A Novel ATP7A Gross Deletion Mutation in a Korean Patient with Menkes Disease

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Abstract. Menkes disease (MD, MIM 309400) is a fatal X-linked recessive disorder that is caused by mutations in the gene encoding ATP7A, a copper-transporting, P-type ATPase. Patients with MD are characterized by progressive hypotonia, seizures, failure to thrive, and death in early childhood. Two Korean patients were diagnosed with Menkes disease by clinical and biochemical findings. We found one missense mutation and one gross deletion in the ATP7A gene in the patients. The missense mutation in Patient 1, c.3943G>A (p.G1315R) in exon 20, was identified in a previous report. Patient 2 had a gross deletion of c.1544-?_2916+?, which was a novel mutation. The patients' mothers were shown to be carriers of the respective mutations. Prenatal DNA diagnosis in the family of Patient 2 was successfully performed, showing a male fetus with the wild-type genotype. The gross deletion is the first mutation to be identified in the ATP7A gene in Korean MD patients. We expect that our findings will be helpful in understanding the wide range of genetic variation in ATP7A in Korean MD patients.

Keywords: Menkes disease, ATP7A copper-transporting ATPase, ATP7A gross deletion mutation

Introduction

Menkes disease (MD, MIM 309400) is a fatal X-linked recessive disorder that is caused by mutations in the gene encoding ATP7A, a copper-transporting P-type ATPase [1,2]. MD patients are characterized by progressive neurological degeneration and death in early childhood [3]. ATP7A pumps copper from the cytoplasm into the secretory pathway for incorporation into secreted enzymes or for export [4]. In Menkes disease, intracellular copper levels increase due to defective export [4]. Affected infants appear healthy at birth and develop normally for 6-8 wk. Subsequently, hypotonia, seizures, and failure to thrive occur, and death by 3 yr of age is typical, although neonatal diagnosis of MD by plasma neurochemical assays and early treatment with copper may improve clinical outcomes [5]. It is known that 4 types of point mutations (deletions/insertions, missense mutations, nonsense mutations, and splice-site mutations) are represented almost equally among ATP7A mutations, and many gross deletions of ATP7A have also been described [4]. The incidences in Western Europe and Japan are estimated to be 1 per 254,000 and 1 per 357,000 live-born babies, respectively [6,7]. There are no reports on the incidence of MD in the Korean population, however. A previous study reported molecular findings in Korean patients with MD, but identified only point mutations, including 3 missense and 2 nonsense mutations, in the ATP7A gene [8]. In the present study, we describe a novel large deletion mutation of the ATP7A gene in a Korean MD patient.
Materials and Methods

Patients. Patient 1 was first evaluated at 3 mo of age because of fever, seizure, and lethargy. Kinky, brittle hair was observed and the infant had intermittent seizures after the fever subsided. Serum copper and ceruloplasmin levels were 1.70 μmol/L (reference range, 1.41-7.22 μmol/L) and 0.04 g/L (reference range, 0.2-0.6 g/L), respectively. The patient died at 14 mo of age. Patient 2, a 5-yr-old boy, was seen because of characteristic features of Menkes disease, including seizures, kinky hair, hypotonia, and developmental delay. Focal motor seizure such as right-sided eye blinking and perioral twitching developed at the age of 4 mo. The patient had kinky hair on his scalp, and the serum levels of copper and ceruloplasmin were 5.18 μmol/L and 0.11 g/L, respectively, at the age of 14 mo. Brain MRI performed at the age of 11 mo revealed remarkable cortical atrophy, and brain MRA performed at the age of 15 mo revealed severe tortuosity of intracranial vessels (Fig. 1). This patient died at 5.5 yr of age. The 2 patients and their family members were screened for ATP7A mutations after obtaining informed consent from the patients’ parents.

PCR and sequencing. Blood samples were collected from the patients and certain family members. Genomic DNA was isolated from peripheral blood leukocytes using a Wizard genomic DNA purification kit following the manufacturer’s instructions (Promega, Madison, WI, USA). The ATP7A gene was amplified by polymerase chain reaction (PCR) using appropriate primers designed by the authors (available upon request) and a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA). Direct sequencing of all coding exons and flanking intronic regions of the ATP7A gene was performed on a ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems).

