E. 結論

遺伝性高チロシン血症１型の酵素反応基質の自作を目標に、ホモゲンチジン酸ジオキシゲナーゼの発現系を作成した。

F. 健康危険情報

特になし

G. 研究発表

1. 論文発表

（発表誌名巻号・頁・発行年等も記入）

なし

2. 学会発表

なし

H. 知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

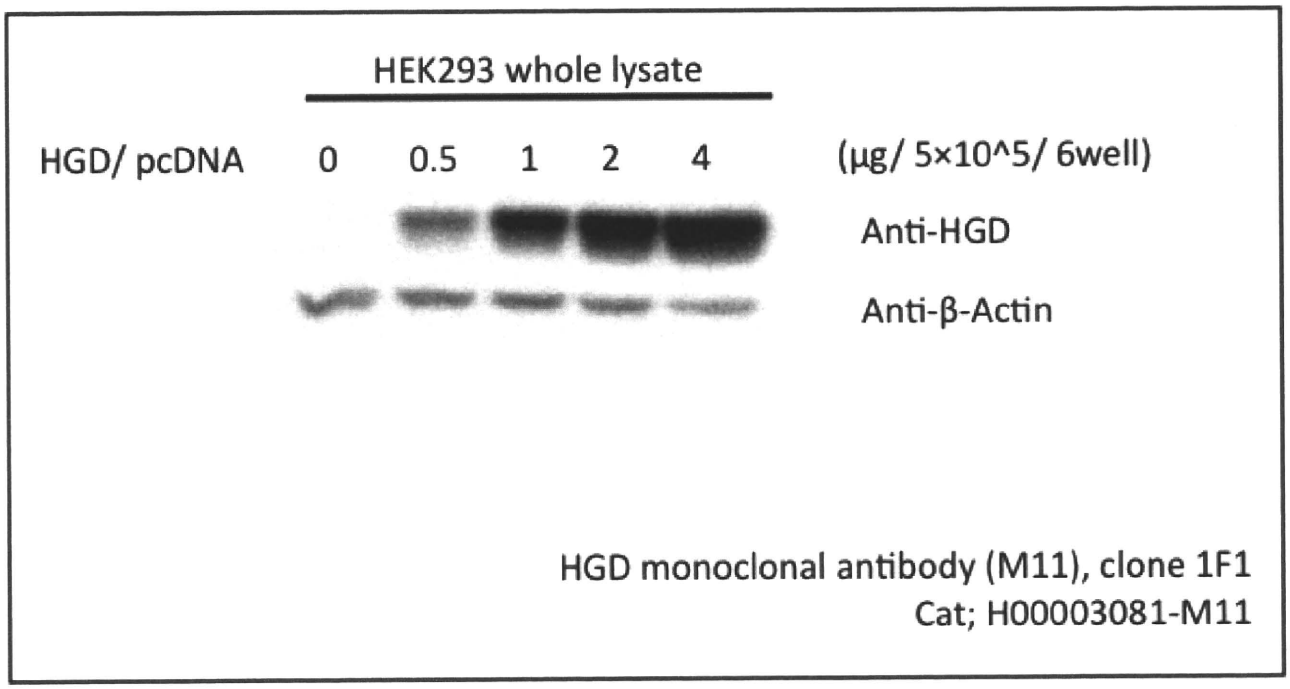

図. ホモゲンチジン酸ジオキシゲナーゼ（HGD）を発現させたHEK293細胞抽出物のwestern blot解析結果

厚生労働科学研究費補助金（難治性疾患克服研究事業）
研究分担報告書

高チロシン血症Ⅰ型に対する生体部分肝移植の効果

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研究要旨

高チロシン血症Ⅰ型は肝硬変、肝不全へと進行し、肝癌を発生する症例も多い。我々は生後７ヶ月で既に肝不全、肝癌を発症していた症例に対して、母をドナーとした生体部分肝移植を行った。移植後の経過は良好で、進行した本症の治療には生体部分肝移植は有用であると思われた。

