Author’s response to reviews

Title: The 4q27 locus and familial prostate cancer risk

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Response to review

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Title: The 4q27 locus and familial prostate cancer risk

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Date: 25 January 2010

Dear Editor,

We thank you for considering our manuscript for publication in BMC Cancer. Please find below our response to the reviewers comments. All changes made to the submitted version of the manuscript have been high-lighted in red for your convenience.

Response to Associated Editor:

1. Consider changing the title as the evidence is only suggestive of a familial prostate cancer effect and results were null overall.

Response: The title has been changed to “The 4q27 locus and prostate cancer risk.” Reference to an association with familial effect has been removed.

2. It would be helpful to have a paragraph in the discussion stating the power of the study, ie what size of effect can be excluded.

Response: The following sentence has been added to the first paragraph of the Discussion “At 0.05 level of significance under a dominant model the sample size of our study allows to detect with 80% power a minimum OR of 1.33 for the most common SNP (rs2069762) and 1.36 for the least common SNP (rs13151961). The corresponding minimum detectable ORs under a recessive model are 1.54 and 2.11.”

3. Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body, which gave approval, with a reference number where appropriate.

Response: The ethics approval, including reference number has been included in the Methods section, under the heading study population. "Ethics approval for
Response to Reviewer 1:

1. It is not clear whether this study is focused on familial or sporadic prostate cancer (PCa). The subject recruitment part did not clearly state about this, but only mentioned that family history of PCa were collected. In addition, the authors stated that “relatively small number of men with a family history of PCa in our study”. So it looks like the study design was to assess the association between genetic variations and the risk of all PCa (sporadic and familial). That is, it is inappropriate to use familiar PCa in the title just because the only significant finding in this study was observed in men with family history.

Response: The title has been changed to reflect better the overall study design. In the Methods section under the subheading “study population” the following reference to familial prostate cancer inclusion has been added, “and included irrespective of familial status”, i.e. the study includes both sporadic and familial (population-based) prostate cancer cases.

2. In Background, 2nd paragraph, the authors stated “Although the etiology of the inflammatory milieu is yet to be determined, …”, which is not true. There are abundant papers about inflammation responses in PCa recently.

Response: The authors have changed this sentence to read, “As an understanding of the role of inflammation in the etiology of prostate cancer begins to emerge, …..”.

3. In background and discussions, the authors stated “extensive linkage disequilibrium within 4q27 has hindered efforts to identify the true causal variants”, which is not true. The common disease/common variants hypothesis clearly tell us that the high LD is actually indirectly facilitate us to establish the association between genetic variations and the risk of PCa through LD between causal SNPs and non-causal SNPs.

Response: In the “Background” additional clarifying words have been added and the sentence now reads, “Although replication studies have confirmed 4q27 as a risk locus for inflammatory disorders [22, 24-29], tight linkage disequilibrium (LD), essentially responsible for identification of the 4q27 region as a prostate cancer risk locus, in turn limits identification of the true causal variant.” In the “Discussion” the following words (in red) are added for clarification “… although a high degree of LD within this gene region has hindered efforts to narrow the locus to its true causal variants.” As a result of this LD and need to identify casual variants, we selected the SNPs to represent potentially functional SNPs within the region.
4. This study population included 23 Asians, to prevent population stratification, the authors should state how the difference the results were when they included only Caucasians and total population.

**Response:** There are 22 men born in Asia (9 cases and 13 controls). In the Methods under “Study Population” the words “including 9 cases and 13 controls of Asian origin” have been included. The Table below shows that the ORs are virtually unchanged when the Asians are excluded from the analysis (figures in bold). Although this Table has not been included, the following sentence has been added to the Results section (second paragraph), “The ORs were virtually unchanged after excluding the 22 men that were born in Asia.”

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIC</td>
<td>0.53</td>
<td>0.45</td>
<td>0.27-0.78</td>
<td>0.029</td>
</tr>
<tr>
<td>GAG</td>
<td>0.47</td>
<td>1.20</td>
<td>0.79-1.84</td>
<td>0.406</td>
</tr>
<tr>
<td>AGG</td>
<td>0.00</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>1.000</td>
</tr>
</tbody>
</table>

5. The author should include whether this study has been approved by IRB in the Method part.

**Response:** See Associate Editor Response above.

6. The selection of the genetic variants was totally based on previous study, which could not represent sufficient genetic information of each gene. In addition, this has limited the ability of performing haplotype analysis in this study. For future study, it is better to perform a systematic selection of SNPs instead of merely replicate the significant SNPs from previous studies since different SNPs
may be associated with the outcome of interest in different populations, even though the population are Caucasians.

**Response:** Comment noted by authors. The rational was that previously associated alleles (although with another disease outcome) may reflect a functionality of the specific variants. Under “variant selection and genotyping” (Methods section) the following sentence has been added, “These associations may reflect an as yet unknown biological effect of these variants.”

7. In Method part, the authors did not state why rs2069762 was genotyped by two different platforms, Sequenom and Taqman.

**Response:** The following sentence has been added, “Study replication was performed for the purpose of platform and chemistry assessment and quantification, as previously published.”

8. Lack of a table illustrates the demographic information of the study population, which is important for the readers to understand the distribution of the study population in term of potential risk factors.

