た肝は軽度の繊維化を認めたのみで悪性所見は見られなかった。その後はほぼ問題なく経過し、蛋白制限、NTBC 投与もなしで良好な経過を取っている。

C. 研究結果

予後不良な疾患で生後早期からの治療でも症状改善せず危険な状態であったが、NTBC 投与により劇的に改善し、食事療法を併用することにより１年半以上、悪性腫瘍発生を認めることなく経過観察が可能であった。

D. 考察

NTBC は４－ヒドロキシフェニルピルビン酸酸化酵素の阻害剤であり、同酵素の欠損症は高チロシン血症 III 型として知られている。III 型は、生命予後は良好で肝機能障害、肝癌の発生は認めないため、I 型の症例にNTBCを投与することで病態はIII型と同様になり、患児の予後改善が期待できる。本症例においても NTBC の効果は著しく、投与後に臨床症状、検査結果とも著明に改善し、本薬剤が無ければ救命できなかった症例と思われた。

E. 結論

高チロシン血症I型に対する治療法としてNTBC投与は非常に有効であり、長期間投与でも副作用などは認めず投与可能であった。同薬剤は現在承認されておらず、今後早期の認可が求められる薬剤であると思われた。

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Ⅲ．研究成果の刊行に関する一覧表

別紙4

研究成果の刊行に関する一覧

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Ⅳ．研究成果の刊行物・別刷

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ARTICLE

Newborn Screening for Lysosomal Storage Disorders

KIMITOSHI NAKAMURA,* KIYOKO HATTORI, AND FUMIO ENDO

Lysosomes are intracellular organelles containing acid hydrolases that degrade biological macromolecules. Lysosomal storage disorders (LSDs) are caused by absent activity of one or more of these enzymes due to mutations of genes encoding lysosomal hydrolases or enzymes that process, target, and transport these enzymes. The specific signs and symptoms of each LSD derive from the type of material accumulated within the lysosome, the site (organ) of accumulation and the response of the body (sometimes in the form of an inflammatory or immune response) to the accumulated material. Interest for inclusion of these disorders in newborn screening programs derives from the availability of effective therapy in the form of enzyme replacement or substrate reduction therapy and bone marrow transplant that may improve long-term outcome especially if started prior to irreversible organ damage. Based on the availability of therapy and suitable screening methods, Gaucher disease, Fabry disease, Pompe disease, mucopolysaccharidosis I and II, Niemann–Pick disease, and Krabbe disease are candidates for newborn screening. Pilot newborn screening projects have been performed for some of these conditions that indicate the feasibility of this approach. This review will provide insight into these screening strategies and discuss their advantages and limitations. © 2011 Wiley-Liss, Inc.

KEY WORDS: tandem mass spectrometry; multiplex assays; mucopolysaccharidosis; Fabry disease; Pompe disease,

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INTRODUCTION

A lysosome is an intracellular organelle containing acid hydrolases that degrade proteins, glycoproteins, proteoglycans, lipids, and other complex macromolecules from phagocytosis, endocytosis, and autophagy [Futerman and van Meer, 2004; Fletcher, 2006; Eckhardt, 2010]. These macromolecules are degraded to smaller molecules through the action of various acid hydrolases. The resulting small molecules are then catabolized or recycled by the cell after export to the cytoplasm by passive diffusion or through the use of transporters. For some pathways, these recycled metabolites play a major role in the synthesis pathway. For example, almost 90% of sphingolipids are synthesized in this recycled pathway in many cells [Fredman, 1998; Gillard et al., 1998]. Lysosomal hydrolases are transported from the endoplasmic reticulum to the lysosome by a vesicular transporter. This vectorial transport is dependent on the presence of mannose 6-phosphate residues on their oligosaccharide chains attached to the lysosomal enzyme by a Golgi-localized phosphotransferase complex [Kollmann et al., 2010]. Mannose-6-phosphate receptors capture these processed enzymes into transport vesicles of the *trans*-Golgi network and deliver them to the lysosome. These enzymes can be endocytosed again by neighboring cells and delivered to the lysosome. This latter pathway plays a key role in allowing enzyme replacement therapy (ERT) to reach the lysosome of target cells.

More than 40 LSD are known and have a total estimated incidence of 1:7,000–1:9,000 [Meikle et al., 1999; Fletcher, 2006]. Symptom severity and disease onset of most LSD vary. This heterogeneity can be explained to some extent by the difference in organs affected and, in part, by the type of mutation. In general, mutations leaving very low residual enzyme activity cause the most severe early onset forms of the diseases. In contrast, higher residual enzyme activity delays disease onset [Kolter and Sandhoff, 1999]. Disease severity and onset are remarkably different in the late-onset forms of LSD and can vary even between siblings with identical mutations [Clarke et al., 1989; Wenger et al., 2000; Zhao and Grabowski, 2002]. The major lysosomal storage disorders (LSDs) for which a therapy is

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available and newborn screening is at different stages of development will be briefly described.

SELECTED LYSOSOMAL STORAGE DISORDERS

Fabry Disease

Fabry disease is an X-linked LSD that was initially described in 1898 [Anderson, 1898; Fabry, 1898]. Women can also have symptoms, but onset is generally later than for men and life expectancy is reported better. Fabry disease is caused by α-galactosidase A (Gal A) deficiency [Desnick et al., 2001]. The enzymatic defect leads to progressive accumulation of glycosphingolipids such as globotriaosylceramide (GL-3), especially in the brain, heart, kidney, eye, and skin. The classic disease phenotype consists of angiokeratomas, acroparesthesias, hypohidrosis, and corneal opacities during childhood. Accumulation of GL-3 in the vascular endothelium leads to renal and cardiac failure and cerebrovascular disease. Late-onset cardiac and renal variants with residual Gal A activity have been identified in individuals lacking some or all of the early classic manifestations mentioned above. Patients with the cardiac variant present with left ventricular hypertrophy (LVH), arrhythmia, and/or cardiomyopathy [Nakao et al., 1995], whereas patients with the renal variant develop proteinuria and end-stage renal disease (ESRD) [Kotanko et al., 2004] after 50 years of age. In addition, some patients with acute strokes after adolescence were found to have previously undiagnosed Fabry disease, 30% of whom had, retrospectively, classic manifestations. Fabry disease is diagnosed by measuring enzyme activity in white cells or plasma in males. Females can have normal enzyme activity and DNA testing is necessary to confirm or exclude the diagnosis in them.