Results

Two patients were diagnosed with Menkes disease by clinical and biochemical findings before molecular analysis. Two mutations, a missense mutation and a gross deletion, were found in the ATP7A gene (Table 1). Patient 1 had a missense mutation c.3943G>A (p.G1315R) in exon 20, inherited from his carrier mother, which was identified in a previous report [9]. Patient 2 had a gross deletion of c.1544-2_2916+?, which was a novel mutation. His mother was a carrier for the gross deletion, but his sister showed the wild-type genotype. Prenatal DNA diagnosis in the family of Patient 2 was successfully performed on a male fetus with the wild genotype.

Discussion

The biochemical result of low copper concentrations in Menkes disease is reduced activities of numerous copper-dependent enzymes such as ceruloplasmin, dopamine-beta-hydroxylase, peptidylglycine alpha-amidating monooxygenase, cytochrome C oxidase, ascorbate oxidase, lysyl oxidase, superoxide dismutase, and tyrosinase [10,11]. There is little genotype-phenotype correlation in MD, and the clinical courses of MD patients may differ within a family despite identical genetic changes [12].

![MRI studies of the brain and cranial vessels of Patient 2.](image-url)
Biosynthesis of ATP7A results in its localization to the trans-Golgi network (TGN), where homeostatic mechanisms maintain its traffic from the TGN to vesicles and to the plasma membrane [13]. p.G1315Gly, the missense mutation in Patient 1, is located in the P domain, one of several conserved regions. In addition, this region is very close to a GDGIND motif that is important for Mg2+ binding, phosphorylation, and phosphoenzyme hydrolysis [9]. Phosphorylation of the P domain and release of ADP is one of the primary steps of the general ATPase catalytic cycle [14].

Unlike the relatively mild Menkes phenotypes usually observed when mutations are in the stalk regions or transmembrane domains (TMD), classic MD is characterized by mutation of residues in the P domain [9]. Thus, it is likely that the p.G1315R mutation may influence the ATPase activity of ATP7A and therefore the patient’s phenotype and clinical course. As noted earlier, Patient 1 with the p.G1315R mutation died at the age of 14 mo.

Type IX Ehlers-Danlos, otherwise known as occipital horn syndrome (OHS, MIM 304150), represents a less severe variant of Menkes disease [10]. Patients with OHS have an unusual facial appearance, chronic diarrhea, genitourinary abnormalities, and skeletal abnormalities [15]. In patients with OHS, a normally processed transcript could be detected, albeit in severely reduced amounts corresponding to 2% - 5% of the normal level, but normal ATP7A mRNA was completely absent in the patients with MD [16]. The mildness of the neurological effects and connective tissue disease of MD patients might be because of a properly localized, partially active protein in the TGN that is able to deliver some copper to lysyl oxidase [17].

Gross deletion of the ATP7A gene is known to be the disease-causing mutation in 14.9% of MD patients [4]. This study is the first report of a gross deletion of ATP7A in a Korean MD patient. This patient was diagnosed with MD by clinical and laboratory findings at the age of 15 mo. Although the patient was hospitalized several times because of variable uncontrolled symptoms, he lived to the relatively advanced age of 5.5 yr. Although 9 exons (exons 6-14) covering the regions encoding the A domain and TM domain of ATP7A were deleted in the allele described in this study, the lifespan of the patient did not appear to be influenced by the gross mutation. In patients with gross deletions of ATP7A, 89.4% (51/57 subjects) were classified as classical MD patients and most of them (38/45 subjects) died at a young age (< 3 yr) [4]. The age of death for 6 patients was unknown. Only 2 of 57 patients had mild symptoms, and their molecular defects were deletion of exon 1 and exons 3-4, respectively [4]. As expected, mothers of our 2 patients were carriers for the identified mutations. A male fetus in the family of the gross deletion allele patient did not have the ATP7A gross deletion by prenatal screening, was born healthy, and has shown no clinical symptoms by the age of 12 mo.

In summary, we identified 2 mutations in ATP7A, a missense mutation and a gross deletion. A deletion of c.1544-2_2916+6del in the ATP7A gene is a novel mutation and the first gross deletion to be identified in a Korean MD patient. This study contributes towards our understanding of the wide range of genetic variation found in ATP7A.
References


