Author's response to reviews

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

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Author's response to reviews:

We again thank the reviewers for their insightful comments which we have implemented in this revised manuscript.

Reviewer John Fyfe:

There is extensive literature demonstrating that some patients that are clearly cobalamin deficient do not exhibit megaloblastic anemia, and some patients present with only neurologic signs. Severe combined degeneration of the dorsolateral columns of the spinal cord is a well recognized result of cobalamin deficiency, but a less well known signs include psychosis, seizures, or obsessive compulsive disorder in the absence of hematological signs. Most of the neurologic signs are responsive to parenteral cobalamin supplementation. Therefore, the fact that Övunc’s patients did not have megaloblastic anemia does not speak to their cobalamin status. In fact, Övunc reported that one of them had seizures since 5 months of age, perhaps due to cobalamin deficiency, but there are many causes of pediatric seizures that are not cobalamin deficiency.

- The authors agree and the paragraph has been changed accordingly.

Here we are in total disagreement. As above, absence of megaloblastic anemia means nothing. The mechanism of nonsense mediated decay relies on molecular
marks made on the mRNA during splicing. The exon 53 mutation described in Ovunc is not at all near the 3' end of the message; there are 13 splice junctions from the premature stop codon in exon 53 to the end of the message. Therefore, it is NMD unless proven otherwise and is not compatible with truncated protein. A nearly identical, and clearly I-GS mutation of CUBN (exon 53 single base deletion ending the open reading frame at the same place as the Ovunc mutation) was described in dogs (1) while your manuscript is in review. The affected dogs have drastically reduced CUBN mRNA, and the very small amount of residual CUBN protein is not truncated.

- Based on the novel report on a similar CUBN exon 53 single base pair deletion identified and investigated in this dog model, the authors agree and the paragraph has been changed accordingly.

You had me going there for a moment because the reference 38 that you mention should be reference 48, but in rereading the reference 38, I realized that it is everything that the Ovunc paper should have been. They found proteinuria in the absence of signs of cobalamin deficiency, but they were not wearing the nephrologist's blinders and discovered the accompanying cobalamin malabsorption in the patients’ early history. They reinforce for the benefit of the medical community and patients that the finding of low grade proteinuria in the absence of hypertension or diabetes should prompt concern for cobalamin absorption leading to correct diagnosis and proper treatment. But as you point out, Tanner et al, in reference 48, does quote the Ovunc paper. I believe, and in email discussion with Dr. Tanner, he believes that he should not have done so in that exact context. In Stephan's defense, in the sentence before the mention of Ovunc's paper they emphasize that the distal mutations must be compatible with expression of stable protein, which the Ovunc mutation is not.

- Reference 38 has been changed to reference 48.
- The paragraph has been changed according to recommendations.

One can certainly speculate on the possibility of mutations that cause proteinuria without cobalamin malabsorption, but the Ovunc mutation should not come into it as support. Even the sequence variation described by Böger is weak because as I previously pointed out and as was pointed out in a published viewpoint (2) that accompanied the Böger publication, the sequence variant they described was an association only. By random chance it was a single nucleotide polymorphism included in the genome wide study and is no more associated than any of likely very many other variants nearby that they did not assess. Neither is the predicted amino acid change likely to alter function cubilin function. But as a class of cubilin mutations, one can imagine there may be some missense changes that could alter interaction with albumin or with megalin and not affect IF-B12 uptake in the intestine, where megalin does not seem to function. It is simply that neither the Ovunc nor Böger variants are good examples to support the conjecture.

- The paragraph has been changed according to reviewer's recommendations.
You also mention that Tanner et al did not find CUBN mutations more distal than exon 29 in 154 cases, but that denominator is quite misleading; only 53 of those patients demonstrated CUBN mutations at all, and only 32 distinct mutations are described (20 cases were FM1 (see table 1 in reference 48). If you use this statement in your revision, please give it the correct reference number (not 38).

- The statement has been revised and does no longer include a specific number of cases, patients or mutations.

Reviewer C. Böger:
The authors have implemented all the recommendations in the particular section of the discussion.