CASE REPORT

A Truncating De Novo Point Mutation in a Young Infant with Severe Menkes Disease

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Menkes disease is a rare neurodegenerative disorder caused by mutations in ATP7A gene. Deficiency in copper-dependent enzymes results in the unique kinky hair appearance, neurodegeneration, developmental delay, seizures, failure to thrive and other connective tissue or organ abnormalities. Other than biochemical tests, DNA-based diagnosis is now playing an important role. More than two hundred mutations in ATP7A gene were identified. Early copper supplementation can help improve neurological symptoms, but not non-neurological problems. Further molecular studies are needed to identify additional mutation types and to understand the mechanism of pathogenesis. This may help in discovering the possible treatment measures to cure the disease. We present a case with the clinical features and biochemical findings, abnormal brain magnetic resonance imaging as well as the effects of treatment with copper-histidine. Direct sequencing of ATP7A gene revealed a de novo point mutation which resulted in an early stop codon with truncated protein.

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1. Introduction

Menkes disease (MD; MIM# 309400) is a disorder of copper metabolism caused by mutations in ATP7A gene. The clinical manifestations are progressive neurologic deterioration, seizures, hair and connective tissue abnormalities. Early diagnosis is difficult. Up-to-date, effective cure has not been reported; however, early copper supplementation can improve neurological outcomes. We demonstrate a case whose gene sequencing revealed a de novo truncating mutation.

2. Case report

A four-month-old male infant was brought to our Pediatric outpatient clinic because of seizure. The pattern of seizure was head shaking with upward gaze, lasting for one minute in each episode, and five to six episodes a day for two days. The infant was delivered via cesarean section at 36 3/7 weeks’ gestation with birth weight of 2,620 gm at a local obstetric clinic. The Apgar score was 9 and 10 at 1 and 5 minutes, respectively. The prenatal examination of the 37-year-old G2P2 mother was normal. No other family members had seizure history or hereditary disease. The results of newborn screening tests were normal.

This was his fourth time of admission. Histories of hyperbilirubinemia, bilateral inguinal hernias, pectus excavatum, left side grade III vesicoureteral reflux, diffuse multiple urinary bladder diverticula, and normocytic anemia were documented in the previous three courses of hospitalization.

On admission, physical examination revealed flat occiput, flat face and depressed nasal bridge with bilateral large floppy ears. The scalp hair was hypopigmented, sparse, curly and brittle (Figure 1). The color of the iris was normal. He had prominent funnel chest, cutis laxa and loose joints. Deep tendon reflexes were increased with hypotonia of four limbs. He failed in reaching the normal loose joints. Deep tendon reflexes were increased with normal. He had prominent funnel chest, cutis laxa and brittle.

Phenobarbital and valproic acid were prescribed and seizure subsided after the 3rd day of hospitalization. Initial laboratory findings above, together with his clinical manifestations, made the diagnosis of Menkes kinky hair disease highly suspicious. Molecular study then confirmed a point mutation in ATP7A gene (c.3502 C>T) leading to an early stop codon with truncated protein (p.Gln1168X). The mother is not a carrier of the mutation. Treatment with daily subcutaneous injections of copper-histidine was started from the age of eight-months. On follow-up at twelve months of age, MRI and magnetic resonance angiography showed brain atrophy (Figure 3A), and tortuosity of bilateral internal carotid arteries and M1 segment of right middle cerebral artery (Figure 3B). He received surgery for left distal femur fracture and laparotomy for intestinal obstruction at the ages of twelve and fourteen months, respectively. Under the treatment, the patient showed improved alertness, better response to external stimulation, and darker and less brittle scalp hair. Social smile was observed at the age of ten months. He is now sixteen months old and still has hypotonia and head lag. Speech delay was also noted. The seizure pattern has changed to infantile spasms from the age of seven months. The anticonvulsants used currently are levetiracetam, topiramate and vigabatrin.

