The T1048I mutation in \textit{ATP7A} gene causes an unusual Menkes disease presentation

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Abstract

**Background:** The *ATP7A* gene resides on the chromosome Xq13.2-q13.3 and encodes the ATP7A protein, which is a trans-Golgi network transporter of copper that is expressed in brain and in other organs. Mutations in this gene cause disorders of copper metabolism, such as Menkes disease. The T1048I mutation in *ATP7A* gene is a novel and unusual mutation, which only has been found in one child, and it has not been analyzed previously. This mutation is localized in the conserved DKTGT$^{1048}$ phosphorylation motif involved in the catalytic activity of the ATP7A protein. The description of this mutation and the response of the child to the treatment with copper-histidinate will contribute to a growing body of knowledge about treatment response in Menkes disease.

**Case Presentation:** An 11-month-old male caucasian infant was studied because of hypotonia identified in early life, cerebellar ataxia and a global developmental delay. He showed low levels of serum copper and ceruloplasmin, and was hemizygous for a missense p.T1048I mutation in the catalytic site of the ATP7A protein, therefore we expected a complete loss of functional ATP7A and a classical Menkes disease presentation. However, the clinical course of the patient was as a moderate Menkes disease, probably due to the conservation of a partial activity of ATP7A protein. The treatment of the patient with copper-histidinate began late, until the age of 18 months. Currently, he is 8-years-old and his developmental regression was arrested and replaced by a slight progression.

**Conclusion:** This case emphasizes the importance of knowing the relationship between the genotype and the phenotype of Menkes disease. The prognosis and the response to the treatment of patients with Menkes disease is associated with its early detection, early initiation of treatment, and with the conserved of some partial ATP7A activity.
We propose that, the copper-histidinate treatment should be initiated immediately after the diagnosis of Menkes disease, before the occurrence of irreparable neurodegeneration.

**Key words:** ATP7A, Menkes disease, Copper transporter, Cu-His treatment
**Background**

Menkes disease (MD) (OMIM #309400) is a genetic disorder of copper metabolism that is inherited as an X-linked recessive trait. MD is caused by mutations in the *ATP7A* gene [1]. There are different clinical variants of MD: classical MD (90–95% of patients), mild MD, occipital horn syndrome (OHS) [2], and a new syndrome characterized by an isolated distal motor neuropathy without signs of copper deficiency, and often adult onset [3]. Classic MD is characterized by neonatal neurological degeneration, hypopigmentation, coarse and twisted hair, seizures and death by the age of three years [2]. Mild MD is a moderate form in which cerebellar ataxia and moderate developmental delay predominate [4]. OHS is the mildest form of MD and is characterized by connective tissue abnormalities [2].

The *ATP7A* gene encodes a transmembrane P-type ATPase that delivers copper to copper-dependent enzymes in the trans-Golgi network (TGN). P-ATPases, ATP7A and ATP7B are membrane proteins that hydrolyse ATP for the active transport of cations across cellular membranes, forming an acylphosphate intermediate [5]. The loss of ATP7B function causes the Wilson disease [6]. ATP7A protein, resides in the TGN but also trafficks to the basolateral membrane of polarized cells in response to rising extracellular copper concentrations [7], it contains: an N-terminal tail with six metal-binding sites (MBS1–6), eight transmembrane segments (TMS), an ATP-binding domain (ATPBD), an A-domain, and a C-terminal tail. The MBS accept copper from cytoplasmic carriers and deliver it to the channel formed by the TMS for its transport across the membrane into the TGN. The ATPBD contains the nucleotide-binding and the phosphorylation domains (N- and P-domains, respectively); the P-domain contains the aspartate 1044 residue of the D\(^{1044}\)KTGT motif [5], conserved in all P-type ATPases [8], which is involved in the
catalytic reaction as the acylphosphate intermediate. The A-domain is a phosphatase that hydrolyses the acylphosphate after the transport of copper [5, 9] (Figure 1A).