共同研究者

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A. 研究目的

高チロシン血症Ⅰ型は新生児期から乳児期早期にかけて発症する肝障害をきたす疾患として知られているが、肝細胞障害は肝硬変、肝不全へと進行し、肝癌を発生する症例も多い。我々は多発性肝腫瘍で発見され、精査の結果高チロシン血症Ⅰ型と診断され、生体部分肝移植を行った症例を経験したので報告する。

B. 研究方法

症例主訴：肝腫瘍

家族歴：血族結婚なし。父、心臓弁膜症のため弁置換術施行。

現病歴：前医にて、在胎 37 週 3 日、出生体重 3608g、正常分娩で出生。初期嘔吐、低血糖のため日齢 10 まで小児科入院となったがその後回復して退院。1ヶ月時、発熱と肝脾腫あり、軽度肝機能異常（AST:75～100 U/l, ALT:35～44 U/l 程度）を認めたため腹部 CT 検査を行われたが異常なく、ウイルス感染症を疑われ経過観察されていた。5 ヶ月時、発熱、咳嗽あり、腹部膨満著明となったため再入院。腹部画像診断で肝内多発性腫瘍を認めたため当科紹介受診となった。

入院時現症：身長：62.5 cm、体重：9.08 kg、
体温：37.0 ℃　心拍数：148／分
呼吸数：52／分　意識：清明
胸部：喘鳴聴取、心音：清
腹部：腹満著明（最大腹囲：55.5 ｃｍ）
肝：肋骨下縁６ｃｍ触知

腫瘍性病変、α-フェトプロテインの異常高値が認められたことから肝芽腫が疑われ、血液腫瘍専門医へ紹介となった。化学療法を検討され、確定診断前に短期間抗癌剤投与も行われた。病型確定のための肝生検も予定されたが肝不全進行のための凝固能悪化がみられ肝生検は断念された。多発性腫瘍であったこと、両側腎腫大も認めたことから代謝異常症の精査が行われ、チロシン血症Ⅰ型と診断された。

C. 研究結果

予後不良な疾患で肝腫瘍があり、肝障害が進行することから生体部分肝移植が考慮された。各種検索の結果、母をドナーにした生体部分肝移植が生後 7 ヶ月時に施行された。摘出肝には多発性に腫瘍を認め、病理組織検査では肝細胞癌の所見を示す部位も認めた。摘出肝を用いたフマリルアセト酢酸ヒドラーゼ活性は、正常の 4%と低下していた。移植後経過は順調で蛋白制限の必要はなく、良好な臨床経過をとっている。

D. 考察

本症例の肝組織は生後 7 ヶ月にして既に肝癌の所見を呈していた。肝不全状態も進行しており肝移植が施行出来な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

与の有用性が知られているが、肝不全、肝癌発生など
のリスクが危惧される例では生体部分肝移植を考慮
すべきであると思われた。

F. 健康危険情報

特記すること無し。

G. 研究発表

1. 論文発表

なし

2. 学会発表

なし

H. 知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧

雑誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Nakamura K, Hattori K, Endo F.	Newborn Screening for lysosomal disorders.	Am J Med Genet.	157	63-71	2011
中村公俊	アミノ酸代謝異常症、尿素サイクル異常症、糖原病の新しい治療法	小児内科	42	1191-1194	2010
中村公俊、遠藤文夫	先天性アミノ酸代謝異常症　高チロシン血症	日本臨床　別冊 肝・胆道系症候群　I 肝臓編（上）		486-489	2010
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Ⅳ. 研究成果の刊行物・別刷

American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 157:63–71 (2011)

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.

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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＜進化する治療法＞

アミノ酸代謝異常症，尿素サイクル異常症，糖原病の新しい治療法

中村公俊*

はじめに

アミノ酸代謝異常症，尿素サイクル異常症，糖原病はそれぞれ多くの先天代謝異常症を含んだ疾患群である。そのなかには治療法の進歩によって予後が改善した疾患も少なくない。たとえば，フェニルケトン尿症におけるテトラヒドロビオプテリン（BH_4），遺伝性高チロシン血症Ⅰ型におけるニチシノン，リジン尿性蛋白不耐症や尿素サイクル異常症におけるシトルリン，尿素サイクル異常症におけるアルギニン，安息香酸ナトリウム，フェニル酢酸ナトリウムなどによる治療法があげられる。また，肝不全をきたすアミノ酸代謝異常症，尿素サイクル異常症，糖原病の一部は，肝臓移植の適応となる。わが国では，高チロシン血症Ⅰ型，オルニチントランスカルバミラーゼ（OTC）欠損症，カルバモイルリン酸合成酵素Ⅰ（CPSⅠ）欠損症，古典型高シトルリン血症やシトリン異常症，アルギニノコハク酸尿症，アルギニン血症，糖原病Ⅰ型，Ⅳ型などで生体肝移植が行われている。