ERT for Fabry disease was approved in Eng et al. [2001] and clinical trials are ongoing for pharmacologic enzyme enhancement therapy [Desnick and Schuchman, 2002]. The estimated incidence of classic Fabry disease is 1 in 50,000 males. Screening of males in hemodialysis, cardiac, and stroke clinics by determination of plasma Gal A activities detected previously undiagnosed Fabry disease in 0.25–1% of males undergoing hemodialysis, in 3–4% of males with LVH or hypertrophic cardiomyopathy, and in 5% of males with acute cryptogenic strokes [Brouns et al., 2010].

Newborn screening using a fluorometric enzyme assay in 37,104 males in Italy with follow-up mutation analysis identified 1 in 3,100 patients with Fabry disease. The mutations identified in this cohort predicted later-onset rather than classic Fabry disease with an 11:1 ratio [Spada et al., 2006]. In Japan, a newborn screening pilot program for Fabry disease has been carried out by Nakamura et al. (submitted for publication) using the fluorometric enzyme assay and subsequent mutation analysis. The incidence of the disease was approximately 1 in 4,700 males, with 88% of mutations being associated with a later-onset phenotype. In Taiwan [Sands and Davidson, 2006], a newborn screening pilot program for Fabry disease using the fluorometric enzyme assay found an incidence of approximately 1 in 1,250 males [Hwu et al., 2009; Lin et al., 2009]. All these studies suggest that Fabry disease may be underdiagnosed, especially the late-onset variants.

Mucopolysaccharidoses

Mucopolysaccharidoses (MPS) are LSDs that are characterized by the accumulation of glycosaminoglycans (GAGs) in urine, plasma, and various tissues. Primary treatment options for MPS include hematopoietic stem cell transplantation (HSCT) and ERT. ERT is now available for MPS I, MPS II, and MPS VI [Kollmann et al., 2010]. ERT reduces GAG accumulation, improves the clinical status and quality of life. Clinical trials of ERT for other types of MPS are underway.

Newborn screening for these conditions can be accomplished by measuring urinary GAG or directly by measuring enzyme activity in blood spots. Methods have been proposed for the quantification and qualitative evaluation of GAGs in urine by LC-MS/MS. This method can screen for MPS I, II, and VI by quantifying dermatan sulfate (DS) and heparan sulfate (HS) in urine.

In blood spots, eight lysosomal enzymes (α-L-iduronidase, iduronate sulfatase, arylsulfatase B, β-D-glucuronidase, β-D-galactosidase, α-D-mannosidase, α-L-fucosidase, and β-hexosaminidase), including those involved in selected MPSs, can be assayed. This can screen for MPS I, MPS II, MPS VI, MPS VII, GM1 gangliosidosis, galactosialidosis, MPS IV B, α-mannosidosis, fucosidosis, Sandhoff disease, and mucolipidosis II and III [Chamoles et al., 2001a,b, 2004]. Unfortunately, there are still no methods described for multiplexing these assays.

More recently, specific substrates have been developed to allow the use of MS/MS [Duffey et al., 2010a,b]. The advantage of this approach is that it allows multiplexing with simultaneous assays for MPS I, MPS II, MPS IIIA, and MPS VI.

Pompe Disease

Pompe disease, also known as glycogen storage disease type II, is an autosomal recessive disorder caused by deficiency

of the enzyme α-glucosidase (GAA), resulting in the accumulation of lysosomal glycogen in the skeletal muscles and heart [Kishnani et al., 2006]. This disorder causes a steady accumulation of glycogen substrate that leads to progressive muscle damage and organ failure. The rates of substrate accumulation and tissue damage are variable and reflect the residual enzyme activity and immune response to the accumulated material. In 2006, alglucosidase alfa was approved as the ERT for Pompe disease. A pilot program for Pompe disease newborn screening was started in Taiwan in 2005 that measures GAA activity using a fluorometric assay [Chien et al., 2009]. A thorough examination was performed to screen positive newborns. A diagnosis of Pompe disease was made clinically after the onset of symptoms. Screening revealed five severely affected infants with an incidence of approximately 1 in 41,000 screened newborns. ERT for Pompe disease was started in the five severely affected infants. In unscreened infants, the clinical diagnosis of Pompe disease was made later, at an average of 4 months of age. Initiation of earlier treatment of infants after newborn screening resulted in normal cardiac function and growth and acquisition of age appropriate milestones.

Krabbe Disease

Krabbe disease (globoid cell leukodystrophy) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme galactosylceramide β-galactosidase (GALC). This results in the accumulation of galactosylceramide and psychosine that in most cases cause abnormalities of the brain white matter. Most patients present early in life with an early infantile or "classic" phenotype. Symptoms usually appear before 6 months of age and death occurs before 2 years of age. Other patients can present later in life with an attenuated phenotype. HSCT is the only available treatment for infants with early infantile Krabbe disease and must be performed prior to neurodegeneration. Newborn screening has been performed for Krabbe disease [Duffner et al., 2009]. Newborns treated with HSCT can have progressive central myelination and continued gains in developmental skills and cognitive function, whereas children who undergo transplantation after symptom onset experience minimal neurologic improvement. Transplantation is not effective in all cases of Krabbe disease and some transplanted patients have experienced developmental delays. Screening involves GALC activity detection by a fluorescent assay and subsequent DNA mutation analysis. Molecular analysis of the GALC gene is used for diagnostic confirmation.

THERAPEUTIC ADVANCES FOR LYSOSOMAL STORAGE DISORDERS

Hematopoietic Stem Cell Transplantation

Allogenic HSCT was one of the first therapies attempted in LSDs to introduce metabolic cross-correction. Therapy may also be useful for neurodegenerative LSDs because microglia cells are derived from hematopoietic stem cells [Asheuer et al., 2004; Boelens, 2006]. Clinical trials of HSCT have suggested that cells migrate across the blood–brain barrier. In animal models, it has been shown that donor cells produce the defective enzyme and that donor macrophages replace microglial cells in the brain [Kennedy and Abkowitz, 1997; Malatack et al., 2003]. Repopulation of transplanted cells in the brain is relatively slow because of the long lifespan of microglia [Kennedy and Abkowitz, 1997].

