Author’s response to reviews

Title: Familial Xp11.22 microdeletion including SHROOM4 and CLCN5 is associated with intellectual disability, short stature, microcephaly and Dent disease

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Author’s response to reviews:

Dear Prof. Pasini,

Thank you very much for reviewing our manuscript “Familial Xp11.22 microdeletion including SHROOM4 and CLCN5 is associated with intellectual disability, short stature, microcephaly and Dent disease” and considering it to the publication in BMC Medical Genomics. Below we quote each of the reviewer’s comments and describe our way of implementing the suggestion into the revised version of the manuscript. All changes are indicated by track changes.

We now hope for your positive decision considering our manuscript.

Sincerely yours,

Denise Horn
Dear Prof. Horn,

Your manuscript "Familial Xp11.22 microdeletion including SHROOM4 and CLCN5 is associated with intellectual disability, short stature, microcephaly and Dent disease" (MGNM-D-18-00275) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Medical Genomics.

Their reports, together with any other comments, are below. Please also take a moment to check our website at https://mgnm.editorialmanager.com/ for any additional comments that were saved as attachments. Please note that as BMC Medical Genomics has a policy of open peer review, you will be able to see the names of the reviewers.

If you are able to fully address these points, we would encourage you to submit a revised manuscript to BMC Medical Genomics.

Once you have made the necessary corrections, please submit online at:

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If you have forgotten your password, please use the 'Send Login Details' link on the login page at https://mgnm.editorialmanager.com/. For security reasons, your password will be reset.

Please include a cover letter with a point-by-point response to the comments, describing any additional experiments that were carried out and including a detailed rebuttal of any criticisms or requested revisions that you disagreed with. Please also ensure that all changes to the manuscript are indicated in the text by highlighting or using track changes.

Please also ensure that your revised manuscript conforms to the journal style, which can be found at the Submission Guidelines on the journal homepage.

A decision will be made once we have received your revised manuscript, which we expect by 10 Dec 2018.

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I look forward to receiving your revised manuscript and please do not hesitate to contact us if you have any questions.

Best wishes,

On behalf of

Matteo Pasini
BMC Medical Genomics
https://bmcmedgenomics.biomedcentral.com/

Technical Comments:

Editor Comments:

BMC Medical Genomics operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:
Arend B Bökenkamp (Reviewer 1): Danyel et al describe a patient with a micro-deletion involving the SHROOM4 and CLCN5 genes resulting in a combined phenotype of Dent disease and X-linked intellectual disability and short stature. The clinical manifestations in the patient presented overlap with the phenotype of a patient with a microdeletion in the same region described by Armanet et al.

The paper is relevant for the genetic counseling of families with this deletion. While the renal phenotype of CLCN5 mutations is well-known the neurological findings attributed to the deletion of SHROOM4 are relevant as there is only a small number of publications on SHROOM4 mutations.

Major comments:
1. There is considerable overlap of the clinical picture between the observed microdeletion and Lowe syndrome due to OCRL1 mutations. The comparable renal phenotype, growth failure and neurological symptoms/retardation should be discussed as a clinical pitfall as the clinical picture of Lowe may be incomplete (cf. review Ped Nephrol 31 (2016): 2201 - 2212)
Thank you for this important comment. We added the following paragraph in the discussion:
“There is a considerable clinical overlap with patients affected by the oculocerebrorenal syndrome, also referred to as Lowe syndrome. The oculocerebrorenal syndrome caused by OCRL1 mutations is mainly characterized by the triad congenital cataracts, intellectual disability and renal tubular dysfunction. However, the ocular manifestations can be missing or can occur later in life, in the second or third decade. Therefore, the oculocerebrorenal syndrome represents an important differential diagnosis of the condition described here.”
We also included the corresponding reference.

2. A number of reports show a comparable neurological phenotype in patients with microduplications in the region involving SHROOM4 (e.g. Am J Med Genetics 2015, Am J Med Genetics 2016). I am not aware of the renal phenotype of these patients but find it remarkable that the duplications apparently lead to a phenotype which is comparable to the deletion reported here.

We added a paragraph about the corresponding microduplications and the phenotype of these patients in discussion:
“The known Xp11.23p11.2 microduplication can be divided into the 4.5 Mb recurrent duplication which includes the SHROOM4 gene, and atypical microduplications of different sizes. Within the recurrent 4.5 Mb microduplication the SHROOM4 gene is considered to be one of the candidate genes for intellectual disability. In addition to intellectual disability carriers of this microduplication are affected by seizures and early onset of puberty in female patients.”

We also included the corresponding reference.

3. I find the description regarding physical examination and neurological findings rather lengthy while the renal findings are very scant. Here, quantitative findings on kidney function, fractional excretions of electrolytes, low-molecular proteinuria/albuminuria and hypercalciuria should be presented.

We included more parameters of the renal phenotype of the patient here described. To be very clear, we created the following table, which we added to the supplemental material:
Supplementary Table 1. Quantitative findings on renal function. Abnormal laboratory parameters are bolded. All parameters were assessed during therapy with Ramiril 1.25 mg.

