Author’s response to reviews

**Title:** The T1048I mutation in ATP7A gene causes an unusual Menkes disease presentation

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**Version:** 2  **Date:** 20 July 2012

**Author’s response to reviews:** see over
Dear Editor:

Please find in the attached files the revised version of the manuscript entitled "The T1048I mutation in ATP7A gene causes an unusual Menkes disease presentation", by: Gregorio de León-García, Alfredo Santana, Nicolás Villegas-Sepúlveda, Concepción Pérez-González, José M. Henríquez-Esquíroz, Carlota de León-García, Carlos Wong and Isabel Baeza.

We have addressed all the points raised by the reviewers, and we wish to thank them for their comments and suggestions and their critical reading of our manuscript.

**Reviewer's report**

**Title:** The T1048I mutation in ATP7A gene causes an unusual Menkes disease presentation

**Version:** 1 Date: 18 June 2012

**Reviewer:** Lisbeth B Møller

**Reviewer's report:**

The paper by Gregorio Leon-Garcia et al., describe a new mutation the p.T104I mutation in the ATP7A in a patient with Menkes disease. The paper is interesting because it shows that treatment might be useful.

I have the following minor comments

1) The language might not be 100% correct concerning English Grammar. It is easy to understand but for example is is correct to say:
In abstract, case presentation. The treatment of the patient with copper-histidinate began late, until the age of 18 months (until?) ...and with the conserved of some partial ATP7A activity (conserved??).
In conclusion: therefore it is essential a detailed newborn screening as a mechanism for early detection and treatment of Menkes disease...should it be:
therefore a detailed newborn screening is essential for early detection and treatment of Menkes disease.
It would be nice if a person with English as first language could read and correct the paper.

The English grammar was corrected throughout the paper.

2) Nomenclature missense mutations are described by a p. before the mutation, please correct.

The nomenclature of the mutation was corrected: p. 2, paragraph 1, line 4; and p. 4, paragraph 1, line 11.

3) Please include the mutation at the c.level also c.XXXX>T>C

We added the requested information: c.3288 C>T, in p. 5, paragraph 2, lines 4-5; and in the legends of figures 1 and 2.

4) Figure 1 is very compact. In Figure C I don’t think it is necessary to include the mother. And if included the nucleotide should not be a T but C/T (heterozygous.)

Figure 1 was divided into two figures, Figure 1 and Figure 2; in the new Figure 2B we removed the mother’s data.

5) It is interesting if the mutated protein still contains activity, and of course the paper would improve if this was proven by complementation in yeast. It is still only speculative. In the result it is written: Despite the fact of (that?) T1048 does not form H-bonds with the ATP. How is this known??

It has not been formally shown that T1048 does not form hydrogen bonds with ATP. However, we deduce that T1048 does not form hydrogen bonds with the phosphate groups of ATP from a molecular modelling analysis of ATP bound to the N-domain of ATP7B [20], and from an analysis of the crystal structures of a bacterial Cu²⁺-transporting P-type ATPase, which is orthologous to ATP7A [22].

This background, and references 20 (Efremov et al., 2004) and 22 (Tsuda et al., 2009), had already been mentioned in the original manuscript (p. 6, paragraph 2, lines 9-11).

We changed the following sentence: “Despite the fact of T1048 does not form H-bonds with the ATP”, to: “The analyses also rule out the participation of the T1048 residue of ATP7A in these hydrogen bonds with ATP, but T1048 could still form hydrogen bonds with amino acid residues in the N- and A-domains of ATP7A” (p. 6, paragraph 2, lines 13-15).

6) What is the IQ of this patient is he going to a special school? Does he have any language?

Our patient was evaluated by a group of six health professionals: a psychologist, a pedagogue, a special education teacher, a physiotherapist and two speech therapists. The assessment was performed at the Early Care and Child Development Centre of the Disabled People’s Association of Lanzarote (ADISLAN), and included an evaluation of neurocognitive processes, psychosocial skills, motor skills, communication and autonomy (see attached document). Because our patient
has motor impairment and lacks oral language, it was not possible to use standardized tests for his assessment (WPPSI, WISC, BENDER or DENVER), and it was not possible to determine his IQ. Likewise, tests for the standard assessment of emotional and social aspects of the child could not be used. In contrast, muscular and articular balance could be evaluated with the Daniels-Worthingham scale. Comparing this recent assessment with an assessment made 5 years ago by the same Centre, we were able to observe a general improvement in the child’s development, especially in the cognitive and psychosocial areas. Psychomotor development, autonomy and expression showed a slow and gradual improvement, and the patient had a notable increase in muscle tone and a decrease in ataxic movements.

