Reviewer's report

**Title:** Birt-Hogg-Dube renal tumors are genetically distinct from other renal neoplasias and are associated with up-regulation of mitochondrial gene expression

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**Reviewer:** Laura Schmidt

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Klomp and colleagues have used comparative gene expression profiling analyses of BHD-derived renal tumors and sporadic renal oncocytopas and chromophobe renal tumors to determine the extent to which these histologically related renal neoplasms share genetic profiles or display distinct gene patterns. They confirmed that BHD-associated renal tumors do not share the same cytogenetic profiles as the sporadic counterpart tumors. However they do share some gene expression profiles that are similar, the most important of which is a high expression for mitochondrial and oxidative phosphorylation associated genes. Furthermore, using gene set enrichment and pathway analysis approaches, the authors identify an inverse relationship between FLCN expression and PGC-1# activation and conclude that a deregulation of AMPK-PGC-1# axis is a result of FLCN inactivation in the BHD-derived renal tumors and perhaps other tumors as well.

This is an interesting study with a unique approach for elucidating the consequences of FLCN inactivation. The data are sound and generally support the conclusions although certain statements in the conclusions may be too definitive without further confirmation by molecular methods (see below). The paper is well written and figures/data are clearly presented.

Minor essential revisions:

1) Abstract, Methods-authors state that results of gene expression analyses were confirmed by qRT-PCR. But only 3 genes (PVALB, CDH19 and RGS20) were tested. Please correct this statement. Were other genes validated in the data set?

2) Abstract, Conclusions- what is the evidence for stating that the results support deregulation of the AMPK-PGC-1a axis? The data presented support activation of PGC-1a but no data is shown that supports AMPK activation. Currently available data only support an interaction of FLCN with AMPK through FNIP1/2; the functional consequences of that interaction and whether FLCN is upstream or downstream of AMPK are not known. If the data are not presented to support deregulation of AMPK-PGC-1a axis, then AMPK should be omitted from that sentence.

3) Background, 2nd paragraph, line 9- insert “BHD worldwide”
4) Background, 3rd paragraph, line 7- “somatic” should be “germline”. BHD is an inherited syndrome caused by inheritance of a germline FLCN mutation with loss of the remaining wild type copy in the tumor.

5) Please state the number of sporadic tumors evaluated for each histologic type in Fig.1A, B, C and E.

6) Is Fig 1C data generated by qRT-PCR? Needs to be stated.

7) Why did the authors select CDH19 and RGS20 for validation by qRT-PCR? HHATL and DAPL1 were more highly expressed in BHD tumors than CDH19, and LRRTM was more highly expressed than RGS20. It would seem important to validate the top genes in Table S2 and more than just the two genes included here.

8) What was the histology and mutation in the outlier BHD tumor that had multiple chromosomal abnormalities? Should state in text.

9) Please state the experimental design for data shown in Fig 4b. Was each gene listed in Fig. 4A evaluated by qRT-PCR in a number of tumors of different types? What was the number of tumors of each type? If all genes were tested in this pathway, this is important data and should be presented at the least in the supplemental section, especially FNIP1, TSC2 and AMPK data.

10) How were the two transcription factors for expression analysis selected? Are PGC-1a and TFAM the only transcription factors involved in mitochondrial biogenesis? If not, were other transcription factors tested?

11) Results, 6th paragraph-it is not entirely clear as to how this experiment was designed. Was this work (HepG2 cell infection with adeno-PGC-1a) performed by the authors or was the data gleaned from the literature? Which two gene sets only overlap 11.8%? This is not clear. The last sentence in that paragraph is too strong. These expression data do not tell us that FNIP2 mediates deregulation of mitochondrial biogenesis. Nor do the data support AMPK as the regulator of mitochondrial biogenesis through deregulation of PGC-1a. Further molecular work needs to be completed to confirm that statement. This is speculative and does not belong in the results section.

**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