Ⅰ 高フェニルアラニン血症のBH_4による治療

BH_4は生体内で補酵素として働いている。高フェニルアラニン血症を示す症例のなかに，BH_4投与の効果がみられる2つの病態がある。ひとつはこの補酵素の欠損であるBH_4欠損症であり，もうひとつはBH_4反応性高フェニルアラニン血症である。BH_4はフェニルアラニン水酸化酵素，チロシン水酸化酵素，トリプトファン水酸化酵素の補酵素であり，BH_4欠損症では上記の3つの酵素の障害が同時に起こるため，神経活性アミンの欠乏も同時に起こる。そのためBH_4欠損症の治療には，BH_4の補充による血中フェニルアラニン濃度のコントロールと，神経伝達物質の補充とが必要である。わが国では，BH_4製剤としてビオプテン顆粒（第一三共アスビオファーマ）が使用できる。BH_4欠損症では，1日あたり2～5 mg/kg，3分割経口投与を行い，血中フェニルアラニンが正常となる量までBH_4を増量する。また，BH_4反応性高フェニルアラニン血症では，1日あたり10 mg/kgを3分割経口投与する。4歳未満の小児ではBH_4投与の安全性が確立されていないため，まずフェニルアラニン制限食を行うことが重要である。

Ⅱ 高チロシン血症Ⅰ型のニチシノンによる治療

遺伝性高チロシン血症には遺伝的に異なった3つの病型がある。Ⅰ型はフマリルアセト酢酸分解酵素，Ⅱ型はチロシンアミノ基転移酵素，Ⅲ型は4-ヒドロキシフェニルピルビン酸酸化酵素の欠損によって発症する。Ⅰ型では肝障害と腎尿細管障害を認め，重症例では乳児期早期から肝障害が進行する。軽症例でも1～3歳で肝移植が必要

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表　尿素サイクル異常症の薬物治療

疾患名		フェニル酢酸 Na (g/kg/日)	安息香酸 Na (g/kg/日)	アルギニン (g/kg/日)	シトルリン (g/kg/日)	カルニチン (g/kg/日)
NAGS 欠損症 CPS I 欠損症 OTC 欠損症	<20 kg	0.45～0.6	0.1～0.25	0.1～0.2	0.1～0.2	20～30
	>20 kg	(9.9～13.0)	(2.2～5.5)	(2.2～4.4)	(2.2～4.4)	
シトルリン血症	<20 kg	0.45～0.6	0.1～0.25	0.4～0.7	—	20～30
	>20 kg	(9.9～13.0)	(2.2～5.5)	(8.8～15.4)	(−)	
アルギニノコハク酸尿症	<20 kg	—	—	0.4～0.7	—	—
	>20 kg	—	—	(8.8～15.4)	(−)	
アルギニン血症	<20 kg	0.3～0.6	0.1～0.25	—	—	20～30
	>20 kg	(6.6～13.0)	(2.2～5.5)	(−)	(−)	

※　>20 kg における投与量 g/m^2/日
※　1 日あたりの維持量。初期投与の場合は上記を 1～2 時間で投与する。

になる。I 型の治療では肝障害の進行を早期に防止することが重要であり，国内未承認薬である 4-ヒドロキシフェニルピルビン酸酸化酵素の阻害薬ニチシノン（Orfadin）を使用し，食事療法（低フェニルアラニン・低チロシン食）を併用する。早期に治療を開始した例では，肝臓移植を回避できる可能性がある。ニチシノンを使用しない例では肝不全に至ることが多く，肝移植が行われる。またニチシノンを使用した例でも，肝臓がんの発生例では肝移植が行われる。

