Original Article

J Mucopolysacch Rare Dis 2016;2(1):19-22
http://dx.doi.org/10.19125/jmrd.2016.2.1.19
pISSN 2465-8936 · eISSN 2465-9452

JMRD
Journal of Mucopolysaccharidosis and Rare Diseases

Prenatal Diagnosis of Mucolipidosis Type II: Comparison of Biochemical and Molecular Analyses

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Purpose: Mucolipidosis type II (ML II), also known as I-cell disease is an autosomal recessive inherited disorder of lysosomal enzyme transport caused by a deficiency of the uridine diphosphate (UDP)-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase). Clinical manifestations are skeletal abnormalities, mental retardation, cardiac disease, and respiratory complications. A severely and rapidity progressive clinical course leads to death before 10 years of age.
Methods/Results: In this study we diagnosed three cases of prenatal ML II in two different at-risk families. We compared two procedures - biochemical analysis and molecular analysis - for the prenatal diagnosis of ML II. Both methods require an invasive procedure to obtain specimens for the diagnosis. Biochemical analysis requires obtaining cell cultures from amniotic fluid for more than two weeks, and would result in a late diagnosis at 19 to 22 weeks of gestation. Molecular genetic testing by direct sequence analysis is usually possible when mutations are confirmed in the proband. Molecular analysis has an advantage in that it can be performed during the first-trimester.
Conclusion: Molecular diagnosis is a preferable method when a prompt decision is necessary.

Keywords: Mucolipidosis type II, Prenatal diagnosis, Lysosomal storage disorder, Alpha/beta GlcNAc-1-phosphotransferase gene

Introduction

Mucolipidosis type II (ML II) – also known as I-cell disease and MIM #252500 – is an autosomal recessive inherited disorder of lysosomal enzyme transport caused by a deficiency of the uridine diphosphate (UDP)-N-acetylglucosamine:lysosomal enzyme N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase) (EC 2.7.8.17)[1]. Clinical manifestations are coarse facial features, skeletal abnormalities, mental retardation, corneal clouding, cardiac disease, and respiratory complications. A severely and rapidity progressive clinical course leads to death before 10 years of age. A deficiency of GlcNAc-phosphotransferase prevents the addition of the mannose-6-phosphate marker to lysosomal acid hydrolases. This consequently impairs the correct transport of lysosomal hydrolases to the lysosomal compartment, leading to a massive secretion of acid hydrolases in the plasma and body fluids[2]. In ML II patients, the level of lysosomal hydrolases is strikingly elevated in serum and other body fluids, and the lysosomal activities in cultured cells are decreased[3]. The prenatal diagnosis of ML II is based on the intracellular reduction in the activity of several lysosomal enzymes in cultured amniotic fluid cells and the extracellular elevation of the activity of these enzymes in the amniotic fluid[4,5]. Tiede et al.[6] report that mutations in *GNPTAB* encoding the alpha/beta GlcNAc-1-phosphotransferase causes ML II. Molecular genetic testing is available for the clinical diagnosis of individuals with ML II and the prenatal diagnosis of at-risk fetuses. To date, more than 80 mutations have been reported in the *GNPTAB* gene[7-11]. When disease-causing mutations are present in a family, a prenatal diagnosis of ML II is possible through an analysis of DNA extracted from fetal cells obtained by amniocentesis or chorionic villus sampling (CVS). In this study, we report the prenatal diagnosis of three cases of ML II. We used biochemical analysis and molecular analysis in two different at-risk families. We compare the use of biochemical

Received May 31, 2016; **Revised** June 10, 2016; **Accepted** June 12, 2016
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analysis with that of molecular analysis for the prenatal diagnosis of ML II disease.

Materials and Methods/Results

1. Case 1 and Case 2

In this family, the first child had died at two months of age because of respiratory failure. This infant boy was diagnosed as having ML II, based on clinical manifestations and elevated serum β-glucuronidase, β-galactosidase, β-hexosaminidase and α-mannosidase activities (Table 1).

Prenatal diagnoses of ML II were determined during the mother's second (Case 1) and fifth (Case 2) pregnancies in 2002 and in 2007, respectively. The mother had two miscarriages after the second pregnancy in the first trimester continuously. The cause of miscarriages is unknown.

