Author’s response to reviews

Title: A rare missense variant in APC interrupts splicing and causes AFAP in two Danish families

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Author’s response to reviews:

Copenhagen, Feb 28, 2020

Dear Mr. Scott.

Thank you for your letter and consideration of publishing our paper “A novel missense variant in APC interrupts splicing and causes AFAP in two Danish families”. Also, many thanks to the reviewers, we are very grateful for their efforts and very useful comments, which will definitely help to improve the paper.

We have now revised the manuscript, and have made the following changes:

1. Since the variant has recently been identified in a Chinese patient, we have changed the title to “A rare missense variant in APC interrupts splicing and causes AFAP in two Danish families”

2. We have cited the manuscript from Wang et al, 2019, who reported the same APC variant, and mention in the paper that the variant has been identified in a patient with a similar phenotype (the paper by Wang et al does not report whether the patient has classic FAP or AFAP, only that the patient was ‘mildly affected’).

3. As mentioned by reviewer #1, we have tried to make it very clear in both the abstract and
discussion, that the occurrence of Caroli Disease and AFAP in the same patient is most likely a coincidence. To our opinion, carrying a pathogenic APC variant could have contributed in the tumorigenesis of the malignant liver tumour in the patient, but we agree with the reviewer that this is hypothetical.

4. As mentioned by reviewer #2, data on allele specific expression is very helpful when interpreting the significance of the variant. We have performed this analysis using primers located in the skipped region, and the results show that only the wt allele is present, supporting the semi-quantitative CE results. This strongly suggests that only the truncated transcript is produced from the mutant allele, which further supports that the variant is pathogenic.

5. We have changed the name of the supplementary table from S2 to S1 and have uploaded a new supplementary table.

6. Finally, we have made minor revisions throughout the paper, which are clearly marked in the text.

The reviewers’ comments are included after this letter with our respond to each comment.

Thank you very much for your consideration,

Best regards,
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Reviewer reports:

Reviewer #1: Very interesting material but it may be coincidence of the both diseases in my opinion authors did not proof the role of the mutations of APC in pathogenesis of Caroli Disease In my opinion most interesting is formation new cryptic acceptor splice site. c.289 is located in the middle of exon and it is very important information for all studding the genetic bases of hereditary disease.

Comment: We completely agree with the reviewer and have tried to make it clear that the APC variant could play a role in the carcinogenesis of Caroli Disease-related cancers, but most likely not in the development of Caroli Disease.

Reviewer #2: This is a clearly written report, which is of interest for everyone who is involved in (molecularly) diagnosing FAP. It is good to have an emphasis on a cryptic splice site mutation, a group of mutations which is in the highly automated genome diagnostics of today easily overlooked. I have two comments:

1. The classification of c.289G>A, p.(Gly97Arg) as a pathogenic mutation and the cause of APC in these families would even be stronger if it was possible to show that from the mutant allele only the (-70) transcript is produced. Is there a SNP close enough to the mutation to distinguish the mutant and the WT allele in a Sanger sequencing experiment?

Comment: This is a very good point. We have carried out an allele specific expression analysis using the c.289G>A variant as informative SNV and included the results in the text and figure 2E.
2. The same mutation c.289G>A, p.(Gly97Arg) in APC was recently described in a Chinese pedigree with mild FAP (Wang (2019) Med Sci Monit 25, 3796). This information should be added to the manuscript, as it is a strong argument against c.289G>A, p.(Gly97Arg) being a rare Danish SNP unrelated to FAP.

Comment: This is of great importance and we have now added this information in the paper.