HSCT has shown efficacy in presymptomatic or mildly affected patients with some LSDs. It has been used in patients with MPS I, II, and VI; Gaucher disease; Wolman disease; metachromatic leukodystrophy; and Krabbe disease. Each LSD responds differently to HSCT, and transplantation timing relative to symptom onset seems critical for some disorders. HSCT is not effective for the patients with Fabry disease because secreted α-galactosidase lacks mannose-6-phosphate residues and the enzyme is seldom taken up by cells with the enzyme defect. Complications after HSCT are common and limit the usefulness of this treatment. These include graft versus host disease, toxicity of the conditioning regimen, and graft failure.

In addition to HSCT, transplantation of neural stem cells to the brain has been performed in an animal model for LSDs. This was first demonstrated in an MPS VII mouse model by injection of neural stem cells overexpressing β-glucuronidase into the ventricles of newborn mice [Snyder et al., 1995]. Clinical improvement has been observed after neural stem cell transplantation in animal models [Lee et al., 2007; Strazza et al., 2009]. There are no human data for this type of therapy.

Enzyme Replacement Therapy

Marked progress has been made in the treatment of LSDs over the past few decades [Brady et al., 1974; Achord et al., 1978; Brady, 2006]. Recombinant DNA techniques have allowed production of lysosomal enzymes in vitro. The recombinant enzymes are transported via the mannose-6 receptor pathway in Fabry disease, MPS I, II, and VI; and Pompe disease. In contrast,

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they are transported by macrophage mannose receptors in Gaucher disease. The exogenous enzymes are internalized by somatic cells and transferred to the lysosome where they degrade accumulated substrate and diminish the burden of the disease. ERT has been approved by regulatory agencies for Gaucher, Fabry, and Pompe disease in addition to MPS I, II, and VI. Before the introduction of ERT, no specific therapy was available for LSD patients, and supportive care and treatment were used only to manage complications. ERT consists in the regular administration of recombinant enzyme intravenously and since its success in patients with Gaucher disease, was extended to other LSDs. Clinical trials have demonstrated the clinical benefit of ERT in Fabry disease [Eng et al., 2001]; MPS I [Kakkis et al., 2001], II [Muenzer et al., 2006], and VI [Harmatz et al., 2005]; and in Pompe disease [Amalfitano et al., 2001].

The usefulness of ERT is limited because the enzyme is not always effective for all clinical symptoms. Clinical studies have shown that many symptoms of LSDs are irreversible in advanced cases despite the use of long-term ERT. Therefore, early diagnosis and treatment is important. In addition, recombinant proteins cannot cross the blood–brain barrier, and ERT has little or no effect on central nervous system (CNS) manifestations. Current clinical trials are assessing the effect of intrathecal enzyme replacement in MPS I and II.

Substrate Reduction Therapy

Substrate reduction therapy partially inhibits the biosynthesis of the accumulated product to reduce substrate influx into the catabolically compromised lysosome. A small-molecule oral substrate reduction therapy, miglustat, is available for Gaucher disease. The efficacy of substrate reduction therapy was evaluated in patients with Gaucher disease [Cox et al., 2000]. Adult Gaucher patients not treated with ERT were treated with *N*-butyldeoxynojirimycin for 12 months. Mean liver and spleen volumes were significantly decreased, and hematological parameters showed slight improvement. The most frequent adverse effect was diarrhea. In the extension study, statistically significant improvement was achieved in all major efficacy end points, indicating that treatment with *N*-butyldeoxynojirimycin was increasingly effective with time [Elstein et al., 2004]. The use of *N*-butyldeoxynojirimycin, known as miglustat (Zavesca), has been approved for Gaucher disease and is considered safe for adult patients, with mild or moderate symptoms, who are unwilling or unable to receive or to continue ERT or for patients with persistent signs of disabling disease activity despite maximal enzyme dosing. The drug may be applied in combination with ERT in these patients.

N-Butyldeoxynojirimycin is also considered an option for patients with Sandhoff disease, Tay–Sachs disease, or Niemann–Pick disease type C (NPC) because the drug is a small enough to cross the blood–brain barrier [Lachmann et al., 2004]. The drug is usually given at higher doses than in Gaucher disease to allow increased entry into the brain. A randomized clinical trial in patients with NPC demonstrated that miglustat improves or stabilizes horizontal saccadic eye movement velocity, a clinically relevant markers of NPC, with improvement in swallowing capacity, stable auditory acuity, and a slower deterioration in ambulatory index [Patterson et al., 2007; Wraith et al., 2010]. An open-label extension confirmed the persistence of clinical benefit that is more marked in patients with milder forms of the disease A lower dose of this drug was not effective in late-onset Tay Sachs [Shapiro et al., 2009]. Nevertheless, further developments in this area have the potential of developing effective an treatment for this condition.

Chemical Chaperons

Chemical chaperones can enhance the residual activity of the defective lysosomal enzyme. Imino sugars, such as deoxynojirimycin can act as both enzyme inhibitors and chaperones, which control the quality of newly synthesized proteins [Sawkar et al., 2002; Fan, 2008]. Under physiological conditions, chaperones help restore the native conformation of misfolded proteins. Chaperone therapy by using small molecules to stabilize and target a misfolded enzyme to the lysosome is in clinical trial for Gaucher, Fabry, and Pompe diseases caused by mutated but catalytically active enzymes. In animal models, these small molecules cross the blood–brain barrier and may be effective for CNS manifestations of LSDs. *N*-(*n*-nonyl)deoxynojirimycin for Gaucher disease and 1-deoxygalactonojirimycin for Fabry disease are good examples of chemical chaperones that show satisfactory response in vitro [Sawkar et al., 2002; Yam et al., 2005]. A similar effect was observed in fibroblasts from adult patients with Tay–Sachs disease and Sandhoff disease [Tropak et al., 2004]. Chemical chaperones may be therapeutically useful for treatment of various LSDs, although they are currently experimental and none is approved for the treatment of any LSD.

