Author's response to reviews

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

Authors:

Tina Storm (tsto@ana.au.dk)
Christina Zeitz (christina.zeitz@inserm.fr)
Olivier Cases (olivier.cases@inserm.fr)
Sabine Amsellem (sabine.amsellem@inserm.fr)
Pierre J Verroust (Pierre.verroust@ana.au.dk)
Mette Madsen (mette@biokemi.au.dk)
Jean-Francoist Benoist (jfbenaus@yahoo.fr)
Sandrine Passemard (sandrine.passemard@noos.fr)
Sophie Lebon (sophie.lebon@inserm.fr)
Iben M. Jønsson (iben.jonsson@ki.au.dk)
Francesco Emma (francesco.emma@opbg.net)
Heidi Koldsø (koldsoe@chem.au.dk)
Jens Michael Hertz (Jens.Michael.Hertz@ouh.regionsyddanmark.dk)
Rikke Nielsen (rn@ana.au.dk)
Erik I. Christensen (eic@ana.au.dk)
Renata Kozyraki (renata.kozyraki@inserm.fr)

Version: 3 Date: 4 January 2013

Author's response to reviews: see over
We thank all reviewers for their constructive and insightful comments which we believe have resulted in a significantly improved paper.

Reviewer 1:

Major compulsory revisions:

1. The authors agree that GIF defects and the FM1 patients today are indistinguishable in the clinical setting and a section depicting this particular issue has been added to the introduction. However, Ralph Gräsbeck continues to classify the FM1 patients as IGS patients without proteinuria in his latest review from 2011[1]. Also, the other major contributors to the genetics of cobalamin malabsorption continue to classify the FM1 patients as IGS without proteinuria in their latest article in which they investigate 154 families with suspected inherited cobalamin malabsorption [2]. Therefore, the authors feel comfortable categorizing the FM1 patients as IGS patients without proteinuria in the present study and agree with Ralph Gräsbeck and Stephan Tanner that to distinguish between GIF deficient patients and IGS patients without proteinuria, correct diagnosis must be made based on genetic testing of the GIF and CUBN genes today.

2. This is indeed a very interesting aspect and a section discussing these issues has been added to the discussion section of the manuscript.

3. The paragraphs in question as well as the title have been changed according to reviewer’s recommendations.

4. The manuscript has been changed according to reviewer’s recommendations.

Minor revisions:

1-4. Text has been changed accordingly

5. Figure 3 have been changed according to reviewer’s recommendations.

Reviewer 2:
The authors agree that nine additional patients is a limited contribution to the genetics of IGS compared to the latest contribution from Tanner et al. 2012 [2]. It is, however, the first detailed investigation of urinary protein excretion in IGS patients that compares proximal tubular function in patients with AMN mutations to the proximal tubular function in patients with CUBN mutations as well as comparing tubular function in patients with different types of mutations in the same gene using in vitro functional analyses.

The Majority of novel reports describing IGS patients today do not include detailed renal phenotypic description with specification of urinary protein excretion. The most recent contribution from Tanner et al.[2] does not contain any renal phenotypic information apart from stating that proteinuria was observed sometimes. Other recent reports including Densupsoontorn et al. 2012, Pediatrics international; Namour et al. 2011, Hematologica; Levin-laina et al. 2011, Nephron clinical practice; Bouuchlaka et al. 2007, Journal of human genetics; Luder et al. 2007, JIMD Short Report; and Broides et al. 2006 et al., J of Pediatr Hematol Oncol. do not contain detailed investigations of the proximal tubular function but only state that proteinuria is observed in these patients. Based on this, the author feels that this study is a valuable contribution to the field and a step towards a comprehensive renal phenotypic understanding of the underlying cellular mechanisms behind the proteinuria observed in many IGS patients.

Regarding the novelty of the proposed hypothesis the statement;

“providing evidence” for a correlation between mutation type and presence of the characteristic low-molecular-weight proteinuria has been changed to “providing additional evidence” for a correlation between mutation type and presence of the characteristic low-molecular-weight proteinuria throughout the manuscript.

Also, “Correlation between the specific disease-causing mutations and the low-molecular-weight proteinuria has not been reported so far but accumulating evidence indicates that functional null mutations of either CUBN or AMN results in low-molecular-weight proteinuria in contrast to observations from patients with the FM1 missense mutation” has been added to the introduction.
Reviewer 3:

**Major compulsory revisions:**

1. A) The title and manuscript has been changed and no longer includes statements of genotype-phenotype correlation. Title and manuscript have been specified to provide additional evidence for a correlation between mutation type and the presence of low-molecular-weight proteinuria in IGS instead of genotype-phenotype correlation. With respect to phenotypic details of the individual patients all clinical data available to the authors have been added as an additional file (additional file 1).

   B) Absolute values of urinary proteins were not identified in the present study as the aim was to identify whether or not patients had low-molecular-weight proteinuria and not to subdivide the proteinuria into categories of e.g. high or moderate proteinuria.

   Furthermore, handling of the collected urines was performed by a number of individuals in a number of different settings and must be regarded as a considerable source of error in respect of urinary protein preservation. Thus, an underestimation of the actual urinary protein content in these patient urines may have occurred if absolute quantitative assessments were to be made.

   The following statement has been added to the methods section for clarification.

   *Urinary protein excretion of a certain ligand was defined as increased when all of the controls despite variability had excretion levels below the excretion levels observed in patients.*

   Spot or 24-hour urines were used in the present study wherefore the urinary protein excretion was normalized using urinary creatinine concentrations. The information has been added to the methods section.
C) The authors agree that this is a very interesting aspect and analyses of urinary cubilin and megalin content have been performed in these patients and compared to healthy controls. However, we have not been able to identify any apparent correlation between type of mutation or gene mutated and urinary content of cubilin and megalin.

2. A) It would indeed be interesting to see the identified mutation’s effects on \( AMN \) transcription. Unfortunately, no patient material was available for RNA analyses and due to the nature of the novel \( AMN \) mutations it would not be possible to do recombinant protein expression/ minigene expression analyses that would provide relevant phenotypic information.

The following statement has been added to the methods section:

*No additional patient material was available for analyses of \( AMN \) splicing.*

B) Table 2 and table legend have been changed according to reviewer’s recommendations.

**Minor revisions:**

1. The novel \( CUBN \) mutation leads to a G1112E aa exchange. This has been corrected in the manuscript text.

2. This is indeed a very interesting aspect and a section discussing the latest reports of \( CUBN \) variations and their association to albuminuria without megaloblastic anaemia has been added to the discussion.

**Discretionary revisions:**

The authors agree that this would be very informative and be an excellent reference and provide the reader with an exceptional overview of renal phenotypes of IGS patients. Unfortunately, most reports of IGS patients does not include detailed analysis of proximal tubular function e.g. the latest very large contribution made by Tanner et al [2] more than doubles the genetic
information on IGS but includes no specific information regarding proteinuria in the individual patients reported. A table summarizing all reported mutations with their respective renal phenotypes would therefore be very incomplete. Hopefully, constructing a table like this may be made possible in the future.

Reviewer 4:

**Major compulsory revisions:**

1. Title has been changed accordingly to “Detailed investigations of proximal tubular function in Imerslund-Gräsbeck Syndrome”.

2. Figure legend has been changed according to reviewer’s recommendations. Indication of the premature stop codon has not been implemented as aberrant stop codon is not reached within the sequence shown in figure 2 (14 aa downstream of Figure 2).

**Minor revisions:**

1-4. manuscript has been changed accordingly.

**Reference List**