In this study, we described the clinical course, diagnosis, response treatment, and the T1048I mutation in ATP7A gene of an infant with MD in whom late copper treatment (beginning at the age of 18 months) was provided.

**Case presentation**

An 11-month-old boy was referred because of hypotonia detected in early life, ataxia, global developmental delay and clonic seizures. He was born after an uncomplicated term pregnancy with a birth weight of 2.5 kg. He exhibited psychomotor retardation, hair changes (scarce, thin, coarse), severe head lag and inability to sit independently. Analysis in serum of glucose, urea nitrogen, creatinine, lactate, K⁺, Na⁺, Cl⁻, Ca²⁺, Mg²⁺, phosphorus, liver enzymes, pancreatic amylase, total cholesterol, HDL, LDL, triglycerides, and total protein, gave normal values.

Initial diagnosis of MD was suggested by the clinical features of the patient and was supported by reduced levels of serum copper (38 µg/dL) and ceruloplasmin (12 mg/dL) (normal range, 90–190 µg/dL and 20–60 mg/dL, respectively) and by copper accumulation in his cultured fibroblasts, at 227 ng/mg protein (normal range, 21–46 ng/mg), which was performed by atomic absorption spectroscopy. Both analytes in the mother and grandmother were within the normal range (Figure 1B).

His family history was not suggestive of an X-linked disorder; however, his mother had a previous spontaneous abort. The mutation analysis of the ATP7A gene of the family was performed by PCR and DNA sequencing, showing that the patient and his mother
carried a point mutation, a change of C for T in exon 16, which results in the replacement of the normal threonine 1048 residue with isoleucine [10,11] (Figure 1C). A specific restriction fragment length polymorphism (RFLP) assay using Hinfl corroborated the presence of the p.T1048I mutation in the patient and in his mother, but not in his grandmother (Figure 1D).

Copper-histidinate (Cu-His) treatment (100 μg/kg/day) was initiated when the patient was 18 months old and was maintained until four months ago. Cu-His treatment was suspended due to increased β-2 microglobulin in urine, an evidence of nephrotoxicity by the copper treatment, which it is reversible [12]. This treatment resulted in an increase of his serum copper and ceruloplasmin levels until normal values (85 μg/dL and 21 mg/dL, respectively). Currently, he is 8 years old and his hair characteristics and muscular tone improved and the frequency of convulsions was reduced.

Only one other mutation has been identified in the DKTGT\textsuperscript{1048} motif, p.D1044G, in a patient with classic MD [13]; additionally, the mutation in D1027 of ATP7B, an analogue residue of D1044, completely prevents the formation of the acylphosphate, which is associated with a complete loss of copper-transport activity of ATP7B in Wilson disease [14,15]. ATP7A mutations at K1045, T1046 or G1047 have not been identified in MD patients; however, directed mutagenesis studies using P-type ATPases reported that mutations in analogous residues lowered the affinity of the P-domain for ATP and disrupted the formation of acylphosphate [16,17]. A molecular-modeling analysis of the ATP bound to the N-domain of ATP7B [18], and to the P-domain of Ca\textsuperscript{2+} ATPase, as well as to CopA, a bacterial orthologous Cu\textsuperscript{2+}-transporting P-type ATPase [19,20], suggest that the D1044, K1045 and T1046 residues of the P-domain of ATP7A may form H-bonds with
the phosphate tail of ATP. Despite the fact of T1048 does not form H-bonds with the ATP, this suggest that it might form H-bonds with residues of N- and A-domains of ATP7A.

In our patient, the change of T1048 residue by isoleucine, an amino acid of different size, charge and hydrophobicity, may alter the interactions between isoleucine and the N- and A-domains, producing an inadequate folding between the P- and N-domains, and the P- and A-domains, which may affect the formation of the phosphorylated intermediate. This may prevent copper-induced relocalization of ATP7A from the TGN to the plasma membrane, affecting the copper transport and lead to mild MD. This conclusion is supported by mutations in an analogous residue, T1031 of ATP7B, found in patients with Wilson disease, which affected copper transport [21-23].