Gene Therapy

Many LSDs respond to HSCT and are excellent candidates for gene transfer therapy [Sands and Davidson, 2006], since they are generally well-characterized single gene disorders, the enzymes defective are usually not subject to complex regulation mechanisms, and enzyme activity even only a little higher than normal should be clinically sufficient. In vivo and ex vivo gene therapy techniques have been developed to administer the gene to defective organs in LSD animal models via the bloodstream or directly to the brain. Gene therapy using adenoassociated viral (AAV) or lentiviral vectors has been tested in small animal models of LSDs and resulted in normalized enzyme activity [Cachon-Gonzalez et al., 2006; Broekman et al., 2007]. However, gene therapy was initiated before the appearance of clinical symptoms in these studies. Testing in large animal models of LSDs is under current study [Haskins, 2009]. After intracerebral injection of

AAV-encoding human arylsulfatase A (ASA) into nonhuman primates, ASA expression could be detected [Colle et al., 2010]. The wide distribution of enzyme expression appears to be mediated by axonal transport and secretion by transduced neurons. At present, gene therapy in humans with their much larger brains has yet to be initiated.

SCREENING FOR DISEASES

Newborn Screening

Newborn screening for metabolic disorders started with Robert Guthrie's study of phenylketonuria (PKU) in the early 1960s. After demonstration that early diagnosis and therapy could prevent mental retardation in PKU, neonatal screening has become routine practice in developed countries as part of a public health program [Guthrie and Susi, 1963; Scriver and Kaufman, 2001]. Newborn screening identifies a high-risk group of patients from normal infants and then thoroughly investigate this group. Initial tests screened for one disorder at a time. The introduction of screening by tandem mass spectrometry permits the measurement of multiple analytes at the same time, allowing the detection of multiple classes of metabolic disorders.

The potential use of MS/MS for newborn screening was first suggested in 1990 [Millington et al., 1990], and early studies soon demonstrated its practicality [Chace et al., 1993; Rashed et al., 1995; Ziadeh et al., 1995]. MS/MS could simultaneously detect a number of disorders, making it possible to screen for some disorders that might otherwise have seemed too rare. Many compounds are initially separated by mass to charge ratio in MS/MS. Each compound is then fragmented for identification. The process requires roughly 2 min per sample and can detect 30 or more inborn errors of metabolism just screening for amino acids and acylcarnitines. At the present time, expanded screening is used to detect disorders of amino acid, organic acid, and fatty acid metabolism. However, the technology can be applied to a much wider range of compounds, and the field appears ready to expand. Table I summarizes the enzymes defective in several LSDs and those for which newborn screening assays have been developed.

Advances in Newborn Screening Technologies for LSD

Enzymatic assays. The initial system to diagnose LSD was the measurement of enzyme activity using a fluorescent artificial substrate [Meikle et al., 2006]. Diagnosis of MPS I is performed on leukocyte or cultured fibroblast homogenates to assay α-L-iduronidase activity by using 4-methyhylumbeliferyl-L-iduronide. For newborn screening, the standard method was adapted to measure α-L-iduronidase activity in dried blood spotted on filter paper [Chamoles et al., 2001a]. A 3-mm-diameter punchout of a blood spot on filter paper is added to elution buffer containing 4-methyhylumbeliferyl-α-L-iduronide as the substrate. Fluorescence of the enzyme product 4-methylumbelliferone is then measured. Methods for detection of other LSD, including MPS II, Pompe, Fabry, Sandhoff, Gaucher, Niemann–Pick (type A/B, not C), and Tay–Sachs diseases have been reported using the revised enzymatic assay of dried blood spot samples [Chamoles et al., 2001b, 2004]. The limitation of these approaches is that each assay uses 4-methylumbelliferone as an indicator of enzyme activity. In these assays, multiplexing is not possible because all assays (for MPS I, MPS II, Pompe, Fabry, Sandhoff, Gaucher, Niemann–Pick, and Tay–Sachs diseases) yield the same product (4-methylumbelliferone) as the fluorescent product of the enzyme reaction.

A variation of this approach includes the use of antibodies to enrich for the enzyme to be tested. In the case of Pompe disease, antibodies against GAA are used to coat microtiter plates. The endogenous GAA from the dried blood spots is eluted, attaches to the antibodies and is assayed for enzyme activity using fluorescent substrate [Umapathysivam et al., 2000]. Hypothetically, microtiter plates could be coated with several different primary antibodies to capture different endogenous enzymes. However, if all of the substrates produce the same fluorescent enzyme product (4-methylumbelliferone), then multiplexing is not possible. These limitations would work against practical newborn screening using this method.

Functional Detection of Enzymatic Products by Using MS/MS

The second advancement in LSD screening technology involves analyzing the activity of endogenous lysosomal enzymes with electrospray ionization-MS/MS [Gerber et al., 2001; Li et al., 2004]. This method, modified from the one for cell lysates for use with dried blood spots, was used in Krabbe disease to detect galactocerebroside β-galactosidase (GALC) activity. The substrate β-Gal-C8-Cer is broken down by GALC to C8-Cer by the enzyme eluted from the dried blood spots. Both C8-Cer and C10-Cer, which is used as an internal standard, are quantified using MS/MS to detect GALC activity. The GALC enzyme on the dried blood spots is stable, allowing for sample transportation. A pilot program for Krabbe disease screening using MS/MS was started in 2006 [Orsini et al., 2009]. Out of 555,000 newborns, 10 were identified at risk for Krabbe disease. MS/MS has the advantage of being able to detect products of different mass to change ratio enabling the analysis of the results of different enzyme reactions. In theory, multiplexed assays can be developed for multiple diseases, including Pompe, Fabry, Gaucher, Niemann–Pick types A/B (NP A/B), Krabbe disease, and MPS-I [Zhang et al., 2008] and for five of them a multiplex assay has been proposed [Gelb et al., 2006]. In reality, the amount of activity measurable in a single blood spot is still limited. The assay for Pompe, Fabry and MPS-I can already be performed on the same blood spot [Duffey et al., 2010a]. MS/MS assays for blood spots have also been reported for MPS-VI [Duffey et al.,

