Author’s response to reviews

Title: The Relationship between Vitamin D Receptor Gene Polymorphism and Deciduous Tooth Decay in Chinese Children

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Author’s response to reviews:

Dear Editor,

Thank you very much for your letter and the review of our manuscript. We have carefully addressed the comments and revised the manuscript as requested. We thank you and the reviewers for your positive comments and concrete suggestions, which provided very helpful to improve our paper. We hope that these changes will make the manuscript acceptable for publication in BMC Oral Health. Our responses to the comments are detailed below. If there are additional changes that we should make, please do not hesitate to inform us.

Thank you very much for your consideration.

Yours sincerely

Qian-zhou Jiang

Response to the comments on our manuscript (OHEA-D-16-00453R1).

1. On the title page please provide author names in the format: First name, Surname.
Respond: Thank you for your comment. We have provided all author names in the right format.

2. In the Methods section please provide references for the World Health Organization protocol recommended for oral health surveys (page 4, lines 17-18) and the DMFT index (page 4, line 28).

Respond: Thank you for your comment. As you suggested, we added the references for the World Health Organization protocol recommended for oral health surveys and the dmft index.

3. It has come to our attention that throughout the manuscript there is significant text overlap with other publications. While we understand that you may wish to express some of the same ideas contained in these publications, please be aware that we cannot condone the use of text from previously published work. We would therefore be grateful if you could provide a justification for the overlap in text between your manuscript and other sources. Please be informed that we cannot proceed with handling your manuscript before this issue is resolved, and the sections of text in question have been reformulated. The report has been attached to further help your revisions.

Please note that the software used to detect overlaps is imperfect and highlights two or more consecutive matching words, therefore, please just focus on those whole sentences/paragraphs highlighted. If this study uses methodology from a previously published work, please provide a summarizing statement in the methodology together with a citation to the original paper.

Respond: Thank you for your comment. As you suggested, we revised the overlap in text between our manuscript and other publications as follow:

(1)Title:
“The Association of Vitamin D Receptor Gene Polymorphism with Deciduous Tooth Decay in Chinese Children” was changed to “The Relationship between Vitamin D Receptor Gene Polymorphism and Deciduous Tooth Decay in Chinese Children”

(2)Abstract:
1) “we investigated the association of vitamin D receptor (VDR)……” was changed to “we explored the link between vitamin D receptor (VDR)……”;

2) “whose genomic DNA was extracted from the buccal mucosa……” was changed to “whose DNA sample was collected from the buccal mucosa……”

3) “VDR gene polymorphisms analysis was performed by polymerase chain reaction-restriction fragment length polymorphism” was changed to “VDR gene polymorphisms was determined by PCR-RFLP”

4) “Logistic regression analysis adjusted for genotypes, age and S. mutans factor demonstrated that BsmI containing the Bb genotype was associated with the risk of deciduous tooth decay”
The adjusted logistic regression analysis demonstrated that BsmI containing the Bb genotype was linked with the increased risk of deciduous tooth decay.

5) “Our results therefore suggested that the BsmI polymorphism of VDR gene might be a genetic factor determining deciduous tooth decay susceptibility in Chinese children.” was changed to “Our results showed that VDR BsmI polymorphism was associated with the risk of deciduous tooth decay in Chinese children aged 4-7 years. However, the specific mechanism remains to further verify through experiment.”

(3) Introduction:

1) “Dental caries is one of the most prevalent chronic pediatric diseases worldwide. The prevalence of dental caries is more than 50% in many countries” was changed to “Dental decay is one of the most common diseases and its prevalence is more than 50% in many countries”

2) “Untreated dental caries can lead to pain, tooth loss, caries related pulpititis and abscesses, or other co-morbidities, which affects growth and well-being in preschool children” was changed to “Untreated dental caries can cause further serious problems, such as caries related pulpititis, abscesses, pain, tooth loss and other co-morbidities, which affects growth and well-being in preschool children”

3) “As a multi-factorial disease, dental caries is caused by a combination of environmental, behavioral factors and gene-by-environment interactions” was changed to “As we known, dental caries is a multi-factorial disease, which is caused by environmental factors, behavioral factors and gene-by-environment interactions”

4) “Despite the acknowledged importance of genetic factors, only a few caries susceptibility genes have been identified and validated thus far” was changed to “Although the importance of genetic factors have been recognized, until now only a few caries susceptibility genes have been identified and verified”

5) “The vitamin D receptor (VDR) gene is a nuclear biological macromolecule that mediates the biological activity of the major metabolite of vitamin D, which plays a major role in tooth formation, especially in the development of the enamel and dentin” was changed to “As a biological macromolecule in nucleus, the VDR gene has the effect of modulating the biological function of the major metabolites of vitamin D, which plays an important role in the formation of teeth, particularly in the calcification of enamel and dentin.”