III 尿素サイクル異常症におけるアルギニンまたはシトルリン治療

高アンモニア血症に対する治療に L-アルギニン，L-シトルリンが用いられる。尿素サイクル異常症として，カルバモイルリン酸合成酵素 I（CPS I），オルニチントランスカルバミラーゼ（OTC），アルギニノコハク酸合成酵素（ASS），アルギニノコハク酸分解酵素（ASL），アルギナーゼ（ARG）のそれぞれの欠損症が知られている。CPS I，OTC，ASS，ASL の欠損症ではアルギニンを補充することで尿素サイクルの代謝が活性化し，尿素，シトルリン，アルギニノコハク酸が尿中に排泄されるため血中アンモニアを低下させることができる。アルギ U 顆粒，アルギ U 注（味の素）が高アンモニア血症の治療適応がある。成長ホルモン分泌刺激試験に用いられるアルギニン注も同様の効果が期待できるため，緊急時には使用されることがある。アルギ U 注は塩酸 L-アルギニンとして 0.2 g/kg を 1 時間程度かけて点滴静注する。その後，0.2～0.6 g/kg/日を 24 時間かけて点滴静注する。アルギ U 顆粒は 0.1～0.2 g を 3 分割経口投与する。また，シトルリンは CPS I 欠損症，OTC 欠損症においてアルギニンより効率的に血中アンモニアを低下させると考えられている。通常は試薬のシトルリンを倫理委員会などの承認を経て用いる。シトルリンは 1 日あたり 0.1～0.2 g/kg を 3 分割経口投与する。

なお，小腸，腎臓などにおける二塩基アミノ酸転送蛋白 y+LAT-1 異常であるリジン尿性蛋白不耐症に対しても，シトルリン 0.1～0.2 g/kg/日の内服治療が有効である。

IV 高アンモニア血症における安息香酸ナトリウム，フェニル酢酸ナトリウムによる治療

安息香酸ナトリウム，フェニル酢酸ナトリウムは尿素サイクル以外の窒素排泄系を利用することで，高アンモニア血症の治療に有効である。

安息香酸は肝臓でグリシン抱合されて尿中へ排泄される。そのため尿素サイクルを介さずに窒素を体外へ排泄できる。過剰投与により肝障害をきたすことがあるので，使用量の設定には注意が必要である。フェニル酢酸ナトリウムは覚醒剤の原料となりうるため手続きや管理が煩雑である。欧

米ではプロドラッグであるフェニル酪酸ナトリウム（ブフェニル）として販売されている。フェニル酪酸ナトリウムはβ酸化によりフェニル酢酸ナトリウムとなり，グルタミンと結合して尿中に排泄される。フェニル酪酸ナトリウムは1モルあたり2モルの窒素を排泄することができる。国内未承認薬であるため，使用の際は個人輸入が必要である。

安息香酸ナトリウムとフェニル酢酸ナトリウムの利用によって，欧米の報告ではアンモニアを効率的に低下させて，高アンモニア血症の生存率を改善することが知られている。CPSⅠ欠損症，OTC 欠損症，ASS 欠損症では，安息香酸ナトリウムまたはフェニル酢酸ナトリウム（0.25 g/kg）を10%グルコースに溶解し，24 時間かけて静脈内投与を行う。試薬を用いた院内製剤として倫理委員会の承認を経て使用することが必要である。同量の試薬を経口投与することも可能である。