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>Result</th>
<th>Unit</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein in serum</td>
<td>7.5</td>
<td>g/dl</td>
<td>6.0-8.0</td>
</tr>
<tr>
<td>Albumin in serum</td>
<td>46.9</td>
<td>g/l</td>
<td>38.0-58.0</td>
</tr>
<tr>
<td>Urea in serum</td>
<td>42.7</td>
<td>mg/dl</td>
<td>10.0-48.0</td>
</tr>
<tr>
<td>Creatinine in serum</td>
<td>0.35</td>
<td>mg/dl</td>
<td>&lt;0.49</td>
</tr>
<tr>
<td>Calcium in serum</td>
<td>2.66</td>
<td>mmol/l</td>
<td>2.10-2.60</td>
</tr>
<tr>
<td>Calcium in urine</td>
<td>2.97</td>
<td>mmol/l</td>
<td></td>
</tr>
<tr>
<td>Calcium/Creatinine ratio</td>
<td>2.84</td>
<td>mmol/mmol</td>
<td>&lt;1.13</td>
</tr>
<tr>
<td>Inorganic phosphate in serum</td>
<td>1.11</td>
<td>mmol/l</td>
<td>0.97-1.94</td>
</tr>
<tr>
<td>Sodium in serum</td>
<td>135</td>
<td>mmol/l</td>
<td>132-145</td>
</tr>
<tr>
<td>Potassium in serum</td>
<td>4</td>
<td>mmol/l</td>
<td>3.1-5.1</td>
</tr>
<tr>
<td>Creatinine in urine</td>
<td>20</td>
<td>mg/dl</td>
<td>40-278</td>
</tr>
<tr>
<td>Protein in urine</td>
<td>693</td>
<td>mg/dl</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Albumin in urine</td>
<td>180.2</td>
<td>mg/dl</td>
<td></td>
</tr>
</tbody>
</table>

4. Which parameters were tested in the mother's urine? Did she have hypercalciuria, LMW proteinuria, nephrocalcinosis (cf recent paper by Li et al, J Pediatr 174 (2016): 204-10)

We got new data of her testing. Low molecular weight proteinuria was confirmed. We added this finding in the case report. Calcium in urine was unfortunately not measured. Also, a statement concerning nephrocalcinosis is not possible.

5. As the the molecular genetic study is central to this paper I believe that the figures presented as supplements should be incorporated in the body of the text. If there are space restraints I believe that the description of the methods could be shortened.

We changed the position of the corresponding figures. They are now included in the manuscript.

6. While the entire CLCN5 gene is missing, only a small part of SHROOM4 is missing. This is different from the patient reported previously in whom the deletion involved the entire SHROOM4 gene. Can the effect of the deletion on gene expression/function be modeled?

This reviewer is right. In the patient reported in the literature the SHROOM4 gene was deleted, in the patient reported here the last three exons of SHROOM4 were missing.

Although the precise breaking points were not assessed, the 3’ breaking point lies between the corresponding exons, as determined by exome sequencing.

SHROOM4 transcript is either prone to nonsense mediated decay or in case of stability of the truncated SHROOM protein, deletion of the last exons would result in a lack of the C-terminal ASD2 domain that is shared within the SHROOM family and important for interaction with the actin cytoskeleton.
The maternal inheritance, intermediate high haploinsufficiency score (44%) and the high pLi score are in concordance with an X-linked recessive mode of inheritance.

Minor comments
1. The authors should consult a native speaker to check the manuscript (e.g. "phosphatise" to indicate phosphate supplementation).

We checked the manuscript together with a native speaker. All changes in the text are indicated using track changes.

Felix Claverie-Martin, Ph.D. (Reviewer 2): It was a pleasure to review the manuscript "Familial Xp11.22 microdeletion including SHROOM4 and CLCN5 is associated with intellectual disability, short stature, microcephaly and Dent disease: A Case Report" by Danyel and col. submitted to BMC Medical Genomics. The authors present the case of a 4-year-old boy with short stature, speech delay, mild intellectual disability, microcephaly, and facial dysmorphism, as well as the clinical characteristics of a rare renal tubulopathy known as Dent disease. By karyotyping analysis, they found a microdeletion Xp11.23p.11.22 that was inherited from the patient's mother. This microdeletion includes the SHROOM4 and CLCN5 genes. SHROOM4 has been shown to be associated with X-linked intellectual disability. On the other hand, the deletion of CLCN5 explains the renal tubulopathy in the patient. Only one previous similar case with Xp11.22 microdeletions that include CLCN5 and SHROOM4 has been reported. The manuscript is well written and the results are clear. I only have a minor comment:

The authors indicate that the patient's mother is asymptomatic. With regard to Dent disease this is normal in the patients' mothers (the patients are usually males), but how do you explain the absence of developmental delay in the mother of this patient?

Thank you for this comment.

Both genes, SHROOM4 and CLCN5, are located on the X chromosome.

As in other X-linked disorders, female carriers may be unaffected or may show a mild phenotype. This effect depends on the random and variable lionization (X inactivation). It is known that female carriers of a CLCN5 mutation are usually asymptomatic, but many show low molecular weight proteinuria and approximately half have hypercalciuria. Only a few data of female carriers with a SHROOM4 mutation is available in the literature. However, one would expect the same effect in female carriers as in other X linked disorders ranging from no phenotypic features to a mild ID phenotype.