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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Version: 1 Date: 05 Feb 2017

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 reviewer’s 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. Major changes made in the text were highlighted in red. Our responses to the reviewer’s 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

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Response to the reviewer’s comments on manuscript entitled “The Association of Vitamin D Receptor Gene Polymorphism with Deciduous Tooth Decay in Chinese Children” (OHEA-D-16-00453).

Reviewer #1:

The authors of manuscript "The association of vitamin D receptor gene polymorphisms with deciduous tooth decay in Chinese children " investigated a possible association between four SNPs in VDR gene and caries susceptibility in a group of 380 subjects with and without dental caries. The authors conclude that VDR BsmI polymorphism can be associated with risk of caries in deciduous teeth in Chinese population. However, I have several concerns about this manuscript and there are many points that should be revised and/or commented.

1. Introduction:

a) Introduction of this manuscript contains several identical information as discussion (e.g. sentences "Dental caries is one of the most common....", "A randomized trial demonstrated that vitamin D deficiency....", "Vitamin D is critical to the maintenance of a constant.........", "The biological function of vitamin D was mediated......", "As reported, the activity of VDR protein......" (this sentence even occurs three times). The same sentences cannot be used firstly in introduction and then in discussion.

Response: Thank you for your comment, we totally agreed with you. As you suggested, we revised the discussion of this manuscript and deleted the same sentences which occurred in the introduction section.

b) Please add the reference after the sentence "The prevalence of dental caries is more than 50%...." - the literature gives 90% prevalence in the population of dental caries

Response: Thank you for your comment. As you suggested, we added the reference (Dye BA, Hsu KL, Afful J: Prevalence and Measurement of Dental Caries in Young Children. Pediatric dentistry 2015, 37(3):200-216). This review article included 87 studies which determined the prevalence of dental caries in primary teeth, and 23 studies found the prevalence of dental caries were more than 50%.

c) The authors mentioned that "Untreated dental caries can lead to pain, tooth loss, and oral infection...." - what oral infection do they mean?
Response: Thank you for your comment. Oral infection means inflammation from caries related pulpitis and abscesses. We changed “oral infection” to “caries related pulpitis and abscesses” in the introduction of our manuscript.

d) The authors should also mention the importance of vitamin D as an immunoregulation factor (not only the factor that affects homeostasis of calcium and phosphate ions.

Response: Thank you for your comment. As you suggested, we mentioned the importance of Vitamin D in immune system, which were highlighted in red in the introduction section as follow, “Vitamin D not only helps regulate calcium levels, it is also an essential component of immune system[13]. Vitamin D deficiency lead to uncontrolled changes in the immune system, which in turn prevents the correct response to microbial infection, including bacterial infection, which occurs in the course of periodontitis or untreated caries disease[14].”

2. Material and methods:

a) Study population: The authors describe that 203 female and 177 male were included in this study. It will be better to write girls and boys (considering the fact that the age of children is 4-7 years).

Response: Thank you for your comment. We totally agreed with your opinion. As you suggested, we changed “203 female and 177 male” to “203 girls and 177 boys”.

b) Inclusion and exclusion criteria for children should be mentioned.

Response: Thank you for your comment. As you suggested, we listed inclusion and exclusion criteria in methods section of the manuscript in detail as follow: “Inclusion criteria involved agreement to comply with the study visits and procedures. 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”.

c) The authors should shortly describe how the levels of S. mutans correspond to the mentioned classification of 0, 1, 2 and 3.

Response: Thank you for your comment. As you suggested, we shortly described the levels of S. mutans correspond to the mentioned classification of 0, 1, 2 and 3 according to the manufacturer’s chart: The levels were classified according to the manufacturer’s instructions, that is: level 0: <10,000 colony forming units (CFU) Mutans streptococci/mL saliva; level 1: 10,000 < CFU/mL < 100,000; level 2:100,000 < CFU/mL < 1,000,000; and level 3: >1,000,000 CFU/mL.

d) If the PCR methods used in the manuscript were taken from the literature, the reference should be sufficient. Only if it is a newly developed method, detailed description should be given.
Response: Thank you for your comment. As you suggested, we deleted the detailed description of PCR, and insert the literature we referred to.

3. Statistical analysis:

a) The authors did not include any data on statistical power. I would like to see sample size and power calculation data included in the study design.

Response: Thank you for your comment. Power calculation was analyzed using the Power Analysis and Sample Size (PASS, version 2008). In the present study, 249 cases and 131 controls can achieve a statistical power of 0.8123 to calculate the risk probability of Bb genotype versus bb, which resulting in an OR of 1.845.

b) Authors should explain why is it possible that when they analyzed BsmI polymorphism with frequencies Bb 152 (61.4%) and bb 97 (38.6%), they did not find BB homozygote - did they try to sequence at least part of the samples which after the restriction analysis were Bb? According to the reviewer’s calculation, this result is highly improbable.

