Author's response to reviews

**Title:** Gorlin syndrome associated with small bowel carcinoma and neuroleiomyomatosis of the gastrointestinal tract: Is two-hit mutational inactivation of PTCH a rare pathomechanism in GI malignancies? Case report and review of literature

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**Author's response to reviews:** see over
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Dear Dr. Shipley,

Please find attached our revised manuscript that we would like to re-submit to BMC Cancer.

We are very grateful for the reviewer’s helpful comments and, according to them, made the following changes:

**Reviewer 1: Mariana Cajaiba**

We agree that neuroleiomyomatosis is not an established term. In fact PubMed lists only one paper in which this term has been used previously. We nevertheless used this term because it (1) best describes the unusual pathological findings in our patient which (2) do not fit any established histopathological entities. In the manuscript we added the information that the term neuroleiomyomatosis is used as a description rather than an established diagnosis.

1. As discussed above, the term neuroleiomyomatosis was used as a description because the histological finding did not fit into any known diagnosis. Therefore the discussion of possible differential diagnoses is rather limited. Leiomyomatosis of the GI is only known for neurofibromatosis and tuberous sclerosis, but in both syndromes no neuronal cell component is present. The results of smooth muscle stains as well as several other stains (i.e. mitotic activity, neuronal cells, mast cells…) have been added. For technical reasons it was not possible to perform ultrastructural studies.

2. The endoscopic evaluation included the stomach, duodenum, terminal ileum and colon but did not include the jejunum. Biopsies were taken from all evaluated parts, and the histopathological findings described as neuroleiomyomatosis were found in duodenum and ileum. The stomach biopsy showed some of the same changes but only in areas close to the section border so that the diagnosis of neuroleiomyomatosis was only tentative. This information has been added to the manuscript.

3. Sorry, there is no answer to this question (see (2)).
4. The diagnosis of GS was first suspected at age 52 years (some months after the GI tumor extirpation) when for the second time multiple BCCs had to be excised. From the dermatological department he was sent to the genetics department where PTCH testing was performed. The text has been rearranged chronologically and subheadings have been added.

5. Yes, an exchange of two adjacent nucleotides (AC>GA) created a stop codon at a position in PTCH not previously described. The mutation is now described in more detail in the manuscript. The sequencing file has been added as figure 4.

6. They were mucosal biopsies taken during the endoscopic procedure. This information has been added to the manuscript.

7. The MIB-1 index of the carcinoma is mentioned because it is unusually high, indicating a high proliferative rate within the tumor. This explanation has been added to the manuscript.

8. The sentence has been changed (see also (10)).

9. The last paragraph has been rewritten to avoid repetitions.

10. The figure shows the macroscopic changes in the stomach (antrum) that showed the above mentioned neuroleiomyomatosis-like changes. The picture from the fundus has been deleted because, as correctly noted by the reviewer, the macroscopically visible changes are too unspecific.

11. Figure 2B now shows an enlargement of the pits from the patient’s right hand.

12. The manuscript has been screened for grammatical and typographical errors.

**Reviewer 2: Vesna Musani**

1. We agree with reviewer 2 that it would be good to show the proposed second mutation. However, we were only able to sequence exon 8 in both the carcinoma and neuroleiomyomatosis tissue, but not the 21 other exons of PTCH. Even if we had been able to do the latter, it would not have been sure that we would have found a second mutation. Studies on other tumor genes show that the second hit is not so often a point mutation but tends to be a deletion, a type of mutation not detectable by sequencing. Unfortunately, MLPA is not yet established in our lab for PTCH, so we are not able to effectively search for the second mutation yet. We therefore followed the reviewer’s suggestion and changed the title and remodelled the discussion.

2. The misspelling of “stop codon” has been corrected through the manuscript.

3. For technical reasons the PTCH staining was not possible.

We hope that we were able to answer all questions raised by the referees and would again like to thank them for their time and valuable comments.

Best regards,

Prof. Dr. Ortrud Steinlein