Author’s response to reviews

Title: Matrix metalloproteinases gene variants and dental caries in Czech children

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BMC Oral Health - Editor

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Dear Editor,

Thank you very much for giving us the opportunity to revise OHEA-D-20-00025 newly entitled “Matrix metalloproteinases gene variants and dental caries in children“ by Petra Borilova Linhartova, Tereza Deissova, Martina Kukletova, Lydie Izakovicova Holla.

Manuscript (MS) was revised according to referees‘ suggestions (all changes were marked with a “text-marker” yellow) and all questions raised were addressed (particular changes made are listed on separate pages). We acknowledge the reviewers’ effort and hope that the revised form the MS will be acceptable for publication in your journal.

Yours faithfully,

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Specific changes made in manuscript OHEA-D-20-00025 - revision

To: Editor

1. STROBE guidelines. In accordance with BioMed Central editorial policies (http://www.biomedcentral.com/submissions/editorial-policies#standards+of+reporting), could you please ensure your manuscript reporting adheres to STROBE guidelines (http://www.strobe-statement.org/) for reporting observational research. This is so your methodology can be fully evaluated and utilised. Can you please include a completed STROBE checklist as an additional file when submitting your revised manuscript. Please note there is a version of the checklist for case-control studies.

Answer: The STROBE checklist was attached. Considering the content of the study, some of the items of Strobe-checklist are irrelevant.

To: Reviewer 1

This manuscript reports on a case-control study to assess the relationship between MMP gene variants and caries status of children in Czech. The study seems to have been properly conducted and the conclusion is valid. However, there are areas for improvement in the reporting of this study.

1. The Abstract - the study aim/objective should be explicitly stated. The contents of the two sentences in the conclusion overlap and they should be combined to form a concise conclusion statement.

Answer: The Abstract was corrected according to the reviewer´s suggestion (page 2).

“The aim of the study was to analyze in Czech children with primary/permanent dentition polymorphisms in genes encoding MMP2, MMP3, MMP9, MMP13, MMP16, and MMP20, which had been previously associated with dental caries in other populations.”

“This study demonstrated the lack of association between the selected SNPs in candidate genes of MMPs and susceptibility to or severity of dental caries in both primary/permanent dentitions.”

2. Introduction - the study aim/objective should be explicitly stated in the last paragraph.

Answer: The guidelines for authors from BMC Oral health impose that the methods section should include the aim.
https://bmcoralhealth.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article

Nevertheless, the aim of this study was transferred to the end of the Introduction part according to the reviewer´s suggestion (page 3).
“The aim of the study was, based on the literature review of studies performed in other populations [19-22, 26-29], to select the SNPs in genes encoding MMP2, MMP3, MMP9, MMP13, MMP16, and MMP20 and to determine alleles/genotypes in these SNPs in Czech children with primary/permanent dentitions.“

3. M&M - why did this study took 13 years (2005 to 2018) to complete? This should be explained in the discussion section. Was there any time difference in recruiting the case and the control group of children?

Answer: Our research primarily dealt with dental caries in permanent dentition; nevertheless, we also focused on dental caries in primary dentition in last years. Children with permanent dentition (group 2) were recruited from the European Longitudinal Study of Pregnancy and Childhood in period 2005-2007 and children with primary dentition (group 1) were recruited from 2016 till 2018. In each group, cases and controls were recruited in the same period. The evaluation of the allele and genotype frequencies was performed within each group between cases and controls, so there is no bias in statistical evaluation according to the different periods of subject recruitment. Periods of recruitment of subjects into each group were added into paragraph Characteristics of participants and sampling (page 4). The Discussion was modified (page 7).

“The allele and genotype frequencies were compared within each group between cases and controls, therefore there is no bias in statistical evaluation according to the different periods of the subject recruitment.“

4. M&M - did this study obtain a specific ethical approval from an IRB? If not, why?

Answer: For the research ethics committees in Europe, the term "Research Ethic Committee" (REC) is used, while in the US these are called IRBs. This study was approved by the REC of the Faculty of Medicine, Masaryk University, Brno (3/2004, from March 30, 2004) and St. Anne’s Faculty Hospital (without number, from April 13, 2004, and 1G/2017). This is stated in the Declarations part.

