014) ran an exome-wide association study of TD, and both Cartmel and Flores et al. (2013) examined the association of candidate genes for substance
dependence/addictive behavior in relation to TD and indoor tanning. However, as these are candidate SNPs for addiction, it is reasonable to believe that if TD behaves in a similar way as other addictions (which has been reported) these SNPs could influence TD as well as indoor tanning behavior.

We agree that these points should be raised in the discussion and have added the following sentences to page 18:

“Alternatively, the outcome of interest (ever used indoor tanning) in the study by Flores et al. [14] may have not been a good measure of tanning dependence and neither study have validated their results nor demonstrated evidence of an association after correction for multiple comparisons.”

2. Is data on mother's sun exposure behavior available? Looking at association between maternal SNPs and mother's behaviors, association between mother's and children's behaviors, and associations between maternal SNP and children's behaviors adjusting for mother's behavior (potential mediator) would make a more comprehensive analysis.

RESPONSE

Unfortunately, we have limited data on maternal sun exposure. We have a measure of sun bed use in early pregnancy but only 1.5% of the full sample (including those without complete data) stated they had used a sun bed at this time and less than 100 mothers with genetic data. Furthermore, the measure is before the child was born and it is how the mother’s genotype directly influences the child’s exposure that we are most interested in. With this data, we wouldn’t be able to do any meaningful analysis.

3. Could the authors also try other methods to correct multiple comparison? Would the reported genetic markers still be significant? Page 9, lines 41-46, they wrote "10 sun exposures were not included in the correction as they are not independent measures". Please show correlation matrix for the 10 variables.
As can be seen in the correlation matrix (Supplementary Table 2), some of the sun exposure variables are moderately correlated with each other. We have further explained our reasoning for omitting the sun exposure variables from the correction in the following sentence on page 11:

“Bonferroni correction only takes account of the 17 different SNPs, the 10 sun exposures were not included in the correction as some were moderately correlated (correlation coefficients ranging from 0.01-0.57, most coefficients>0.20, Supplementary Table 2) and so were not considered independent measures. Furthermore, as these are exploratory analyses rather than hypotheses driven, the focus of these results is not on p-value thresholds [18].”

4. The sun-exposure variables are self-reported, please discuss the validity of the data and how it may affect the estimations of association being studied. Children aged ~8 yrs old usually spend most of their time at school, how accurate is the measurement of sun exposure if the information was solely from their moms.

RESPONSE

Many thanks for this suggestion. The self-reported sun-exposure variables strongly correlate with pigmentation genetic scores (Bonilla et al., 2014). Children who are genetically fairer skinned tend to be more protected from the sun. We would speculate that self-reporting inaccuracies would make it harder to detect an effect. We have updated the discussion with the following sentences on page 18:

“However, the use of maternal reports of sun-exposure may reduce the validity of the data as mothers may not be aware of their child’s exposure when they are at school where children spend the majority of their time. Although it could also be argued that children spend the majority of their time inside during the school day, mothers can protect their child from sun exposure during the day by applying sun cream before school, and mothers may observe sun burns when their child returns from school.”
5. Indoor tanning is more popular among females, and boys and girls may have very different sun exposure behavior patterns. Could the authors conduct stratified analysis by gender to detect potential effect modification? The same to pigmentation traits.

RESPONSE

This is a very good point. Due to the limited sample in some of pigmentation categories, we were unable to run the stratified analysis. We have however, rerun the analyses including sex and pigmentation of both mother (eye color [skin pigmentation data not available]) and child (skin pigmentation) in the analyses. We have updated the methods section to reflect this. In general, the main findings were weaker after this adjustment, however, this analysis reduces the sample by roughly 50%, meaning we have less power to detect an effect. We have included the following statement on page 15 (and a similar statement in the discussion on page 17) to illustrate this:

“In the secondary analyses, where sex and pigmentation were included in the analyses, the findings were similar but weakened in terms of the effect size, strength of the effect or both (Table 3). However, the inclusion of these covariates resulted in a dramatic reduction in sample size (roughly 50%) and therefore power to detect an effect.”

We were able to stratify the analyses by sex (Supplementary Table 6). Only two SNPs/sun exposure associations were found when the analyses were stratified. The mother’s rs2073478 SNP was associated with males but not females using a lower star-rated sun cream or block (same direction as main finding). However, this finding is based on just 346 individuals. For the other main findings, the results were similar but with weaker evidence of the effect in both sexes. The rs29132 SNP in children was associated with males but not females having been badly burnt up to the age of 8. The differences found here are likely due to the reduction in sample size and power by stratifying the results, so we feel it is more appropriate to present the analyses adjusted for sex in the text.

