PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Determining the frequency of pathogenic germline variants from exome sequencing in castrate resistant prostate cancer patients
AUTHORS	Hart, Steven; Ellingson, Marissa; Schahl, Kim; Vedell, Peter; Carlson, Rachel; Sinnwell, Jason; Barman, Poulami; Sicotte, Hugues; Eckel Passow, Jeanette; Wang, Liguo; Kalari, Krishna; Qin, Rui; Kruisselbrink, Teresa; Jimenez, Rafael; Bryce, Alan; Tan, Winston; Weinshilboum, Richard; Wang, Liewei; Kohli, Manish

VERSION 1 - REVIEW

REVIEWER	Zsofia Kote-Jarai The Institute of Cancer Research UK
REVIEW RETURNED	09-Nov-2015

GENERAL COMMENTS	This paper looks at a subset of 157 candidate genes selected from WES data on 69 mCRPC cases and report 12 likely pathogenic mutations in 9 genes, with BRCA2 (x3) and ATM (x2) the only genes mutated in >1 patient. However, the introduction and discussion only really refer to germline variants in BRCA2, for which they go into comparatively high detail, and so by omission it gives the impression that less has previously been reported elsewhere than is the case (this is summed up by the fact that in the intro, of their 10 references, 5 are for BRCA2, 3 to castrate resistant PrCa, and 2 to PrCa incidence – with nothing else there even mentioned). They need to improve this as below: - SU2C (Robinson et al. 2015) reported similar observations for mCRPC in a larger set of 150 cases. They only mention this study as pretty much a footnote to their discussion at the end of a whole paragraph of intimating how they've observed a "striking enrichment" (at 4.2%) compared to other studies (Our finding is consistent with a recent report in which germline mutations in BRCA2 were found in 5.3% of 150 mCRPC cases [19].) and not at all in the intro, where they make the statement (In contrast to PC, very little is known about the prevalence of inherited pathogenic germline variants in patients who have progressed to mCRPC stage after initial treatments). The authors need to acknowledge this study in the introduction as well as give greater prominence in the discussion to the strong agreement and confirmatory aspect of their observations with the larger previous study, as elaborated on again lower down. - 5 references to germline variants in BRCA2 but none to even mention any other genes is imbalanced given what they later present in their results. The authors should reference Leongamornlert et al, 2013, paper since not only was that probably the earliest mutli-gene germline PrCa sequencing study published, but still covers possibly the most relevant panel of genes relative to the ones they've selected, and also has broadly similar findings.

	They should also mention the HOXB13 variant in the same context, as this is reported to be associated with FH but not aggressiveness, and they include HOXB13 in the panel of genes they analyse. The methods are generally clear but 2 minor points: - Should state the size of the cohort within the methods (even though prominently mentioned in various other parts of the manuscript), and also state that clinical characteristics of the cohort can be found in table 1 at the same point. - They mention their Agilent bait kit in consecutive paragraphs in the methods. The first relates to the enrichment step and is the correct place for this, whilst the second is about sequencing and doesn't make sense there so should be removed. However the second mention states that it's a modified all exon library with extra AR gene content, which the mention in the first paragraph doesn't state, so that should detail should also be added to the proper description in the first paragraph. The first paragraph of the discussion isn't well written at all: sentences need to be reshuffled and re-phrased: 1. "Our finding is consistent with a recent report in which germline mutations in BRCA2 were found in 5.3% of 150 mCRPC cases [19]." Given that that this paper not only relates to a larger set of mCRPC cases which is the focus of this paper but also reports broadly similar findings, it's unfair to give so little prominence to that and squeeze it in at the end. That part should be discussed before the comparison with familial sets and not in the minimal way they do here at the end. 2."The pathogenic BRCA2 variant frequency (4.2%) in patients with mCRPC is much higher than has been reported in the context of familial prostate cancer. In a large study of 266 familial prostate cancer cases, pathogenic BRCA2 variants were not observed .." this is again unfair comparison as this study was in a specific population testing for 1 variant! 3. Generally the order of paragraph has to be re-arranged to reflect published information correctly! They appear to have decent family history information for their cohort, yet don't present this data in any way. Ideally family history of cancers should be added to Table 1 for a better overview of the cohort, and a comparison of their set for overlap with FH for carriers and non-carriers made. This would be an interesting extra strand to their findings and relatively simple to do.

