autosomal dominant, and apnea attacks and developmental delay were a relatively common complication in addition to muscle stiffness and startle responses in patients with hyperekplexia.[3] In our study, apnea attacks and developmental delay were seen in only a small number of cases, and umbilical hernia was a frequent complication of hyperekplexia. Autosomal dominant mutations were most commonly identified. The differences between the report by Thomas et al.[3] and our study may depend on ethnic factors. Furthermore, some studies suggest that the detection rate of gene mutations is approximately 60% in patients with hyperekplexia,[11] even now. Further studies of genetic background are required with more cases of hyperekplexia.

CONCLUSIONS

This is the largest report of Japanese patients with hyperekplexia, and the first to highlight potential delays in diagnosis. Delayed diagnosis of hyperekplexia because of an incorrect diagnosis, such as epilepsy and adult-onset anxiety neurosis, may result in improper treatment and/or unnecessary examinations. Consistent with previous reports, all patients with hyperekplexia in the present study experienced a neonatal onset. For early detection, muscle stiffness, startle responses, and a positive nose-tapping test from the neonatal period are important points to note. A low dose of clonazepam will be most effective, although the outcome of the startle responses varied. The majority of Japanese patients have *GLRA1* mutations. Genetic analysis of glycinergic neurotransmission-associated genes, including *GLRA1*, could provide an appropriate diagnosis of hyperekplexia, and is helpful for prompt and appropriate treatment. Genotype–phenotype correlations are partially observed in hyperekplexia although other factors may regulate their clinical course.

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Letter to the editor

Amelioration of acylcarnitine profile using bezafibrate and riboflavin in a case of adult-onset glutaric acidemia type 2 with novel mutations of the electron transfer flavoprotein dehydrogenase (*ETFDH*) gene

Keywords:
Glutaric acidemia type 2
Acylcarnitine profile
Adult onset
Bezafibrate

1. Introduction

Multiple acyl-coenzyme A dehydrogenase deficiency (MADD), also known as glutaric acidemia type 2 (GA2), was first described in 1976 [1]. GA2 is a rare autosomal recessive disorder whose biochemical abnormalities result from a deficiency of one of the two electron transfer flavoproteins (ETF and ETFDH) that transfer electrons from acyl-CoA dehydrogenases to the respiratory chain [2]. The disorder affects multiple metabolic pathways involving branched amino acids, fatty acids, and tryptophan, and results in a variety of distinctive organic acids being discharged. The heterogeneous clinical features of patients with GA2 fall into three subclasses: two neonatal-onset forms (types I/II) and a late-onset form (type III) [3]. The late-onset form is typically characterized by intermittent vomiting, hypoglycemia, hepatomegaly, metabolic acidosis, and/or hyperammonemia, symptoms that are often triggered by general infections or catabolic conditions [4].

Here, we describe the case of a man with lipid-storage myopathy, low muscle carnitine, and an adult-onset form of GA2 with two novel mutations in the *ETFDH* gene. In this case, a combination of a hypolipidemic drug (bezafibrate), riboflavin, and L-carnitine was effective in treating the disease.

2. Case report

A 31-year-old man was referred to our hospital because of muscle weakness and limb fatigability. Nine months earlier, he had gradually developed proximal muscle weakness and fatigability. He exhibited normal psychomotor development. His relatives had no history of neuromuscular disease. Physical examination on admission showed a normally developed, well-nourished man (185 cm, 73 kg) without hepatosplenomegaly. Neurological examination revealed mild muscle weakness in his left iliopsoas muscle (grade 5 −). Muscle amyotrophy and myalgia were not noted. The following serum biochemistry markers were elevated: creatine kinase (CK), 689 U/L (normal <230); creatine kinase-MB, 50 U/L (<10); aldolase, 8.9 IU/L (<5.9); myoglobin, 107 ng/mL (<72.0); and triglycerides, 315 mg/dL (<149). The full blood count, blood glucose, renal and thyroid function, immunoglobulins, inflammatory markers, and antinuclear antibodies were normal. Echocardiography, pulmonary function tests, and a brain MRI were normal. Abdominal echography revealed only the fatty liver. A muscle MRI showed a high-density area in the bilateral lower limb muscles in short-T1 inversion recovery (STIR) (Fig. 1A). Atrophy of the biceps was suspected based on a muscle CT scan. Electromyography of the left vastus lateralis muscle and the tibialis anterior muscle displayed myopathic patterns. In the muscle biopsy specimen from the biceps brachii, neither lymphocytic infiltration nor endomysial fibrosis was observed (Fig. 1B), although some fibers contained many vacuoles. These were positively stained with Oil Red O, suggesting a lipid storage myopathy (Fig. 1C).

