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:

Major compulsory revisions:
1. The paragraph has been changed accordingly.
2. The authors do not agree with the reviewer’s interpretation of the data presented in reference 40 (Wahlestedt-Fröberg et al.). It is true that three (out of a total of 10) FM1 patients showed either mild or clear-cut proteinuria (two clear-cut and one mild). As proteinuria is not uncommon in the general population it is expected that a minor group of the FM1 patients would have proteinuria unrelated to their CUBN phenotype. Also, the authors of reference 40 do also state in the methods section (patient information) and in discussion section, that:
   a. One FM1 patient had been nephrectomized due to hydronephrosis (this patient belonged to the clear-cut or mild proteinuria patients, which group is not specified)
   b. One of the patients had a child that presented with benign proteinuria but no B12 malabsorption
c. A large number of “additional functional polymorphisms” segregating with the disease were identified in the original genetic study of Finnish patients by Aminoff et al. [1]

d. The patients are aged from 25-63 years of age

According to a, the proteinuria in one of the three FM1 patients listed with proteinuria is most likely not a result of the CUBN phenotype. Regarding the remaining two FM1 patients, unfortunately, the authors of reference 40 do not state the age of the individual patients (renal function generally decreases with age) nor do they state which of the patients had a child with mild proteinuria and no B12 malabsorption or which polymorphisms was identified in the individual patients. Regardless, it is most likely that the proteinuria identified in the remaining two FM1 patients is consistent with the prevalence of (CUBN unrelated) proteinuria in the general population or a result of any of the circumstances listed in b-d and not the CUBN phenotype. Thus, the authors feel confident to say that the urinary protein excretion data presented in the manuscript are in fact in line with the data presented in reference 40.

The statement “Alternatively, the low-molecular-weight proteinuria may be unrelated to CUBN” has been removed from the section according to reviewer’s recommendation.

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.

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. Consequently, the authors do not feel that the speculations listed on cubilin CUB domains 20-22 in the discussion are irrelevant.
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).

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.

4. The paragraph has been changed according to recommendations.

Reference List