Reviewer's report

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

Version: 4 Date: 2 July 2013

Reviewer: John Fyfe

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The authors of this manuscript have responded appropriately to my major compulsory changes except for point 3. I have integrated my further critique into their response (between <> below).

<3. The authors do not agree with the reviewer’s interpretation of the data presented in reference 49 (Ovunc et al.). Ovunc et al. clearly states throughout the article that both patients did NOT have megaloblastic anaemia and that both hemoglobin levels and red blood cell indices were normal. It is true that Ovunc et al. did not do detailed investigations of intestinal cobalamin absorption (Schilling’s test) in the patients but based on their clinical picture there was no medical rationale to do so. The statement “causing only albuminuria but not intestinal IF-B12 malabsorption and IGS” has been specified to “causing only albuminuria and not megaloblastic anaemia” to fit the precise statements from Ovunc et al. The comment on possible beneficial effects on B12 supplementation in these patients made by Ovunc et al. is not referring to possible beneficial effects on the hematological state of the patients but is a speculative parallel to the beneficial effects on neurological symptoms previously observed in a case report on a German IGS patient by Hauck et al., 2008.>

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 Ovunc’s patients did not have megaloblastic anemia does not speak to their cobalamin status. In fact, Ovunc 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.

<Ovunc et al. did not perform any functional analyses of the single base pair
deletion identified in the two patients wherefore there is no evidence for neither nonsense mediated decay of the transcription product nor for production of a stable translation product. Although the mutation results in a premature stop codon it is possible, due to the position near the 3’ end of the CUBN gene (exon 53 of a total of 67), that the deletion does not result in nonsense mediated decay but in a truncated cubilin protein with the IF-B12 binding site and amnionless interaction region intact. Although speculative, it is possible and consistent with the absence of megaloblastic anaemia in these patients.

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.

The authors do not feel that Tanner et al. is misquoted as the authors themselves use reference 49 as an argument for mono-symptomatic proteinuria due to CUBN mutations in reference 38 (Tanner et al.). Tanner et al. furthermore reach similar conclusions as presented in this manuscript regarding the effects of CUBN mutations on renal function (reference 49 was published during the review of this manuscript).

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.
Regarding the general relevance of the section speculating on the functional consequences of the positions of the sequence variations associated with proteinuria and not megaloblastic anaemia the authors were urged by reviewer C. Böger to include this section. The authors agree with C. Böger but to accommodate this point of critique it has been specified in the particular section that it is speculations.

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.

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).


http://jasn.asnjournals.org/content/22/3/404.long

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'