Total and free carnitine concentrations in muscle specimens were severely decreased at 3.5 (control 15.7 ± 2.8) and 1.7 (12.9 ± 3.7) nmol/mg non-collagen protein (NCP), respectively. Activity of acyl-CoA dehydrogenases was normal. Analysis of urinary organic acids showed increased 2-OH-glutarate, ethylmalonate, and 3-OH-propionate. The acylcarnitine profile of the patient's serum showed a broad-range elevation of acylcarnitines, but no abnormalities were observed in the amino acid profile. This indicated a multiple-dehydrogenation abnormality, which is consistent with GA2. After receiving informed consent, the patient's skin fibroblasts were isolated and cultured, as described previously [5]. Genetic analysis identified novel, compound heterozygous missense mutations in the *ETFDH* gene (890G > T/W297L and 950C > G/P317R). Western blot analysis showed decreased production of ETFDH in the patient's fibroblasts (Fig. 1D). This indicated that the mutations would be pathogenic.

Following treatment with L-carnitine alone, the patient's serum CK reached nearly normal levels. However, his serum acylcarnitine profile remained abnormal (Fig. 1E, left panel). The L-carnitine treatment was then supplemented with riboflavin at 105 mg/day or with bezafibrate (BEZ; 600 mg/day) because the patient showed mild hyperlipidemia, and because this hypolipidemic drug was effective for adolescent GA2 patients [5]. However, the combined treatment of L-carnitine and riboflavin, or L-carnitine and BEZ, failed to improve the acylcarnitine profile (Fig. 1E, left panel) and the patient's symptoms remained stable. For the next 7 months, the patient was treated with L-carnitine alone. During this period, he felt fatigability and his serum CK increased mildly. BEZ was again added to his treatment regimen. His serum acylcarnitine profile improved, but his serum CK remained high and he occasionally complained of fatigue (Fig. 1E, right panel). After 15 months, riboflavin was added to the L-carnitine and BEZ. His serum CK and acylcarnitine profile returned to normal within one month, and his symptoms completely disappeared. This amelioration has continued beyond 6 months.

3. Discussion

We diagnosed a patient with GA2 based on observations of the muscle pathology, acylcarnitine analysis, and *ETFDH* gene mutations. In the

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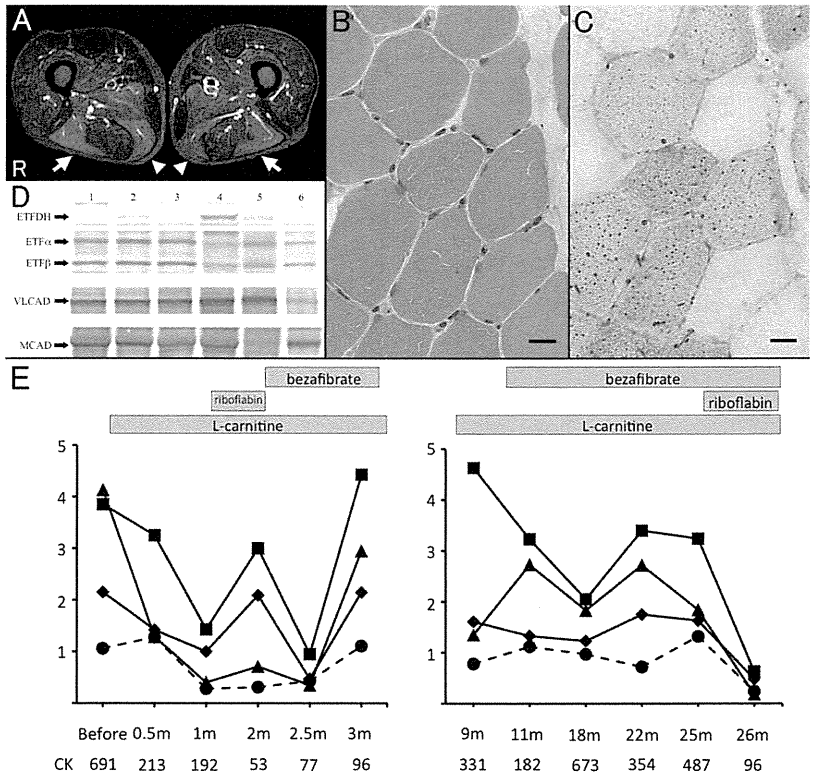

