Author's response to reviews

Title: Single Nucleotide Polymorphisms in DNA Repair Genes as Risk Factors Associated to Prostate Cancer Progression

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Author's response to reviews: see over
December 5, 2014

Dear Sir,

Thank you for considering our original paper entitled “Single Nucleotide Polymorphisms in DNA Repair Genes as Risk Factors Associated to Prostate Cancer Progression” for publication in BMC Medical Genetics.

As suggested, we have carefully considered all the comments of the Referees and the manuscript has been accordingly changed. Please, find enclosed the reviewed version of the manuscript and our comments to the Referees further down.

We feel that the quality of the paper was highly improved following the Reviewers’ considerations.

Yours sincerely,

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REVIEWER’S COMMENT

Reviewer #1
In the present manuscript, Henriquez-Hernandez et al evaluated the effect of single nucleotide polymorphisms in 6 genes involved in DNA repair and prostate cancer progression. The methods are appropriate. The study design is adequate. Although the study lacks novelty, the results might be of importance in the field of prostate cancer research. The results are interesting and may give a light on the process of carcinogenesis of prostate cancer. However the work and the manuscript have several limitations and imperfection that should be considered and corrected.

Authors’ comments
We thank the effort made by the reviewer to improve the quality of the manuscript.

Major Compulsory Revisions
1. The association of polymorphisms and prostate cancer risk has been intensively studied. The authors point out that the population is of Spanish origin. An argument should be provided as to why major differences in genetic risk underlying disease phenotype would be expected in a population that is relatively closely related to other populations on the European continent.

Authors’ comments
We agree with the referee in this point. In our opinion, the real role of some SNPs would be underestimated by the fact that the genotype distribution of polymorphisms could be different among people with the same ethnic origin. We have demonstrated this fact in our population (PLoS One. 2013 Jul 23;8(7):e69735. PMID: 23936089). We have also postulated that this not-taken-into-account variable could be an important confounding factor responsible for the lack of validation of SNPs associated with radiation-induced toxicity (Lancet Oncol, 13 (2012), pp. 65–77). Thus, we consider that this type of studies, where the role of SNPs as risk factors are studied, must be carried out in populations that present no different SNPs genotype distribution. Moreover, the literature is full of SNPs that are risk factor for different diseases among Chinese population but not among African or Caucasian populations. A clear example is the role of GGN and CAG repeat polymorphisms in the exon-1 of the androgen receptor gene as risk factor for prostate cancer among black population (J Hum Genet. 2008;53(3):220-6. Int J Androl. 2008 Feb;31(1):25-30).

Nonetheless, following the recommendation of the reviewer, we have included a paragraph in the Introduction section to clarify this point.

2. Authors use a threshold P value 0.05. This does not take into account the fact that several SNPs are addressed over several phenotypes. This increases the likelihood of false positive results and would be avoided by applying a correction for multiple testing.
Authors’ comments
We absolutely agree with the referee. In fact, according to the Radiogenomics Consortium guidelines (STROGAR), it is recommended the use of statistics corrections (i.e. Bonferroni correction) to avoid false positive results (Radiother Oncol. 2014 Jan;110(1):182-8). Nonetheless, this fact is encouraged in GWAS studies and other high-throughput technologies.

Anyhow, we insist that we agree with the comment from reviewer. However, we humbly think that the p-values showed in our study are robust (< 0.0001 for the main results), and they are reproducible in different genetic and statistical models. We hope this is enough to convince the reviewer and the readers of the validity of the results.

3. The rs25489 and rs1799782 in XRCC1 and rs1805388 and rs1805386 in Lig4 seems to be in perfect linkage disequilibrium, thus only on SNP from both genes are needed to be genotyped and presented in all the text and tables. This argument should be analyzed.

Authors’ comments
This is a high quality observation that reflects the level of knowledge of the reviewer. We absolutely agree with the reviewer. We would like to provide information about as much SNPs as possible, and for that reason, we would like to keep as much information as possible. Although one SNP from both genes (for any case) are needed, we wanted to include all the information regarding to the genotype and allelic distribution, and all the potential relations with clinical variables. Nonetheless, we would include this key information in the footnote of Table 2 (Table 1 in the old version of the manuscript).

Minor Essential Revisions
Table 1: instead of percentage, the number for respective alleles, OR and 95% CI should be presented.

Authors’ comments
The genotypic and allelic frequencies were determined using an online tool named SNPator (developed by CeGen (Spain’s National Genotyping Center) and INB (National Institute for Bioinformatics)). SNPator gives an output file containing only percentages. For genotype distribution, it is easy to include the number of subjects carrying each specific genotype, but it is not as simple for the allelic frequencies. Moreover, presenting allelic frequency as percentages allows the comparison with the MAF (minor allele frequency), a fact that gives information about the allelic distribution in the reference population.

Thus, we request approval of the reviewer to maintain the table in its current format. If she considers that our arguments are insufficient, we will try to correct this table subsequently.

