**Author's response to reviews**

**Title:** Paraganglioma and pheochromocytoma upon maternal transmission of SDHD mutations

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**Version:** 2  
**Date:** 26 August 2014

**Author's response to reviews:** see over
Dear Editor,

We thank the reviewers for their comments, which we discuss below. Our replies are indicated in bold, italics. New text inserted in the manuscript is indicated in italics.

Reviewer’s report
Title: Paraganglioma and pheochromocytoma upon maternal transmission of SDHD mutations
Version: 1 Date: 28 May 2014
Reviewer: Ales Vicha
Reviewer’s report:
This article describes transmission of SDHD mutations via the maternal line, which in rare cases, lead to development of paragangliomas or pheochromocytoma. Although, mutations in this gene show a remarkable pattern of paternally-transmitted mutation related tumorigenesis. Despite this finding, the overwhelming majority of carriers of maternally-transmitted mutations will remain tumor-free throughout life. This article brings new information about tumorigenesis trough maternally-transmitted mutation. Therefore, this article is very needed for improving knowledge about pattern of paternally-transmitted SDHD mutation related tumorigenesis. Together with author, I hope that this article will stimulate clinicians to re-evaluate carriers of maternally-inherited mutations, leading to the recognition of further cases. Therefore, I recommended this article for publication.
I have only discretionary revision
1. BMC journals are only on-line journals with more space than hard copy journals. Therefore, it can be better to situate haplotype analysis markers and primers details for allele specific loss of SDHD in to manuscript or as a supplement.

We will be happy to supply these as a supplement at the editor’s discretion.

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Reviewer’s report
Title: Paraganglioma and pheochromocytoma upon maternal transmission of SDHD mutations
Version: 1 Date: 26 May 2014
Reviewer: Takeshi Usui
Reviewer’s report:
The manuscript showed the three cases of “apparently maternally transmitted” SDHD related paraganglioma. The case 3 had been concludes as a photocopy of PGL (sporadic paraganglioma in an SDHD mutation carrier).
In case 2, although immunohistochemical analysis may suggest the pathogenicity of SDHx, the authors could not demonstrate the LOH in adrenal medullary tissue (as the paraganglioma tissues were not available). Therefore, authors could not
conclude that the paragangliomas in this case arise from the maternally transmitted Asp92Tyr SDHD mutation itself. As this mutation is reported as a founder mutation in Netherlands, they must be very careful about the pathogenicity of this mutation in Netherland. The authors should discuss this point.

We understand the point the reviewer is making, but would like to point out that this single inconclusive case does not alter general conclusions on the pathogenicity of this mutation for the many hundreds of paraganglioma patient-carriers. A huge weight of evidence conclusively proves the pathogenicity of this mutation and we did not wish to imply that this was in dispute. We have now added some further discussion of the patient 2-related evidence to clarify this point.

“This result could be due to admixture of normal cells with tumor cells, thereby masking loss of SDHD/chromosome 11 in tumors cells – a phenomenon common in paragangliomas. Another possibility, and one suggested by the profound loss of SDHB staining in the adrenal medulla, is that a non-genetic mechanism is mediating SDHD/SDHB loss in this tissue. While inconclusive in terms of SDHD p.Asp92Tyr-mediated pathogenicity, these data do show that this case of adrenal hyperplasia is SDH-related. However, speaking conservatively, we cannot definitively conclude that the patient’s p.Asp92Tyr mutation, a mutation proven by its dominant role in paraganglioma patients in the Netherlands and worldwide to be profoundly pathogenic, is the cause of the adrenal hyperplasia in this case. A firm conclusion will have to await the availability of new tumor tissue."

Location: bottom page 8

In case 1, the authors describe that “maternal family of this patient had history of paraganglioma”. Who are the patients in this family? Please indicate the affected members in Fig 1a. Also, the authors should describe the precise clinical phenotype of the parents of case 1.

As no immediate relatives of this patient have paraganglioma (carriers of maternally-inherited mutations), we have altered this phrase to specify that we are referring to members of a very extended family. Showing a very extended pedigree would serve no clear purpose. Our description of the immediate relatives was unclear and appears to have caused confusion. We have now altered the phrasing to, hopefully, better specify the situation.

“Although distant maternal relatives of this patient have a history of paragangliomas and carry a known pathogenic SDHD gene mutation, no immediate maternal relative is affected, probably due to the inheritance of the mutation via the maternal line for several generations. The paternal family had no known history of paraganglioma. Due to the maternal inheritance of the mutation, the patient was not immediately suspect for a SDHD-related paraganglioma.”