TABLE I. Lysosomal Storage Disorders Amenable to Newborn Screening

Disease	Protein defect	Availability of screening strategies	Chromosomal localization	OMIM
Defects in glycosaminoglycan degradation (mucopolysaccharidoses)				
MPS I (Hurler, Scheie)	α-Iduronidase	Fluorometric, immune-quantification, multiplex	4p16.3	607015
MPS II (Hunter)	Iduronate sulfatase	Fluorometric, immune-quantification, multiplex	Xq28	309900
MPS IIIA (Sanfflippo A)	Heparan *N*-sulfatase	Immune-quantification, multiplex	17q25.3	252900
MPS IIIB (Sanfflippo B)	*N*-Acetylglucosaminidase	None	17q21	252910
MPS IIIC (Sanfflippo C)	Acetyl-CoA transferase	None	8p11.1	252930
MPS IIID (Sanfflippo D)	*N*-Acetylglucosamine-6-sulfatase	None	12q14	252940
MPS IVA (Morquio A)	*N*-Acetylgalactosamine-6-sulfatase	None	16q24.3	253000
MPS IVB (Morquio B9)	β-Galactosidase	None	3p21.33	230500
MPS VI (Maroteaux–Lamy)	*N*-Acetylgalactosamine-4-sulfatase	Fluorometric, MS/MS, immune-quantification, multiplex	5q11–13	253200
MPS IX	Hyaluronidase	None	3p21.3	601492
Defects in glycoprotein degradation (oligosaccharidoses)				
α-Mannosidosis	α-Mannosidase	None	19q12	248500
β-Mannosidosis	β-Mannosidase	None	4q22	248510
α-Fucosidosis	α-Fucosidase	None	1q34	230000
Sialidosis	α-Sialidase	None	6p21.3	608272
Galactosialidosis	Cathepsin A	None	20q13.1	256540
Aspartylglucosaminuria	Aspartylglucosaminidase	None	4q32	208400
Schindler disease, Kanzaki disease	α-Acetylglucosaminidase	None	22q13.1	104170
Others				
GM1-gangliosidosis	β-Galactosidase	None	3p21.33	230500
GM2-gangliosidosis (Tay–Sachs)	α-Subunit of β-hexosaminidase	Fluorometric	15q23	606869
GM2-gangliosidosis (Sandhoff)	β-Subunit of β-hexosaminidase	Fluorometric	5q13	606873
GM2-gangliosidosis (variant AB)	GM2 activator protein	None	5q31	272750
Gaucher disease	β-Glucocerebrosidase	Fluorometric, MS/MS, immune-quantification, multiplex	1q21	606463
Fabry disease	α-Galactosidase	Fluorometric, MS/MS, immune-quantification, multiplex	Xq22.1	301500
Pompe disease	Acid α-glucosidase	Fluorometric, MS/MS, immune-quantification, multiplex	17q25.2–q25.3	232300
Niemann–Pick type A and B	Sphingomyelinase	Fluorometric, MS/MS, immune-quantification, multiplex	11p15.2	607808
Krabbe disease	Galactosylceramidase	Fluorometric, MS/MS, immune-quantification, multiplex	14q31	245200

2010b] and Gaucher disease [Legini et al., 2011]. One issue with newborn screening is the identification of patients whose phenotype is not clear. For example, most patients identified by screening for Fabry disease have late-onset variants [Spada et al., 2006] and it is unclear whether they would have had clinical symptoms without treatment.

SUMMARY

Newborn screening is a major public health achievement that has improved the morbidity and mortality of inborn

errors of metabolism. The introduction of newborn screening for LSDs presents new challenges. The first is to be able to design a multiplex assay for multiple enzymes applicable to the limited amount of enzyme present in blood spots. These new assays must be validated in large numbers of newborns to confirm sensitivity and specificity. The second challenge is to have a better understanding of which forms of these diseases need treatment. This will allow us to determine if and when to start therapeutic interventions. In the absence of a family history, presymptomatic detection of an LSD can be achieved only through a newborn screening program. The efficacy and cost of the currently available therapies and the detection in newborns of diseases with later onset, often in adulthood, may raise ethical issues. The advancement of therapeutic options for treatment of LSD, especially in the field of small molecules, capable of entering he brain offers new hopes to affected patients in whom a timely diagnosis will become even more essential.

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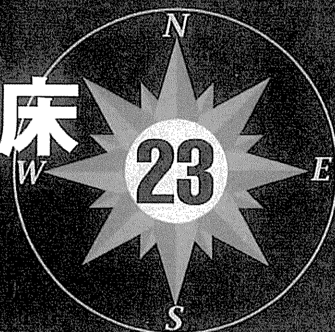

見逃せない
先天代謝異常

総 編 集◉五十嵐　隆 東京大学
専門編集◉高柳　正樹 千葉県こども病院

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機能検査・負荷試験

代謝のプロファイリング

中村公俊

- 先天代謝異常症の診断では，まず代謝異常を疑うこと，それから先天代謝異常症を専門とする医師に相談することが大切であるとされる．しかし，多くの鑑別疾患のなかの一つとして先天代謝異常症を考えたとしても，特異的な検査を行わなければ診断へのアプローチが困難と思われることが少なくない．そのため，専門とする医師への相談を躊躇する場合もある．
- ところが，最近の分析化学の進歩により，簡便な検査によって多くの先天代謝異常症を網羅的に診断することが可能になってきている．代謝プロファイリングにより，先天代謝異常症の診断はより身近なものになっている（❶）．

代謝プロファイリングとは

- 簡便な前処理によって得られた試料を，単一の分析機器を用いて一斉分析する方法である．分析機器によって違いはあるが，数～数百種類の代謝物質を同時に定性・定量分析することが可能になっている．
- たとえば，血中アミノ酸分析では血漿を試料とし，陽イオン交換樹脂を用いた液体クロマトグラフィー法によって約40種類のアミノ酸を一斉分析している．
- タンデム質量分析を用いたタンデムマススクリーニングでは，新生児マススクリーニングにおけるGuthrie濾紙血を試料として，高速液体クロマトグラフィー（HPLC）と四重極タンデム質量分析計を用いたアシルカルニチンやアミノ酸の一斉分析を行っている．
- 血中アミノ酸分析やタンデム質量分析は，アミノ酸代謝異常症，尿素サイクル異常症，脂肪酸代謝異常症や有機酸代謝異常症などの診断に用いられる．このようにして代謝プロファイリングは先天代謝異常症の診断に応用されている．