We added the following sentence: …”the patient does not speak and he attends a special school for children with different capabilities” (p. 7, paragraph 2, lines 7-8).

**Referee 2 comments:**

*The authors have identified a novel mutation in the copper transporting P-type ATPase encoded by the ATP7A gene. This missense mutation (T1048I) occurs within a conserved phosphorylation motif that is required for the catalytic activity of ATP7A. The moderate presentation of Menkes disease (MD) in the patient suggested that partial copper-transport activity was preserved, and copper-histidinate treatment led to an arrest of developmental regression and an improvement of neurological and cognitive function.*

*Beyond the initial identification of the mutation by sequencing and RFLP analysis, the authors clearly document the importance of the residues preceding the mutation site and hypothesize that T1048 may not be directly involved in formation of the acylphosphate. This seems plausible in light of the patient’s moderate presentation. Instead, they propose T1048 may form hydrogen bonds with residues in the N- and A-domains of ATP7A. They go on to suggest that the mutation could result in miss-folding of the cytoplasmic domains, which could further disrupt copper-induced trafficking to the plasma membrane. It would be interesting to see this hypothesis tested directly, perhaps by assessing the cellular localization of ATP7A in the patient’s cultured fibroblasts, although such experiments seem beyond the scope of this paper.*

*Figure 1B is somewhat difficult to interpret. It could be more intuitive by reformatting the data (age, copper, ceruloplasmin) into a table and moving the labels from the center to one side. In the Table, please also provide specific values for urine beta 2 microglobulin during treatment, since increased excretion of this metabolite is mentioned in the text.*

The data of Figure 1B was included into a Table in the new Figure 1, and we added the specific value for urine beta 2 microglobulin during treatment (> 1450 µg/24 h). In the legend of Figure 1B, we added the following information: “The urine level of beta 2 microglobulin was determined 6.5 years after the administration of Cu-His (normal range, 30– 370 µg/24 h)” (p. 14, paragraph 1, lines 9-10).
The proposal to apply copper-histidinate treatment immediately after diagnosis of MD seems plausible, but the authors do not address the question of achieving an early diagnosis, except for a mention of newborn screening. It would be interesting for the authors to speculate about the effect of early copper-histidinate treatment on mitigating neurodegeneration in the context of the T1048I mutation. In general, the authors make their case that a better understanding of the relationship between genotype and phenotype of MD could lead to improved prognosis and treatment response in this difficult illness.

We added … “which probably does not completely block copper transport across the blood brain barrier and therefore results in moderate neurological impairment” (p. 6, paragraph 1, lines 3-4).

Some minor changes would improve the paper:

Include additional references to occipital horn syndrome (e.g., a family with mutation in the nucleotide binding domain of ATP7A - see Tang et al. reference below).

We included the references of Tang et al. 2006 and Donsante et al. 2007 (references 5 and 6, respectively), and we added: … “and some neurological alterations [2]; in this form of the disease, the ATP7A protein retains some of its activity [5, 6]” (p. 3, paragraph 2, lines 9-10).


The spectrum of epilepsy in Menkes disease (White et al).

We included the reference of White et al., 1993, reference 27 (p. 7, paragraph 2, line 3).


And the issues to consider in copper treatment of older symptomatic subjects with Menkes disease, such as this patient (Sheela et al.)

We added (p. 7, paragraph 2, lines 9-10 and p. 8, paragraph 1, lines 1-2). … “In most cases, the neurological improvements of patients with MD that start their treatment so long after birth are poor [28]; however, it is advisable to administer the copper treatment anyway, because it prolongs survival, reduces the frequency of seizures and improves the patient’s quality of life [29,30]”.

We included the references of Kaler, 1994, Sheela et al., 1994, and Kirodian et al., 2002 as references 28, 29 and 30, respectively.


2) Please provide the patient’s age at time of diagnosis, the total duration of copper histidine treatment, and source of the copper. These elements are not clear in the current version of the manuscript.

We added: … “The diagnosis was confirmed when the patient was 18 months old, and treatment with copper-histidinate (Cu-His) was started immediately” (p. 2, paragraph 2, lines 3-5); and we added … “Treatment with 100 µg/kg/day of copper-histidinate (Cu-His) (Carreras Pharmaceutical Laboratories, Barcelona, Spain) was initiated when the patient was 18 months old and was maintained for 6.5 years” (p. 5, paragraph 3, lines 1-3).

The copper histidinate is synthesized in the Carreras Pharmaceutical Laboratories in Barcelona, Spain, under the rules of the Spanish Health Department.

We hope to have satisfactorily addressed all the Referee’s comments. Thank you for a prompt review and for your help and efforts in the review process.

Sincerely yours

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