The amniotic fluid samples and cultivated amniotic cells underwent lysosomal enzyme assay for the prenatal diagnoses of ML II in both fetuses. These results are summarized in Table 1.

In Case 1, the activity of five lysosomal enzymes - including β-glucosidase, β-glucuronidase, β-galactosidase, β-hexosaminidase and α-mannosidase - were measured and their activity was compared with normal control samples. Four lysosomal enzyme levels were reduced by 54% to 73%, compared with the control samples. Only the level of β-hexosaminidase was substantially elevated in cultivated amniotic cells. By contrast, the activity of all five enzymes in the amniotic fluid samples was a few times higher than their activity in normal controls. On the basis of these results, this fetus was not diagnosed as having ML II or as being a possible asymptomatic carrier. The pregnancy continued and a normal child was born after a successful pregnancy. In Case 2, the activity of nine lysosomal enzymes–including α- and β-galactosidases, α- and β-glucosidases, α- and β-mannosidases, β-hexosaminidase, α-fucosidase and β-glucuronidase–was measured in cultivated amniotic cells that had been derived from at-risk fetuses and compared with a normal control. The activity of all nine enzymes was slightly reduced in the cultivated amniotic cells, but retained 36% to 95% of the activity found in the normal control. The levels of β-glucosidase, β-galactosidase, β-hexosaminidase, α-mannosidase, α-fucosidase and α-galactosidase in the amniotic fluids were measured and compared with a normal control. Four of six lysosomal enzymes were slightly increased. Based on these results, the fetus was diagnosed as not having ML II and a normal child was born.

Table 1. The results of biochemical analysis in Case 1 and Case 2

Lysosomal enzyme activity in cultured amniotic cells (nmol/mg protein/h)				
	Case 1.	Case 1. control (n=1)	Case 2.	Case 2. control (n=2)
β-galactosidase	263	329	222.7	344.7
β-glucosidase	34	63	20.6	32.7
β-hexosaminidase	980	319	1,416	1,938
α-mannosidase	45	71	42.4	44.2
β-glucuronidase	11	15	9.4	17.9
α-fucosidase			123.8	236.7
α-galactosidase			27	29.7
α-glucosidase			7.5	20.6
β-mannosidase			36.1	71.2

2. Case 3

At 10 months old, a first-born girl was diagnosed as having ML II, based on the abnormal intracellular-extracellular distribution of the activity of lysosomal enzymes. The activity of plasma α- and β-galactosidases, β-hexosaminidase and α-mannosidase was grossly elevated, whereas the activity of the lysosomal enzymes was marginally diminished in her leucocytes (data not shown). A mutation analysis of the *GNPTAB* gene (performed when she was one year and four months old) showed that she was a compound heterozygote of nonsense mutations (Q104X; c.310 C>T and R1189X; c.3565 C>T). These mutations have been already reported in the medical literature[7,8]. Molecular genetic testing on the child's parents revealed that the Q104X mutation presented in the maternal allele and the R1189X mutation presented in the paternal allele. Her parents were not consanguineous. She died at the age of 4 years 8 months due to a respiratory infection.

For the second pregnancy, the parents requested a prenatal diagnosis. Therefore, CVS was performed at 11 weeks and 1 day of gestation. The disease-causing mutations were not detected in either of the alleles. The fetus was diagnosed as not having ML II, and a healthy baby was born after a successful pregnancy.

Discussion

We diagnosed three cases of prenatal ML II in two families whose first child had ML II. In Case 1, the prenatal diagnosis resulted from a lysosomal enzyme assay of the mother's amniotic fluid samples and cultivated amniotic cells. Genetic testing for

ML II was unavailable at that time since *GNPTAB* gene sequencing has only been available since 2005. Therefore, the prenatal diagnosis depended on a biochemical analysis of the activity of lysosomal enzymes in the amniotic fluid samples and cultivated amniotic cells. Based on the slight elevation in the activity of the enzymes in the mother's amniotic fluid samples, the fetus was a presumed carrier. In Case 2, a prenatal diagnosis again relied on biochemical analysis since we had not identified the disease-causing mutations in the proband of this family.