Only two decimals should be used in all the text and tables.
Authors’ comments
We have revised all the text and tables and only two decimals were included (except for p values, that are classically expressed with three decimals).

**When genes mentioned, the name of the gene should be in italics.**

Authors’ comments
We have revised all the text and tables and this topic was properly addressed.

**Table 2 and 3 should be placed in supplementary documents.**

Authors’ comments
We thank the referee all the effort made to simplify our manuscript and improve its understanding. We placed table 3 as supplementary material. We decided to keep table 2 in its present form because referee 2 gave much importance to the exhibition of the clinical data of the series. Please, note that Table 2 is Table 1 in the new version of the manuscript (accordingly to the comments made by referee 2).

**Figures 1 and 3 should be also placed into supplementary documents**

Authors’ comments
Following the instructions of the referee, figures 1 and 3 were placed into supplementary documents.
Reviewer #2:
MAJOR COMPULSORY REVISIONS

1) Abstract: cT2b is hardly an aggressive tumour. I would focus on T3-4 tumours. The conclusions must not repeat the results of your study. You should rather stress your take-home message.

Authors’ comments
We thanks to referee 2 all the comments made to improve the quality of the manuscript, especially in its clinical side.

We agree with the referee in that cT2b tumors are hardly aggressive tumors. However, the aggressiveness of prostate cancer can not be addressed taken into account the clinical tumor size, exclusively. It is possible to have a cT2b tumor with a Gleason score 9 that would be a very aggressive disease. However, cT2b tumors are considered intermediate risk tumors, according to de D’Amico classification. In our analysis, we tried to segregate patients accordingly to the D’Amico classification (a fact that can be observed when patients are segregated according to Gleason score (<7 vs. ≥ 7) or PSA at diagnosis (< 10 vs. ≥ 10 ng/mL)). Nonetheless, we absolutely agree with the referee; thus, we decided to delete the word “aggressive” in that sentence to avoid misunderstanding and change as follows: “It is need to develop new biological markers associated with the tumor behavior which would be valuable to better individualize treatment”.

Following the comment of the referee, the conclusions of the abstract were changed as follows: “Conclusions. Genetic variants at DNA repair genes are associated with prostate cancer progression, and would be taken into account when assessing the malignancy of prostate cancer”. We humbly think that this is the most important take-home message of our study.

2) Introduction: in the first paragraph, I would suggest to remove the epidemiological data, which are well known in the urological community. You could use these words to better explain the background of study in terms of genetic findings up-to-date.

Authors’ comments
Following the comment of the referee, this section was deleted and the introduction has been rewritten.

In the study aim you should stress why you focused on these particular 10 SNPs (one sentence explaining the link between the six genes and tumour aggressiveness would do).

Authors’ comment
We agree with the referee. These 6 genes have been classically associated to prostate cancer risk, and even to radiation-induced toxicity (a phenomenon highly determined by the efficiency of DNA repair pathways). All these SNPs were explored in a previous publication in the same series, as stated in the introduction section of our manuscript (PLoS One. 2013 Jul 23;8(7):e69735). Following the comment of
the referee, we added a sentence and a reference at the aim section to explain this topic. We included a hypothesis of the study to improve the understanding of our study.

I would rephrase the sentence about PSA screening, which is a very complex problem not limited to overdiagnosis and overtreatment.

Authors’ comments
Following the opinion of the referee, we decided to delete the sentence regarding to PSA screening. It is a very complex problem that should not be exposed in one sentence.

Please revise the manuscript for grammatical errors (i.e. 12 men HAVE to be...).

Authors’ comment
The manuscript was carefully revised before the resubmission. Grammatical errors were detected and corrected in the new version of the manuscript.

3) Methods: how was clinical stage assessed? With DRE, with imaging..?

Authors’ comment
This is a very good observation. We included this important information in the Methods section as follows: “Clinical tumor size was assessed by digital rectal examination (DRE) followed by transrectal ultrasonography (TRUS) and magnetic resonance imaging (MRI); PSA serum levels were assessed by chemiluminescence in an Architect i2000 analyzer (Abbott Laboratories, IL, USA); Gleason score was determined in the biopsy specimen by a pathologist.”

I would avoid to report in the M&M section every institution participating in study, as they are already shown in the title page.

Authors’ comment
Following the instructions of the reviewer, this section was accordingly deleted in the new version of the manuscript.

Rather, you should briefly explain which was the study protocol followed for each patient: baseline infos collection, blood samples, analyses, and so on...

Authors’ comment
Following the instructions of the reviewer, some sentences were added at the end of the last paragraph of section 2.1: “After collecting demographic and clinical data, a blood sample was taken after the signature of informed consent. All samples were sent by courier to the Hospital Universitario de Gran Canaria Dr. Negrín for DNA isolation and genotype analyses as follows”.
4) Results: you should describe the baseline patient info! Clinical data of your patients are essential, better if resumed in a table. In my opinion, the whole section should be modified, reporting in a more effective way your results.

