Review Article

J Mucopolysacch Rare Dis 2016;2(1):13-16
http://dx.doi.org/10.19125/jmrd.2016.2.1.13
pISSN 2465-8936 · eISSN 2465-9452

JMRD
Journal of Mucopolysaccharidosis and Rare Diseases

Molecular Genetics and Diagnostic Approach of Mucolipidosis II/III

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Mucolipidosis (ML) II/III are autosomal recessive diseases caused by deficiency of post-translational modification of lysosomal enzymes. The mannose-6-phosphate (M6P) residue in lysosomal enzymes synthesized by N-acetylglucosamine 1-phosphotransferase (GlcNAc-phosphotransferase) serves as recognition marker for trafficking in lysosomes. GlcNAc-phosphotransferase is encoded by *GNPTAB* and *GNPTG*. Mutations in *GNPTAB* cause severe ML II alpha/beta and the attenuated ML III alpha/beta. Whereas mutations in *GNPTG* cause the ML III gamma, the attenuated type of ML III variant. For the diagnostic approaches, increased urinary oligosaccharides excretion could be a screening test in clinically suspicious patients. To confirm the diagnosis, instead of measuring the activity of GlcNAc phosphotransferase, measuring the enzymatic activities of different lysosomal hydrolases are useful for diagnosis. The activities of several lysosomal hydrolases are decreased in fibroblasts but increased in serum of the patients. In addition, the sequence analysis of causative gene is warranted. Therefore, the confirmatory diagnosis requires a combination of clinical evaluation, biochemical and molecular genetic testing. ML II/III show complex disease manifestations with lysosomal storage as the prime cellular defect that initiates consequential organic dysfunctions. As there are no specific therapy for ML to date, understanding the molecular pathogenesis can contribute to develop new therapeutic approaches ultimately.

Keywords: Mucolipidosis II, Mucolipidosis III, GlcNAc-phosphotransferase, *GNPTAB*, *GNPTG*

Introduction

Mucolipidosis (ML) II/III are autosomal recessive diseases caused by deficiency of post-translational modification of lysosomal enzymes. Targeting of newly synthesized lysosomal enzymes to lysosomes is mediated mainly by mannose-6-phosphate (M6P) receptor, which recognizes the phosphate at the end of the sugar chain on lysosomal enzymes. The recognition marker is synthesized by N-acetylglucosamine 1-phosphotransferase (EC 2.7.8.17), commonly termed GlcNAc-phosphotransferase, that is the responsible defect of ML II/III[1]. GlcNAc-phosphotransferase is α2β2γ2 hexameric peptide complex[2]. In 2000, Raas-Rothschild et al.[3] reported that the γ subunit is encoded by the *GNPTG* gene and contributes to the pathology of ML III gamma. The cloning of cDNAs for α/β subunits showed that it is encoded by a single gene *GNPTAB*[4]. Mutations in *GNPTAB* cause both the severe type of ML (ML II alpha/beta, ML II, I-cell disease [MIM 252500]) and the attenuated type of ML (ML III alpha/beta, ML IIIA, Pseudo-Hurler polydystrophy [MIM 252600])[4]. Mutations in *GNPTG* cause the attenuated type of ML (ML III gamma, ML IIIC, ML III variant [MIM 252605])[5]. In patients, targeting of many lysosomal enzymes to the lysosome is impaired and levels of overflowed lysosomal enzymes are elevated in the serum and body fluids[1]. These findings could be a diagnostic clue of ML.

In this review, the molecular basis of ML and diagnostic approach are covered. Although the ML is rare in occurrence, they have provided important insight into the transport of lysosomal hydrolases[6].

Received June 8, 2016; **Revised** June 12, 2016; **Accepted** June 16, 2016
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Biochemical and Molecular Basis of Mucolipidosis II/III

