Author's response to reviews

Title: Association of vitamin D receptor polymorphisms with the risk of prostate cancer in the Han population of Southern China

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Author's response to reviews: see over
Dear Dr Melissa:

Thank you very much for reviewing our manuscript entitled: “Association of vitamin D receptor polymorphisms with the risk of prostate cancer in the Han population of Southern China” (Manuscript ID 4910149112673367) for BMC Medical Genetics. We were pleased with the overall positive reviews of our paper. We are now able to respond to these major, as well as minor, but important issues brought up by the two reviewers. Enclosed please find our revised manuscript.

Reviewer: Sonja Berndt

We were pleased that the reviewer felt that our “findings are important to those with closely related research interests”. We have corrected both the major and minor points, and thank the reviewer for his careful notations on our manuscript.

Major Compulsory Revisions

1) Background: The author state that the relationships of age, race, genetics and geography with prostate cancer incidence is not clear. However, I would argue that the relationship of factors, such as age, is clear, it is just that the underlying mechanisms or reasons why this is true are not clear. Also, the authors state that polymorphisms in the 3’ end of the gene may correlate with VDR mRNA stability and gene transcription; however, not all studies have observed this correlation.

Answer:

Thank you for your comments about the sentence “the relationships of age, race, genetics and geography with prostate cancer incidence are not clear”. As you argued, the relationship of factors, such as age, is clear, but the underlying mechanisms or reasons why this is true are not clear. Thus, this sentence should be replaced by “Age, race, and geographical factors are well-established risk factors of prostate cancer. However, these factors may not completely explain the differences between different ethnic groups in prostate cancer rates. Therefore, genetic variation in certain genes, including genes controlling vitamin D activity, could play a role in determination of susceptibility to prostate cancer”.

In our manuscript, we stated that “polymorphisms in the 3’ end of the gene may correlate with VDR mRNA stability and gene transcription”. After evaluating previous studies, we found it was the absence of evidence to show the variants in the 3’region of the VDR gene correlating with VDR mRNA, but the variants may interact differently with other upstream sequences to regulate transcription, translation, or RNA processing. These evidences can be obtained from many studies as follows:
2) Results p.9: It is not clear what is meant when the authors mention the “irrelevance of survival to old age.” I assume the authors are referring to the fact the SNPs are not associated with age as the authors do not appear to have done a survival analysis, but this is not clear.

Answer: No significant association in the distribution of age was found in our study among different genotype ($P>0.05$) based on the Independent-sample T test (Table 3). Maybe here, the expression in English is not clear and accurate, and we correct it to the sentence “Considering no association with the age distribution among different genotypes based on the Independent-sample T test ($P>0.05$, Table 3), we here hypothesized the BsmI ‘B’ allele might have a protective effect against tumorigenesis”.

3) Results: How do the minor allele frequencies reported in this study compare to other studies of Asian populations? In most studies, the ‘A’ allele at BsmI is rare in Asian populations. Here the ‘G’ allele at BsmI is rare, which is inconsistent with other databases.

Answer: Yes, exactly. In most studies, the ‘A’ allele at BsmI is rare in Asian populations, not the G allele. In our manuscript, we made a mistake that BsmI “BB” genotype is for GG genotype, not for the AA genotype. Therefore, we would correct these errors and all genotypes and alleles were all re-expressed. In the revised version of the manuscript, FokI, BsmI, Tru9I, ApaI, and TaqI, are reported according to the standard nomenclature in which lowercase and uppercase letters represent the presence or absence of a restriction site, respectively. The FokI T and C alleles are represented by $f$ and $F$, the BsmI G and A alleles by $b$ and $B$, the Tru9I G and A alleles by $U$ and $u$, the ApaI T and G alleles by $A$ and $a$, and the TaqI T and C alleles by $T$ and $t$, respectively. Thank you very much for the suggestion with the proper and thorough examinations.

4) Results p.10: Not all studies of BsmI have observed the same association with prostate cancer. A more comprehensive review is warranted. Also, the reference for the Japanese population (1st line of page 10) appears to be incorrect.

Answer: In the review titled “A systematic review of vitamin D receptor gene polymorphisms and prostate cancer risk”, you performed a systematic meta-analysis of population-based studies to investigate the relationship between the polymorphism of vitamin D receptor gene and the
susceptibility of prostate cancer, and no association was observed between the BsmI polymorphic
variant and cancer risk. Although the results from different populations were inconsistent, in most East-
Asians such as Taiwanese population (Huang et al. ref 29), Japanese population (Habuchi et al. ref 32)
and Southern Chinese population (our study), the BsmI “B” allele was observed to have a protective
role against the onset of prostate cancer, We hypothesized this association with disease risk might be
linked with the similar geographical distribution, habits and customs.
As to the second error, we had revised it and the reference for the Japanese population [22] (Ma et al.)
would be changed to [32] (Habuchi et al.).

