Author’s response to reviews

Title: Case report: Recurrent pituitary adenoma has increased load of somatic variants

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Answers to the specific comments raised by reviewer (MS Word formatted version available at the end of the revised cover letter RPeculis_cover_letter_20191119.docx)

Response to the comments of Reviewer 2
Comment 1.
As we know, the leftover of tumor is a critical factor to induce recurrent PA. While whether the resident tumor regrowth is the reason for recurrence needs to do further analyse. The imaging data can be a candidate to assess the likelihood of recurrence. Please support the MRI after the first resection and assess the result briefly

Answer: We agree that additional MRI images would add some incremental information to the paper,
however, the postoperative MRI (taken three months after surgery) images were not available in the electronic medical records of the patient and therefore the only available information from the clinical record was included in the paper. In order to clarify this information, we have included the following additional information to the Results section of the manuscript:

“The first postoperative MRI was performed three months after surgery. A clinical record indicates that MRI showed a large residual tumour at the left side of the cavernous sinus and a cystic structure that was also suprasellar. No images were available in the electronic medical records of the patient for this procedure.”

We would like to stress out that absence of the MRI images does not influence the main conclusions on the observed genetic differences between the tumors, demonstrating that somatic variant acquisition and possibly clonal expansion is occurring in the PAs similarly to other types of tumors.

Comment 2.
There are enough evidences for PA recurrence in the paper, while it is insufficient for rapid growth. Malignant tumor has the ability of invasion and rapid proliferation. Compare and summarize with some proliferous genes of malignant tumor to highlight the likelihood of rapid proliferation via the brief generalization of various SNPs. And there are too many pleomorphisms about the other five SNPs attribution, and the potential function of gene in PAs is not highlighted. Why do the authors choose these SNPs found in other cancers?

Answer: There was no indication of malignancy in the presented PA case and therefore we did not include additional genes in the prioritization strategy to analyse genetic variants (see below). We also would like to stress out that we did not included SNPs in this analysis, but only variants with frequencies below 0.01

We performed additional literature search to identify new publications that would provide additional information of involvement of these genes in PA but did not revealed any new information. To clarify the potential roles of the genes with identified variants in PA and, we made following changes in the manuscript:

“Three of the genes JPH2, OR5M1 and NPTXR have had a somatic variant (although different position) in previous PA exome sequencing studies in the literature (Bi et al. 2017, Ronchi et al. 2016) and further functional studies are needed to evaluate the role of these gene variants on pituitary cell functionality. Recurrence of deleterious rare SNVs across different PA sequencing studies could indicate that they could be part of Knudson’s two hit hypothesis in PA, especially when taking into account other genetic and/or environmental factors. ZFYVE26 has been shown to harbour somatic mutations in hereditary non-polyposis colorectal cancer and is expressed in hepatocellular adenocarcinoma (Yu et al. 2018), but the potential relation to PA development is discussable. HRAS is widely described proto-oncogene which predominates in head and neck squamous cell carcinoma (Hobbs et al. 2016) but is also found in salivary duct (Chiosea et al. 2014), bladder urothelial carcinoma and acute myeloid leukaemia (Hobbs et al. 2016). Having been implicated in other carcinogenesis cases HRAS could be investigated as a novel candidate that influence PA. Cosmic somatic mutation (COSM4825382) found in our PA patient has been previously found once in a cervix cancer (Zerbino et al. 2018).”

Concerning the choice of variants, we would like to emphasise the use of our variant prioritization strategy which relies on two commonly used algorithms to predict variant effect and we did not deliberately “chose” any of the described variants. The detailed description of the choice of target genes and SNP filtering is described in section “Exome sequencing results”:

“We also performed a targeted analysis of SNPs located in a gene set compiled from literature data about PA genetics, tumour suppression and genome and exome sequencing. By searching literature with keywords “pituitary adenoma genetics”, “pituitary adenoma exome sequencing”, “pituitary adenoma gwas” and “tumour suppressor genes” on 15 th of May 2018, list of 403 genes were compiled
Targeted SNP analysis was performed in this region which encompasses 47,370,914 base pairs (including introns, which are sparsely represented in exome data) (Figure 3). 252 missense SNPs were found in 133 genes. SIFT and Polyphen prediction algorithms rated 23 missense SNPs both “deleterious” and “probably damaging” or “possibly damaging”.

“A frequency filter with minor allele frequency threshold 1% (gnomAD database, non-Finnish European population) left five SNP as top candidates possibly contributing towards characteristics and development of PA (Table 2).”

Comment 3.
Did the authors exclude the effect of treatment on mutation load, such as radiotherapy?

Answer: We thank reviewer for rising the awareness about possible impact of radiotherapy on variant amount in the recurrent tumors in PA, but in our case, patient did not agree to radiotherapy as stated in the manuscript section “Case presentation”: “Subsequent radiotherapy was declined.”. However, to emphasise this we have added the following to the discussion section: “We also would like to notice that patient did not receive radiotherapy during any state of the treatment that would stimulate additional mutagenesis.”