Author’s response to reviews

Title: Identification of viral infections in the prostate and evaluation of their association with cancer.

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Version: 4 Date: 6 June 2010

Author’s response to reviews: see over
Ms. Iratxe Puebla.

Subject: MS: 1903186081317681- Associations of Prostate Cancer Risk with the RNASEL R462Q Polymorphism and Viral Infection.

Enclosed you will find the re-structured article by Martinez Fierro, et al. MS: 1903186081317681 (MS: 1903186081317681) and the list of modifications as requested. For this new version, we carefully attended all the observations of the Reviewer 2, since the other reviewers did not request further changes.

We hope this new article version will satisfy the demands of the Reviewer and will be ready for publication. Thanks a lot for all your attentions and hope to hear from you soon,

Sincerely yours,

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REVIEWER REPORTS.

REFEREE 2.

Reviewer's report
Title: Associations of Prostate Cancer Risk with Viral Infection and the RNASEL R462Q variant.
Version: 3 Date: 10 March 2010
Reviewer: Nicole Fischer

Reviewer's report:
The authors submitted a new version of the paper addressing most of the points raised by the reviewers. In general, the manuscript still needs major improvement with regard to the main focus, discussion of the data (in this current version the discussion is not well supported by the data) and “take home message” of the manuscript, which is not Prostate Cancer risk and RnaseL R462Q variant. Furthermore, XRMV and prostate cancer risk is discussed too lengthy, the study does not include R462Q homozygous variants and hardly any XMRV positive cases were identified. This is not a technically advanced paper which could add significant information/clarification to the current discussion about technical differences in XMRV detection in the field.
The title of the manuscript still is misleading and should be changed: “Analysis of viral infections in Prostate Cancer”.
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.

LIST OF MODIFICATIONS.

Attending the reviewer’s considerations, the following modifications were included.

1. **Title (first line):** “Associations of Prostate Cancer Risk with Viral Infection and the RNASEL R462Q variant” **was changed to** “Identification of viral infections in the prostate and evaluating their association with cancer.”

2. **Abstract (Background section):** “Several viral infections in prostate tissue with oncogenic potential have been reported. Recently, the Xenotropic Murine Leukemia Virus-related gammaretrovirus (XMRV) was associated with prostate cancer (PC) in patients who were homozygous for the Q identified variant in the RNASEL gene (R462Q/Q). Association studies for the R462Q allele and non-XMRV viruses have not been reported. We analyzed possible associations among prostate cancer, prostate viral infections [polyomaviruses BKV, JCV, and SV40; human papillomaviruses (HPVs), human cytomegalovirus (HCMV), and XMRV] and the RNASEL 462Q allele in Mexican cancer patients and controls” **was changed to** “Several viruses with known oncogenic potential infect prostate tissue, among these are the polyomaviruses BKV, JCV, and SV40; human papillomaviruses (HPVs), human cytomegalovirus (HCMV), and XMRV] and the RNASEL 462Q allele in Mexican cancer patients and controls” **was changed to** “Several viruses with known oncogenic potential infect prostate tissue, among these are the polyomaviruses BKV, JCV, and SV40; human papillomaviruses (HPVs), human cytomegalovirus (HCMV), and XMRV] and the RNASEL 462Q allele in Mexican cancer patients and controls.”
non-XMRV viruses have not been reported. We assessed associations between prostate cancer, prostate viral infections, and the RNASEL 462Q allele in Mexican cancer patients and controls”.

3. Abstract (Results section): “HPV sequences were detected in 11 (20.0%) cases and 4 (5.3%) controls” was moved from line 41 to line 40.

4. In line 47, the word “proven” was replaced for “evaluated”.

Modifications in Background section.

5. The paragraph “Infections of the prostate with polyomaviruses (BK, JC, and SV40), human papillomaviruses (HPVs), and members of the herpesvirus family (HHV-8, HCMV, Epstein Barr virus) have been previously described [4, 7-11]. Viral products such as the large T antigen of polyomaviruses, or the E6 and E7 proteins of HPVs are able to induce cell transformation and interact with the signaling capacity of the interferon pathway in a synergistic manner [10]” was moved from line 61 to line 52.

6. In line 61, the paragraph “The R462Q variant of the RNASEL gene has been reported to occur 13% of sporadic cases of PC [11]. Urisman et al. identified the presence of the Xenotropic Murine Leukemia Virus-related gammaretrovirus virus (XMRV) in 40% (8/20) of PC cases homozygous for the 462Q allele of RNASEL [12]. The R462Q variant reduces the enzyme activity by about two-thirds and this may impact cellular response against viral infection [13]” was replaced for “The R462Q variant of the RNASEL gene has been reported to occur in 13% of sporadic cases of PC [16]. The enzyme activity of this variant is reduced about two-thirds and this may impact cellular response against viral infection [17]. The RNASEL variant R462Q is suggested to increase susceptibility for PC and has been associated with an increase in prevalence of the Xenotropic Murine Leukemia Virus-related gammaretrovirus (XMRV) [7, 9, 11]. No study has reported relationships among the variant, other viral infections, and PC. In the present study the association between viral infection, prostate cancer and the RNASEL variant are assessed.”