5) Results p. 10: Ma et al found that the BB (or AA) genotype was associated with higher plasma
1,25(OH)D, not the GG genotype.
Answer: Yes, Ma et al. found that the BB (or AA) genotype was associated with higher plasma
1,25(OH)D, not the GG genotype. In our manuscript, we made a mistake that BsmI “BB” genotype is
for GG genotype, not for the AA genotype. Thus, in the revised version of the manuscript (page 10), we
corrected this errors and all genotypes and alleles were all re-expressed in the type of the restriction
site.

6) Discussion p 10-11: It is not clear what SNPs are included in the haplotypes being discussed.
Answer: In the revised manuscript, we had supplemented the order what SNPs are included in the
haplotypes on page 11. Thank you for your suggestion.

7) Table 3: I am not sure that giving the mean age for each genotype is useful. It would be more useful
to give the odds ratios for the association with prostate cancer as this is the objective of the study.
Answer: Exactly. It would be more useful to give the odds ratios for the association with prostate
cancer as this is the objective of the study. Thus, we modified them in Table 4. In this study, we
stratified the age to two groups (>71 and ≤71 years) and so that we could access the association
modified by the age. Among the elderly men, the OR value for the “B” allele was 0.09 (95%CI: 0.01-
0.68) compared with the “b” allele (Table 4), whereas the OR value among the younger men was 0.64
(95%CI: 0.25-1.66), suggested that the elder men with the “B” allele had lower risk of prostate cancer.

Minor Essential Revisions

8) Methods: Please clarify the definition of localized and aggressive cancer. Were any samples
replicated for quality control?
Answer: We accept the reviewer’s comment. In the revised version of the manuscript, the definition of localized and aggressive cancer has been supplemented on page 7. To control the experiments, a total of 80 samples were randomly selected and genotyped and confirmed by DNA sequencing by a second investigator. This part has been added on page 8.

9) Table 2: Is the age the age at diagnosis? Please clarify.
Answer: In our country, for various reasons, it is very difficult to make early diagnosis of the onset of prostate cancer. Thus, the age is the age at diagnosis, not the age of onset. It has been shown in the revised version (Table 2).

10) Table 4: Please give the number of cases and controls with each genotype for the different strata in the table, so that the reader can assess the basis and stability of the odds ratios presented.
Answer: Thank you for your reminding. In the revised version of the manuscript, the number of cases and controls with each genotype for the different strata has been listed in Table 4.

11) Table 6 and abstract. It is not clear what the reference group is for the haplotypes. Is each haplotype being compared with all other haplotypes or is each haplotype compared with one reference haplotype? It is usually most useful to choose one haplotype as the referent and to compare each haplotype to the referent haplotype. Also it is not clear what the order of the SNPs is in the haplotype.
Answer: It is usually most useful to choose one haplotype as the reference and to compare each haplotype to the referent haplotype. In our study, we compared the haplotype with non-haplotype, and we did not set a reference group. The aim we just did was to observe the association to cancer risk with each haplotype. In the revised manuscript, the order what SNPs are included in the haplotypes has been supplemented on page 11. Thank you for the suggestion.

Reviewer: Arslan Akhmedkhanov
We were pleased that the reviewer felt that our results are “interesting”, and “the strengths of the study include the well-defined study question and the appropriate methods that are well described”. We have corrected both the major and minor points, and thank the reviewer for his careful notations on our manuscript.
Major Compulsory Revisions

1. Background, page 5, end of the first paragraph. The authors state that “Although age, race, genetics, and geography may all be significant risk factors for prostate cancer [6], the relationships of these factors with disease occurrence are not yet clear.” It is not clear what the authors trying to say here. Age, race, and geographical factors are well-established risk factors of prostate cancer. I would recommend modifying this sentence to reflect that these factors may not completely explain the differences between different ethnic groups in prostate cancer rates. Therefore, genetic variation in certain genes, including genes controlling vitamin D activity, could play a role in determination of susceptibility to prostate cancer.
Answer: Thank you very much for your suggestion. Actually, We want to say that the relationship of factors, such as age, is clear, but the underlying mechanisms or reasons why this is true are not clear, and therefore as you suggested, we have changed to “Age, race, and geographical factors are well-established risk factors of prostate cancer [6]. However, these factors may not completely explain the differences between different ethnic groups in prostate cancer rates. Therefore, genetic variation in certain genes, including genes controlling vitamin D activity, could play a role in determination of susceptibility to prostate cancer.”

2. Methods, page 7. Although the authors have stated that “controls met the same eligibility criteria, except that they had never been diagnosed with cancer”, it is not clear what were the eligibility criteria for this study. The eligibility criteria should be clearly defined.
Answer: We agree that the eligibility criteria should be clearly defined. In the revised version of the manuscript, the eligibility criteria “The controls were screened to ensure that there had never been diagnosed with cancer and had low plasma prostate-specific antigen (PSA) levels (total PSA<4.00ng/ml). The controls were also checked for cancer history based on their past medical records and/or asked directly for their cancer history” has been added on page 7.