6. Page 6, line 21-26, the authors spent some words explaining the relationship between sun protective behaviors and TD. Mothers with TD protect their children and themselves less than mothers without TD. In Table 3 and Supplementary table 2, are the directions of association consistent with the hypothesis? It would be helpful if the authors could summarize the effect
alleles and ORs for the association between selected SNPs with TD from the original publications (ref 13 and 14).

RESPONSE

This is a good point. We have discussed this on pages 16 and 17 but have added this additional sentence to make our stance clearer:

“This is consistent with the hypothesis that mothers with TD may protect their child less from UV damage than mothers without TD.”

The known effect alleles and odds ratios for the association between selected SNPs and TD are now included in Supplementary Table 3 (previously Supplementary Table 2).

In the footnote of Table 3 we have added: “Note: RA = Risk allele (increases the risk of becoming TD, allele used based on findings from Cartmel et al. [13]).”

Reviewer 2:

Gloria Ribas (Reviewer 2): The manuscript is based on The Avon Longitudinal Study of Parents and Children (ALSPAC) which is a large, prospective mother-child cohort study set on 1992 with Local Research Ethics approval and informed consent from child's mothers. They recruited more than 14500 pregnant women and followed them and their children for years. When the children reached the age of 8 years old, about 7300 mothers responded to a long questionnaire (it contains some questions related to sun exposure, sun protection and related habits). The cohort has been extensively used in different studies, the web page of the association shows the full list of the articles published.

Although the cohort is impressive and the initiative has given rise to hundreds of articles in other subjects, in this particular context, the results identified are not strong and even the authors called them weak findings. These make me have some concerns about the hypothesis and aims.
The hypothesis is based on the attractiveness of becoming tanned, a process that has severely increased in the last decades. Tanning, then, can be seen for some (particularly women) as a way to be healthier and more attractive, and several studies have shown that those individuals can become tanning dependent (TD). In this particular case, the authors tried to demonstrate the influence of maternal genotype, in addition to the genotype of the children, at tanning dependence-related SNPs on sun exposure in childhood. The hypothesis is based on the fact that the tanning dependent behavior of the mothers may regulate their children's sun exposure.

- The design of the experiment is not clear: For one side, authors declare to have selected 20 SNP-genes based on previous published studies; in the next paragraph they specified the use of GWAS data for the children and genotyping arrays for the mothers. In the following paragraph the authors mention that the final number of SNPs studied was of 17 located in 13 different genes (they explain the reduction from 20 to 17). Authors need to make clear that as I have guessed; they have accessed to hundred thousands of SNPs but only used the data from a few. This could have statistical implications. I would like to have this clear in the text.

RESPONSE

This is true, mothers and children in ALSPAC were genotyped for hundreds of thousands of SNPs across the genome and millions of genotypes were imputed afterwards using the 1000 Genomes panel as a reference. We picked out the SNPs of interest from this set. We have included the following statement on page 7 to address this comment:

"From the genome-wide data we selected 20 SNPs for analysis (listed in Supplementary Table 1) based on previous findings from Cartmel and colleagues [13] and Flores et al. [14]."

- The whole cohort presents several weaknesses, some are mentioned in the text others are ignored:

a - I missed information on pigmentation characteristics both from mothers and kids (can be different from mothers). Pigmentation (skin, hair, eyes, freckles, tanning ability, etc) has been previously involved in susceptibility to sunburns and tanning. Several SNPs related to pigmentation are well documented and could have been available in the GWAs and genomic data. Mothers with genetic (and phenotypic) susceptibility to sunburn should be more prone to
protect their children. None of these is neither studied nor mentioned in the manuscript and to my opinion is very relevant.

RESPONSE

This is an interesting point and we have addressed it by adding information on pigmentation of the mothers and their children to Table 1. We also ran extra analyses to confirm that these pigmentation characteristics are not associated with the TD SNPs associated with sun exposure (Supplementary Table 5) and have completed additional analyses in which we adjusted for pigmentation.

b - Fathers are not included in the equation. Both their behavior and also their pigmentation may introduce some confusion to the results.

RESPONSE

Unfortunately, data from the mother’s partners (whether they are the biological father or not) in ALSPAC is limited and for these phenotypes is unavailable. In our discussion, we briefly discuss that future research should include data from fathers (page 19).

c - They report the number of days the child was in the sun for 4 or more hours at 8 years. Maximum value is 40. Is that during the week, on weekends? In holidays? Holidays in the same country or abroad? In spring-summer? For the whole year? All this information should be better explained in the manuscript. Chronic sun exposure has been related with protection to sunburns, however, acute sun exposure is indeed a risk activity. All this can introduce biases to the responses. Authors should explain better the subgroups and the conditions.