Ⅴ 糖原病

糖原病はグリコーゲンの代謝に関係する酵素の異常によって，肝臓や心筋，骨格筋などにグリコーゲンが蓄積する。主に肝臓の異常が起こる肝型糖原病と，心筋，骨格筋の異常が主にみられる筋型糖原病とに分けられる。肝型糖原病としては，Ⅰ型，Ⅲ型，Ⅳ型，Ⅵ型，Ⅸ型がある。以前Ⅷ型とよばれていた肝臓，筋，脳の phosphorylase kinase（α，β，γサブユニット）異常症は，現在ではこのⅨ型に分類されている。肝型糖原病の主な症状は，空腹時の低血糖と肝腫大である。糖原病Ⅰ型では glucose-6-phosphatase が欠損しているⅠa型と glucose-6-phosphatase transporter が欠損しているⅠb型とが存在する。Ⅰa型では低血糖と高乳酸血症の予防が重要である。診断はグルコース負荷によって血糖値が上昇し乳酸値が低下すること，遺伝子診断などによって行われる。早期に診断される症例が増えたことで，長期の予後も改善してきた。とくに幼児期は糖質を頻回に摂取し，夜間に未調理のコーンスターチ投与や糖液の持続胃内栄養を行うことなどが有効である。Ⅰb型はⅠa型の症状に加えて好中球の減少と機能障害が特徴である。糖原病Ⅲ型では debranching enzyme が欠損しており，Ⅰ型と同様の低血糖に対する治療を行う。予後は良好なことが多い。糖原病Ⅳ型は branching enzyme が欠損しており，進行性の肝障害によって幼児期に肝不全にいたる。予後が不良であるため肝臓移植の適応となる。糖原病Ⅵ型は肝型 phosphorylase が欠損している。低血糖は比較的軽度であり，予後は良好である。筋型糖原病であるⅡ型，Ⅴ型，Ⅶ型では，筋力低下や易疲労性，筋肉痛などが主な症状である。糖原病Ⅱ型（Pompe 病）はリソゾームの異常により全身の細胞にグリコーゲンが蓄積する。酵素補充療法が可能となり，良好な予後がみられる症例もある。糖原病Ⅴ型は筋 phosphorylase が欠損している。激しい運動を避けることが有効である。糖原病Ⅶ型は筋 phospofructokinase が欠損している。Ⅴ型と同様に激しい運動を避けることが有効である。

Ⅵ 肝移植

肝不全をきたすアミノ酸代謝異常症，尿素サイクル異常症や糖原病は肝臓移植の適応となる。上記の疾患群においてわが国では，高チロシン血症Ⅰ型，OTC 欠損症，CPSⅠ欠損症，古典型高シトルリン血症やシトリン異常症，アルギニノコハク酸尿症，アルギニン血症，糖原病Ⅰ型，Ⅳ型などで生体肝移植が行われている。尿素サイクル異常症では，新生児期早期に発症しアンモニア値が高い症例は早期に肝臓移植が必要であるが，移植前に意識障害が進行し死亡する例も少なくない。乳幼児期に発症する症例や，学童期以降に発症し，高アンモニア血症をくり返しながら悪化していく症例は肝移植治療の適応と考えられる。遺伝性高チロシン血症Ⅰ型は特殊ミルクと，国内未承認薬であるニチシノン（Orfadin）による治療が行われるが，肝細胞がんの発症が知られており，肝移植の適応が考慮される。

これらの疾患以外にも，先天性代謝異常症では，α_1アンチトリプシン欠損症，Wilson 病，Crigler-Najjar 症候群，原発性高シュウ酸血症Ⅰ型，メチルマロン酸血症，プロピオン酸血症などが肝移植

の適応となる。移植の適応とドナー選定の基準に絶対的なものはなく，移植可能な施設において症例ごとに十分な検討を行うことが必要である。

Ⅶ 肝細胞移植

ドナーの肝臓から肝細胞を採取・分離し，レシピエントの門脈からカテーテルを用いて注入する移植法である。採取，分離されたドナー肝細胞は凍結保存できるため，くり返し移植を行うことが可能である。肝臓移植が可能となるまでの「つなぎ治療」として，肝不全状態を改善することができる。欧米では肝細胞移植の治験が行われており，わが国でも臨床応用されることが期待されている。

Ⅷ 救急室での対応

本稿で取り扱っている疾患群のなかで緊急の対応が必要と考えられる疾患は，アシドーシス，高アンモニア血症，低血糖，高乳酸血症などを呈するものがあげられる。診断と並行して治療を行うことが重要であり，異化を防ぐために十分なグルコースを補給すること，高アンモニア血症が高度である場合には，血液浄化治療が可能な高度医療施設との連携を行うことが必要である。

おわりに

先天代謝異常症の多くは希少疾患であり，これらの治療に用いられる薬剤にはオーファンドラッグとして治療薬の承認や適応拡大が求められているものが少なくない。治療にあたっては先天代謝異常症を専門としている施設に遠慮なく尋ねていただきたい。日本先天代謝異常学会のホームページ（http://square.umin.ac.jp/JSIMD/），日本先天代謝異常学会セミナーなどが，それぞれの施設の専門分野を知るのに有用と思われる。