6) “Vitamin D is critical to the maintenance of a constant relationship between calcium and phosphate ions, which are key factors in the strengthening and protection of teeth” was changed to “Vitamin D is pivotal to maintaining a stable relationship between phosphate and calcium ions, which are known as vital factors in protecting and strengthening the teeth”

7) “A randomized trial demonstrated that vitamin D deficiency during pregnancy was associated with enamel hypoplasias in the offspring. In turn, enamel hypoplasias was related to caries risk in cohort studies” was changed to “A randomized trial have found that lack of vitamin D during
pregnancy is associated with enamel dysplasia in children. Further cohort study found that enamel dysplasia was associated with risk of dental caries”

8) “Vitamin D not only helps regulate calcium levels, it is also an essential component of immune system” was changed to “Vitamin D is not only involved in the regulation of calcium and phosphate levels, but also an important part of the immune system”

9) “which in turn prevents the correct response to microbial infection, including bacterial infection, which occurs in the course of periodontitis or untreated caries disease” was changed to “which then blocks the correct immune response to oral microbial infections that occur during periodontitis or untreated caries”

10) “The biological function of Vitamin D is mediated by VDR protein, via interaction with the VDR protein” was changed to “The biological function of Vitamin D is modulated by VDR protein, through interaction with the VDR protein”

11) “As reported, the activity of VDR protein is influenced by polymorphisms in the VDR gene” was changed to “As reported, the activity of VDR protein is affected by VDR gene polymorphisms”

(4) Methods:

1) “The exclusion criteria were as follows: (i) parents/guardians who did not authorize the child’s participation in the study; (ii) patients with syndromes and who were systemically compromised” was cited from the published study performed by Antunes et al. Thus we changed this sentence to “The exclusion criteria were according to published study performed by Antunes et al.”

2) “The children underwent dental examination at teacher's office of the kindergarten…….The kappa values for inter-examiner’s reliability to distinguish between sound and enamel/dentinal caries were 1” was changed to “All individuals underwent dental examination by two dentists with uniform training at teacher's office of the kindergarten. Deciduous tooth decay was diagnosed by visual examination and confirmed using a modified protocol recommended by World Health Organization. All examination results were used to calculate the Kappa value, which used to determine the inter-examiner’s reproducibility were 1.”

3) “The results were noted in a standardized examination chart…….based on caries experience” was changed to “The decayed, missing and filled teeth (dmft) index was used to assess deciduous tooth decay. According to dmft, all individuals were separated into two groups based on caries experience. The results were indicated in the standardized checklist marked with a continuous reference number”

4) “The results were recorded by a classification of 0, 1, 2, 3, that is……and level 3: >1,000,000 CFU/mL” was changed to “The results were recorded by a classification of 0, 1, 2, 3 according to the manufacturer’s instructions’
5) “The epithelial cells for DNA extraction were obtained by rubbing the buccal mucosa with a swab. Genomic DNA was extracted with TIANamp Swab DNA Kits (TIANGEN BIOTECH, BEIJING, China) according to the manufacturer’s instructions. DNAs were stored at -20℃” was changed to “DNAs were obtained from epithelial cells in buccal mucosa and were extracted with TIANamp Swab DNA Kits (TIANGEN BIOTECH, BEIJING, China) according to the manufacturer’s instructions, then stored at -20℃.”

6) “The four PCR fragments generated were subjected to restriction enzyme digestion……under UV light (Transiluminator 4000; Stratagene La Jolla, CA, USA)” was changed to “The BsmI, TaqI, ApaI and FokI fragments were digested by restriction endonuclease. The digestion products of four VDR gene polymorphisms were separated, stained, and recorded under UV light (Transiluminator 4000; Stratagene La Jolla, CA, USA).”