HPLC：high performance liquid chromatography

❶ 先天代謝異常症の診断に有用な代謝プロファイリングを用いた検査

検査名	測定物質	値の変化	主な対象疾患
タンデム質量分析	アシルカルニチン アミノ酸	増加，比の変化 増加	脂肪酸・有機酸代謝異常症 アミノ酸代謝異常症 尿素サイクル異常症
尿中有機酸分析	尿中代謝産物	増加	脂肪酸・有機酸代謝異常症 アミノ酸代謝異常症 尿素サイクル異常症
血中・尿中アミノ酸分析	アミノ酸	増加，減少	アミノ酸代謝異常症 尿素サイクル異常症
乳酸・ピルビン酸	乳酸/ピルビン酸	増加	ミトコンドリア病など
血中ケトン体分画	3-OHBA/AcAc	増加，減少	ミトコンドリア病など

3-OHBA：3-hydroxybutyric acid

AcAc：acetoacetic acid

- 臨床診断や研究を目的として用いられている代謝プロファイリングには，ガスクロマトグラフィー，液体クロマトグラフィー，それらと組み合わせた質量分析（GC/MS, LC/MS），タンデム質量分析（LC-MS/MS），Fourier変換イオンサイクロトロン共鳴質量分析（FT-ICRMS），核磁気共鳴装置（NMR）などが用いられている．

GC/MS：gas chromatography-mass spectrometry（ガスクロマトグラフィー質量分析）

LC/MS：liquid chromatography-mass spectrometry（液体クロマトグラフィー質量分析）

FT-ICRMS：Fourier transform ion cyclotron resonance mass spectrometry

NMR：nuclear magnetic resonance

血中・尿中アミノ酸分析

- 血中アミノ酸分析では血漿を試料とし，除タンパク液をpH2～3の緩衝液で酸性とし，陽イオン交換樹脂を用いて分離，ニンヒドリン反応を用いて検出している．この方法により約40種類のアミノ酸の一斉分析が可能である．
- 血中アミノ酸分析によって，代謝障害部位の前駆物質であるアミノ酸の上昇や，代謝産物であるアミノ酸の低下を検出することができる．その結果，フェニルケトン尿症におけるフェニルアラニンの高値，メープルシロップ尿症における分枝鎖アミノ酸（BCAA）の高値，シトルリン血症1型におけるシトルリンの著明な上昇とアルギニンの低下など，アミノ酸代謝異常症や尿素サイクル異常症の診断がほぼ確定できる疾患がある．
- 一方，フェニルアラニン，チロシン，メチオニンなどは，先天代謝異常症のほかに非特異的な肝障害などにおいて高値になることがある．そのため，尿中有機酸分析など他の代謝プロファイリングを用いた検査や，酵素活性の測定，遺伝子解析などが診断の確定に必要となる．
- そのほかに，アミノ酸を利用した代謝プロファイリングの応用として，Fischer比やBTR（BCAA/チロシン比）がある．
- Fischer比はBCAAのバリン＋ロイシン＋イソロイシンと，芳香族アミノ酸（AAA）であるチロシン＋フェニルアラニンとの比を検出するものである．重症肝障害時に高アンモニア血症の代償作用としてBCAAが減少し，肝代謝能の低下のためフェニルアラニン，チロシン代謝経路が障害されAAAが上昇することによりFischer比が低下することを利用している．
- 同様にBTRは重症肝障害時にBCAAの減少とチロシンの上昇により比が低下する．これらは代謝プロファイリングを利用した肝障害の指標として知られている．

BCAA：branched chain amino acid

BTR： BCAA and tyrosine ratio

AAA：aromatic amino acid

タンデム質量分析と尿中有機酸分析

- タンデムマス検査の普及によって，これまで診断が困難であった有機酸代謝異常症，脂肪酸代謝異常症，アミノ酸代謝異常症などの早期診断が可能になってきた．
- この方法は新生児のスクリーニング検査に応用されており，米国，ヨーロッパ，アジアの一部の国々などに普及している．わが国でも厚生労働省の班研究を中心としたパイロットスタディにおいて一部の地域でこのスクリーニング検査が行われている．
- タンデム質量分析は，濾紙血抽出液や血清などを試料として，オートイン

ESI：electro spray ionization

ジェクターを備えたHPLCと，エレクトロスプレーイオン化（ESI）インターフェースをもつ四重極タンデム質量分析計を用いてアシルカルニチンやアミノ酸の一斉分析を行う検査法である．衝突解離室を介して質量分析計を直列（タンデム）に接続することで，分析対象物質を特異的に測定することができる．

- アミノ酸分析では，断片化イオンの異性体が多数存在することや解離パターンが類似していることなどから，精度良く分析できるアミノ酸には限りがある．
- 尿中有機酸分析では，ガスクロマトグラフィー質量分析装置（GC/MS）を用いた検査が行われている．安定同位体を内部標準物質として用いることで定量化が可能となっている．

その他の代謝プロファイリング検査

- 糖原病や高乳酸血症の鑑別に，血糖，乳酸，ピルビン酸，血中ケトン体分画などが利用されている．
- 血糖，乳酸値は血液ガス分析で同時に測定が可能な施設もあり，先天代謝異常症の診断を進めるうえで簡便な代謝プロファイリングを用いた検査の一つである．

NADH：reduced nicotinamide adenine dinucleotide（還元型ニコチンアミドアデニンジヌクレオチド）

NAD^+：nicotinamide adenine dinucleotide

- 乳酸/ピルビン酸比（L/P比）は細胞内NADH/NAD^+比を反映しており，ミトコンドリアの電子伝達系の障害ではNADHの蓄積が増加しL/P比が上昇する．
- ケトン体とその分画は，エネルギー源としての糖質と脂肪酸の利用度の指標として用いられる．

TCA：tricarboxylic acid

- 先天代謝異常症においては，3-ヒドロキシ酪酸（3-OHBA）とアセト酢酸（AcAc）の比が高乳酸血症の鑑別の指標として用いられる．3-OHBA/AcAc比が上昇していれば呼吸鎖やTCAサイクルの代謝異常症を疑う．
- 血中，尿中アミノ酸分析，タンデム質量分析，尿中有機酸分析などはいずれも先天代謝異常症の診断に有用な検査である．結果がわかるまで数日かかることがあるが，先天代謝異常症を疑う場合には必ず考慮すべき代謝プロファイリング検査である．

