Author's response to reviews

Title: A multinodular goiter as the initial presentation of a renal cell carcinoma harbouring a novel VHL mutation.

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Re: MS 1234633136111046 entitled "A MULTINODULAR GOITER AS THE INITIAL PRESENTATION OF A RENAL CELL CARCINOMA HARBOURING A NOVEL VHL MUTATION"

Dear Doctor Sandra Le:

We are, herein, re-submitting the revised version of the above manuscript. Corrections and modifications were performed according to the reviewers' comments and criticisms as well as to the editor's notes.

We would like to thank both reviewers for their contributions. As required, we responded directly to the points raised by each reviewer:

Reviewer #1 (Dr David N Poller)

* Typographical errors (page 8 lines 8 and 9) were corrected.

* We do agree that the suggested references are pertinent for the discussion and as so were included in the present version (Section Conclusions, page 9).
* As a matter of fact, an abdominal CT scan was performed based on the FNAC results, as stated in page 4/1st version "FNAC results prompted a clinical and radiographic investigation. An abdominal CT scan revealed a tumor of the left kidney measuring in greatest diameter 10 cm". We rephrased the abstract to make this point more clear, since the first version could be misleading.

* A list of other primary clear-cell tumors was already included in the first version of the manuscript (page 7): "Although the kidney is the most common primary anatomic site for clear-cell tumor, other primary sites include the lung, salivary gland, breast, ovary, endometrium, cervix, vagina, pancreas, liver, adrenal gland and thyroid [6]."

* The frequency of somatic VHL mutations in patients with clear cell renal cancer is now reported in page 3, last paragraph: "... the von Hippel-Lindau tumor suppressor gene (VHL) is mutated or silenced in more than 50% of sporadic renal cell carcinomas of the clear cell type [8-12], ...".

* To screen for VHL mutations in thyroid aspirates, 2.5 ul of first-strand cDNA was used as a template for PCR using primers designed by us: F-5'-TCAGAGATGCAGGGACACAC-3', R- 5'-TGACGATGTCCAGTCTCCTG-3', (VHL mutation analysis, page 5). To amplify somatic DNA we used primers previously described (reference 14). This is clearly stated in page 6, 2nd paragraph, first version of manuscript.

* To overcome technical limitations, we used different approaches to seek for VHL mutations. SSCP was a screening method. To further characterize the abnormal pattern (2 bands) observed in the SSCP - exon 3, PCR purified products were either sequenced directly or subcloned and subsequently sequenced. We did not cut any band from the SSCP, for sequencing analysis. Moreover, sequencing results were further confirmed by restriction analysis.

* Actually, we did not perform immunohistochemical studies for the VHL gene product. Retrospectively, we do not consider it of great value. The presence of a mutation creating a premature stop predicting a truncated pVHL makes unpredictable the results in renal tumor samples. Moreover, immunoreactivity for VHL in smears from thyroid would not allow concluding for a renal origin, since VHL is expressed in nonneoplastic lesions and differentiated tumors derived from follicular epithelium (Hinze R, Endocr Pathol 2000).

* VHL is a tumor suppressor gene based on both genetic and functional criteria. Therefore, biallelic VHL mutations are expected in sporadic renal cell carcinomas of the clear-cell type. Evidence for a heterozygous mutation in exon 3, in the absence of other abnormalities in the remaining exons, made us to consider the possibility of coexisting events, not tested, such as hypermethylation.

Sincerely,
Maria Joao Martins Bugalho