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Menkes Disease with Discordant Phenotype in Female Monozygotic Twins

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Abstract

Menkes disease (MD) is a rare X-linked recessive disorder caused by mutations in the *ATP7A* gene. This neurodegenerative disorder typically affects males and is characterized by impaired copper distribution and the malfunction of several copper-dependent enzymes. We report clinically discordant female monozygotic twins (MZT) with a heterozygous *ATP7A* mutation. One twin girl is healthy at the current age of 4 years, whereas the other twin girl developed classical MD, showed disease stabilization under copper histidine treatment but died at the age of 3 years. Presumably, the affected girl developed MD due to skewed X inactivation, although this could not be demonstrated in two tissues (blood, buccal mucosa). This case is a rare example of an affected girl with MD and shows the possibility of a discordant phenotype in MZT girls. As speculated in other X-linked diseases, the process of monozygotic twinning may be associated with skewed X inactivation leading to a discordant phenotype.

Keywords

Menkes disease; female monozygotic twins; discordant phenotype; *ATP7A*

INTRODUCTION

Menkes disease (MD) is a rare X-linked neurodegenerative disorder caused by mutations in the *ATP7A* gene leading to impaired copper distribution and malfunction of several copper-dependent enzymes. As first described by Menkes et al. [1962], MD typically affects males and is characterized by sparse and kinky hair (*pili torti*), distinctive facial appearance, failure to thrive, hypotonia, and deterioration of the nervous system with seizures, developmental

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delay and intellectual disability. Children with MD typically begin to develop symptoms during infancy, and death usually occurs by the age of 3 years. Initial diagnosis is based on clinical phenotype and reduced levels of serum copper and ceruloplasmin, definitive diagnosis is based on molecular genetic testing. Early subcutaneous copper histidine supplementation may modify disease progression [Kaler et al., 2008; Tümer and Møller, 2010; Kaler, 2013,2014], and addition of working copies of *ATP7A* by viral gene therapy is a promising new therapeutic approach in development [Donsante et al., 2011]. MD manifestations in females have been reported, mostly associated with X-autosome translocations interrupting the *ATP7A* gene [Kapur et al., 1987; see also review in Møller et al., 2012].

We report female MZTs discordant for MD with a heterozygous *ATP7A* mutation.

CLINICAL REPORT

The MZT girls were born at term by cesarean section to healthy, non-consanguineous parents after an uneventful pregnancy (healthy twin: weight 2,450 g, length 48 cm, OFC 33 cm; affected twin: weight 2,600, length 48 cm, OFC 34cm, all normal). They have a healthy elder brother. One twin girl is healthy at the current age of 4 years showing normal development and growth (weight 17,5kg, height 106 cm, OFC 51cm). The normal neurodevelopmental evaluation included a clinical-neurological examination as well as formal developmental assessment with a norm-referenced test (ET-6–6-R developmental test for children aged 6 months to 6 years by Pearson Assessment, 2013). She has no apparent sight difficulties or abnormalities of eye movement; formal eye assessment has not been performed. About 30% of her hair is kinky, scattered amongst normal hair. She has patchy hypopigmentation of the skin on the trunk, dry skin on the extremities and shows rather rigid facial expressions.

The other twin girl developed classical MD. She required inpatient treatment for neonatal infection, but otherwise pediatric findings were normal neonatally. After the first 4–8 weeks, she developed recurrent infections, feeding difficulties with sucking weakness, long periods of sleep, almost no motor development and onset of severe hypotonia, never achieving free sitting.

The diagnosis MD was suspected at the age of 10 months due to severe developmental delay with regression, hypotonia, ataxia, “poor” feeding, cutis laxa, hypopigmented kinky hair, and pudgy cheeks (Fig. 1). Serum copper and ceruloplasmin concentrations were decreased (serum copper: 28 µg/dl [normal: 90–190 µg/dl]; ceruloplasmin: 152mg/L [normal: 310–910 mg/L]). Light microscopy confirmed pili *torti*. Abdominal sonography showed bladder diverticulae. Repeat cranial magnetic resonance imaging (MRI) and magnetic resonance angiography (MR-A) revealed progressive brain atrophy and vascular tortuosity with a “corkscrew” appearance of cerebral vessels (Fig. 2).

Regression in motor skills, for example, head control and motor traction, was observed at 13 months. According to the mother, the patient’s eye contact and social reactivity improved after initiation of copper histidine treatment at 13 months. A gastrostomy tube was placed at

15 months. In the course of the disease, the patient developed nystagmus with headnodding and spastic and dystonic symptoms in addition to the predominantly hypotonic and ataxic cerebral movement disorder. She never had seizures, though the electroencephalography (EEG) was abnormal, showing sharp wave foci. Adequate caloric intake became difficult due to vomiting when larger amounts were fed by tube. Growth parameters dropped to the lower normal range (at age 10 months: weight, OFC on 25th percentile and length on 50th percentile; at age 3 years: weight, OFC on 3rd-25th percentile and length on 25th percentile). At 3 years, an inguinal hernia was diagnosed. Over all, the clinical disease course was rather stable with no overt psychomotor regression under copper histidine treatment until the patient died of a febrile infection at the age of 3 years. Autopsy was not performed.

