Author's response to reviews

Title: Prevalence and predictors of germline CDKN2A mutations for melanoma cases from Australia, Spain and the United Kingdom

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Reply to Referee's Comments

We thank the reviewers for their time and effort in appraising the manuscript. Our answers to the queries raised are below.

Referee 1: Veronica Höiom

Essential revisions
1) In additional file 3, the classification of a variant as “pathogenic” or “non-pathogenic”, is not consistent. For example, the D156A is classified as non-pathogenic despite an overall score of 2, no data on function or segregation in melanoma families. Other variants with similar outcome have been classified as pathogenic (for example G111S).

   Dr Höiom is correct, the classification of the variants is not consistent in 'additional file 3'. On careful examination we identified two errors in this table. The variant D156A should have been marked as 'wild type' in the functional information column; and the variant G139R should have been marked as 'No' for segregation in melanoma pedigrees. These have been corrected, and all variants have now been re-checked and confirmed to follow the classification flow chart in 'additional file 1'.

2) For classification of intronic variants, were any analyses done on for example effect on splicing, CADD-scores etc?

   All non-coding variants were checked for possible effect on splicing using the Berkeley splice site prediction server. This is now mentioned in the text (page 9).

Discretionary revisions
1) Was there any difference in prevalence regarding predicted non-pathogenic variants?

   The analyses had also been conducted with all missense variants included (except A148T). However only 5 missense variants have been classified as non-pathogenic and their inclusion had little effect on the prevalence.

2) For the Spanish patients, were all patients diagnosed with invasive melanoma and were the controls sex-matched? Were the gender known for the Spanish controls? Because, mutations were about 3 times more common among male carriers, the proportion of males among Spanish controls might be of importance.

   An increased incidence of mutations in males has been reported previously in Spanish MPM cases [1,2]. We agree that the high incidence of CDKN2A mutations in male Spanish cases is interesting, although in this study it is only marginally statistically significant and so may be due to chance (p=0.06). Most Spanish patients (94%) were diagnosed with invasive melanoma. Spanish controls were not sex-matched. Gender is known for all cases and controls (although not presented). The percentage of males in Spanish controls (79%) is higher than that observed in the Spanish cases (42%). However, the percentage of male Spanish cases is similar to that seen in UK (41%) and Australian cases (37%).
3) The p.A148T seems to be a low-risk variant. Was this variant seen alone or present with another (pathogenic) CDKN2A-mutation?

   The A148T variant is found both on its own or, in rare instances, in combination with a pathogenic mutation. As a low penetrance variant A148T might be hypothesised to increase the effect of the pathogenic mutation, but with very small numbers of individuals carrying both A148T and a pathogenic mutation we were not able to look at this specifically.

Referee 2: Robert McWilliams

Specific comments
Page 7, end of 2nd paragraph: With regard to recruitment of participants, it states "Cases were asked about family history of melanoma and previous melanomas". What about controls?

   Controls were also asked about family history of cancer and previous melanomas. We have changed the sentence to clarify this in the text (page 7).

References