Key Points

❶ 高フェニルアラニン血症では BH_4 治療によって血中フェニルアラニン値が低下する症例がみられる。

❷ 尿素サイクル異常症などの高アンモニア血症の治療における L-アルギニン，L-シトルリン，安息香酸ナトリウム，フェニル酢酸ナトリウムの効果が明らかになってきた。

❸ 糖原病Ⅰ型では早期診断と早期治療によって，予後が改善してきている。

❹ 肝臓移植の適応とドナー選定の基準に絶対的なものはなく，移植可能な施設において症例ごとに十分な検討を行うことが必要である。

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VIII　遺伝と代謝

先天性アミノ酸代謝異常症
—高チロシン血症I型—

Inborn errors of amino acid metabolism
—Hereditary hypertyrosinemia type I—

Key words：チロシン，肝移植，アミノ酸，ニチシノン，肝臓癌

中村公俊
遠藤文夫

1. 概念・定義

チロシンは食事に含まれるアミノ酸の一つとして，またフェニルアラニンの代謝産物として得られる．生体内でフェニルアラニンはフェニルアラニン水酸化酵素によってチロシンへと変換される．チロシンは，チロシンアミノ基転移酵素によって4-ヒドロキシフェニルピルビン酸，続いて4-ヒドロキシフェニルピルビン酸酸化酵素によってホモゲンチジン酸，ホモゲンチジン酸酸化酵素によってマレイルアセト酢酸，マレイルアセト酢酸イソメラーゼによってフマリルアセト酢酸へと代謝され，フマリルアセト酢酸ヒドラーゼによってフマル酸とアセト酢酸に分解される(**図1**)．このチロシン代謝系の異常である遺伝性高チロシン血症は，遺伝的・酵素学的に3つの病型に分類されている(**表1**)．これらは，臨床症状出現の機序も異なる．遺伝形式はいずれも常染色体劣性である．遺伝性高チロシン血症I型[MIM276700 HEREDITARY TYROSINEMIA TYPE I]はフマリルアセト酢酸ヒドラーゼ(FAH: EC 3.7.1.2)が欠損することで発症する．同じチロシン代謝系の異常である遺伝性高チロシン血症II型[MIM276600 HEREDITARY TYROSINEMIA TYPE II]は細胞質チロシンアミノ基転移酵素(TAT: EC2.6.1.5)の欠損症で，眼皮膚型高チロシン血症，Richner-Hanhart症候群とも呼ばれる．また，遺伝性高チロシン血症III型[MIM276710 HEREDITARY TYROSINEMIA TYPE III]は4-ヒドロキシフェニルピルビン酸酸化酵素(HPD: EC1.13.11.27)が欠損している．また，ホーキンシン尿症もHPDのヘテロの異常により発症する常染色体優性遺伝性疾患である．

2. 疫　　学

高チロシン血症はMedesによって1932年に初めて報告されている．この報告では重症筋無力症患者の尿中にチロシン代謝産物の著明な排泄増加を認めている．この患者の病型は明らかにされていない．遺伝性高チロシン血症I型の原因がフマリルアセト酢酸ヒドラーゼの異常であることが判明したのは1980年代の初めのことである．遺伝性高チロシン血症II型は1960年代末にチロシンアミノ基転移酵素の欠損が原因であることが明らかにされた．1980年代の初めには，これらの酵素欠損とは異なる高チロシン血症を示す症例が遠藤らによって報告され，この欠損酵素は4-ヒドロキシフェニルピルビン酸であることが判明した．この酵素異常は高チロシン血症III型と分類された．それぞれまれな疾患であり，頻度は明らかにされていない．カナダ，ケベック州のSaguenay-Lac St. Jean地区には遺伝性高チロシン血症I型の集積を認める．この地域の患者頻度は約1,800人に1人と大変高いことが報告されている．

3. 病　　因

(1) I型ではフマリルアセト酢酸ヒドラーゼ酵素欠損によって細胞内に蓄積するフマリルア

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