Location: bottom page 6

The data of LOH is ambiguous by both Sanger sequence analysis and microsatellite analysis (Fig 2a, b). Both data indicate incomplete LOH in the tumor. Please discuss this point (contamination of normal tissue?). The tissue should be obtained by laser
In some fields of tumor analysis these findings might appear ambiguous, but in paraganglioma analysis these findings are typical and this level of evidence has been acceptable as an indication of LOH for the past two decades (1). Paragangliomas are unusually complex tumors that largely maintain their normal tissue morphology and thus contain significant amounts of normal cells that are intimately associated with tumor cells in the so-called ‘zellballen’ structures. All paraganglioma tissue analysis suffers from this problem and ‘micro’ dissection techniques such as laser microdissection lack the resolution to effectively separate these different cell types. We have used laser microdissection in the past, but with disappointing results. This type of result is so well known to be affected by contaminating normal cells within the paraganglioma research community that this issue is often left unmentioned. We realize that we have also been guilty of this omission. We have now added this sentence to highlight this issue.

“While not showing complete loss, this result is typical of LOH in paraganglioma which show complex and significant admixture of normal cells, largely maintaining their normal tissue architecture and cellular composition of normal cell types, which also proliferate together with tumor cells.”

Location: mid page


In Fig 2b and 2c, the authors should show all the profiles of microsatellite markers alleles.

These marker illustrations were provided as examples of this well-known and largely routine type of analysis. In our opinion adding a large number of extra illustrations would make an already complex figure entirely unwieldy, and serve no useful purpose.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.

Reviewer’s report
Title: Paraganglioma and pheochromocytoma upon maternal transmission of SDHD mutations
Version: 1 Date: 23 May 2014
Reviewer: Guiomar Perez de Nanclares
Reviewer’s report:
Bayley and cols present three cases of SDHD-paraganglioma/pheochromocytoma putatively caused by maternally inherited alterations. Due to the rarity of this
inheritance, this MS is quite interesting for reporting. However, there are some points that, in my opinion, can help to prove this inheritance.

Major Compulsory Revisions

Even if initially imprinting at SDHD was suggested (as indicated by the authors), more recent papers suggest that the deletion of the maternal 11p copy is enough for the development of the PGL/PHE as many other tumors. In fact, in a recent paper pat11pUPD has been suggested as the underlying mechanism for the tumorigenesis.

I think that if authors can confirm the absence of the maternal chromosome at 11p, they could confirm that the three tumors are due to SDHD mutations. In fact, in other cancers as BRCA1-breast cancer, it has been demonstrated that when LOH is present both native or mutant alleles can remain in the tumor. I think this idea can be discussed.

We are not aware of ANY publication that claims to prove SOLE loss of maternal chromosome 11 as the cause of PGL/PC. All papers on this subject have produced suggestive, global evidence of LOH of many chromosomes, but with a prominent contribution of Ch11. In some cases, the parental origin has been determined and this appears to be predominantly maternal. More careful studies of tumors with a known SDHD mutation generally show loss of the maternal allele and loss of chromosome 11 in cases where this has been studied. We have studied the presence or absence of chromosome 11 in this paper. With all respect to the reviewer, this was one of main purposes of this paper and is described in detail.

Patient#2:
Microsatellite typing of 11p in tumor and germline DNA should be performed to discard/confirm the absence of maternal chromosome as the underlying causative mechanism of tumorigenesis.

This was one of the main goals of this paper and has therefore already been carried out. Results are described on page 8 and shown in fig. 2.

Is there a real and proven need of only mutated allele to develop disease?
How do authors explain the absence of IHQ in this patient with normal allele present?

All previous carefully conducted tumor studies of paraganglioma have shown loss of the wild type allele and SDH genes are therefore considered to be classic Knudson tumor suppressor genes. We have discussed the second part of this question in reply to reviewer Usui.

Patient#3
Microsatellite typing of 11p in tumor and germline DNA should be performed to discard/confirm the absence of maternal chromosome as the underlying causative mechanism of tumorigenesis

This was one of the main goals of this paper and has therefore already been carried out. Results are described on page 9 and shown in fig. 3.
Minor Essential Revisions

1. Authors’ filiations should be listed in a orderly fashion

Affiliations have now been reordered.