Author’s response to reviews

Title: Identification of compound heterozygous patients with Primary Hyperoxaluria Type 1: Clinical Evaluations and In Silico Investigations

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Version: 1 Date: 15 Mar 2017

Author’s response to reviews:

Dear Editor,

Thank you very much for your decision letter and advice on our manuscript entitled “Identification of compound heterozygous patients with Primary Hyperoxaluria Type 1: Clinical Evaluations and In Silico Investigations”. We also thank the reviewers for the constructive and positive comments and suggestions. We have revised the manuscript according to the comments and suggestions.

We hope that the revision is acceptable for publication in your journal.

Look forward to hearing from you soon.
Response to reviewers comments

Reviewer #1:

Comments to the Author

Kanoun and colleagues examined the impact of compound heterozygous mutations in 4 patients from 2 non-consanguineous PH1 families on clinical phenotype... Overall, the study is well executed but the authors have not clearly stated in the discussion section the relevance of those results and how would they affect future research in this area. What are their recommendations? It is generally known that there are more than 150 known mutations for AGXT, with the three most common mutations c.33_34insC, c.508G>A, and c.731T>C and that genotype phenotype variability are common like any other genetic disorder. Compound heterozygous mutations have also been identified in patient with PH. As such, I would appreciate a statement from the authors in the discussion regarding novelty and expected future applicability of their findings.

Response: Thank you for your thoughtful suggestion. In fact, the c.731T>C mutation was previously described as the first causative mutation of PH1 in Tunisia. In the present study, we highlight the presence of the compound heterozygous cases with variable severity which increases the frequency of this disease in our country. The identification of the mutational spectrum of PH1 provides a rapid diagnosis and an appropriate management for affected patients. Besides, as shown in the manuscript, two variations were detected in heterozygous state and described for the first time as the cause of PH1.

A correction has been made in the revised manuscript (a statement has been added in the end of the manuscript).

Other suggested revisions

Abstract/Background section: “In the present work, we reported clinical study, molecular analysis of AGXT gene and in silico investigations performed in four patients with PH1 among two non consanguineous families” is changed to “In the present work, we aimed to analyze
AGXT gene and in silico investigations performed in four patients with PH1 among two non consanguineous families.

Introduction/background section, line 25, page 25: “The” in the beginning of the paragraph is deleted.

Subjects and Methods section, line 47, page 6: "mean clinical features" is changed to "mean clinical characteristics".

Results section, line 45, page 9: "is" is deleted before the word occurred.

Discussion section, line 32, page 12: "In the present study, we reported clinical and molecular analysis ....." is changed to "In the present study, we report the clinical and molecular analysis....".

Discussion section, line 40, page 12: "In Tunisia, one of countries" is changed to "In Tunisia, one of the countries".

Discussion section, line 47, page 12: "the" in the sentence "in exon 10 were detected in the patient P1" is deleted.

Discussion section, line 52, page 12: "Family F2 presented only two known" is revised to "Family F2 presented with only two known".

Discussion section, line 34, page 13: the sentence "However, the coexistence of this mutation with c.32C>T (p.Pro11Leu) substitution causes …" is divided into two sentences "However, the coexistence of this mutation with c.32C>T (p.Pro11Leu) substitution causes a stable interaction between the AGT protein and molecular chaperones and thus lead to aggregation and rapid degradation of this enzyme. AGT activity is decreased to less than 5% in the presence of these two variants".
Discussion section, line 44, page 14: "we expected that causes alterations in the protein structure" is changed to "and may cause alterations in the protein structure".

Discussion section, line 54, page 15: "They present the disease early before 1 year of age" is changed to "Disease was presented early before 1 year of age" and the sentence "under the age of 1 year and presented a" from the following line is replaced with "with".

Discussion section, line 22, page 16: "The" and "respectively" in the sentence "The patients P1 and P4 presented less severe phenotypes respectively" is deleted and "with" is added before the word "less".