Response: Thank you for your comment. In the present study, we did not find BB homozygote, and we haven’t sequenced the samples which after the restriction analysis were Bb, as your suggestion, we should take this examination. But we believe that this result is reliable, because many studies in Chinese population neither found BB homozygote. A systematic review performed by Yu et al. showed 6 studies in BsmI polymorphism didn’t found BB homozygote in Chinese population (Meng Yu et al., Kaohsiung Journal of Medical Sciences). Also, Li et al. neither found BB homozygote in Chinese patients with generalized aggressive periodontitis (LI et al., J Periodont Res). Moreover, we thank you for pointing out the mistakes we made in calculating the frequency of BsmI polymorphism, the frequency of Bb should be 152 (61%) and bb 97 (39%), which we have revised in table 2.

4. Results:

a) Please describe number of caries in a group of children with caries (as mean ± SD or median ± quartiles).

Response: Thank you for your comment. As you suggested, we described number of caries in the group of children with caries (6.6±4.4).

5. Discussion:

a) Discussion is confused; authors jump from one issue to another and repeat a lot of information from the introduction.
Response: Thank you for your comment. As you suggested, we revised the discussion and deleted the same sentences which occurred in the introduction section of this manuscript, major changes made in the discussion were highlighted in red.

b) It should be useful to compare allele frequencies of VDR SNPs with the frequencies found within other studies in the Chinese population [26], for example, in BsmI the frequency highly differs.

Response: Thank you for your comment. As you suggested, we compare allele frequencies of VDR SNPs with the frequencies found within other studies in the Chinese population, which were highlighted in red. The frequencies of VDR SNPs in our study (performed in Guangzhou, southern China) were different with the frequencies found within other study by Li et al. (performed in Nanjing, eastern China) [26], for example in BsmI the frequency in our study highly differed with Li’s study, we speculated it was caused by geographic variations (Guangzhou versus Nanjing).

c) Some potential limitations to the present study should be considered.

Response: Thank you for your comment. We totally agreed with your opinion and added some potential limitations to the present study as follow: we only investigated the association between VDR polymorphism and caries susceptibility of children in Guangzhou, China; however, dental caries was likely to be influenced by multiple genetic factors and environment. The mechanism of the possible effect of VDR on susceptibility to dental caries is unknown, further research in people with different ethnic backgrounds will be required to delineate the true role of the VDR gene in caries incidence, and its interactions with the environment and other susceptibility genes.

6. References:

a) The selection of the literature is not completely representative - 3 studies about VDR polymorphism and dental caries have been published to date - the authors should add the third study (Izakovicova Holla et al., Caries Res), which is in compliance with their findings of “no association of TaqI VDR polymorphism with caries”. Similarly besides Bayram et al., also other studies exist confirming the differences in the caries genetic determination in the deciduous and permanent dentition (e.g. Borilova Linhartova et al., Caries Res 2016)

Response: Thank you for your comment, we totally agreed with your opinion. We inserted these representative literatures to our manuscript, and revised the discussion as follow: “According to studies conducted by Bayram M et al.[32] and Borilova Linhartova et al.[33], genetic influences on dental enamel that impacted caries differ between the primary and permanent dentitions. Thus, the inconsistency between our results and other reported study may be caused by difference between the primary and permanent dentitions. Cogulu et al.[34] found TaqI genotypes in VDR gene might be used as a marker for determining dental caries susceptibility in Turkish children, however our study and other study conducted by Izakovicova Holla et al.[35] found no association of TaqI VDR polymorphism with caries, this difference may reflect the geographic and ethnic variations.”
7. Fig. 1 and 2 are not necessary (could be omitted).
Response: Thank you for your comment. We omitted Fig. 1 and 2.

8. Minor comments:

*In title: Title should be "The association between vitamin D receptor gene polymorphism (not polymorphisms) and deciduous tooth decay in Chinese children" - only BsmI SNP was associated with caries.

*For enzymes, the abbreviation which forms the first three letters of the name of the restriction enzyme is written in italics and the following number is not in italics - e.g. TaqI

*There should be a gap between S. and mutans

*Page 6, paragraph 1: Correct SRBY GREEN as SYBR GREEN

*Page 6, paragraph 2: Correct New England Biolabbs as New England Biolabs

*Table 3: Values 0.000 should be corrected (values are not given without any valid number)

Response: Thank you for your comment. As you suggested, we changed the mistakes of this manuscript and highlighted them in red.