**Response:** A Table illustrating the characteristics of the RFPC study has been added (Table 1) and referenced in the Methods under “Study Population”. Note that all other Table numbers have changed per original submission due to this addition. Total 3 tables.

9. In statistical analysis, it is not clear what the difference between the following statements. The authors should rewrite the sentences to clarify the purpose of each analysis to prevent confusion. “Polytomous logistic regression models were used to estimate odds ratios (OR) by tumor stage (stage I-II and stage III-IV tumors) and grade (moderate grade and high grade tumors). Tests for association between genotypes and case-control status were performed under dominant, recessive, co-dominant, and per allele models.” and “Heterogeneity in the ORs by tumor stage (stage I-II (0) and stage III-IV (1)) and grade (moderate-grade (0) and high-grade (1)) were tested using polytomous logistic regression models.”

**Response:** To avoid the repetition about polytomous logistic regression, the paragraph in red (manuscript) has been changed to read, “Case-control analyses were conducted using unconditional logistic regression and OR estimates and their 95% confidence intervals (CI) were derived under likelihood theory. We fitted dominant, recessive, co-dominant, and per allele models. Heterogeneity in the odds ratios by age and family history of prostate cancer in first-degree relatives (0 or at least one) was tested by including an interaction term in the logistic model. One polytomous logistic regression model was used to estimate odds ratios (OR) for stage I-II and stage III-IV tumors and another to estimate ORs for moderate-grade and high-grade tumors. In order to test for heterogeneity
in the ORs by tumor stage or grade, the likelihood from these models was compared with that from polytomous logistic regression models with ORs constrained not to vary by tumour stage or grade.”

10. It is highly possible that the null findings in this study is a result of inappropriate selection of SNPs. For example, “In this study we selected variants that were most strongly associated with autoimmune disease susceptibility and are the first, to our knowledge to investigate variants within the IL-21 gene region for prostate cancer risk.” The SNP selected may be strongly associated with autoimmune disease but not with PCa, so the link of these two by merely inflammation response is too weak.

Response: As mentioned above, the selection of SNPs based on previous disease-based association, may reflect a functional (direct) association. We would therefore strongly argue that previously associated disease (albeit a different disease, similarly associated with an inflammatory response) variants provide ideal targets. In addition, the distribution of the SNPs across the region (although not specifically reflecting tagSNPs), were highly representative of previously established haplotype blocks.

11. It is not true that “Although prostate cancer GWAS have thus far failed to implicate the 4q27 region as a candidate risk locus, the complex and heterogeneous nature of prostate cancer may suggest that multiple, low to moderate risk alleles (possibly not identified by GWAS) will be implemented in the disease.” The SNPs identified by GWAS tend to have OR between 0.67 to 1.50, which are definitely not strongly associated with the outcome.

Response: The sentence has been slightly changed to better clarify and avoid confusion in interpretation. It reads as follows, “Although prostate cancer GWAS have thus far failed to implicate the 4q27 region as a candidate risk locus, the growing number of candidate gene regions already identified in these large-scale studies, highlights the complex and heterogeneous nature of prostate cancer. Thus, multiple, low to moderate risk alleles (not yet identified by GWAS) may still be implemented in the disease.”

Response to Reviewer 2:

Minor Essential Revisions:

1. The criteria for family history of prostate cancer, which were not shown in the “Methods-Study Population”, should be described in details.

Response: The family history of prostate cancer was for first degree relatives and is high-lighted in the following sentence in the Methods, Study population section, “Information including age, family history of prostate cancer (in first-
degree relatives), country of birth, and lifestyle were recorded during a face-to-face interview.”

2. In the “discussion (the second paragraph)”, the sentence “The potential of…and suggestions that..., implicates...” contains some grammar error. The author should check it and make it clear.

**Response:** The sentence now reads as follows “The potential role of chronic inflammation in prostate cancer development, together with an observed association between the 4q27 region and susceptibility to autoimmune disease and in turn immune dysfunction, implicates the cytokine rich 4q27 region as a candidate prostate cancer risk locus.”

3. In the “Figures Legend-Figure 1”, it should be figured out which NCBI Build Version was used for the physical position in Figure 1 (top).

**Response:** The following sentence has been added to Figure 1 legend, “The reference genome assembly used to determine physical SNP position was NCBI Build version 36.3.”

**Discretionary Revisions:**

4. In the “Results (the second paragraph)” or the “Discussion (the 6th paragraph)”, it would be better if the authors add some words to show the risk allele of rs13119723 with disease risk and its position in the intron of KIAA1109.

**Response:** The following words in red were added to the “Results”. Analysis by family history of prostate cancer (Table 3) however showed evidence that the G allele for the intronic KIAA1109 variant rs13119723, was significantly more frequent in men with a family history compared to those with no family history (P-value for interaction = 0.02). The gene identifier (KIAA1109) was added to the Discussion.

5. Are the PSA data available for the case and control samples the authors used in this study? It is worthy to test whether IL-2/IL-21 variants are related with the PSA level in pca cases.

**Response:** The PSA data was available. We have fitted linear regression between log(PSA) (outcome) and genotype for all SNPs and found no association (all P-values > 0.1). The following words in red have been added in the Results section, “No significant association was observed between genotypes and tumor stage or grade, age of prostate cancer diagnosis, prostate-specific antigen (PSA) levels, or country of birth for any of the variants we assessed in this study.”
Kind regards,
Vanessa Hayes