We consider that mutations in residues that are involved directly in attachment and hydrolysis of ATP, formation and hydrolysis of acylphosphate and in attachment of copper may cause complete loss of functional ATP7A and results in classical MD; in contrast, point mutations that are not involved in these processes may cause partial loss of function.

It seems that some partial ATP7A activity is an essential prerequisite for a response to Cu-His therapy in MD [12]. The milder phenotype of our patient and his long survival, together with his response to the Cu-His treatment suggest that the p.T1048I mutation only causes partial loss of ATP7A function.

In our patient, the Cu-His treatment did not normalize completely neurological manifestations but some affections improved, although Cu supplementation was initiated 7 months after MD diagnosis, the epilepsy pattern was always multifocal and myoclonic, as previously reported for this age [24]. We also observed a decrease in frequency of seizures
and ataxic movements, an increase in motor activities and muscle tone, which its initial score was of 2 and increased to 3, being 5 the maximum score on the Daniels–Worthingham scale. Additionally, an important improves in the cognitive and psychosocial areas were observed. The copper and ceruloplasmin levels have increased steadily, until normal values.

**Conclusion**

We concluded that the missense mutation p.T1048I localized in the conserved DKTGT$^{1048}$ phosphorylation motif probably causes partial loss of ATP7A function. The partially preserved activity of ATP7A and the Cu-His treatment, although late, led to the patient's long survival. This case emphasizes the important correlation between the genotype and the response to Cu-His therapy in Menkes disease. The prognosis and the response to treatment of patients with MD is associated with its early detection, early initiation of Cu-His treatment, and the conserved of some partial ATP7A activity, therefore it is essential a detailed newborn screening as a mechanism for early detection and treatment of Menkes disease. We considered that, the Cu-His treatment should be initiated immediately after the diagnosis of Menkes disease, before the occurrence of irreparable neurodegeneration.
Abbreviations

PCR: Polymerase chain reaction; H: Hydrogen; MD: Menkes disease; TGN: Trans-Golgi network; Cu-His: Copper-histidinate.

Acknowledgments

The ethics review committee of the Hospital of Lanzarote approved the study. Written informed consent was obtained from parents of the patient for publication of this Case report. A copy of the written consent is available for review by the Series Editor of this journal.

Authors’ contributions

All authors contributed to conception and design, acquisition of data, analysis or interpretation of data and gave final approval of the version to be published. In detail: GL and CW designed the study, oversaw the biochemical analysis, and wrote the first draft of the manuscript. AS and NV carried out the molecular studies, assisted in the interpretation of the results and contributed significantly to the final draft. CP, JH, and CL provided long term specialty care of the patient, evaluated the patient, participated in the acquisition and analysis of the data, and helped to draft the manuscript. IB reviewed the manuscript for important intellectual content and gave final approval of the version to be published. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.
References


Figure legends

Figure 1. A) Trans-membrane organization of the human ATP7A protein. This schematic representation was doing taking in account some structural studies of ATP7A [5]. ATP7A contains five regions: i) The N-terminal tail with six MBS motifs (1–6). ii) Eight transmembrane TMS. iii) The ATP-binding region containing the N-domain (with the ATP binding motif) and the P-domain (with the DKTGT$^{1048}$ phosphorylation motif). iv) The A-domain, and v) The C-terminal tail. B) ATP7A gene mutation (T1048I) and copper and ceruloplasmin levels in the patient and in members of his family. The levels of copper and ceruloplasmin of the patient were measured while he was receiving Cu-His (100 µg/Kg/day). C) The DNA sequence shows part of 16 exon of ATP7A; the mutation is in red color. D) Exon 16 of the ATP7A gene was amplified using specific primers and the PCR product was digested using HinfI enzyme. The mutation introduces a HinfI restriction site that divides the 107 bp fragment into fragments of 79 and 28 bp. udPCR, undigested PCR.