REVIEWER	Paolo Radice Fondazione IRCCS Istituto Nazionale dei Tumori Nilano, Italy
REVIEW RETURNED	27-Nov-2015

GENERAL COMMENTS	In their manuscript Hart and colleagues report the results of an exome-wide sequencing analysis of 69 patients affected with metastatic castrate resistant prostate cancer (mCRPC). This is an interesting and well conducted study. Unfortunately, the authors seem to have paid little efforts to discuss their results. In my opinion, the study would benefit from a more in depth critical evaluation of the observed findings. 1) The authors quote a previous study describing a genomic investigation of mCRPCs at both germline and somatic level (Robinson et al., Cell 2015). It would be interesting if they could

	provide a more detailed comparison of the outcomes of the two studies (in addition to the observed BRCA2 mutation frequencies). 2) The association with prostate cancer risk of variants in the genes listed in table 2 has not been established (with the only exception of BRCA2). Therefore, in the discussion it should be acknowledged that the clinical relevance of the alterations observed in these genes remains at present undefined. Consequently, although these alterations are (or are likely to be) deleterious (i.e., affect protein function), I'd like to suggest the authors to avoid referring to them as "pathogenic". 3) The choice to restrict the variant investigation to those in known (or putative) cancer predisposition genes, although justifiable, is another limitation of the study that need to be discussed, since it is entirely possible that variants in other not investigated genes are responsible for prostate cancer risk increase. Additional points: 1) The methods used in the study are largely standardized. Therefore, the materials and methods section can be substantially reduced and authors can refer to previous publications. 2) Although interesting, the observation that none of the patients eligible for return of results ultimately opted not to receive them, does not substantially add to the relevance of the study. In addition, the very limited number of patients documented does not allow to draw any robust conclusion. Therefore, I would suggest to remove such information from the manuscript.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1, Zsofia Kote-Jarai
The Institute of Cancer Research, UK

This paper looks at a subset of 157 candidate genes selected from WES data on 69 mCRPC cases and report 12 likely pathogenic mutations in 9 genes, with BRCA2 (x3) and ATM (x2) the only genes mutated in >1 patient. However, the introduction and discussion only really refer to germline variants in BRCA2, for which they go into comparatively high detail, and so by omission it gives the impression that less has previously been reported elsewhere than is the case (this is summed up by the fact that in the intro, of their 10 references, 5 are for BRCA2, 3 to castrate resistant PrCa, and 2 to PrCa incidence – with nothing else there even mentioned). They need to improve this as below:

We added text to the introduction clarifying the role of other predisposition screens that have been performed in the familial PC setting.

- SU2C (Robinson et al. 2015) reported similar observations for mCRPC in a larger set of 150 cases. They only mention this study as pretty much a footnote to their discussion at the end of a whole paragraph of intimating how they've observed a "striking enrichment" (at 4.2%) compared to other studies (Our finding is consistent with a recent report in which germline mutations in BRCA2 were found in 5.3% of 150 mCRPC cases [19].) and not at all in the intro, where they make the statement (In contrast to PC, very little is known about the prevalence of inherited pathogenic germline variants in patients who have progressed to mCRPC stage after initial treatments). The authors need to acknowledge this study in the introduction as well as give greater prominence in the discussion to the strong agreement and confirmatory aspect of their observations with the larger previous study, as elaborated on again lower down.

We have added sections to both the introduction and discussion comparing our results to that of the recent SU2C paper.

- 5 references to germline variants in BRCA2 but none to even mention any other genes is imbalanced given what they later present in their results. The authors should reference Leongamornlert et al, 2013, paper since not only was that probably the earliest mutli-gene germline PrCa sequencing study published, but still covers possibly the most relevant panel of genes relative to the ones they've selected, and also has broadly similar findings. They should also mention the HOXB13 variant in the same context, as this is reported to be associated with FH but not aggressiveness, and they include HOXB13 in the panel of genes they analyse.

We apologize for the oversight and have added a reference and discussion to Leongamornlert et al. As for HOXB13, we did not include it in the discussion because we did not observe any clinically relevant variants in this gene.

The methods are generally clear but 2 minor points:
- Should state the size of the cohort within the methods (even though prominently mentioned in various other parts of the manuscript), and also state that clinical characteristics of the cohort can be found in table 1 at the same point.

We added the number of cases and a pointer to Table 1 to the Patient eligibility paragraph.