3. The authors state that the age-matched controls were “recruited from employees at the First Affiliated Hospital of Wenzhou Medical College”, whereas the cases were recruited from 4 different hospitals in Southern China. The authors should address the possibility of selection bias since the cases and controls were recruited from different hospitals. In addition, Table 2 indicates that there were 59 controls aged 70-79, and 18 controls aged greater than 80 years. Are these elderly controls also the current employees of the First Affiliated Hospital of Wenzhou Medical College? Please explain.
Answer:
The age-matched controls were “recruited from employees at the First Affiliated Hospital of Wenzhou Medical College”, whereas the cases were recruited from 4 different hospitals in Southern China. In fact, the First Affiliated Hospital of Wenzhou Medical College, which is also one of the 4 different hospitals in Zhejiang area, owned the similar range of patients and the common population who come to take physical examination. In other words, the probability is about the same for the people go to a hospital. Thus, the control subjects recruited from employees at the First Affiliated Hospital of Wenzhou Medical College may match the cases.

To match the age between cases and controls, we selected the elderly controls aged over 80 years, which are recruited from employees at the First Affiliated Hospital of Wenzhou Medical College. It may increase to some extent the bias of sampling, and make the representativeness weak. However, each SNP in the VDR gene was in Hardy-Weinberg equilibrium. Thus, it is acceptable for the subjects been recruited, we believed, to access the relationship between the VDR polymorphic variants and the risk of prostate cancer.

4. Results and Discussion, page 9. Please remove “in prostate cancer subjects” at the end of paragraph 1.
Answer: We agreed with the reviewer, and we have removed “in prostate cancer subjects” in the revises manuscript. Thank you very much for the suggestion

5. Results and Discussion, page 9. The first sentence of paragraph 2 states: “Considering the distribution of the BsmI allele among subjects and the irrelevance of survival to old age, which was not significant among alleles (Table3)…”. Please explain what you mean here by irrelevance of survival to old age. This sentence needs clarification.
Answer: We agree that the sentence is not clear and accurate, and therefore we have changed to “owing to no association in the distribution of age was found among different genotype (P>0.05) based on the Independent-sample T test, we here hypothesized the BsmI ‘B’ allele have a protective effect against tumorigenesis.”

6. Results and Discussion, page 10. Please indicate the order of SNPs in haplotype sequences listed here and in Table 6.
Answer: Thank you very much for the suggestion. In the revised manuscript, we had supplemented the order what SNPs are included in the haplotypes on page 11.
7. Results and Discussion, page 10. Here you state that ‘CGGTT” haplotype is associated with a decreased risk of prostate cancer. However, the provided OR=5.17 (95% CI: 1.13-23.75) suggest an increased risk. Please correct the statement or the OR (95% CI) here and in table 6.
Answer: In the revised manuscript, we corrected the OR (95%CI) in Table 6, and the correct value were 0.19 (0.04~0.89). In addition, we also revised the ORs (95%CI) of other haplotypes.

8. Results and Discussion, page 10. The authors mentioned that the allelic frequency of TaqI ‘T’ in the southern Chinese Han population was significantly higher than in white Americans and Portuguese populations. It would be also interesting to compare whether the allelic frequency of BsmI ‘G’ allele that is found to be associated with lower risk of prostate cancer, is higher in the Han population compared to the published data for Caucasian populations.
Answer: In our manuscript, we made a mistake that BsmI “B” allele for G allele, not for the A allele. Thus, we would correct these errors and all genotypes and alleles were all re-expressed. In the revised version of the manuscript, FokI, BsmI, Tru9I, ApaI, and TaqI, are reported according to the standard nomenclature in which lowercase and uppercase letters represent the presence or absence of a restriction site, respectively. The FokI T and C alleles are represented by f and F, the BsmI G and A alleles by b and B, the Tru9I G and A alleles by U and u, the ApaI T and G alleles by A and a, and the TaqI T and C alleles by T and t, respectively. Thank you very much for the suggestion.
In our studies, we compared the allelic frequencies in the Han population with those in Caucasian populations, and the allelic frequencies of FokI “F”, BsmI “B”, Tru9I “U”, and ApaI “A” were found in the Han population significantly lower than those in Caucasian populations, but TaqI “T” for higher. Oddly enough, why the lower incidence of prostate cancer in Asian population is inconsistent with the lower allelic frequency of BsmI “B”? We hypothesized the single polymorphic variant of BsmI may be linked to decreased cancer risk, but prostate cancer is a complex, multi-gene disease, and its etiology and pathogenesis may be linked not only to the BsmI, but also to the near SNPs, and even to other genes. Thus, the SNPs with Chinese characteristics may have own peculiar way of pathogenesis of prostate cancer.

Minor Essential Revisions
9. Replace “Peking” to “Beijing” on page 5.
Answer: Thank you very much for the suggestion. The word “Peking” has been changed to “Beijing” on page 5.

10. Remove word “protein” after “(VDR)” on page 5.
Answer: The “VDR” itself expressed as a protein, and we need remove the word “protein” here. Thank you very much.

11. Change “high” to “higher” in the second paragraph on page 10.
Answer: The word “high” has been changed to “higher” in the revised manuscript.

12. Change “Divided” to “Stratified” in the legend of Table 4.
Answer: The word “Divided” has been changed to “Stratified” in the revised manuscript.