We changed the title “The Association of Vitamin D Receptor Gene Polymorphisms with Deciduous Tooth Decay in Chinese Children” to “The Association of Vitamin D Receptor Gene Polymorphism with Deciduous Tooth Decay in Chinese Children”.

We changed all abbreviations in italics except the following number.

We added a gap between S. and mutans.

We corrected SRBY GREEN as SYBR GREEN.

We corrected New England Biolabbs as New England Biolabs.

We corrected values 0.000 as “<0.001” Table 3.

9. The MS must be carefully edited, numerous stylistic inaccuracies and errors (gaps, brackets - e.g. Tab.2: BsmI and TaqI in comparisons with ApaI and FokI, misspellings) corrected and paper readability improved.

Response: Thank you for your comment. As you suggested, we carefully edited the manuscript and corrected numerous stylistic inaccuracies and errors.
Reviewer #2:

The manuscript is very valuable and interesting, however, I suggest following amendments:

1. The study design could be described in details - How were children recruited? Where did you examine the children (e.g. classroom, dental surgery, etc)? How were the children's teeth examined? It was mentioned that a calibration was done for examiners. Do the Authors have a kappa value? How was the diagnosis of dental caries confirmed?

Response: Thank you for your comment. As your suggestion, we described the study design in details. Children were recruited from the five kindergartens and two primary schools of Liwan district in Guangzhou city. The children underwent dental examination at teacher's office of the kindergarten. Children were seated in a chair, and the examiners used a natural light, tongue depressors, and gauze. The kappa values for inter-examiner’s reliability to distinguish between sound and enamel/dentinal caries were 1. Caries was diagnosed in primary teeth by visual examination and confirmed using a modified World Health Organization protocol recommended for oral health surveys.

2. Please check if this sentence is correct: Page 5 lines 6-9: The restriction fragment length polymorphism technique was performed in a final reaction volume of 20μL, by using 0.5μL BsmI, TaqI, ApaI and FokI (New England Biolabbs, Beijing, China) and a 5μL aliquot of polymerase chain reaction products, BsmI, TaqI, ApaI and FokI were digested at 65°C for 2, 2, 16 and 3 hours respectively.

Response: Thank you for your comment. As your suggestion, we changed “The restriction fragment length polymorphism technique was performed in a final reaction volume of 20μL, by using 0.5μL BsmI, TaqI, ApaI and FokI (New England Biolabbs, Beijing, China) and a 5μL aliquot of polymerase chain reaction products, BsmI, TaqI, ApaI and FokI were digested at 65°C for 2, 2, 16 and 3 hours respectively” to “The restriction fragment length polymorphism technique was performed in a final reaction volume of 20μL reaction mixture, containing 0.5μL BsmI, TaqI, ApaI and FokI (New England Biolabbs, Beijing, China), a 5μL aliquot of polymerase chain reaction products and 14.5μL ddH2O. The four PCR fragments generated were subjected to restriction enzyme digestion using BsmI, TaqI, ApaI and FokI restriction endonuclease, respectively”.

3. Could you add the number of the approval of the Institutional Ethical Committee of Guangzhou Medical University?

Response: Thank you for your comment. As you suggested, we added the number of the approval of the Institutional Ethical Committee of Guangzhou Medical University (No.2014018).

4. Could you write in “Authors' contributions”section who contributed to obtaining: the funding, the oral swab collection, the clinical genetics investigation and who performed the bioinformatics analysis?
Response: Thank you for your comment. We agreed with your opinion. As you suggested, we added Authors' contributions section as follows:

Kong Yuan-yuan wrote the first draft of the manuscript. Zheng Jian-mao wrote the final draft of the manuscript. Jiang Qian-zhou contributed to obtaining the funding. Yang Xue-chao and Zeng Su-juan worked the oral swab collection. Kong Yuan-yuan, Zhang Wen-juan and Yu Miao implemented the clinical genetics investigation. Kong Yuan-yuan and Zheng Jian-mao performed the bioinformatics analysis. All authors critically reviewed the final draft of the manuscript.

5. Please correct some minor grammar/language mistakes.

Response: Thank you for your comment and pointing out the mistake we made. We corrected all the minor grammar/language mistakes. Major changes made in the text were highlighted in red.

Reviewer# 3

Dear Braun,

I have just reviewed the manuscript entitled "The Association of Vitamin D Receptor Gene Polymorphisms with Deciduous Tooth Decay in Chinese Children". First, I would like to thank you for giving this opportunity. According to my opinion, it can be acceptable without any revision. Attach you can find the form.

Response: Thank you very much for your positive comments.