Author’s response to reviews

Title: CTNS mRNA MOLECULAR ANALYSIS REVEALED A NOVEL MUTATION IN A CHILD WITH INFANTILE NEPHROPATHIC CYSTINOSIS: A CASE REPORT

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Reviewer reports:

John A. Sayer (Reviewer 1): This is an interesting case report describing a novel mutation (large del) in a patient with cystinosis.

More detail is required regarding the initial gene tests. What did the report say - did exon 3 and 4 fail PCR, was MPLA or other investigations suggested at this time by the genetics centre.

Thank you for the question. We clarified this part in the case presentation and discussion in the manuscript: Abstract, line 33-38, p 3 and Case presentation, line 47-52, p 7

To confirm the diagnosis we performed a direct Sanger sequencing and RFLP analysis of the common in the Northern European population 57-kb deletion of proband’s DNA which revealed no mutations in the coding part of the CTNS gene…

PCR failed to amplify exons 3 and 4. MLPA is not evaluable in our genetic laboratory.

The delay in recognition and molecular genetic diagnosis is very important. What lessons can be learnt to prevent further delays in the future with similar cases?
Thank you for the question. The answer to the question what lessons can be learned from our case, in our opinion, is in the final part of our manuscript: Discussion, line 52-57, p 11 and line 1, p 12

…In some patients with characteristic clinical features of the disease mutations in the CTNS gene are not detected by analysis of genomic DNA. It does not necessary exclude the diagnosis of nephropathic cystinosis in all cases and can be complemented by the examination of the CTNS mRNA transcript, which might finally establish the genetic defect in an additional number patient…. 

What is the ethnicity of the family, was there any effort to find similar families - ie is this deletion a founder mutation?

Thank you for the question. The family is Caucasian. e.g. Abstract, line 23, p 3 and Case presentation, line 47, p 5

It is difficult to define if this deletion is a founder mutation because other members of this family and their relatives are not accessible for segregation analysis.

Some clinical photos / data would be welcome as a new figure

Thank you for your suggestion. We have included a table with clinical data in the manuscript: Case presentation, line 16-56, p 6 and line 1-38, p 7

How many cases of cystinosis are genetically unsolved or have just a single heterozygous change - could this deletion (heterozygous) allow other cases to be solved.

Thank you very much for this question. The answer is reflected in the discussion part in the manuscript: Discussion, line 1-8, p.11

…Shotelersuk et al. failed to identify mutations in the CTNS gene in 19% of American cystinosis patients because the CTNS promoter was not analyzed [24]. Similar studies with analysis of the CTNS promoter region showed heterozygous or no mutations in 18% of Italian patients and in 6% of French patients with nephropathic cystinosis…

Has the mutation been uploading into LOVD or a similar genetic database?

Thank you. We put this information to the manuscript: Case presentation, line 16, p 10

LOVD variant ID : 0000597339 https://databases.lovd.nl/shared/variants/0000597339

Roser Torra (Reviewer 2): This a nicely written case report highlighting the use of unidentified mutations in the CTNS gene. I have very few comments.

Abstract:
Please mention why do you perform RNA analysis instead of DNA. It is explained in the manuscript but it sounds very strange in the abstract not explaining this, as RNA sequencing is not the usual first approach.

Thank you for your notice. We clarified in the abstract that initially DNA analysis was performed, but no mutation was detected. Then the RNA sequencing was performed.

Abstract, line 33-38, p 3

Careful with the name of the gene: CNTS instead of CTNS

Thank you. We have fixed it. Abstract, line 45, p 3

Manuscript:

Mention if NGS could have detected the mutation without need of RNA analysis. Also consider mentioning that for suspected genetic diseases with no clear clinical diagnosis NGS may be of great help.

Thank you for your notice. We have added a paragraph concerning RNA analysis and NGS in the discussion part of the manuscript: Discussion, line38-55, p 10 and Case presentation, line 23-28, p 8

….Primary Sanger sequencing didn’t reveal any pathogenic variants in the CTNS gene. Conventional Sanger sequence analysis can reliably detect small genetic lesions, including point mutations and small insertions/deletions, but does not detect heterozygous exonic deletions, duplications, or other rearrangements [22]. Due to this fact we decided to perform RNA analysis as a powerful diagnostic approach to detect splice site mutations and allelic dysbalance due to regulatory mutations….

"Taking into consideration that cystine crystals can be determined after 16-18 months of age, we initially performed Sanger sequencing of the CTNS gene using genomic DNA which did not show anomalies because only part of the required genetic data were obtained."

This sentence is very confusing:

What's the relation between having crystals at 16-18 months and using genomic DNA?

What part of genetic data was missing?

Thank you for your question. We excluded this part from our manuscript.