7) “Data was analyzed using the Statistical Package for the Social Sciences 20.0 software platform (SPSS 20.0)” was changed to “Data analysis was performed by the SPSS 20.0 software platform”

8) “Odds ratio (OR) calculations and chi-square test were performed to compare……allele and genotype frequency differences.” was changed to “Odds ratio (OR) calculations and χ² test were performed to evaluate differences of allele, genotype, gender, age and S. mutans between caries-experience groups with control groups. p<0.05 was indicated statistically significant difference.”

9) “The binary logistic regression was adjusted for genotype……from Hardy-Weinberg equilibrium” was changed to “The binary logistic regression analysis was performed after adjusting by genotype, age and S. mutans factor. The deviation from Hardy-Weinberg equilibrium was tested by standard Chi-square test”

(5)Discussion:

1) “Dental caries is a lifestyle-related disease involving complex interplay between heredity and environment” was changed to “Dental caries is caused by a complex interaction between genetics and the environment”

2) “40-65% of caries risk has been attributed to genes factor……oral hygiene, and host factors” was changed to “more than 40% of caries risk was attributed to genetic factors. Published studies found that the caries risk may be determined by patients’ DNA, in addition to environmental factors related to the risk of dental caries, such as oral hygiene, diet, bacteria and host factors”

3) “Mutations in genes linked with enamel formation such as enamelin (ENAM), amelogenin(AMELX), matrix metalloproteinase-20 (MMP-20)” was changed to “Mutations in some genes involved in enamel formation, such as enamelin (ENAM), amelogenin(AMELX), matrix metalloproteinase-20 (MMP-20)”

4) “Also, genetic variation in MMP-13 and MMP-20 was linked to caries susceptibility during the initial process of enamel development” was changed to “Also, genetic variation in MMP-13 and MMP-20 was involved in increased caries risk during the enamel development”
5) “Vitamin D is a key hormone regulating the calcium metabolism, and promotes the deposition of calcium onto the enamel” was changed to “Vitamin D regulates the metabolism of calcium and promotes calcium depositing onto the enamel”

6) “genetic influences on dental enamel that impacted caries differ between the primary and permanent dentitions” was changed to “influences of genetic factors on enamel caries differ between the primary and permanent teeth”

7) “In this study, we only investigated the association between VDR polymorphism and caries susceptibility of children in Guangzhou……and its interactions with the environment and other susceptibility genes” was changed to “The limitation of study was that we only investigated the relationship between VDR polymorphisms and caries risk of children in Guangzhou, China; however, dental caries was caused by multiple genetic factors and environment. The mechanism of the possible effect of VDR on susceptibility to dental caries remained unclear, further research in people with different ethnic backgrounds should be performed to reveal the mechanism of VDR gene in caries incidence, and clarify its interactions with other susceptibility genes and environmental factors”

(6) Availability of data and materials:

“The datasets used and/or analysed during the current study available from the corresponding author on reasonable request” was changed to “The data and materials of the present study were available from the corresponding author”

(7) Acknowledgments:

“We thank Qian Zhao from the Guangzhou Medical University and Hongbing Guan from the University of South Carolina School of Medicine for their positive comments and correction to improve our manuscript”

4. Please copy the statement “All procedures were performed with adequate understanding and written consent of the parents/guardians” (page 4, lines 34-35) and include it in the Ethics approval and consent to participate section of the Declarations.

Respond: Thank you for your comment. As you suggested, we included the statement “All procedures were performed with adequate understanding and written consent of the parents/guardians” in the Ethics approval and consent to participate section of the Declarations.

5. Please use initials only rather than full names in the Authors’ contributions section.

We would also like to ask for you to provide more justification for the contributions of Jiang Qian-zhou, as currently they do not automatically qualify for authorship. Contribution to obtaining funding, alone, does not usually justify authorship.
Respond: Thank you for your comment. As you suggested, we used initials in the authors' contributions section. And we have provided more justification for the contributions of Jiang Qian-zhou.

6. Please provide a list of all the abbreviations used in the manuscript. This list should be placed just before the Declarations section. All abbreviations should still be defined in the text at first use.

Respond: Thank you for your comment. As you suggested, we have provided a list of all the abbreviations used in the manuscript in the Abbreviations section.

7. Please submit your revised manuscript as a clean copy without any tracked changes, coloured or highlighted text, as these are no longer required at this stage of the editorial process.

Respond: Thank you for your comment. We have submitted our revised manuscript as you required.