Reviewer's report

Title: Genotype-phenotype correlation of proximal tubular function in Imerslund-Gräsbeck Syndrome

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Reviewer: John Fyfe

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The manuscript, Genotype-phenotype correlation of proximal tubule cell function in Imerslund-Gräsbeck syndrome by Tina Storm et al., is a report of newly determined mutations in AMN and CUBN that cause selective intestinal malabsorption of vitamin B12 (cobalamin) with selective proteinuria. It is generally well written and presents interesting findings, but there are some issues that, if addressed, will make it a much better manuscript. I am not suggesting that new experiments are required for publication, but that the significant revision be done to bring the introductory statements, conclusions, and discussion in line with the data.

Major compulsory revisions:

1. AMN and CUBN form a heterodimeric, multi-ligand, apical membrane receptor, called cubam, of the intestinal and renal proximal tubule epithelia. The receptor mediates uptake of dietary cobalamin in complex with intrinsic factor in the intestine and a variety of low-molecular weight proteins from the glomerular ultrafiltrate for reabsorption by renal tubules. I-GS was independently described in 1959, by the two individuals whose names are memorialized, in Norwegian and Finnish patients, respectively. Both descriptions included selective cobalamin malabsorption and proteinuria, thus defining the disorder as a syndrome affecting multiple organ systems and having a set of defining clinical signs. Currently, a problem exists because some authors insist (mistakenly in my view) on also calling selective cobalamin malabsorption but without proteinuria as I-GS syndrome. I doubt either Olga Imerslund or Ralph Gräsbeck would agree. Selective cobalamin malabsorption without proteinuria is no different clinically than defects of GIF synthesis or function. The inattention to diagnostic terminology abrogates the clinical value of recognizing the disorder and the combination of signs as a syndrome. The observation of clearly defined proteinuria along with cobalamin malabsorption removes intrinsic factor defects, cbl F disease, and most recently, ABCD4 mutations from the list of diagnostic possibilities for selective cobalamin malabsorption. Since much of the authors' discussion rests on the lack of proteinuria in the FM1 Finnish mutation, they need to take the opportunity to make this distinction. Because the current state of the literature is a bit confusing at present, Storm et al. can well use the introduction to help bring some clarity to it, particularly for a journal of medical genetics. The authors of reference 29, where the functional consequences of the FM1 mutation were demonstrated, quite carefully used the term MGA1, indicating the first
mapped I-GS locus on HSA 10, only subsequently determined to be occupied by CUBN. FM1 and FM2 are CUBN alleles rather than I-GS alleles. MGA1 is an unfortunate term because it does not acknowledge the disease mechanism and only one of several clinical signs. Note that OMIM already subdivides MGA1 (now used to denote all of I-GS) into Finnish type, for CUBN mutations, and Norwegian type, for AMN mutations, but this is also already obsolete (and one hopes will be changed soon).

2. I would argue that what the authors would like to call type 1 I-GS in the current submission is actually not I-GS at all, even if it is due to the most common of the Finnish CUBN mutations. The reverse of this problem exists in the reference 42 in which it is claimed a CUBN mutation causes proteinuria without cobalamin malabsorption. Do the authors believe we should also call this I-GS? I would add that the evidence in the Ovunc paper for an “albuminuria-only” CUBN mutation is quite poor. Suggestion that it represents a third type is very much premature and must be removed from the second last paragraph of the discussion. How, for instance, would you classify a mutation that allows cubilin cell surface expression but also presents with selective low-molecular weight proteinuria of the CUBN-deficient type? I can imagine mutations in AMN or as yet unknown gene products that would do so.

3. A main theme of the article is to correlate the renal phenotype, represented by selective low molecular weight proteinuria with the CUBN and AMN genotypes of I-GS patients. Their conclusion is that any mutation, whether in AMN or CUBN, which results in failure of luminal CUBN expression in the proximal tubules, will cause proteinuria due to failure of the protein reabsorption function of CUBN. This is entirely reasonable, and there is good evidence for it already from AMN knockouts in model systems as well as in humans with CUBN (ref 20) or AMN (ref 21) mutations, as they have pointed out in the introduction. There are several references to reports of human patients with demonstrated mutation genotypes and presence of proteinuria. Why, therefore, is this manuscript providing the first such correlation, as they claim in the last paragraph of the introduction? Is it because this paper is the first to compare an FM1 patient to the others? I’ve already argued that the FM1 mutation does not cause I-GS, and so would require a change in the title.

This also presents a problem because there is only one FM1 patient analyzed, and because the authors report some excretion of all the cubilin ligands they examined. Something is needed in the discussion of the authors’ idea of why the CUBN p.P1297L mutation that disrupts IF-B12 binding results in traces of multiple CUBN ligands in urine when, according to the heterologous expression system, it does not affect surface expression of CUBN. Their reference 34 suggests that there may well be variation in degrees of proteinuria unrelated to CUBN genotype. [Suggestion: The correlation they want to make would be better done in the context of a review article or the current data but with an explicit review of this aspect in the literature, rather than making the case for it solely on their own data.]

4. At the very least, the absence of any direct demonstration of failed cubilin cell
surface expression in renal tubules dictates that the last column in Table 2 must be relabeled as, “Predicted cubilin cell surface expression”. A footnote in the legend is not sufficient. In family 5, the data shown (figure 4) are suggestive, but expression of cubs 5-8 in a heterologous system may not be fully representative of what is happening in tubule epithelial cells in the context of the much larger full-length protein and when chaperoned by AMN. Interpreting the fig 4 experiment as, “affected structural integrity of cubilin and consequently also cubilin cell-surface expression” is too definite a statement for the evidence shown and should be modified or removed from the abstract.

Minor essential revisions:
Introduction:
1. A specific problem occurs in the first line of paragraph 3 in the introduction, in which the second part of the sentence suggests that the first mutations, rather than the disease, were reported in the 1960s.

Results:
2. “In Family 3,….” should be the start of a new paragraph.
3. Fourth line from the bottom of that paragraph, the word “coding” should be “codon”.
4. The second to last sentence of (what should be) the 3rd paragraph of the results presents a conceptual problem. If, as stated, a frame shift does not create a new stop codon in the coding sequence, by definition, it does not invoke nonsense mediated decay of the mRNA. Have the authors not considered that the altered amino acid sequence would either make an unstable protein or at least one lacking the crucial functions of AMN?
5. Figure 3 would be very much improved by removing the CUB 6 and G1112 lables from panel A and the IF #-domain symbol from panel B.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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.