Authors’ comment
Clinical data of patients are resumed in Table 2. The paragraph containing all the clinical info was moved to the first paragraph of the Results section to increase its importance. Thus, Table 2 is now Table 1. All the genotype information was located below and we think that, thanks to the referee’s comment, the Results section is more understandable and ordered.

You speak about two SNPs: what about the others? You say that only 2 SNPs were significantly differently distributed according to clinical variables (only stage and Gleason?): you should better report your findings (all SNPs, all clinical variables).

Authors’ comment
All the 10 SNPs were analyzed following the same statistical tests. This kind of studies give a great amount of not meaningful information, and authors should simplify the results to be presented simply and understandable throughout the manuscript. In the new version of the manuscript, and following the recommendation of Reviewer 1, some tables and figures were placed as supplementary files, trying to simplify the results and highlighting the truly important findings. We humbly think that tables and figures have to highlight the most important results, and should be avoided the inclusion of worthless information that contributes to confusion. We hope we have convinced the reviewer with these arguments.

I think that reporting only the distributions is not enough. If you speak about associations between SNPs and clinical variables, you should report crosstabs showing p-values and statistical tests used.

Authors’ comment
Genotype and allelic distribution is just the first stage of the analyses, and are contained in Table 2 (Table 1 in the old version of the manuscript). Table 3, Figure 1 and all the supplemental material reported all the significant associations between genetic and clinical variables. Even more, Supplementary file 1 is a crosstab table showing these significant associations. Figure legends, table footnotes and supplementary materials include the statistical tests employed to reach the significant results. Taken together, we humbly think that we fit properly to the requirements of the reviewer.

I understand that you focused on rs11615 and rs17503908 as they were the only one to show an association with clinical variables: again, you should better report your results! Did you perform chi-square analysis to calculate an OR?

Authors’ comment
We think that we answered to this request above. Throughout the text and also in the footnotes of tables and figure legends, we included the statistical test employed to reach the results. Thus, chi-square test and binary logistic regression (a statistical test that gives an OR), among other statistical tests, were used in our study.

The risk of developing T2b - T4 tumours includes a huge variety of tumours: why you did not focus on T3-T4 (the high risks)? Did you find any associations between SNPs and higher values of PSA?

Authors’ comment

The risk of developing T2b – T4 tumors is just one result. Supplementary file 1 contains the association between rs11615 and all the cT, separately. The referee can see the same approach in Supplementary file 2, plot A. We group the cT for analyses contained in Table 3 because we performed at this stage a binary logistic regression; thus, it is needed to dicotomize the variable. For that reason, and trying to avoid biased presentation of results, we complemented the analyses comparing genetics vs. D’Amico risk groups.

We did not observe any statistical association between the 10 SNPs and PSA serum levels. This negative finding was noted in the last sentence in the Results section.

Do not speak of “bigger Gleason scores”! Rather, you should say “Gleason scores 8-10”. I think that the association with D’Amico groups is not necessary if you perform a thorough analysis on PSA, Gleason score and stage, which are the factors included in the D’Amico classification.

Authors’ comment

We absolutely agree with the reviewer. The words “bigger” was changed in the new version of the manuscript.

About the associations with D’Amico groups, we consider those findings as a positive control to the previous results, and it is the result that has the real clinical translation (contained in Figure 1). D’Amico classification offers to the urology a prognostic tool about the evolution of the disease. It has to be taken into account that the variables considered for D’Amico classification are not collinear. Thus, a cT1a tumor with a Gleason score > 7 or PSA levels > 10, it is not a good prognostic disease. The fact that a certain SNP is associated to, for example, Gleason score, and the same SNP is also associated to the D’Amico classification means that this particular SNP would have a clinical role far beyond the simple association with a particular clinical variable. In our study, we observed that the combination of the two significant SNPs have a more robust association with the D’Amico classification than the SNPs taken one by one (Figure 1). In our humbly opinion, the association with the D’Amico classification is of great value, and we hope that also the referee thinks the same after our explanation.
5) Discussion: the discussion should be done according to the previous modification. I would suggest to provide a theory about the biological reasons of your findings on a possible association between certain SNPs and more aggressive cancer features.

Authors’ comment
We absolutely agree with the referee. A new sentence was added at the end of the first paragraph of the Discussion section as follows: “These genetic variations would influence the nucleotide excision repair and double-strand break repair mechanisms of DNA, possible favoring genomic instability and the development of more aggressive cell phenotypes that would cause the appearance of tumors of poor prognosis (i.e. D'Amico high-risk tumors)”.

6) Conclusions: as for the abstract, the conclusion should not repeat your results. You should rather provide a clear take home message.

Authors’ comment
We absolutely agree with the referee. The Conclusions were modified as follows: “We found that genetic variants at DNA repair genes are associated with clinical variables of poor prognosis for prostate cancer. Prospective studies are required to validate our results”.