Author’s response to reviews

Title: INFORMING PATIENTS ABOUT THEIR MUTATION TESTS: CDKN2A c.256G>A IN MELANOMA AS AN EXAMPLE

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Author’s response to reviews:

To Professor Evgeny Imyanitov
HCCP

Dear Evgeny,

Thank you for your positive comments on our paper, and may thanks are due also to the reviewers for their helpful comments. We believe that all the comments are covered by the changes in the manuscript, except that we did not want to extend the pedigree (Reviewer 1) because that would expose the family (many members that were left out are still living, and they are only marginally relevant to the conclusions).

Finally, we think this is a research article rather than a case report. Extensive in silico analyses were conducted on the detected variant, summarized in Table 1.

Best wishes,

Kari

REBUTTAL ON HCCP-D-20-00021
We thank for the expert comments, which have led to the improved revised version. The changes are shown by underlining. Additionally the language has been edited throughout.

Reviewer #1: This is a interesting write up of a single melanoma family found to have a likely pathogenic variant in CDKN2A. This manuscript was submitted as a research article though it seems to fit better into the case report category. This is a useful article as more variant-specific information is needed to help clinicians determine the most appropriate recommendations for patients and their families with regards to germline genetic findings. Penetrance of all variants are not all equivalent.

We have no strong views on this but prefer a research article category. Actually the sequencing results and bioinformatics/in silico analyses are novel, cf Table 1. A few specific notes on items in the discussion section of this manuscript:

* Page 8, Line 6: The assumption of 50% of the 16 FRD and SDR of the proband's mother being carriers is an overestimate. 50% of the siblings (N=2) may be presumed carriers and 25% of the siblings' offspring (N=3), making 7 of the 16 FDR/SDR likely carriers of the described variant. Other possibilities should be mentioned that may describe the incomplete penetrance in this family (e.g. de novo variant in proband's mother, possibility of proband's mother being the only one in her sibship that inherited the variant (3% likelihood)).

Thanks, this was a miss, revised in the penultimate paragraph on p. 8.

* Page 8, Line 27: To accommodate no-nuclear family structures (e.g. adopted individuals and those estranged from their relatives), the wording of the sentence "All patients have biological families..." could be reworded.

‘Biological’ was deleted, the last paragraph. Additionally, it would be useful if the authors modified Fig 2 to include the maternal cousins of the proband as these individuals are discussed as part of the results and discussion points made by the authors, including a possibly-pertinent cancer diagnosis. As many of these individuals are still alive we are reluctant to have a full pedigree. Thatement added to the last full paragraph on p 5.

Overall, this paper describes the case well and the authors were thorough in obtaining genetic status on as many close relatives as possible (deceased or alive) in order to inform their conclusions. Methods were appropriate, though it is not clear why only two of the three living individuals who received genetic testing also received genetic counseling. Explained, the last line in p. 5 (living abroad).

Reviewer #2: Thank you for describing this interesting case.

Please could you elaborate on the rationale for describing this particular case? It is already well established that guidelines for variant interpretation with scientific and clinical input should be used, and that in silico prediction tools should not be used in isolation to assign pathogenicity. Are
you wanting to highlight the importance of taking a family history and encouraging probands to find out as much as possible about their family? It would be helpful to raise this as an important point for mainstream clinicians (outside genetics) to consider. However, as it seems you have accepted the Class 4 likely pathogenic status of the variant, the lack of family history should not change the advice given to family members.

We wanted to add to the motivation with 2 new sentences at the end of Introduction, p. 5.

Could you consider noting that everyone should be aware of the risk factors for melanoma, even without a family history? The screening advice for this family would likely not change dramatically with the predictive test results; whether they test positive or negative they will be advised to avoid sunburn and be vigilant about skin changes. In the UK carriers would be advised to have an annual appointment with a Consultant Dermatologist, but there might be different guidelines in your country.

This is also the case in Finland, see addition to the Sequencing and counseling section, p. 5/6,

General comments about the manuscript:

Abstract:
In the first sentence it says 'suspected as a course of cancer'. Should this be 'suspected as a cause of cancer'?
Could you change 'deleterious' to pathogenic?
There are several small errors, examples below. The manuscript should be checked throughout for these please.
line 27: real word example - should be real world example
32: also disposes to pancreatic - should be predisposes

We have corrected the above errors, thanks.

Reviewer #3: The authors use the example of the CDKN2A:c.256G>A variant to illustrate an important point, which is that it is critical to have defined rules in place for the return of genetic testing information to a patient.
However, it is my opinion that the present manuscript does not report work that is suitable for publication as a "research paper". It is merely reporting the genetic testing of a few individuals from the same family and offering some discussion points.

Also extensive in silico analysis of the variant…Table 1!
I find this manuscript highly confusing. I do not understand who the target of this report is: genetic counsellors? genetic testing labs? policy makers? Are the authors suggesting a change in the
national guidelines for the return of genetic information? If so, they need to present in the manuscript what the current recommendations are.

What is the message the authors try to convey? That several levels of evidence are required to make a "pathogenicity" call? This is not novel and is actually widely agreed on (it is already part of several frameworks for variant classification (ACMG, ClinGen expert panels etc) for instance).

As we point out in the first paragraph of Introduction there are expert recommendations on how to deal with sequence variants but we want to give an example on how they work in practice. We summarize the outcome at the end and emphasize the readily available information on extended families.

A few other points that I would like to bring to the authors attention, should they decide to work further on this manuscript.

1) It is unclear what the context was for the genetic testing of this family. Who ordered the test? If a genetic counsellor ordered the test, then it is their responsibility to follow international and national guidelines for reporting the result. In the absence of any other evidence than in-silico predictions, the genetic counsellor should not have returned a "pathogenic" call on this test. If the test was conducted at the initiative of the proband, then a genetic consultation should have led to the same conclusion. Genetic testing must be conducted within a strict and controlled framework to avoid "unnecessary fear", this is something we all agree on.

This now detailed in Methods, p.5 and 6.

2) It is unclear if Blue Genetics or the authors themselves performed the in-silico analyses. Did Blue Genetics provide the evidence used to classify the genetic variant as "pathogenic? If they did not, and are an accredited genetic testing lab, the lack of explanation for the result should be fed back to them and they should probably not be used in the future for genetic testing! If they did, why do the authors suggest that it was "probably" due to the prediction tools (page 7, line 51)?

Our detailed in silico analysis is described on p. 6. A further explanation of the 'pathogenic' classification is on p. 7.

3) The authors should review relevant literature to assess whether patient-reported family cancer history is reliable. In any case, family history alone cannot be used to assess the penetrance of the mutation, contrarily to the authors' claim in the discussion.

We thank the reviewer for reminding us of the reliability of the family history, a theme which we have been engaged in during the last 30 years. In this case the family history is water tight, last sentence last full paragraph p. 5. We agree about family history and penetrance, and changed the word in summary (indicate to suggest).

4) "Functionality" is not assessed by in-silico prediction tools. The word is used correctly here. Functionality is assessed by proper functional assays, which measure active/functional protein levels, none of which are mentioned here.

We changed the wording.

5) Overall, the text would benefit from being read by an English native speaker to improve syntax, grammar and overall comprehension.
Was edited throughout.