**Reviewer's report**

**Title:** Detailed investigations of proximal tubular function in Imerslund-Grasbeck Syndrome

**Version:** 4  **Date:** 2 October 2013

**Reviewer:** C Böger

**Reviewer's report:**

In my previous review, I have indicated that the current revision is suitable for publication.

One of the other reviewers (John Fyfe) has raised a number of concerns in how the authors dealt with his comment nr 3, and I have been asked to comment. The key issue is the hypothesis raised by Storm et al in the discussion on page 19 (last para): classifying identified IGS causing mutations as either a) affecting IF-B12recognition or b) affecting both IF-B12 recognition in the small intestine and decreased prox tubular readsoption of cubilin ligands. the authors’ own data in this manuscript are in support of this hypothesis, while the reviewer Fyfe does not agree with the arguments in support of this hypothesis. Probably the main disagreement is on the role of mutations not covered by this paper, (giving this dissense a tragic note): the authors’ proposition that CUBN mutations beyond CUB domain 8 cause only prox tubular dysfunction and not decreased B12-IF adsorption (and mutations before cub domain 8 cause b12 deficiency also). They support this statement with the immensely vast data set published in Tanner et al (Orphanet J Rare dis 2012: 33 CUBN mutations in 54 families/cases), and also the Ovunc paper (ref 49), where two individuals were identified with mutations in exon 53/cub domain 20 that had proteinuria but normal full blood count. While Fyfe contests correctly that the presence of normal erythrocytes does not prove that the 2 individuals in the Ovunc paper had no b12 deficiency (b12 was not determined), I do not fully agree with Fyfe that Storm et al misquotes Tanner et al. I think Tanner’S conclusion that no IGS cases are observed if mutations are beyond exon 28/cub8 is correctly quoted, but Storm et al should include the important half sentence "as long as the protein is stable".

This half sentence is important because of the next disagreement between Fyfe and Storm et al: the authors and Fyfe are in disagreement of the functional consequences of the mutation observed by Ovunc et al in exon 53 (cub domain 20) -- Storm et al hypothesise that such a mutation may lead to a truncated, but partially functional cubilin protein, while Fyfe contradicts this position by citing his own work in dogs where a mutation in exon 53 leads to "drastically reduced" CUBN mRNA and protein expression in kidney and intestine (Mol Genet Metab 2013, Fyfe et al). Perhaps it is most prudent to state that currently, it appears that more work is needed to clarify the effect of mutations in this exon on cubn expression and/or function.
Storm et al did not identify the mutation in exon 53 in their work, so I think it would be very unfortunate if their very important contribution to CUBN and AMN genetics were not accepted for publication solely for the discussion on a mutation seen in other publications. I think the hypothesis proposed by Storm et al and by Tanner is an enticing one. I would propose to keep the discussion on it in the manuscript, but ask the authors to add the half sentence from Tanner cited above, cite the new Fyfe paper and point out the potential discrepancy to the discussion on its functionality in the Ovunc paper, and to tone down the language, e.g by adding something like "further functional analyses are required to further investigate this hypothesis" at the end of the paragraph 1 on page 20. If it helps to appease reviewer Fyfe, the passage citing Böger et al in JASN 2011 can be removed or more careful wording used when referring to this paper.

**Level of interest:** An article of outstanding merit and interest in its field

**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'