Published in final edited form as:

Pediatr Neurol. 2014 April ; 50(4): 417–420. doi:10.1016/j.pediatrneurol.2014.01.005.

A novel two-nucleotide deletion in the *ATP7A* gene associated with delayed infantile onset of Menkes disease

Takahito Wada^{1,*}, Marie Reine Haddad², Ling Yi², Tomomi Murakami¹, Akiko Sasaki¹, Hiroko Shimbo¹, Hiroko Kodama³, Hitoshi Osaka¹, and Stephen G. Kaler²

¹ Department of Pediatric Neurology, Kanagawa Children's Medical Center, Yokohama, Kanagawa, Japan

² The Unit on Human Copper Metabolism, Molecular Medicine Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, USA

³ Department of Health and Dietetics, Faculty of Health & Medical Sciences, Teikyo Heisei University, Tokyo, Japan.

Abstract

BACKGROUND—Determining the relationship between clinical phenotype and genotype in genetic diseases is important in clinical practice. In general, frameshift mutations are expected to produce premature termination codons, leading to production of mutant transcripts destined for degradation by nonsense-mediated decay. In X-linked recessive diseases, male patients with frameshift mutations typically have a severe or even lethal phenotype.

PATIENT—We report a case of a 17-month-old boy patient with Menkes disease (NIM#309400), an X-linked recessive copper metabolism disorder, caused by mutations in the *ATP7A* copper transporter gene. His clinical presentation involved unexpectedly late onset and milder symptoms.

STUDY and RESULT—His genomic DNA showed a *de novo* two-nucleotide deletion in exon 4 of *ATP7A*, predicting a translational frameshift and premature stop codon, and a classic severe phenotype. Characterization of his *ATP7A* mRNA showed no abnormal splicing.

CONCLUSION—We speculated that translation re-initiation could occur downstream of the premature termination codon, and produce a partially functional *ATP7A* protein. Study of the patient's fibroblasts found no evidence of translation reinitiation, however the possibility remains that this phenomenon occurred in neural tissues and influenced the clinical phenotype.

Keywords

Menkes disease; *ATP7A*; translation reinitiation; premature termination codon

Introduction

Menkes disease (MD; NIM#309400) is an infantile onset X-linked recessive, lethal neurodegenerative disorder of copper metabolism, caused by mutations in the *ATP7A* gene, which encodes a transmembrane copper-transporting P-type ATPase (ATP7A) [1-3].

The *ATP7A* gene consists of 23 exons, spanning a 140-kb genomic region. The second exon includes the ATG translation start codon, and the last exon includes the TAA translation termination codon [4]. The ATP7A copper transporter has six homologous cysteine-rich metal binding domains (MBDs 1-6) at the cytoplasmic amino-terminus, eight hydrophobic transmembrane domains (TM 1-8), and A-, P-, and N-domains. ATP7A has the dual function of transporting copper to cuproenzymes in the secretory pathway (biosynthetic function), and of exporting excess copper from the cell (homeostatic function) [5,6]. It is involved in absorption of copper across the intestinal mucosa as well as transport across the blood-brain and blood-cerebrospinal fluid barriers.

Patients with MD appear healthy until two to three months of age, when loss of developmental milestones, hypotonia, and failure to thrive occur. Death usually occurs by three years of age. Many affected patients develop seizures by 2 to 3 months of age. Subcutaneous copper-histidine treatment before ten days of age can help to normalize developmental outcomes in some children and improves the neurologic outcomes in others [7]. To date, more than 300 different disease-causing variations of ATP7A have been reported.

Here, we report a case of a 17-month-old boy patient with MD associated with a novel two-nucleotide deletion (delTG) in the fourth exon of the *ATP7A* gene, in which the onset was delayed and clinical phenotype unexpectedly mild.

Case Report

The patient had a forceps delivery at 38 weeks of gestational age due to weakness of labor and the birthweight was 2708g. He manifested hypotonia, multiple cranial bone fractures, a cephalohematoma, lightly pigmented hair, respiratory distress, and disseminated intravascular coagulation (DIC), and was referred to the neonatal intensive care unit. He recovered and was discharged home at 30 days of age.

At five months of age, he demonstrated good head control and could roll over. By seven months of age, he sat independently for brief moments (Figure 1). Subsequent neurodevelopment stalled and some regression of gross motor skills was noted. He received outpatient follow-up at a local hospital for his delayed neurodevelopment. Mitochondrial encephalomyopathy was first suspected as his diagnosis.

At 10 months of age, he was referred to our medical center for the evaluation of delayed development. His weight, height, and head circumference were 7.77 kg (−1.4 S.D.), 69 cm (−1.5 S.D.), and 45.5 cm (−0.1 S.D.), respectively. He showed generalized hypotonia, and attenuated deep tendon reflexes. He could no longer hold his head up or sit. His skin was loose and dry, his hair was hypopigmented and coarse, and his joints were hyper-extensible. He could raise his arms and grasp a toy. While able to eat food, his consumption was insufficient, and tube feeding was ultimately required. He was unable to produce any meaningful words, but showed enjoyment and laughed when watching television programs. There was no history of seizures or episodes of hypothermia.