GENETIC ANALYSIS

Molecular genetic testing confirmed the clinical diagnosis of MD in the patient: a pathogenic deletion of exon 6 of the *ATP7A* gene was detected by multiplex ligation-dependent probe amplification analysis (MLPA). The deletion was also present in the healthy twin sister, and excluded in the mother. X-autosome translocation was excluded in both girls by chromosome analysis with fluorescence in situ hybridization (FISH) with X-chromosome specific DNA-probes. Monozygosity was demonstrated by short tandem repeat marker analysis. X inactivation studies were carried out using the methylation status of the humane androgen receptor (HUMARA) gene as an indicator for X chromosome inactivation. Both girls showed normal X inactivation in peripheral blood and buccal mucosa. X inactivation studies could not be performed in fibroblasts, as the patient's cells failed to grow.

DISCUSSION

This patient showed the typical findings of classical Menkes disease, for example, developmental delay with regression and hypotonia, brain atrophy and cerebrovascular tortuosity, “jowly” face, hypopigmented *pili torti*, bladder diverticula, and low serum copper and ceruloplasmin. To date, only 18 females with MD phenotypes have been reported excluding the present report [see review in Smpokou et al., 2015], with classical MD being described in only two other female patients [Kapur et al., 1987; Smpokou et al., 2015]. To our knowledge, this is the first report of female MZTs discordant for MD.

As the patient developed no overt regression since commencement of treatment and did not develop seizures, we postulate that her course was stabilized through copper histidine treatment. Treatment has shown the greatest benefits when begun in young, as yet asymptomatic neonates. Treatment outcomes also depend on the individual *ATP7A* mutations: patients with mutations shown to possess partial copper transport capacity showed dramatically better improvement than patients with severe loss of function *ATP7A* mutations [Kaler et al., 2008; Kaler, 2013]. Reduced irritability and lower incidence of seizures are potential benefits of copper replacement, even if treatment is initiated comparatively late as in our patient [Sheela et al., 2005; Kaler, 2011]. We assume that the limited scope of treatment benefit in our patient was related not only to the presence of

neurological symptoms when treatment began, but also due to her mutation, which disrupts the reading frame and is therefore expected to lead to severe loss of *ATP7A* copper transport function [Kaler et al., 2008].

Female carriers of MD usually are asymptomatic. Scattered *pili torti* and skin hypopigmentation as found in our patient's healthy twin sister have been described in about 50% of female MD carriers [Moore and Howell, 1985]. Favorably skewed X inactivation has been shown to account for neurological sparing in female MD carriers [Desai et al., 2011]. We propose that the discordant phenotype in our patient and her twin sister related to unfavorably skewed X inactivation in the affected twin. Normal X inactivation in both girls in blood could be explained by inter-fetal blood exchange leading to skewed X inactivation in blood in the affected twin being masked by the sister's normal X inactivation in blood. Normal X inactivation was also shown for both twin girls in buccal mucosa, an ectodermally derived tissue not exchanged between twins. As patterns of X inactivation can deviate between different tissues [Ørstavik, 2009], we suspect X inactivation studies would have revealed skewing in fibroblasts, at the blood-CSF barrier (choroid plexus epithelia), blood-brain barrier (brain capillary endothelia and neuroglia) or in the brain of the affected girl; these tissues were unavailable for study.

X inactivation and twinning have repeatedly been linked, with unequal lyonization proposed as a trigger for monozygotic twinning [Burn et al., 1986; Machin, 1996, 2009; De Gregorio et al., 2005]. According to this hypothesis, random X inactivation produces two clusters of cells in the initial cell mass with opposite X inactivation patterns, leading to twinning and resulting in MZTs that show inverse skewing of X inactivation [Burn et al., 1986; Machin, 1996]. According to another hypothesis, twinning with asymmetrical splitting of the inner cell mass occurs after X inactivation due to unrelated causes, leading to catch-up growth and skewed X inactivation in the twin with fewer cells and random X inactivation in the twin with the larger fraction of cells [Nance, 1990; Lupski et al., 1991; Machin, 1996]. Due to lack of X inactivation studies in fibroblasts or other tissues affected in MD, our case report cannot respond to the question of whether one or the other hypothesis might apply. However, the discordant phenotype in female MZTs denotes a strong connection between female twinning and X inactivation and a possibly higher risk for X-linked disorders in female MZTs.

In conclusion, we describe a rare case of an affected girl who demonstrated the characteristic findings of classic MD. Furthermore, our case report shows the possibility of a discordant phenotype in MZT girls presumably due to skewed X inactivation, which may be associated with the process of monozygotic twinning.

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FIG. 1. Phenotype. A: Discordant phenotype in the twin girls (affected girl on the viewer's left). B: Affected twin girl at age 10 months. Above: Abnormal hair growth with wearing off on sides and back of head, and "jowly" facial appearance. Below: Hypopigmentation and twisted hair shafts. [Color figure can be viewed in the online issue, which is available at [http://onlinelibrary.wiley.com/journal/10.1002/\(ISSN\)1552-4833](http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833)].

FIG. 2. Magnetic resonance imaging of the brain and cerebral vessels of the patient at 21 months. A: Angiogram showing marked cerebrovascular tortuosity. B: Sagittal view showing global brain atrophy (T-2 weighted image). [Color figure can be viewed in the online issue, which is available at [http://onlinelibrary.wiley.com/journal/10.1002/\(ISSN\)1552-4833](http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833)].