5. M&M - a brief description of the inclusion and exclusion criteria for the Group 2 children (those with permanent dentition) should be given. Referring the readers to another paper is not sufficient.

Answer: The inclusion and exclusion criteria for group 2 were added (page 4).

“The inclusion criteria for group 2 were: age 13-15 years, general good health and willingness of the parents to enter their children in the study [32]. The exclusion criteria for group 2 were: previous or concomitant therapy with orthodontic appliances, family relationship between children, and other than Czech Caucasian ethnicity.“

6. M&M - the mentioning of gingival index, plaque index and calculus indices is not needed as these parameters are not reported in this manuscript.
Answer: The description of the clinical assessment in the part M&M was corrected (mentioned indices were deleted) as suggested by the reviewer (page 4).

7. Results - The age and gender distribution of the children in the case and control subgroups should be reported separately and statistical tests should be performed to show that the two groups did not differ significantly.

Answer: Group 1 (young children): in controls with dmft=0, age was $3.92 \pm 1.04$ years and gender 24 boys and 27 girls, in patients with dmft&gt;0, age was $3.51 \pm 0.88$ years (59 boys and 42 girls), P&gt;0.05 both for age and gender distributions. All children from group 2 (ELSPAC) were examined at the age of 13-15 years, therefore we did not find exact data (mean age ± SD), Boy/girl ratio for DMFT=0 was 83/72, for DMFT&gt;0 it was 253/221, P&gt;0.05. This data was added to results (page 5, the last paragraph).

8. Results - the statistical tests used to generate the p-values should be mentioned.

Answer: The Fisher-exact test was used for testing the differences in the allele frequencies; the chi-square test ($\chi^2$) for calculating Hardy-Weinberg equilibrium (HWE) and the differences in the genotype frequencies between the cases and controls in each group.

9. Result/Discussion - information on the 1006 EUR children included in Table 2 should be provided.

Answer: The European (EUR) population mentioned in Table 2 is from the 1000 Genomes Project super population [40]. This population (no children) includes Utah Residents (CEPH) with Northern and Western European Ancestry (CEU), Toscani in Italia (TSI), Finnish in Finland (FIN), British in England and Scotland (GBR) and Iberian Population in Spain (IBS). The reference was added into the manuscript (page 7).

10. Conclusion - the present paragraph under the heading of conclusion should be used as the last paragraph of the Discussion section. A concise conclusion statement with reference to the study objective should be added.

Answer: The mentioned paragraph was transferred as the last paragraph of the Discussion section as suggested. A concise conclusion statement was added in Conclusion:

“In conclusion, this study demonstrated the lack of association between the selected MMPs gene variants and susceptibility to or severity of dental caries in both primary/permanent dentitions in the Czech population.”
11. Please use the STROBE statement (for case-cohort studies) to check for completeness of information to be included in reporting this observational study.

Answer: We used the STROBE statement and the hypothesis was added, periods of subject recruitment, inclusion and exclusion criteria, characteristics of subjects were completed also according to the reviewer’s suggestions.

12. A thorough proof reading will enhance the English language use in this manuscript.

Answer: The manuscript was checked and minor mistakes corrected.

Thank you again very much for your comments that as we hope helped improve quality of our article.

To: Reviewer 2

I have read the manuscript "Matrix metalloproteinases gene variants and dental caries in children: a case control study" with a particular interest. This is an important study with 2 large groups of children included that evaluated 9 SNPs in MMPs.

1. Remove case-control study from the title. This is not a "classical case-control.

Answer: The guidelines for authors from BMC Oral health require the author to state a title that includes the study design.
https://bmcoralhealth.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article
Nevertheless, the text was removed from the title according to the reviewer’s suggestion.

2. The introduction is too short and should be expanded.

Answer: We expanded the text in Introduction according to the reviewer’s suggestion (page 3, paragraph 1-3).

3. The aim of the study should be placed in the introduction. Should also have references which are the "previous studies".