3. Discussion

MD is a rare neurodegenerative disorder caused by mutations in ATP7A gene (MIM# 300011), leading to malfunction of copper-dependent enzymes. The incidence estimated ranges from 1 in 50,000 to 1 in 300,000 live births.1,2 In Asia, one survey in Japan calculated the incidence as 1 in 354,507 live births.3 Most patients are males for the X-linked recessive inheritance, though some female patients have been reported.4

The location of ATP7A gene is on the long arm of the X chromosome between positions q13.2 and q13.3. It encodes a copper-transporting P-type ATPase. The 8.5 kb

Figure 1 The scalp hair is hypopigmented, sparse, curly and brittle.
transcribed sequence of \textit{ATP7A} is organized in 23 exons. To date, more than 270 different mutations have been reported, and some of them are not disease-related.\textsuperscript{5} About one-third of cases arise from de novo mutations.\textsuperscript{6} However, the mutation types are not obviously correlated to the clinical course. This patient had a c.3502 C>T point mutation in exon 17, leading to an early stop codon with truncated protein (p.Gln1168X). The location of this mutation is on the nucleotide-binding domain (N-domain) of \textit{ATP7A} gene. Since N-domain is one of the characteristics of P-type ATPases, this mutation interferes with normal recognition and binding of copper for further transport and translocation.\textsuperscript{5} Previous reports on Menkes mutations on the related locations of exon 17 of \textit{ATP7A} gene all showed classic phenotype of this disease, including missense mutations of c.3299 T>C (died at 4.5 years)\textsuperscript{7} and c.3353 G>A (died at 1.5 years),\textsuperscript{8} as well as frameshift mutation of c.3492delT (died at 4 months).\textsuperscript{9}

There were limited reports on Menkes mutations in the Taiwanese population. Mak et al.\textsuperscript{10} reported two de novo mutations in the Taiwanese Menkes patients: c.2519 C>T, resulting in a nonsense mutation, and c.3681delC, resulting in frameshift mutation. The former patient died at 17 months old from respiratory failure. Our patient had acute intestinal obstruction and received emergent laparotomy at fourteen months old. The intestine was obstructed by a subserosal hematoma, which could have been caused by the abnormal connective tissue and blood vessels in MD. Neuroimages showed the progressive change from encephalomalacia to brain atrophy, which was suggestive of a neurodegenerative process as reported.\textsuperscript{11}

Three stages of epilepsy have been identified.\textsuperscript{6} The first stage presents as focal seizures at 3 months old. Three to 8 months later, the intermediate stage appears, with infantile spasms. The late stage, at the mean age of 20 to 25 months old, is characterized by multifocal seizures. Our patient currently appears to be in the intermediate stage, with presentations of infantile spasm.

The benefits of early diagnosis and treatment with copper-histidine in neonatal period have been reported,\textsuperscript{12,13} including reduced seizure frequency and improved EEG pattern. Some patients had normal neurodevelopment and brain myelination. The recommended dosage of copper-histidine is 200–1000 \text{\mu g}/day, once per day or 2–3 times per week.\textsuperscript{14} Despite better prognosis in some series, copper-histidine is ineffective in some other patients.\textsuperscript{15} Our patient did not receive the treatment in the neonatal period. However, four months after the initial

\begin{figure}
\centering
\includegraphics[width=\textwidth]{brain_mri.png}
\caption{Brain MRI. A, B: T2 FLAIR sequence revealed encephalomalacia in bilateral temporal and left frontal lobes. C: T2WI sequence revealed symmetric faint hyperintense areas in bilateral head of caudate nucleus and putamen. D: T1 FLAIR sequence revealed dysgenesis of the genu and anterior body of corpus callosum.}
\end{figure}
treatment, he was found to be more physically active and to have more facial expression. After five months of treatment, the frequency of seizures decreased. Long-term follow-up is needed to assess the neurodevelopment in motor and language areas.

Conflicts of interest statement

The authors have no conflicts of interest relevant to this article.

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References