Discussion section, line 46, page 16: the sentence "20 to 50% of patients had advanced chronic kidney disease or even ESRD at the time of diagnosis" is changed to "At the time of diagnosis, 20 to 50% of patients had advanced chronic kidney disease or even ESRD ".

Table 1, the title "Clinical features in studied patients" is changed to "Patients' Baseline Demographics and Key Clinical characteristics".

References were formatted to BMC nephrology style.

Reviewer #2:

Comments to the Author:

An interesting study by Kanoun and colleagues to report the identification of AGXT gene compound heterozygous mutations in two PH1 non-consanguineous families. They also come across the clinical characterization of the four affected patients from the above unrelated two families. That being said the authors need to elaborate more on clinical phenotyping of the reported patients.

Response: Thank you for your thoughtful suggestion. These limitations were considered. In fact, the clinical characterizations of the four affected patients including the age of onset, radiological and clinical presentation, the responsiveness to pyridoxine and outcome of disease, were summarized in table 1. But it is true that more elaboration of the clinical phenotype in the
manuscript is needed. So, in the results section, particularly in the “clinical Severity evaluation of the compound heterozygous studied patients” part, more detailed was added.

“Crystalluria performed in these two patients showed monohydrated calcium oxalate crystal, or whewellite, in the urine with a crystalline volume exceeding 200/mm3. Interestingly, P2 and P3 respond to pyridoxine treatment and thus their oxalate elimination declined after three months of treatment.” was added in this section, line 56, page11.

“Palliative treatment with vitamin B6 didn’t show any response for P1 and P4” was also added in this section, line 26, page12.

Further comments/concerns:

Abstract section, line 52, page 3: "Two patients were compound heterozygous for the c.731T>C, c.32C>T, c.1020A>G and c.33_34insC and presented the disease with recurrent urinary tract infection" is changed to "Two patients were compound heterozygous for the c.731T>C, c.32C>T, c.1020A>G and c.33_34insC and presented clinically with recurrent urinary tract infection".

This suggestion was applied also in the discussion section; line 54, page15 in the revised manuscript. The sentence "They present the disease early before 1 year of age with recurrent urinary tract infection, multiple urolithiasis and nephrocalcinosis under the age of 1 year and presented a persistent hyperoxaluria at the age of diagnosis" is changed to "Disease was presented early before 1 year of age with recurrent urinary tract infection, multiple urolithiasis and nephrocalcinosis with persistent hyperoxaluria at the age of diagnosis."

Results section, line 44, page 11: the dot is deleted in the end of "Clinical Severity evaluation of the compound heterozygous studied patients".

In this section and as it was suggested by the reviewer, we have more elaborated the clinical phenotype of the compound heterozygous studied patients.
Comments to the Author:

More detailed information are needed as to the value of in silico investigation in the current study and its link to clinical phenotypes of the studied patients/families.

Response:

Thank you for your thoughtful comment. Bioinformatic tools were used to assess the impact of the mutated alleles on RNA or protein and therefore on clinical phenotype of the patients.

For example, in silico investigations showed two deficient proteins for P2 and P3 and explain their severe phenotype. In discussion section, line 56, page 15: we said "This severity is correlated with the presence of two mutated alleles in compound heterozygous state leading to production of two deficient proteins".

For patient P4, we explained that the two variants found (previously described as rare polymorphism) occurred in an important region of the protein, were slightly conserved and for c.65A>G, I found that it introduced important changes in the overall RNA secondary structure and we correlated with the phenotype for this patient. In the discussion section, line 39, page 14, we said: So the coexistence of these missense changes Thr9Asn and Asn22Ser in an important region of AGT protein can explain the phenotype the patient P4 and we hypothesize that these changes are of functional significance and we expected that causes alterations in the protein structure.

An English linguistic review was made by a native English spoken person as recommended by the reviewers and I hope that the quality of written English of the revised manuscript is acceptable for publication.