Author’s response to reviews

Title: GERMLINE c.1A>C HETEROZYGOUS PATHOGENIC VARIANT IN SDHA REPORTED FOR THE FIRST TIME IN A YOUNG ADULT WITH A GASTRIC GASTROINTESTINAL STROMAL TUMOUR (GIST): A CASE REPORT

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Author’s response to reviews:

Dear Editor:

First of all, thank you for your comments and reviewers´ suggestions.

I would like to submit the manuscript with the suggested corrections. Point-by-point responses have been added.

Sorry for the nuisance. Thank you in advance,

Sergio Carrera.
Reviewer reports:

Reviewer #1: In the paper by Carrera et al., the authors identify a heterozygote germline SDHA c.1A>C, p.(Met1?) variant in a young adult with a GIST negative for c-KIT or PDGRFA mutations. Family history revealed that the paternal grandfather died as a consequence of a pituitary adenoma. Genetic analysis moreover revealed that the patient's brother did not have the variant, while the patient's father and paternal uncle both had the variant. According to the authors this is the first description of a GIST tumor in a SDHA c.1A>C carrier. Carrera et al., conclude that all patients with GIST without c-KIT or PDGFRA mutations should be referred to genetic counselling regardless of the age at presentation or the absence of a family history.

Major points:

1) The authors should include the methodology used for mutational analysis of c-KIT and PDGFRA genes. Which exons are examined?

Analysis of exons 9, 11, 13 and 17 of KIT gene and exons 12, 14 and 18 of PDGFRα gene were performed in tumor samples using amplification of the exons of interest by polymerase chain reaction (PCR) followed by direct sequencing (Sanger method) of amplification products.

2) The authors state that the sequencing data is processed taking into account the SDHA and SDHC pseudogenes. How is that done?

Despite of presence of pseudogenes of SDHC and SDHA genes, there are enough intronic mismatches to differentiate them. We designed paired of primers specific for SDHC and SDHA genes, and not to their pseudogenes, obtaining amplification from SDHC and SDHA genes only. However, we have realized that the place that occupies de sentence “taking into account the SDHA and SDHC pseudogenes” is not really correct. We have changed it in the manuscript.

3) c.1A>C should not be described as a missense variant (it's a start codon variant). At the protein level it should be described as p.(Met1?). This should be changed throughout the manuscript, including the discussion.

The correction has been made.
4) I would suggest that the authors clearly indicate in Fig. 1 individuals that are carriers and non-carriers (brother) using +/- . Moreover, remove the color in the father and uncle. Finally, include a more comprehensive figure legend (e.g. explain squares and circles, carriers etc.).

This figure has been corrected. More comprehensive legend has been added.

5) In the results section (line 46-47) the authors state that "a new upstream state codon, resulting in an mRNA transcript that will be different from the original". This is only correct if the variant affects splicing and should therefore be rewritten.

Our group have not performed mRNA analysis, but we have commented what other group suggest based on their studies. Parfait B, Chretien D, Rötig A, Marsac C, Munnich A, Rustin P. Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. Hum Genet. 2000 Feb;106(2):236-43.

6) What does the authors mean by "true" in the sentence "a true negative/positive result" (results section, line 48-51).

We have changed “true positive” into “carrier” and true negative” into no carrier of the variant.

7) The authors state in the result section (line 51-54), that "these results confirmed that the parental grandfather … was obligate carrier. I do not agree in this and would not use the term obligate carrier. In line with this, currently I do not agree that the variant co-segregates with the disease (mentioned in the discussion and conclusion section). It is true that the grandfather has a phenotype, but you cannot rule out that the variant was inherited from the grandmother. I would suggest that you test the grandmother - and if possible tissue from the grandfather.

We have tested the grandmother and confirmed that she does not carry the variant. “Subsequently, we performed predictive tests to her relatives. Her brother was found to be no carrier of the variant; her father and paternal uncle were carriers (confirming paternal inheritance) and her paternal grandmother was no carrier of the variant. These results suggest that the paternal grandfather, who died as a consequence of a pituitary adenoma, could be obligate carrier of the c.1A>C (p.Met1?) pathogenic variant in the SDHA gene.

8) Have the hepatic metastases been examined for c-KIT and PDGFRA variants?

Hepatic metastases were not examined for KIT and PDGFRA variants.
9) The pathogenic classification in ClinVar by OMIM is based on data from ref 29. Therefore, the paragraph in the discussion and conclusion section should be rewritten. Moreover, the authors do not need to describe the variant once more but focus on the putative consequence and the functional data.

This part has been rewritten.

10) The manuscript should be read thoroughly by an English-speaking person.

We have sent the manuscript again to a native English speaking person.

Minor points:

11) Please use italics when genes are described.

We have made this correction.

12) Please include information regarding the frequency of the c.1A>C variant in the general population (e.g. gnomAD).

This variant is absent in gnomAD (genome aggregation database). It has been added in Results.

13) If space allows I would suggest that the authors include a figure with an electropherogram showing the SDHA c.1A>C variant.

It has been added.

Reviewer #2: The manuscript by Carrera et al. deals with a very interesting and remarkable case of a 20 years old female patient presenting with a gastric gastrointestinal tumor (GIST). The manuscript is written in a plain and manner and all steps are comprehensible

However there are some points of criticism that should be answered in a revised version of the manuscript:
1) The authors should indicate which edition of the TNM classification was used and they should add the risk classification (Armed Forces Institute of Pathology (AFIP)). In addition, a pathologist should be added in the list of authors because of the diagnosis and its associated aspects.

“This corresponded to pT1pN0 (0/1) high mitotic rate - stage II - of the eighth edition of TNM classification. Armed Forces Institute of Pathology (AFIP) criteria calculate the risk of this tumor relapse and/or progression as zero, due to the low number of published cases”.

2) Because the question of the pathogenicity of the detected mutation is the crucial point, in addition to the reference (27), the underlying reference (29) should be included in "RESULTS" as well.

We have made this correction.

3) Was an immunohistochemical analysis of SDHA protein performed in order to examine a potential loss of expression of the protein in the tumor? If not, why?

The analysis was not performed because the technique was not available in our hospital.

4) The complete manuscript should be revised by an academic native speaker because of the need of improvement quality of English in some phrases.

We have sent the manuscript again to a native English speaking person.

Therefore, publication of this study in "Hereditary Cancer in Clinical Practice" can be recommended after minor revisions, only.