Reviewer's report

Title: Ser80Ile mutation and a concurrent Pro25Leu variant of the VHL gene in an extended Hungarian von Hippel-Lindau family

Version: 1 Date: 11 December 2007

Reviewer: Rob B van der Luijt

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- Major Compulsory Revisions

1. The authors suggest that the Pro25Leu variant may present a protective variant for VHL disease caused by the Ser80Ile mutation. This is solely based on the observed compound heterozygosity in individual II.1 in the pedigree (Figure 1) who is clinically unaffected. However, it cannot be excluded that individual II.5, who has clinically proven VHL syndrome, is a compound heterozygote as well. This is based on the observation of the Pro25Leu variant in individual III.6 and of the Ser80Ile mutation in individuals III.7 and III.8. Moreover, no molecular basis (e.g. based on functional analysis of VHL proteins carrying the respective amino acid changes) is provided by the authors to support such a protective effect. Therefore, the suggestion(s) that Pro25Leu is a possible protective variant for VHL should be removed in its entirety from the manuscript.

2. The symbols used in Figure 1 are not according to standard conventions for pedigree drawing. This may be confusing. Clinically affected persons should be shown as filled symbols and unaffected persons as open symbols. Slashed symbols are used by the authors to identify individuals not tested, however this symbol is usually used for deceased persons. Persons not tested may be labelled as â##NTâ## e.g.

To indicate whether a person carries the Ser80Ile mutation and/or the Pro25Leu variant, the authors could use an asterisk combined with a different symbol.

3. The resolution of Figures 2A and 2B (at least, in the PDF-version that I have downloaded and printed) is far from optimal, the text cannot be read properly, especially in 2B.

4. The authors do not use the standard HGVS nomenclature for the description of sequence variants, while it is generally recommended to do so. At least, the authors should provide a full description of the VHL changes identified in their patients, i.e. including the nucleotide changes as well as the predicted amino acid changes.

The author must respond to these before a decision on publication can be
reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

- Minor Essential Revisions

5. Page 2, line 7: downstreem = downstream
6. Page 3, line 13: 1:36000 and 1:85000 = 1:36,000 and 1:85,000 in
7. Page 3, line 18: germline mutation or deletions = germline mutation or deletion
8. Page 4, line 9: downstreem = downstream
9. Page 10, line 7: Ser802Ile = Ser80Ile
10. Page 14 (legend Figure 2), line 2. gene = protein

The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

- Discretionary Revisions

11. It would be useful to run the Align GVGD software tool for both Ser80Ile and Pro25Leu. Align-GVGD is a freely available, web-based program that combines the biophysical characteristics of amino acids and protein multiple sequence alignments to predict where missense substitutions in genes of interest fall in a spectrum from enriched deleterious to enriched neutral. Align-GVGD is an extension of the original Grantham difference to multiple sequence alignments and true simultaneous multiple comparisons. Data from this program may help to confirm the pathogenicity of Ser80Ile and the neutrality of Pro25Leu.

12. Extension of the multiple alignment analysis of the vhl protein from different species by including orthologs from evolutionary more distant species, such as fugu and drosophila, can be informative. The depth of the alignment provided by the authors may be insufficient.

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

Please note that both the comments entered here and answers to the questions below constitute the report, bearing your name, that will be forwarded to the authors and published on the site if the article is accepted.

What next?

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Based on your assessment of the validity of the manuscript, what do you advise should be the next step?

- Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest
- An article whose findings are important to those with closely related research interests

Quality of written English
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- Acceptable

Statistical review
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Is it essential that this manuscript be seen by an expert statistician?
- No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests
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'I declare that I have no competing interests’