Author’s response to reviews

Title: A genetic polymorphism of the osteoprotegerin gene is associated with an increased risk of advanced prostate cancer

Authors:

Naofumi Narita (narita@doc.med.akita-u.ac.jp)
Takeshi Yuasa (yuasa@doc.med.akita-u.ac.jp)
Norihiro Tsuchiya (tsuchiya@med.akita-u.ac.jp)
Teruaki Kumazawa (kuma@doc.med.akita-u.ac.jp)
Shintaro Narita (nari6202@gipc.akita-u.ac.jp)
Takamitsu Inoue (takamitu@doc.med.akita-u.ac.jp)
Mitsuru Saito (mitsaito@med.akita-u.ac.jp)
Yohei Horikawa (horikawa@doc.med.akita-u.ac.jp)
Shigeru Satoh (shigerus@doc.med.akita-u.ac.jp)
Osamu Ogawa (ogawao@kuhp.kyoto-u.ac.jp)
Tomonori Habuchi (thabuchi@doc.med.akita-u.ac.jp)

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Author’s response to reviews: see over
Dear Editor and Reviewers

We were very happy to learn that we have been given the opportunity to respond to the reviewers and revise our manuscript entitled “A genetic polymorphism of the osteoprotegerin gene is associated with an increasing risk of advanced prostate cancer.” by Naofumi Narita et al. We would like to re-submit our manuscript with the appropriate modifications and revisions for publication in the genetics section of *BMC Cancer.*

We believe that our manuscript, which has been greatly improved by the reviewers’ helpful comments, will provide general urologists as well as urologic oncologists with valuable information. Your consideration of the revised manuscript for publication in *BMC Cancer* is greatly appreciated.

Sincerely yours,

Takeshi Yuasa M.D.Ph.D.
Department of Urology
Akita University School of Medicine
1-1-1 Hondo, Akita, 010-8543, Japan
Phone: +81-18-884-6156
Fax: +81-18-836-2619
E-mail: yuasa@doc.med.akita-u.ac.jp

Dear Dr. Jovanny Zabaleta
Thank you very much for your interest in our manuscript. We are grateful for your helpful comments. We believe that our manuscript has been greatly improved by your helpful comments.

Minor:
Q1: I suggest doing a general grammar checking to the manuscript, especially to the abstract section.

To accommodate the Reviewer’s comments, we did a general grammar checking to the manuscript. We appreciate very much if the Reviewer notices an attached certification of Dr. Patrick Hughes, Bioedit LTD.

Q2 -Since these two SNPs (149T/C and 950T/C) are at the promoter region, I suggest denoting them as -149T/C and -950T/C to indicate that they are upstream of the transcription start site.

Various investigators used these names, 149 T/C and 950 T/C. In order to let the readers better understand, we express these SNPs, 149 T/C (rs3134071, 1024 bp upstream of the translation initiation site) and 950 T/C (rs2073617, 223 bp upstream of the translation initiation site)

Q3 -The authors should state if the 950T/C SNP is in LD with some neighboring SNPs. This is an important point to understand the role of this SNP in the transcription of the OPG gene.

To accommodate the Reviewer’s comments, we added the following sentence in the Results section. Page 11, line 6
“No SNP has been reported, which is in strong linkage disequilibrium with 950 T/C.”

Q4 -The method for the OPG genotyping is a little repetitive. I suggest cutting it a little in order to make it easier to read.

To accommodate the Reviewer’s comments, we shortened the OPG genotyping in Materials and Methods section.

Q5 -In the section dedicated to patient description, please do it consistently with the order in Table 1 (A through D).

To accommodate the Reviewer’s comments, we did it consistently with the order in Table 1.
Q6 - In Tables 1 and 2, in the footnote, the labels a, b, and c refer to what in the Tables?

To accommodate the Reviewer’s comments, we labeled a, b, and c in Table 1 and 2.

Q7 - Why were the levels of OPG determined only in 66 patients with localized PC?

We measured all of 66 serum samples, which we could measure.

Q8 - In the genotyping of the OPG gene: the authors said that the 950T/C SNP is located at the promoter of OPG, but in this section they say it is 801 bp downstream of the 149T/C SNPs (also located in the promoter region). This means that the 950T/C would be located in the coding region of the gene. In other words, there is an inconsistency here that needs to be clarified.

149T/C SNP is located at 801 upstream of the 950T/C SNP. Both of these SNPs are located at the putative promoter regions.

Q9 - It looks that the common allele for both polymorphisms is the T allele. So, I wonder if the authors did the same analysis using the TT genotype as reference and if so, what results they obtained.

When we did use T allele as reference, C allele could be considered as the protective allele. Therefore, we described it in the Discussion section, as below.

The presence of the variant C allele may have a considerable protective effect against metastasis or advanced disease status in patients with PCa.

