Reviewer's report

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

Version: 3 Date: 20 February 2013

Reviewer: John Fyfe

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Major Compulsory Revisions

1. Results, 3rd paragraph, page 15: There is no biological rationale for the mutation (c.1041_1042delinsCTC) to create an unstable transcription product. Without laboratory evidence, this supposition must be withdrawn.

2. Results, last paragraph (Urinalyses), page 16: The statement, “In line with a previous report of urinary protein excretion in FM1 patients [40], analyses of urinary protein excretion in the index patient of family 6, however, did not show……” in the middle of the paragraph, actually ignores the findings in the referenced article. The same problem appears in the discussion on page 19, top paragraph. Reference 40 clearly demonstrates excessive protein and cubilin ligand excretion in some FM1 patients (Figure 1, patients 5, 6, and 8), but not in others. Obviously, FM1 patients all have the same CUBN genotype, but their renal tubule phenotype differs drastically. There appears to be no correlation. The results section is not the place for it, but the authors must use and discuss the reference honestly. The concluding statement of that discussion paragraph that, “Alternatively, the low-molecular-weight proteinuria may be unrelated to CUBN.”, while quite likely, is too abrupt and is not helpful to the reader.

3. Discussion, top paragraph, page 20: This new paragraph entirely misinterprets three recent papers and must be removed. While Ovunc et al [49] did report the exon 53 single base pair deletion mutation as causing only proteinuria, that conclusion is almost certainly wrong. They did not evaluate cobalamin absorption in their patients, and you will note that near the end of the paper, they do concede that the patients might benefit from cobalamin administration. The mutation they report is a frameshift mutation that predicts an early stop codon and very likely results in nonsense mediated decay of the mRNA. Conjecture on how alteration of the CUB 20 structure could affect megalin or albumin binding is, therefore, irrelevant.

The authors misquote Tanner et al. [38]; he does not postulate that CUBN mutations beyond exon 28 do not cause IGS. He says that, based on his observations, mutations beyond exon 28 that are compatible with a stable protein may not cause cobalamin malabsorption (IGS). The Ovunc mutation is not compatible with a stable message, much less a stable protein.
Boger et al. [50] reported an association of microalbuminuria with a cubilin SNP that is a missense variation in CUB 22. There is no experimental evidence of altered expression or albumin binding or such. An association study is only that, and the Boger study is just as indicative of association of microalbuminuria with any of many possible nearby CUBN sequence variations that are in LD with the identified SNP. Limitations of the study were pointed out in an accompanying essay (O'Toole JF and Sedor JR 2011JASN 22: 404-406). Therefore, speculation on how the I2984V sequence variation may cause albuminuria is not relevant in this manuscript.

4. Discussion, last paragraph, page 19. As indicated above, the data in your reference [40] presents a huge problem trying to classify cubam mutations into B12 malabsorption-only vs. B12 malabsorption with proteinuria. Having the FM1 mutation is not predictive of renal tubule function phenotype. The supposed genotype-phenotype correlation presented in this manuscript is so weak that it is not useful to construct an artificial classification into types 1 and 2 IGS, and this suggestion must be removed.

Level of interest: An article of limited interest

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'