Answer: The guidelines for authors from BMC Oral health impose that the methods section should include the aim.
https://bmcoralhealth.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article
Nevertheless, the aim of this study was transferred to the end of the Introduction part and the references were added according to the reviewer’s suggestion (page 3).

We also added references for “previous studies”.
“The aim of the study was based on the literature review, to select the SNPs in genes encoding MMP2, MMP3, MMP9, MMP13, MMP16, and MMP20, which had been previously associated...
with dental caries in other populations [19-22, 26-29] and to determine genotypes in these SNPs in Czech children with primary/permanent dentitions."

4. The inclusion and exclusion criteria for group 2 should also be described here.

Answer: The inclusion and exclusion criteria for the group 2 were added (page 4).

“The inclusion criteria for group 2 were: age 13-15 years, general good health and willingness of the parents to enter their children in the study [32]. The exclusion criteria for group 2 were: previous or concomitant therapy with orthodontic appliances, family relationship between children, and other than Czech Caucasian ethnicity."

5. In table 1, the group with "Caries affected children (DMFT≥6)" were compared with which group? A note should be added in the table.

Answer: The note was added.

6. More data regarding how and where clinical examination was performed is needed.

Answer: We added more data regarding how and where clinical examination was performed (page 4, paragraph 3 – 4)

“To determine the DMFT index, in each child, all present teeth were examined in the dentist's chair using a mirror and a dental probe, the teeth were firstly dried and then examined in a good light. The data was entered into a standard medical record. The radiograph examination was not performed as it was not part of routine dental care for these adolescents and would therefore be deemed unethical.”

7. Why gingival index (GI), plaque index (PI) and calculus index (CSI) were evaluated if was not used?

Answer: These indices are usually examined in patients with permanent dentition, however, these were not statistically evaluated in this study. Based on the suggestion of the other reviewer, this information was deleted from the manuscript.

8. In the results, the authors should demonstrate age and gender distribution among all groups.

Answer: Group 1 (young children): in controls with dmft=0, age was 3.92 ± 1.04 years and gender 24 boys and 27 girls, in patients with dmft&gt;0, age was 3.51 ± 0.88 years (59 boys and 42 girls), p&gt;0.05 both for age and gender distributions
All children from group 2 (ELSPAC) were examined at the age of 13-15 years, therefore we did not find exact data (mean age ± SD), Boy/girl ratio for DMFT=0 was 83/72, for DMFT&gt;0 it was 253/221, p&gt;0.05.
This data was added to results (page 5, the last paragraph)

9. Where is the allele distribution results?
Answer: Allele distributions (together with P values) were added into Table 1.

10. Why the authors did not test dominant and recessive models?

Answer: The Fisher-exact test was used for testing the differences in the allele frequencies; the chi-square test ($\chi^2$) for calculating Hardy-Weinberg equilibrium (HWE) and genotype frequencies between the cases and controls in each group. Only the values of P below 0.05 were considered statistically significant. Bonferroni correction was used to adjust the level according to the number of independent comparisons to the overall value of 0.05. According to our statistician, it is not meaningful in this case (absolutely negative results) to use more complex statistical methods with testing dominant or recessive models.

11. Table 2 needs a better explanation in the results section.

Answer: The comment to Table 2 was added to Results (page 5).

“Minor allele frequencies (MAF) of all nine studied MMPs SNPs were similar between European population (EUR, N=1006, data obtained from NCBI database) and Czech population (P>0.05, see Table 2). In MMP16 SNP (rs2046315) was the highest difference in MAFs (6.4 % between EUR population and Czech children with dmft=0). In contrast to EUR population MAFs of specific MMPs SNPs in Bulgarians, Turks, Brazilians, Chinese and/or Saudi Arabians are more or less frequent (difference to 22.7 %). Also allele distribution of MMP20 (rs1784418) in Polish Caucasians differ from frequency in EUR population (difference to 7.6 %), while similar frequencies were found in both MMP16 SNPs (rs2046315 and rs10429371) in COHRA1 including Caucasians northern Appalachian families and EUR population, see Table 2.”

12. This manuscript is missing this reference bellow:

Answer: The reference was added into the manuscript (page 3 and Table 2).

Thank you again very much for your comments that as we hope helped improve quality of our article.