RESPONSE

We have updated pages 7 and 8 with more details on the subgroups and conditions.
The study was initially intended to study the mothers. And so, different platforms have been used, and most probably different set of SNPs have been analyzed. Genotyping has been performed at distinct institutions: children, at the Wellcome Trust Sanger Institute, and the Laboratory Corporation of America-23andMe; mothers at the Centre National de Génotypage, France. The genotyping more likely has been done at different times. All this together with different technologies and sets of SNPs may add some difficulty in the combination of the data. Could the authors comment on that and how they have minimize any bias for all the mentioned above.

RESPONSE

The ALSPAC genotype data, generated using the Illumina HumanHap550 quad (children) and Illumina human660W quad (mothers), has been jointly imputed to the 1000 genomes reference panel (Version 1, Phase 3, Dec 2013 Release). This helps reduce the differences between mothers and children that result from the different chips, labs and times of genotyping. We have updated the methods to discuss the imputation methods in more detail.

e - Mothers responded on child habits and exposure to sun, which is highly modulated by mothers themselves, independent tanning addiction in children, is difficult to evaluate. When children of the cohort reach adulthood would be more accurate to evaluate their addictiveness.

RESPONSE

We agree. A questionnaire asking about outdoor and indoor tanning in the children of the cohort who are now approximately 26 years old has been distributed to participants in 2017. We should have data available on these variables towards the end of this year (at the earliest) which we can use to assess this question in further studies.

For the present study, we have added the following sentences to page 19 to address this point:

“The cohort are now 26 years old, meaning that if we can acquire this data we could more accurately assess their TD without their mothers’ mediating effects and assess the association between their genotypes and TD as well as the association between their behaviors in childhood and TD.”
- Table 1 "Characteristics of the children in the study population". Title should be changed. Which characteristics are described? The table summarizes the information related to sun exposure, sunburns and sun protection habits of the children in the ALSPAC cohort given by their mothers. Refer to characteristics to the children induce to errors. The table should show statistical significance for the different subgroups in all categories.

RESPONSE

We have made the following adjustment to the title of Table 1: Sex, age, sun exposure and pigmentation of the children and mothers in the study population.

We have also added the following note to the table: “Note: Sun exposure variables were reported by the children’s mothers.”

- They highlighted the findings of two SNPs located in two corresponding genes that were associated to several sun exposure variables, and a third variation was associated to an increased likelihood of using sun cream, the conclusions shown weak evidence to tanning dependence. Taking into account that the results have been obtained using more than 7000 samples from the kids and their corresponding mothers, I think the significance obtained is pretty low.

RESPONSE

Although the sample size is large, after adjustment, the sample is reduced to around 3,500. We agree that our findings are not strong considering our sample size but we wouldn’t expect tanning dependence to be having a great effect until the children have greater autonomy nor would we expect mothers’ tanning dependence to have a large effect on the child’s exposure. The fact that we have found an association, although weak, at such an early age is surprising. This study builds on the work of ref. 13, which found a nominal association with tanning dependence with SNPs in VAPA, SPATC1, OPRM1, and ALDH1B1, loci that were also nominally associated in our study. Replication is needed, of course, but we have increased the evidence available.
- Table 2: Is difficult to understand. Authors should described which are the sun exposure variables they have obtained, those explained in table 1? In the text is said that the 13 genes studied are shown in table 2, however nothing more is said about number of sun exposure variables associated to exactly what? The genes? Why one of the genes, LY75, then has zero

RESPONSE

On Page 11, we have added the following sentences in order to make the text clearer (note that Supplementary Table 3 was formerly Supplementary Table 2):

“Out of the 17 SNPs examined, 15 SNPs in 8 genes in the children (11 SNPs in 9 genes after adjusting for the mother’s genotype) and 8 SNPs in 6 genes in the mothers (11 SNPs in 8 genes after adjusting for the child’s genotype) showed evidence of association with at least one of the 10 children’s sun exposure variables before applying a Bonferroni correction for multiple comparisons (p < .05; Table 2 and Supplementary Table 3).”

To clarify, the sun exposure variables in Table 2 are those that are displayed in Table 1. The number of SNPs in brackets previously referred to the number of SNPs in which associations were found for each gene. However, we realize this was misleading and have adjusted the column to reflect the number of SNPs in total that were tested in each gene.