Modifications in Methods section.

7. Line 72: “All patients provided written informed consent for participation”, was modified to “All patients provided written informed consent prior to participation”.

8. Line 75: “serum specific prostate antigen” was replaced for serum prostate specific antigen”.

9. Line 82: “The control group was constituted by subjects who underwent a TRB or TURP, but had no pathological evidence for PC” was replaced for “The control group consisted of subjects who underwent a TRB or TURP, but had no pathological evidence of PC”.

10. Line 102: “The evaluation of R462Q variant (rs486907) of RNASEL was performed by Taqman assay” was replaced for “The evaluation of the R462Q variant (rs486907) of RNASEL was performed using the Taqman assay”.

11. Line 108: The paragraph “This was achieved through amplification by conventional PCR, using serial dilutions of control plasmids for each virus from 600,000 to 60 viral copies as template (Table 1 of Supplementary Material). The obtained products were visualized on
3% agarose gels stained with ethidium bromide” was corrected and rewritten as “This was achieved through conventional PCR amplification, using serial dilutions of control plasmids for each virus consisting of 600,000 to 60 viral copies (Table 1 of Supplementary Material). The reaction products were electrophoresed through 3% agarose gels and visualized by ethidium bromide staining”.

12. **Line 113**: “Briefly, 500 ng of DNA isolated from the prostate were subjected to amplification for polyomaviruses and HPV screenings” was replaced for “Briefly, 500 ng of DNA isolated from the prostate were subjected to PCR amplification designed to detect polyomaviruses and HPV”.

13. **Line 116**: “The HCMV detection was performed in two amplification rounds using sets of specific primers for the UL3 region, which amplified segments of 420 and 188 bp, consecutively” was replaced for “The HCMV detection was performed in two amplification rounds using sets of primers specific for the UL3 region, which produced amplicons of 420 and 188 bp, consecutively”.

Modifications in Results section.

14. **Line 142**: “A total of 130 men (55 prostate cancer cases and 75 controls) were entered into the study” was replaced for “A total of 130 men (55 prostate cancer cases and 75 controls) were enrolled into the study”.

15. **Line 144**: “47.3% of men with PC vs. 24.0% of controls were older than 70 years; mean age for cases was 71 years (range: 36-88) and mean age of controls was 66 years (range: 50-88), P=0.003. There was a statistical difference in PSA values between case and control groups (P<0.001). Median of PSA values were 18.30 ng/ml (range: 0.16-1062.00 ng/ml) for cases and 6.70 ng/ml (range: 0.20-25.99 ng/ml) for controls. Most men with PC had PSA values >10.0 ng/ml (Table 1)” was replaced for “For men with PC, 47.3% were older than 70 years of age while 24.0% of controls were older than 70 years. The mean age for cases was 71 years (range: 36-88) and the mean age for controls was 66 years (range: 50-88), p=0.003. There was a statistical difference in PSA values between case and control groups (p<0.001). The median PSA value was 18.30 ng/ml (range: 0.16-1062.00 ng/ml) for cases and 6.70 ng/ml (range: 0.20-25.99 ng/ml) for controls. Most men with PC had PSA values >10.0 ng/ml (Table 1)”.

16. **Line 157**: “All DNA/RNA samples isolated showed amplification of the GAPDH gene and they were therefore used for subsequent determinations. Frequencies of genotypes R/R, R/Q, and Q/Q for RNASEL R462Q were 0.62, 0.38, 0.0 for cases and 0.69, 0.24, and 0.07 for controls, respectively. Genotype frequencies for the control group had no departures from Hardy-Weinberg equilibrium by exact test (P= 0.1), and due the non representation of the Q/Q genotype in the PC cases in the study population, the association between the RNASEL R462Q variant and PC could not be determined. We also tested the association between PC or viral infection and R462Q genotypes identified in this study. For this, frequencies of the R/Q and Q/Q genotypes were grouped and compared with the R/R frequencies. No associations with PC (P=0.48) or viral infection (P=0.34) were found in this subset analysis” was corrected and replaced for “All DNA/RNA samples demonstrated amplification of the GAPDH gene and were therefore used for subsequent analyses. Frequencies of the RNASEL R462Q genotypes R/R, R/Q, and Q/Q were 0.62, 0.38 and 0.0 for cases and 0.69, 0.24 and 0.07 for controls, respectively. Genotype frequencies for the
control group did not depart from Hardy-Weinberg equilibrium by the exact test (p= 0.1),
and due to the lack of representation of the Q/Q genotype in the PC cases, the association
between the RNASEL R462Q variant and PC could not be determined. We also tested the
association between PC or viral infection and R462Q genotype. For this association,
frequencies of the R/Q and Q/Q genotypes were grouped and compared with the R/R
frequencies. No associations with PC (p=0.48) or viral infection (p=0.34) were found in this
subset analysis.”