- They mention their Agilent bait kit in consecutive paragraphs in the methods. The first relates to the enrichment step and is the correct place for this, whilst the second is about sequencing and doesn't make sense there so should be removed. However the second mention states that it's a modified all exon library with extra AR gene content, which the mention in the first paragraph doesn't state, so that should detail should also be added to the proper description in the first paragraph.

We decreased the number of details that are related to Agilent's standard operating procedure, which may have inadvertently introduced confusion.

The first paragraph of the discussion isn't well written at all: sentences need to be reshuffled and re-phrased:
1. "Our finding is consistent with a recent report in which germline mutations in BRCA2 were found in 5.3% of 150 mCRPC cases [19]." Given that that this paper not only relates to a larger set of mCRPC cases which is the focus of this paper but also reports broadly similar findings, it's unfair to give so little prominence to that and squeeze it in at the end. That part should be discussed before the comparison with familial sets and not in the minimal way they do here at the end.

We have reorganized the paragraph as the reviewer suggested and have emphasized the similarities between the two results.

2."The pathogenic BRCA2 variant frequency (4.2%) in patients with mCRPC is much higher than has been reported in the context of familial prostate cancer. In a large study of 266 familial prostate cancer cases, pathogenic BRCA2 variants were not observed .." this is again unfair comparison as this study was in a specific population testing for 1 variant!

The reference cited was incorrect. It should have referenced the Agalliu et al 2007 paper in which full gene sequencing of BRCA2 was performed. This has been corrected in the text.

3. Generally the order of paragraph has to be re-arranged to reflect published information correctly!

They appear to have decent family history information for their cohort, yet don't present this data in

any way. Ideally family history of cancers should be added to Table 1 for a better overview of the cohort, and a comparison of their set for overlap with FH for carriers and non-carriers made. This would be an interesting extra strand to their findings and relatively simple to do.

We have made the family history data available in Table 1, and added an explanation in the Results section.

Reviewer: 2

Reviewer Name
Paolo Radice

Institution and Country
Fondazione IRCCS Istituto Nazionale dei Tumori Nilano, Italy

Please state any competing interests or state 'None declared':
None declared

Please leave your comments for the authors below In their manuscript Hart and colleagues report the results of an exome-wide sequencing analysis of 69 patients affected with metastatic castrate resistant prostate cancer (mCRPC).
This is an interesting and well conducted study. Unfortunately, the authors seem to have paid little efforts to discuss their results. In my opinion, the study would benefit from a more in depth critical evaluation of the observed findings.
1) The authors quote a previous study describing a genomic investigation of mCRPCs at both germline and somatic level (Robinson et al., Cell 2015). It would be interesting if they could provide a more detailed comparison of the outcomes of the two studies (in addition to the observed BRCA2 mutation frequencies).

This suggestion was echoed by the first reviewer and has been updated accordingly.

2) The association with prostate cancer risk of variants in the genes listed in table 2 has not been established (with the only exception of BRCA2). Therefore, in the discussion it should be acknowledged that the clinical relevance of the alterations observed in these genes remains at present undefined. Consequently, although these alterations are (or are likely to be) deleterious (i.e., affect protein function), I'd like to suggest the authors to avoid referring to them as "pathogenic".

We agree that the clinical relevance of most of these genes are currently not well defined for mCRPC risk, and this has been reflected more in the discussion. The use of the term "pathogenic" is appropriate in this context according to the ACMG guidelines. All variants were reviewed by multiple genetic counselors to ensure we conform to those standards. It is important that we distinguish these results from predictions of deleteriousness, as each is well supported to be causative variants.

3) The choice to restrict the variant investigation to those in known (or putative) cancer predisposition genes, although justifiable, is another limitation of the study that need to be discussed, since it is entirely possible that variants in other not investigated genes are responsible for prostate cancer risk increase.

We have added this limitation to the "Strengths and Limitations" section of the manuscript.

Additional points:
1) The methods used in the study are largely standardized. Therefore, the materials and methods

section can be substantially reduced and authors can refer to previous publications.

Per this and the comment from Reviewer #1, we have trimmed down the detail of the methods section.

2) Although interesting, the observation that none of the patients eligible for return of results ultimately opted not to receive them, does not substantially add to the relevance of the study. In addition, the very limited number of patients documented does not allow to draw any robust conclusion. Therefore, I would suggest to remove such information from the manuscript.

We removed the return of results part out of the discussion.