治療を急ごう

知っていなければいけない治療法

肝移植

笠原群生

- 肝移植は1963年3月1日に米国のStarzlにより開始された．当初は手術合併症や拒絶反応などで散々な結果であったが，周術期管理の向上，臓器保存液の開発，免疫抑制薬の進歩，外科手術手技の向上により，その成績は飛躍的に向上した．
- わが国における18歳以下の肝移植成績は，5年生存率83.2%で，代謝性疾患に対する肝移植成績は81.8%である*1．
- 先天代謝異常症に対する肝移植の歴史は古く，Duboisらにより1971年にWilson病に対して肝移植が行われたのが最初である[2]．❶に2006年度までのわが国における小児代謝性疾患に対する肝移植症例を示した[3]．
- 全体で115例に生体肝移植が行われており，Wilson病42.6%，オルニチントランスカルバミラーゼ欠損症（OTCD）15.7%，高チロシン血症10.4%，高シュウ酸尿症6.1%などが適応であった．その他には，プロピオン酸血症（PA），メチルマロン酸血症（MMA），Crigler-Najjar症候群，家族性肝内胆汁うっ滞症（FIC），高リポタンパク血症，プロトポルフィリン症，カルバミルリン酸シンターゼ（CPS）I欠損症，アルギニノコハク酸シンターゼ欠損症，Dubin-Johnson症候群などが適応とされている（❶）．

*1 国立成育医療センターにおける患者生存率は92%（100例，3年）で，24例の代謝性肝疾患に対する肝移植成績は95.8%であった[1]．

OTCD：ornithine transcarbamylase deficiency

PA：propionic acidemia

MMA：methylmalonic acidemia

FIC：familial intrahepatic cholestasis

CPS：carbamyl phosphate synthase

代謝性疾患の肝移植適応

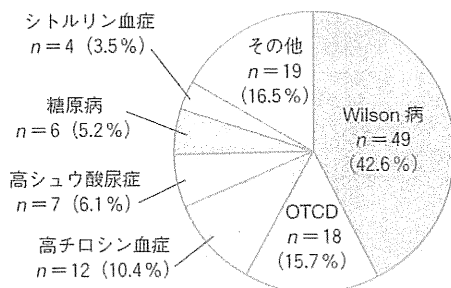
- Wilson病のように非代償性肝硬変に対する肝移植と異なり，肝硬変をきたすことが少ない代謝異常症に肝移植を適応することには，いまだに大きな議論がある*2．
- 移植技術の進歩と同様に，代謝性疾患に対する内科的治療も日覚ましい進歩を遂げている*3．
- 代謝性疾患に対する肝移植の適応は，① 完成された肝硬変，② 内科治療でコントロール不良のmetabolic crisis，③ 発育・発達遅延，④ 著しいQOL

*2 すなわち，肝移植を行う結果得られる患者・家族のQOLが，手術により死亡する危険性を上回るか否かである．

QOL：quality of life

*3 注意深く経過観察すれば，metabolic crisisは回避できるというのが多くの小児科医の意見であろう．

❶ 小児代謝性肝疾患に対する肝移植



（2006年日本肝移植研究会年次報告[3]より改変）

❷ 肝移植適応になりうる小児代謝性疾患

1. 酵素欠損・異常により肝硬変に至る疾患
   α_1アンチトリプシン欠損症，Wilson病，ヘモクロマトーシス，高チロシン血症，FIC（胆汁酸代謝異常），NICCD
2. 肝実質細胞に著明な病変はないが，酵素欠損・異常により肝外に重篤な症状をきたす疾患
   高リポタンパク血症，Crigler-Najjar症候群，血友病，プロテインC欠損症，糖原病，プロトポルフィリン症，シトルリン血症（2型），尿素サイクル異常症（OTCD，CPS I欠損症，アルギニノコハク酸シンターゼ欠損症），ガラクトース血症，シュウ酸症，有機酸血症（MMA，PA）

低下，と考えている．これら肝移植適応条件と肝移植の危険性を比較・検討し，肝移植適応を考慮する*4．

- 代謝性肝疾患は，① 酵素欠損・異常により肝硬変に至る疾患，② 肝実質細胞に著明な病変はないが，酵素欠損・異常により肝外に重篤な症状をきたす疾患，に分類される（❷）．
- とくに後者では高アンモニア血症，高ビリルビン血症，低血糖，アシドーシスなどによる metabolic stroke，骨髄抑制，精神発達遅滞で著しい QOL の低下がみられる場合がある．患者の自然予後を考慮し，肝移植により長期的な QOL・生命予後の改善が見込まれるならば，早期の移植が好ましいと考えている[4]．

*4
肝移植を行うことで食事制限，持続注入などから解放され，好きなものを食べることができ，家族で自由に旅行ができるようになっている現実を見るたびに，筆者らは "QOL の改善" が十分に肝移植の適応になりうると考えている．

小児科医に求められる肝移植の基本知識

レシピエント

- 代謝性疾患は，生直後からさまざまな metabolic crisis で発症することが多く，血液濾過透析，血漿交換を含めた内科治療が適応される．内科治療で second attack は回避できることが多いが，繰り返し metabolic crisis を発症する場合，将来的な発達への影響を考慮して，肝移植*5 を検討すべきである．
- 代謝性疾患に対する肝移植では，通常の肝硬変患者と違い，吻合する動脈径が細いため，術前体重は重いほうがよい*6．
- 代謝性疾患の metabolic crisis 時は，血液凝固能を含めた肝機能が軽度上昇することが多く，濾過透析・血漿交換が適応となることも多いため，感染症に陥りやすい．血液培養が陽性の場合，菌種を同定し速やかに抗生物質を投与すべきである．
- 肝移植後に免疫抑制薬投与を要するため，培養が 48 時間以上陰性化してから肝移植を施行している．免疫抑制薬使用に伴い，肝移植後に予防接種を行うことには注意を要する．可能な限り術前に接種を行いたい．
- レシピエントは術後免疫抑制薬（カルシニューリン阻害薬）とステロイドを用いた拒絶反応の治療を原則的に生涯にわたって必要とする（❸）．退院までの平均入院期間は約 1 か月半である．