Q10 - It has been shown that PSA induces OPG production (Yonou H et al, Prostate 67(8):840, 2007), did the authors check the correlation of PSA and OPG?

In this study, we did not check the correlation of PSA and OPG. To accommodate the Reviewer’s comments, we will analyze this correlation among the control subject and prostate cancer in a relatively large scale in the next study.
Dear Dr. Yen Ching Chen

Thank you very much for your helpful comments. We believe that our manuscript has been greatly improved by the reviewers’ helpful comments.

Q1 Why the authors believe that only two SNPs in the promoter region could explain the genetic regulation of bone mass? Has the LD structure been analyzed in this gene region? Are these two SNPs strongly linkage disequilibrium with other SNPs? If not, then these two SNPs may only explain part of the effect of this gene and the risk of prostate cancer.

We can agree the reviewer’s helpful comment. To accommodate the Reviewer’s comments, we added the following sentence in Discussion section.

“Alternatively, it is possible that these polymorphism is in linkage disequilibrium with other mutation(s) that alters OPG function.”

Q2 “The prostate specific antigen (PSA) levels of all the controls were measured and men with a PSA level of 4.0 ng/ml or more were omitted from the control group” Does this mean that the authors excluded controls without PSA information?

This means that we did not consider men with a PSA level of 4.0 ng/ml or more as control.

Q3 “A recent report revealed that the OPG polymorphisms in the promoter region, 149 T/C, 209 G/A, and 245 T/G, show complete linkage [9]”. Does the linkage here actually mean “linkage disequilibrium”? Please clarify.

To accommodate the Reviewer’s comments, we changed the following sentence in Results section.

“A recent report revealed that the OPG polymorphisms in the promoter region, 149 T/C, 209 G/A, and 245 T/G are in strong linkage disequilibrium.”

Q4 “to evaluate the risk of PCa according to the OPG genotype, logistic regression analysis was conducted with an adjustment for age at the time of diagnosis (Table 1 A, B). No significant increased risk was observed among the different genotypes of
patients with PCa and the controls (P=0.939 and 0.294 for 149 T/C and 950 T/C polymorphisms, respectively).” These results were from Table 2 instead of Table 1. In addition, the author only put the results of 950 T/C in Table 2 but lack of the results for 149 T/C. Because these are main results, it is weird only put the results of one SNP.

To accommodate the Reviewer’s comments, we added the following sentences in Results and Discussion section.

Page 13
Regarding the 149 T/C polymorphism, no significant differences in the allele frequencies were found between low/intermediate and high grades or between low and high stages (data not shown).

Page 17
“In this study, no significant differences in the allele frequencies of 149 T/C polymorphism were found in the clinical and pathological variables in the patients with PCa.”

Q5 Page 14, please check the spelling of alkariphosphatase (ALP).
It is odd that the authors automatically delete all results for 149 T/C after Table 1 and in Discussion. The authors should explain why this is the case or they should drop out 149 T/C from the manuscript.

To accommodate the Reviewer’s comments, we changed it to alkaline phosphatase (ALP). Regarding 149 T/C SNP, we added the following sentences in Results and Discussion sections.

Page 13
“Regarding the 149 T/C polymorphism, no significant differences in the allele frequencies were found between the clinical variables of these patients and the genotype (data not shown).”

Page 15
“We also investigated the relationship between the OPG serum level and the 149 T/C and 950 T/C genotypes in healthy controls and patients with PCa; no significant association was found (Figure 2A).”
Page 17
“In this study, no significant differences in the allele frequencies of 149 T/C polymorphism were found in the clinical and pathological variables in the patients with PCa.”

Q6 For figure 1, the author should calculate the p-value comparing survival between different genotyping groups.

We have described these p-values in Results section.

Although the difference was not significant, the cause-specific survival of patients with the CC genotype was better than that of patients with the TC or TT genotype (OR = 2.938 and 3.018 for TC and TT genotypes compared with the CC genotype, P = 0.087 and 0.082, respectively. Figure 1).

Q7 Page 15, …”however, the age of the patients with PCa was also significantly higher than that of the healthy controls.” Please give P-value for this result.

To accommodate the Reviewer’s comments, we added P-value in the following sentence.

“however, the age of the patients with PCa was also significantly higher than that of the healthy controls (P = 3.6E-10).”

Q8 Page 19, the comparison between DNA (SNPs), serum (protein), and mRNA level are interesting. It will be better if the authors could find a reference for mRNA expression level in human instead of in mice.

We have much interest in the comparison between DNA (SNPs), serum (protein), and mRNA level. However, we could not find a reference for mRNA expression level in human instead of in mice.

Q9 The subtitles in RESULTS are too long and may not be necessary.
To accommodate the Reviewer’s comments, we shortened the subtitles in RESULTS.