Blood tests showed low serum copper (4.71 mmol/L; normal range; 11.0-23.6 mmol/L), and low serum ceruloplasmin (100 mg/L; normal range; 230-460 mg/L), and his cerebrospinal

fluid analysis showed an elevated lactate/pyruvate ratio of 17.0 (2.38/0.14 mmol/L). Brain magnetic resonance imaging (MRI) showed delayed myelination, and slightly dilated ventricles, with mild brain atrophy. Magnetic resonance angiography (MRA) showed tortuosity of cerebral blood vessels, and pelvic ultrasonography revealed diverticula of the urinary bladder.

Based on these clinical and biochemical findings, he was diagnosed as having Menkes disease and began treatment with subcutaneous copper-histidine (750 µg every other day) prepared at Teikyo University, Tokyo, Japan. The regimen was changed to 1500 µg once a week (from 11 months of age – present) with 100 mg of oral disulfiram. Serum copper was maintained between 15.5–21.0 mmol/L, and ceruloplasmin from 260–400 mg/L. CSF lactate/pyruvate ratio declined to 14.6 (1.17/0.08 mmol/L) at 17 months.

By 17 months old of age, weight, length, and head circumference were 9.125 kg (–1.0 S.D.), 76 cm (–1.0 S.D.), and 47.5 cm (+0.3 S.D.), respectively. Currently the patient's neurological condition is stable without further regression.

Study

Molecular genetic study

Sequencing of genomic DNA extracted from the patient's peripheral blood showed a previously unreported two-nucleotide deletion (delTG) in the fourth exon of the *ATP7A* gene, predicting a translational reading frameshift after amino acid residue 379 and inclusion of three incorrect amino acids before a stop codon (Figure 2).

Analysis of ATP7A protein using fibroblasts (Figure 3)

Western blot analysis of fibroblast protein from the patient did not detect ATP7A protein (Figure 3a). Reverse transcription-polymerase chain reaction (PCR) to assess the potential presence of ATP7A mRNA showed that the patient's fibroblast cell line did not produce transcripts harboring ATP7A exons 21 and 22 (94 bp RT-PCR fragment) (Figure 3b). Confocal microscopy of the patient's cultured fibroblasts after staining with an anti-ATP7A antibody also showed no ATP7A signal (Figure 3c). In combination, these experimental results suggested little or no translation re-initiation in *ATP7A* in the patient's fibroblasts.

Discussion

Here we report a MD patient with a *de novo* two-nucleotide deletion in the fourth exon of the *ATP7A* gene, predicting a premature stop codon. This mutation should result in a truncated protein, consisting of only 382 amino acids (Figure 2). The mutant protein does not seem likely to contribute to normal copper transport since most of its functionally important domains, including MBDs 4 through 6 are lost. However, the patient's clinical phenotype was notable for atypical later infantile onset, a fact which contributed to his delayed diagnosis and late initiation of copper therapy.

We considered two possible molecular explanations for the late onset of symptoms in the context of a presumably severe molecular defect. The first was that the two-nucleotide deletion might be associated with some abnormal mRNA splicing and result in an in-frame transcript and translation of shorter than normal but partially functional protein [8]. However, characterization of *ATP7A* cDNA from the patient's fibroblasts showed no evidence of aberrant splicing or abnormal transcripts. .

The second molecular possibility was translation reinitiation downstream of the premature stop codon, which could result in translation of some ATP7A protein lacking only the

amino-terminal portion of the gene product. Translation reinitiation could explain this patient's delayed onset of symptoms and clinical phenotype, since a truncated protein with CBDs 5 and 6 intact could yield some copper transport activity. A previous report showed that translation reinitiation led to a partially functional ATP7A protein in a MD patient with deletion of exons 3-4 in *ATP7A* [9]. We were unable to document this mechanism in the present case, since the patient's fibroblasts showed no evidence of an ATP7A species of the sizes expected for any of the likely methionine reinitiation residues (≈ 122 kDa to 132 kDa) on Western blot analysis (Figure 3a). In addition, the apparent absence of any stable transcript or protein containing ATP7A exons 21 and 22 suggested that any mutant ATP7A transcripts were rapidly degraded, presumably due to nonsense-mediated mRNA decay.

While this patient presented with a later than typical disease onset, whether this case can be deemed "mild" should be considered. Another MD patient with a mutation that resulted in no ATP7A protein on Western blotting died at 5.5 months despite very early copper treatment [10]. Although our patient's current medical problems appear consistent with classic MD, his progressive development up until 8 months old, when he could control his head and sit with minimal assistance, in the absence of copper treatment, was atypical [7].

Our patient has not had epileptic seizures or abnormal discharges on electric encephalogram, or hypothermia, although he had almost all other signs and symptoms of MD. His phenotype may simply represent a less common and under-appreciated form of MD. Clinical and biochemical variability in MD was previously described among individuals from the same family who shared specific *ATP7A* mutations [11]. Even with very early copper treatment, Menkes disease patients with complete loss-of-function ATP7A mutations do not achieve normal neurodevelopment but some show longer survival and a lower incidence of clinical seizures [12].