*5
肝移植施設に紹介する場合，身長・体重・血液型などの現症以外に，感染症の有無，予防接種歴，発達評価が必要である．

*6
可能であれば 6kg 以上の体重が好ましいが，小児肝移植専門施設であれば 2.5kg 程度の体重があれば，技術的に肝移植が可能である．

❸ 肝移植後の免疫抑制薬使用方法

タクロリムスのトラフレベル	
術後 0～13 日	12～15 ng/mL
術後 14～27 日	10～12 ng/mL
術後 28 日以降	8～10 ng/mL
メチルプレドニゾロン	
術後 1～3 日	1 mg/kg/日
術後 4～6 日	0.5 mg/kg/日
プレドニゾロン（経口）	
術後 7～27 日	0.3 mg/kg/日
術後 28 日以降	0.1 mg/kg/日
術後 3 か月でプレドニゾロン中止	

ドナー

- 詳細なドナー評価は移植施設で行うべきであるが，年齢，身長，体重，血液型，既往歴は問診をされたい*7．
- 小児肝移植ではレシピエント体重に対して 4％以下の移植肝重量が好ましい*8．最終的に CT 画像で容量測定を行い計測するが，総肝重量はドナー体表面積からおよそ計測可能であるため，移植肝臓がレシピエントに対してどの程度の大きさか予測する[5]．
- 遺伝性疾患の場合，同胞内に同様の家族歴がないか十分に注意する必要がある．保因者であっても，疾患によっては生体肝移植ドナーになりえる[6]．
- レシピエントが 2 歳以上の場合，血液型不適合に伴う液性拒絶反応により患者死亡率が増すため，可能であれば血液型は一致ないし適合しているほうが好ましい．

*7
脳死移植が進まない現状では，小児肝移植のドナーは 3 親等以内の親族が現実的である．自発的臓器提供の意思があるか，生来健康であったかを問診する．

*8
レシピエント体重が 5kg であれば移植肝重量は 200g 以下．

国立成育医療センターでも代謝性疾患に積極的に肝移植を行っている．2009年8月現在までMMA10例，PA1例，糖原病Ib型4例，Wilson病2例，OTCD 5例，CPS I欠損症4例，FIC2 1例，オキシステロール7α-ヒドロキシラーゼ欠損症1例，シトリン欠損による新生児肝内胆汁うっ滞症（NICCD）1例，ミトコンドリア肝障害1例の30例に生体肝移植を実施してきた．

NICCD：neonatal intrahepatic cholestasis caused by citrin deficiency

＊9
MMAでは頻回の嘔吐や代謝性アシドーシス発作により，自然予後は5年生存率20％程度と報告されている[7]．

＊10
報告されている心筋症は，重度アシドーシス発作により惹起されるものであり，肝移植後は認められない[9]．

GSD：glycogen storage disease

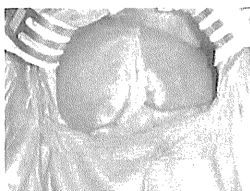
＊11
機序は完全に明らかになっていないが，好中球減少も改善する症例も認められる[10]．

- 通常，ドナーの術後入院期間は10日程度である．

代表的な代謝性肝疾患に対する肝移植

有機酸代謝異常症（MMA，PA）

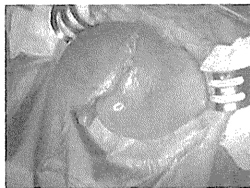
- MMAは有機酸代謝異常症で，メチルマロニルCoAムターゼ完全欠損型が肝移植適応になりうる＊9．
- 肝移植により嘔吐回数が減り経口摂取が可能となる[8]．
- 原疾患に対する内科治療，タンパク制限は肝移植後も継続するが，アシドーシス発作がなくなり，患者のQOLは明らかに向上する．併存する精神発達障害，腎機能障害については，長期経過をみなければならない．慎重な移植適応を要する．
- 同様にPAも肝移植適応になり，重篤な代謝性アシドーシス発作はなくなり，経口摂取可能となる＊10．
- MMA，PAの肝臓は一見正常に見えるが，タンパク制限治療によると思われる軽度脂肪肝および線維化を認めることがある（❹）．



❹ PAの肝臓

糖原病Ib型（GSD Ib）

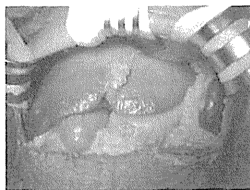
- GSD Ibはグルコース-6-リン酸トランスロカーゼ欠損により低血糖を起こす，常染色体劣性遺伝疾患である．24時間持続経管栄養法などの内科治療で，血糖コントロールは良好となっているが，併存する好中球減少による感染症を繰り返すことが多い．
- 自経例4例を含めて世界で10例の肝移植報告がある．移植適応は頻回の低血糖発作および著しいQOLの低下である．肝移植により血糖は安定し，持続栄養は必要なくなる＊11．
- 肝臓は腫大しており，時に骨盤腔にまで達する（❺）．血液凝固能異常も併存するため，十分な止血を確認しながら手術を進めなければならない．



❺ GSD Ibの肝臓

Wilson病

- 肝細胞から胆汁へ銅排泄障害，銅結合タンパクであるセルロプラスミン合成障害により，銅沈着を起こす遺伝性銅代謝異常症である．
- キレート薬による内科的治療に抵抗性の症例，完成された肝硬変症例，溶血発作で発症するいわゆるWilsonian fulminant hepatic failure，進行性の神経型病変が肝臓移植の適応となりうる[11]．
- 肝臓は暗緑色で門脈圧亢進により脾腫を伴う（❻）．尾状葉が下大静脈を取り巻いていることが多く，下大静脈の処理が困難であることが多い．Wilson病の肝臓は，程度差はあれ線維化を認める．



❻ Wilson病の肝臓

尿素サイクル異常症（OTCD，CPS I欠損症）

- 尿素サイクル異常により，繰り返す高アンモニア血症を原因とする精神発達遅滞を認める．タンパク制限により著明な脂肪肝を呈する（❼）．手術後はタンパク制限，原疾患に対する投薬（安息香酸ナトリウム，フェニル酢酸，カルニチン，アルギニン）は必要ない．
- OTCD男児およびCPS I欠損症では，内科的コントロールで高アンモニア血症がコントロール不良な症例を認める．