In higher eukaryotes, most lysosomal hydrolases are targeted to the lysosomes via the M6P pathway which requires the post-translational modification of newly synthesized lysosomal proteins with M6P as a recognition marker[1]. The biosynthesis of M6P is mediated in two sequential enzymatic pathway. GlcNAc-phosphotransferase is responsible for the first step of this pathway[7]. In the second step, the M6P is exposed via removal of the N-acetylglucosamine residue by the enzyme N-acetylglucosamine 1-phosphodiester N-acetylglucosaminidase (NAGPA, EC 3.1.4.45) localized in the trans Golgi network[8]. Subsequently, lysosomal proteins can be recognized by the M6P receptors and transferred to endosomes where they dissociate from their receptors due to the low pH and are further transported to the lysosomes[9]. GlcNAc-phosphotransferase is encoded by two genes, *GNPTAB* and *GNPTG*. *GNPTAB* is located at 12q23.3, contains 21 exons and codes 1256 amino acids and encodes the α/β subunit containing the catalytic activity of the enzyme. *GNPTG* is located at 16p13.3 encodes 305 amino acids and encodes the γ subunit yielding a membrane-bound enzyme complex in *cis* Golgi[3,10]. Mutations in the *GNPTAB* as observed in ML II alpha/beta and ML III alpha/beta result in a reduction of GlcNAc phosphotransferase activity. As a consequence, the lysosomal enzymes lacking M6P residues are unable to bind to M6P receptors, resulting in hypersecretion into the extracellular space and the body fluids instead of lysosomal targeting[6]. Lacking lysosomal hydrolases result in undigested macrocompounds accumulate in the lysosomes. Total or near-total deficiency of GlcNAc phosphotransferase leads to ML II alpha/beta, the clinically more severe form. ML III is genetically heterogeneous[11]. In patients with ML III alpha/beta, GlcNAc phosphotransferase activity is reduced rather than absent, resulting in a milder phenotype compared to ML II alpha/beta[1]. On the other hand, ML III gamma is caused by mutations in the *GNPTG*. This variant is characterized by normal activity of GlcNAc phosphotransferase towards the small pseudosubstrate α-methyl mannoside but reduced activity towards native lysosomal hydrolases as substrates[12]. A study with knock out mice lacking either the *GNPTAB* or *GNPTG* have shown that disruption of *GNPTAB* completely abolishes phosphorylation of high mannose oligosaccharides on acid hydrolases whereas knock-out of *GNPTG* results in only a partial loss of phosphorylation[13]. Therefore it was proposed that the α/β subunits, in addition to their catalytic function, interact with lysosomal hydrolases and that this recognition is enhanced by the γ subunit. Also a possible role of the γ subunit in facilitating protein folding or maintaining enzyme conformation has been reported[14]. Intriguingly, there is no direct evidence of interactions between the γ subunit and lysosomal proteins[14], making an understanding of the pathomechanisms of ML III gamma even more difficult[6].

Diagnostic Approaches

The most characteristic histological features is the enlargement of lysosomes filled with undigested compounds in patient fibroblasts, also called inclusion- or I-cells[15]. The variability in clinical symptoms of the ML result from differences in residual enzymatic activity of GlcNAc phosphotransferase. The clinical spectrum varies from prenatally lethal[16] to progressive ML II alpha/beta and mild adult onset forms of ML III alpha/beta[17]. Residual activities of GlcNAc phosphotransferase in ML III alpha/beta are reported to be 2 to 20% of normal values[18,19].

Clinically suspicious patients can be diagnosed by biochemical and genetic techniques. In patients with ML, urinary excretion of oligosaccharides (OSs) is increased. However, the elevation of urinary OSs is a nonspecific finding of ML. Urinary OSs can serve as a screening test for clinically suspicious patients. Urinary excretion of glycosaminoglycans (GAGs) is normal in patients with ML. Therefore, urinary excretion of GAGs is helpful in distinguishing between ML and mucopolysaccharidosis in neonatal period when the clinical differences between the two disorders may not be obvious. An increased urinary GAG test rules out ML.

In contrast to other storage disorders resulting from deficiency of a single lysosomal enzyme, ML cannot be diagnosed by assay of acid hydrolases in leukocytes. Demonstration of deficiency of GlcNAc phosphotransferase confirms the diagnosis of ML. However, this analysis requires specific substrates, laboratory techniques, and experience. Therefore, instead of measuring the activity of GlcNAc phosphotransferase, measuring the the enzymatic activities of different lysosomal hydrolases are useful for diagnosis. The activities of several lysosomal hydrolases are severely decreased in fibroblasts of affected patients, whereas they are markedly increased in serum or cell culture medium. In ML II, the activity of nearly all lysosomal hydrolases is five- to 20-fold higher in plasma and other body fluids than in normal controls. The following hydrolases are of most interest as their increased activity is relevant in the differential diagnosis of ML:

- β-D-hexosaminidase (EC 3.2.1.52)
- β-D-glucuronidase (EC 3.2.1.31)

- β-D-galactosidase (EC 3.2.1.23)
- α-L-fucosidase (EC 3.2.1.51)
- Arylsulfatase A (EC 3.1.6.1)

Sequencing of the impaired genes (*GNPTAB* and *GNPTG*) could confirm the diagnosis and identify the underlying mutations, which are necessary for genetic counseling including prenatal diagnosis.

Therefore, to confirm the diagnosis in a proband requires a combination of clinical evaluation and laboratory testing. The following order of diagnostic testing is recommended:

1. Identification of characteristic clinical and radiographic findings
2. Assay of oligosaccharides (OS) in urine
3. Assay of several acid hydrolases in plasma
4. Sequence analysis of *GNPTAB* or *GNPTG*

Conclusion

ML II/III show complex disease manifestations with lysosomal storage as the prime cellular defect that initiates consequential organic dysfunctions. The non-lysosomal localization of the affected many lysosomal enzymes resulting from defective post-translational modifications has been demonstrated. As there are no specific therapy for ML to date, understanding the molecular pathogenesis can contribute to develop new therapeutic approaches ultimately.

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