Author's response to reviews

Title: Somatic VHL gene alterations in MEN2-associated medullary thyroid carcinoma

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Author's response to reviews: see over
Dear Editor and Editorial Team:

We would like to thank you and the reviewers for very constructive comments which have helped improve our paper. We responded accordingly and hope that you will consider our revised paper for publication.

**Reviewer 1 (RE):**

Major compulsory revisions:

1. The reviewer is absolutely correct – it would be nice to have a larger sample size. However, we were not able to retrieve more cases (please note that coauthor Prof. Tannapfel, Dept. of Pathology, who provided us with samples from the University of Leipzig, has left for a director position at the University of Bochum, Germany; and Prof. Koch, the lead author, has left the University of Leipzig to take on a new position in the U.S.A.)

2. It is also correct that it would have been better to successfully analyze all samples for somatic \textit{VHL} mutations. Please note that there were several attempts after multiple microdissections to get the DNA to work with the primers used in the other MTC samples. However, we did not have luck with these samples and do not have any DNA left (the microdissection procedure had been carried out with the help of Prof. Vortmeyer at the NIH, who is a coauthor of this study).

3. We included a normal sequence in Fig. 3.

4. All the patients in this study had germline mutations in the \textit{RET} gene and did not show clinical evidence of \textit{VHL} syndrome. We did not analyze the blood DNA for germline mutations in the \textit{VHL} gene. This has been added to the text.

5. Thank you very much for this correction – we rephrased the sentence as follows: Polymorphisms G691S/S904S of \textit{RET} have recently been found to affect the development of MTC and the age at onset of MEN2A in patients with a \textit{RET} germline mutation (32-34). Elisei et al. (33) found a statistically significant higher allelic frequency of G691S polymorphism in MTCs than that found in normal controls. Cebrian et al. (34) carried out an association study in 135 sporadic MTC patients and 533 controls and discovered a strong association between the disease and specific haplotypes of \textit{RET}. 
Minor revisions:

1. We replaced tumorinitiation with tumor initiation
2. We replaced effect with affect
3. We corrected all the spelling and had the paper checked by a native English speaker

Reviewer 2 (AP):

Major compulsory revisions:

The reviewer is absolutely correct: the study size is small, indeed, and the results found may change after extending the investigation. We, therefore, balanced the discussion accordingly and acknowledged this limitation. Unfortunately, we cannot extend this study at the present time, especially because the main investigators, coauthor Prof. Tannapfel from the Institute of Pathology, and lead author Prof. Koch, have both changed their professional location to take on director positions at other universities.

When performing analyses for LOH, we use microdissection of study samples to avoid contamination of tumor tissue with normal tissue as much as possible. However, there is no absolute guarantee (Zhuang Z, Vortmeyer AO: Applications of tissue microdissection in cancer genetics. Cell Vis 1998, 5:43-48). The microdissected tumor DNA prepared for conducting sequencing analyses in the present study may have been contaminated with some normal tissue/cells, therefore delivering the result shown on the chromatogram. We do not have any tumor specimens left to conduct further studies, and we attempted multiple times to get as much tumor DNA as possible to achieve a successful complete sequencing analysis for the VHL gene in the samples number 3 and 4. The “technical” problem with these samples had been addressed by various ways including redesigning the primers, and we feel that the main problem is of human nature: the technician who carried out the initial analyses had left his position and the person following him did not have as much experience and precision. Unfortunately, all the DNA had been used up, as pointed out above. We did not carry out laser capture microdissection but microdissection by hand (Dr. Vortmeyer, NIH, Bethesda).

Minor essential revisions:

We corrected all the spelling errors, i.e. missing bracket on page 6, and had the paper checked by a native English speaker.
We also corrected the references in the style suggested by the journal. Gene names had been written in Italic style except when used in connection with the article “the”, i.e. the VHL gene vs. in VHL.

The legend for figure 3 has been changed to “Somatic mutation of the VHL gene in MEN2A-associated MTC”….”products obtained using PCR conditions described by Ganguly et al.”

The quality of figure 2 could unfortunately not be improved. The authors could submit a high quality glossy paper with the figure printed on it, if that would help.

Table 1: the title has been changed and now includes “mutations of RET”

Reviewer 3 (SF):

Major compulsory revisions:
None

Minor revisions:
None

Discretionary revisions:

The reviewer is absolutely correct: the study size is small and one sample of CCH does not allow to draw major conclusions. Therefore, we added this limitation and rephrased that paragraph on page 8 to “….we suggest that such somatic VHL gene alterations rather play a role in tumor progression than in tumor formation of MEN2-related MTC. This interpretation is based on a single case of CCH and would require confirmation in studies of larger size.”

Minor points:

1. We do not have clinical data on all patients. However, we did add table 2 including the information we had.

2. Yes. We are planning to perform further studies on CCH specimens. At present, we do not have any specimens available, since the pathology coauthor Prof. Tannapfel as well as the lead author Prof. Koch have changed locations/universities.