In Case 3, a mutation analysis of the *GNPTAB* gene had already been performed and the disease-causing mutations in the family had been determined. The DNA analysis showed the fetus had normal alleles. This indicated that this fetus was clearly not affected (Table 2).

In this study, we compared two procedures - biochemical analysis and molecular analysis - for the prenatal diagnosis of ML II. The comparison of these two methods is summarized in Table 3.

Both methods require an invasive procedure to obtain specimens for the diagnosis. In addition, there is a 1% to 2% risk of fetal and prenatal loss. Since the 1970s, a prenatal diagnosis of ML II has relied on measuring the activity of lysosomal enzymes in amniotic fluid samples and cultivated amniotic cells[12]. In our experience, a biochemical analysis requires obtaining cell cultures from amniotic fluid for more than two weeks to provide a sufficient amount of cells for a confidence result. This means that a prenatal diagnosis, involving the use of amniotic fluid samples and cultivated amniotic cells obtained through amniocentesis at 16 to 18 weeks of gestation, would result in a late diagnosis at 19 to 22 weeks of gestation. In addition, the method should be performed in a confined laboratory and require amniotic samples from normal pregnant controls who have been subjected to the same conditions. Nevertheless, this method is still useful.

Biochemical tests have one significant advantage over DNA analysis. Biochemical analysis can directly detect disease-causing abnormalities. Moreover, the measurement of the UDP-*N*-acetylglucosamine: *N*-acetylglucosaminyl-1-phosphotransferase activity has an advantage over measurements based on detecting an abnormal intracellular-extracellular distribution of the activity of lysosomal enzymes.

Molecular genetic testing by direct sequence analysis is usually

Table 2. The results of molecular analysis in Case 3

Molecular analysis based on direct sequencing of GNPTAB gene			
	Age (in years)	Allele 1	Allele 2
Proband	3	Q104 X; c.310 C>T	R1189X ; c.3565 C>T
Father	33	Wild type	R1189X ; c.3565 C>T
Mother	35	Q104 X; c.310 C>T	Wild type
Fetus	12 fetal weeks	Wild type	Wild type

Table 3. Comparison of biochemical and molecular analysis for prenatal diagnosis of ML II

	Biochemical analysis	Molecular analysis
Samples	Samples derived from amniotic cells and amniotic fluid. Amniocentesis is typically performed at 16 to 18 weeks of gestation	Samples derived from chorionic villi. CVS is performed at 10 to 13 weeks of gestation
Cell culture	A cell culture requires a large number of cells for measuring the activity of lysosomal enzymes	A cell culture is not required. A small amount of villi is enough to extract DNA
Turnaround time	Results are ready three to four weeks after amniocentesis (i.e., at 19 to 22 weeks of gestation)	Results are ready one to two week after CVS (i.e., at 13 weeks of gestation without a cell culture and at 15 or more weeks of gestation when a cell culture is required)
Procedure	The activity of several enzymes from the intracellular and extracellular space is compared with at least two to three normal controls	Molecular analyses are based on PCR, PCR-RFLP, and direct sequencing analysis from genomic DNA
Prerequisite condition	A trained center must measure the activity of lysosomal enzymes	Possible to perform at any center, if there are technicians trained in molecular genetic analysis
Technical compliance	Used only in an assigned laboratory with designated and trained technicians	Identification of the disease-causing mutations at the proband
Maternal tissue contamination	Tissue contamination is avoided using cell cultures	Tissue contamination is avoided using GeneScan microsatellite analysis

CVS, chorionic villi sampling; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

possible when mutations are confirmed in the proband. Mutation analysis that can reveal the affected (homozygote), unaffected (normal), and the heterozygote is distinctly preferred for disorders in which enzyme analysis is complicated and miss-prone. For example, the measured activity level of lysosomal enzymes is between that expected for a heterozygote and the patient's range. Molecular analysis has an advantage in that it can be performed during the first-trimester (as early as 11 to 13 weeks of gestation), whereas amniocentesis is usually performed during the mid-trimester. Therefore, molecular diagnosis is a preferable method when a prompt decision is necessary.

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