A possible mechanism that would explain the present patient's early course is that the frame shift mutation affected neuronal cells differently than in the tissue we studied. Generating neuronal cells from iPS cells established from our patient's fibroblast could help to clarify this possibility. Other environmental factors may also have affected his clinical course, e.g., copper intake, maternal copper levels during pregnancy, or milk copper during lactation.

Finally, this patient was treated with a combination of oral disulfiram and subcutaneous histidine-copper treatment from 11 months of age. Whether disulfiram, a copper-binding molecule, can affect the clinical course in MD by enhancing delivery to the brain is yet to be determined [13].

Acknowledgments

This work was supported by research grants from the Ministry of Health, Labour and Welfare (H22-nanchi-ippan-114; T.W. and H.O.) and Kanagawa Pediatric Medical Fund (T.W.), and the Intramural Research Program of the Eunice Kenney Shriver National Institute of Child Health and Human Development (MRH, EYC, SGK).

References

1. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. *Nat Genet.* 1993; 3:7–13. [PubMed: 8490659]
2. Mercer JF, Livingston J, Hall B, et al. Isolation of a partial candidate gene for Menkes disease by positional cloning. *Nat Genet.* 1993; 3:20–25. [PubMed: 8490647]
3. Chelly J, Tumer Z, Tonnesen T, et al. Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. *Nat Genet.* 1993; 3:14–19. [PubMed: 8490646]

4. Tumer Z, Vural B, Tonnesen T, Chelly J, Monaco AP, Horn N. Characterization of the exon structure of the Menkes disease gene using vectorette PCR. *Genomics*. 1995; 26:437–442. [PubMed: 7607665]
5. Tumer Z. An overview and update of ATP7A mutations leading to Menkes disease and occipital horn syndrome. *Hum Mutat*. 2013; 34:417–429. [PubMed: 23281160]
6. Kaler SG. ATP7A-related copper transport diseases-emerging concepts and future trends. *Nat Rev Neurol*. 2011; 7:15–29. [PubMed: 21221114]
7. Kaler SG, Holmes CS, Goldstein DS, et al. Neonatal diagnosis and treatment of Menkes disease. *N Engl J Med*. 2008; 358:605–614. [PubMed: 18256395]
8. Das S, Levinson B, Whitney S, Vulpe C, Packman S, Gitschier J. Diverse mutations in patients with Menkes disease often lead to exon skipping. *Am J Hum Genet*. 1994; 55:883–889. [PubMed: 7977350]
9. Paulsen M, Lund C, Akram Z, Winther JR, Horn N, Moller LB. Evidence that translation reinitiation leads to a partially functional Menkes protein containing two copper-binding sites. *Am J Hum Genet*. 2006; 79:214–229. [PubMed: 16826513]
10. Haddad MR, Macri CJ, Holmes CS, et al. In utero copper treatment for Menkes disease associated with a severe ATP7A mutation. *Mol Genet Metab*. 2012; 107:222–228. [PubMed: 22695177]
11. Donsante A, Tang J, Godwin SC, et al. Differences in ATP7A gene expression underlie intrafamilial variability in Menkes disease/occipital horn syndrome. *J Med Genet*. 2007; 44:492–497. [PubMed: 17496194]
12. Kaler SG. Menkes disease. *Adv Pediatr*. 1994; 41:263–304. [PubMed: 7992686]
13. Ogawa E, Kodama H. Effects of disulfiram treatment in patients with Menkes disease and occipital horn syndrome. *J Trace Elem Med Biol*. 2012; 26:102–104. [PubMed: 22575542]

Figure 1.
Photographs of the patient at eight months old when he sat independently.

Figure 2.

Diagram of the patient's mutation in exon 4 of *ATP7A*. The normal sequence [1] contains two consecutive TG dinucleotides in this region (nucleotides 1282 to 1285), one of which was deleted in this patient. Whichever TG was deleted results in the same translational frameshift, predicting addition of three novel amino acids and then a stop codon (all in red font).

Figure 3.
Characterization of ATP7A protein and transcripts in the patient's fibroblasts.

- (a) Western blot of fibroblast proteins from a well-characterized normal male fibroblast cell line (CRL2076), the patient, and a Menkes disease patient with deletion of exons 20-23. Red arrow denotes normal ATP7A protein (178 kD). Various smaller sized bands are detected in these samples by the C-terminal antibody that do not however correspond to functional versions of ATP7A, and which we consider to denote non-specific binding. The blot was re-probed with an anti-beta-actin antibody as a control for loading (lower panel).
- (b) Reverse-transcription PCR to assess presence of full-length ATP7A transcripts shows that only the normal control cell line produces a stable transcript containing ATP7A exons 21 and 22 (94 bp RT-PCR fragment). Partial length beta-actin transcripts (57 bp RT-PCR fragment) were amplified in the patient as well as controls.
- (c) Confocal microscopy of cultured fibroblasts after staining with an anti-ATP7A antibody shows the typical condensed perinuclear pattern in the normal cell line, and only faint background staining in the patient. Scale bars=10 microns.