Modifications in Discussion section.

17. Line 204: “However, replication studies of both the R462Q variant in PC or viral infections
have yielded conflicting results worldwide. This work analyzed associations among viral
infections, the R462Q variant of RNASEL, and PC” was substituted by “However, studies
designed to replicate the association of either the R462Q variant or viral infections with PC
have yielded conflicting results worldwide. This current study analyzed associations
between viral infections, the R462Q variant of RNASEL, and PC”.

18. Line 215: The paragraph “Our results for the XMRV virus in a Mexican sample differ from
those reported by Urisman et al. [11] who described 8 out of 20 XMRV positive samples in
Q/Q homozygous subjects versus 1 out of 52 positive samples in R/R subjects. XMRV
prevalence in sporadic PC cases has been notoriously lower than reported by Urisman.
Fischer et al. [9] identified two XMRV positive samples in 157 prostatic tissue samples and
none in eleven Q/Q homozygous tissue samples included in the study performed in
German subjects. In an additional report from this country, Hohn et al. [29] could not
demonstrate the presence of the XMRV in 589 prostate tumor samples using real-time
PCR. D’arcy et al. [7] reported no evidence of XMRV in Q/Q Irish PC patients. A recent
report by Schlaberg et al. [30] describes no association between XMRV infection and
RNASEL R462Q genotype; but in contrast, found association between retroviral infection
and PC.” was moved to line 251.

19. Line 216: The paragraph “This study analyzed retroviral DNA integration by qPCR and
expression of retroviral proteins by immunohistochemistry using a XMRV-specific antiserum
in paraffin embedded and frozen prostate tissues. Our results, based on studies on RNA
from fresh frozen tissues could not demonstrate association of XMRV prostate infection
with PC” was deleted.

20. Line 217: The paragraph “In this study, polyomavirus genomes were not detected in any of
the 130 evaluated tissue samples; however, six HCMV positive samples were detected in
the control group. Absence of HCMV, polyomaviruses, and XMRV in tumor prostate tissue
suggests that they are not associated with PC in Mexican population, though positive
control samples (with the exception of the XMRV’s positive sample) demonstrated chronic
prostatitis that may evolve to malignancy, as suggested by some authors [2-6]” was moved
to line 245.

21. Line 217: The paragraph “HPV screening showed that this infection increases the risk of
PC by 3.98 times (P=0.027), and HPV DNA sequences were found in 11/55 cases (20.0%)
and 4/75 (5.3%) controls. Our results are consistent with the data reported by Leiros et al.
[24], who demonstrated a significant association between HPV DNA presence and prostate
carcinomas in Argentina (41.5% prevalence in PC cases); in their study no HPV sequences
were detected in hyperplastic samples” was replaced by HPV DNA sequences were
found in 11/55 cases (20.0%) and 4/75 (5.3%) controls. This infection increases the risk of PC by 3.98 times (p=0.027). These results are consistent with the data reported by Leiros et al. [24], who demonstrated a significant association between the presence of HPV sequences and prostate carcinomas in Argentina (41.5% prevalence in PC cases).

22. Line 266: The sentence “The 462Q/Q RNASEL genotype was not represented in PC cases, therefore the Interactions among prostate viral infections, PC, and the 462Q allele could not be proven” was replaced by “The RNASEL 462Q/Q genotype was not represented in PC cases, therefore the association between prostate viral infections, PC, and the 462Q allele could not be proven”.
Reviewer’s report
Title: Associations of Prostate Cancer Risk with Viral Infection and the RNASEL R462Q variant.
Version: 3 Date: 18 March 2010
Reviewer: Oleg Alexeyev
Reviewer’s report:
I am satisfied with the authors revisions and comments and do not have further comments.
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report
Title: Associations of Prostate Cancer Risk with Viral Infection and the RNASEL R462Q variant.
Version: 3 Date: 11 May 2010
Reviewer: Douglas Curran-Everett
Reviewer's report: I am satisfied with the statistics.
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: I declare that I have no competing interests. DCE.