Fig. 1. A. A muscle MRI showed an area of high intensity in the bilateral biceps femoris muscle (arrow) and semimembranous muscle (arrowheads) in short-T1 inversion recovery (STIR). This indicated that increased water content in these muscles due to cellular lysis or fluid accumulation secondary to inflammation [10]. B, C. Biopsy of the patient's right biceps muscle. (B) Hematoxylin and eosin staining showed multiple optically empty vacuoles. (C) Oil Red O staining revealed excessive lipid droplets. The scale bar represents 20 μm. D. Western blot analyses of proteins in the patient's fibroblasts. The patient's fibroblasts were prepared as described previously [5,11]. For analysis of ETFDH, ETFα, and ETFβ, 25 μg of protein was applied to the gel. For analysis of very long-chain acyl-CoA dehydrogenase (VLCAD) and medium-chain acyl-CoA dehydrogenase (MCAD), 10 μg of protein was applied to the gel. Lane 1, patient's fibroblasts; lane 2, control (normal) fibroblasts; lane 3, ETFDH-defective fibroblasts; lane 4, ETFβ-defective fibroblasts; lane 5, MCAD-defective fibroblasts; lane 6, VLCAD-defective fibroblasts. Note that lane 1 from this patient, and lane 3 from the negative control, lack the band corresponding to ETFDH. This indicates that this patient had no ETFDH protein. Compared to control, the patient's fibroblasts showed no change in the expression of ETFα, ETFβ, VLCAD, or MCAD proteins. E. Changes in blood acylcarnitines with various treatments. The acylcarnitine profile of the patient's serum before treatment showed a broad-range elevation of acylcarnitines, including C6, C8, C10, C12, C14, and C16 acylcarnitine at 1.06 nmol/mL (normal <0.46), 2.15 (<1), 3.84 (<0.8), 4.13 (<0.4), 2.81 (<0.3), and 2.22 (<0.5), respectively. In the left panel, BEZ or riboflavin combined with L-carnitine, partially improved serum CK and serum acylcarnitine levels. Combining all three agents completely restored to normal the patient's acylcarnitine profile (right panel). During the seven-month period between the results shown in panels E and F, the patient was treated with L-carnitine alone. Units for acylcarnitine are nmol/mL and for CK are U/L. "m" indicates month. ●, C4; ✦, C8; ■, C10; ▲, C12.

adult myopathic form of GA2, patients sometimes do not show rhabdomyolysis, and there is no typical biochemical examination that can help us to consider the presence of a fatty acid oxidation disorder (FAO), as was observed here. Muscle biopsy and acylcarnitine analysis provide useful information and should be employed without hesitation.

Intake of L-carnitine has been reported to either exacerbate symptoms or to be effective for GA2 patients [6,7]. In the present case, oral carnitine alone leads to only partial improvement based on amelioration of the patient's muscle weakness and decreases in his serum CK and acyl-CoA. Riboflavin supplementation produces improvements in the symptoms and metabolic profiles of GA2 patients with *ETFDH* mutations, and the late-onset form [2]. BEZ is a hypolipidemic drug that is as an agonist of the peroxisome proliferating activator receptor, and was found to be beneficial in Japanese children with *ETFDH* gene mutations exhibiting GA2 [5]. Several mechanisms for the effectiveness of BEZ for FAO have been reported including upregulating mRNA and the activity of several FAO enzymes [8,9]. In the present case, BEZ, L-carnitine, and riboflavin each showed partial effectiveness and produced partial remission in a patient with GA2. In children, BEZ has been administered at doses from 17 to 25 mg/kg/day [5]. In the current patient, 600 mg/day of BEZ was administered, corresponding to only 8.2 mg/kg/day. This low dose was used because of the limitations of BEZ as a hypolipidemic drug and may explain the limited effectiveness of BEZ for our patient. A combination of BEZ, riboflavin, and L-carnitine produced complete remission in this patient, not only of his symptoms and serum CK, but also of his defect in fatty acid metabolism.

This case supports a new option for the treatment of GA2 patients, even in adults. Additional clinical studies and experimental investigation of the mechanisms of action of these drugs are required.

Conflict of interest

The authors have no conflicts of interest to declare.

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Original article

Carnitine–acylcarnitine translocase deficiency: Two neonatal cases with common splicing mutation and *in vitro* bezafibrate response

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Abstract

Background: Mitochondrial fatty acid oxidation (FAO) disorders are among the causes of acute encephalopathy- or myopathy-like illness. Carnitine–acylcarnitine translocase (CACT) deficiency is a rare FAO disorder, which represent an energy production insufficiency during prolonged fasting, febrile illness, or increased muscular activity. CACT deficiency is caused by mutations of the *SLC25A20* gene. Most patients developed severe metabolic decompensation in the neonatal period and died in infancy despite aggressive treatment.

Patients and methods: We herein report the clinical findings of two unrelated cases of CACT deficiency with mutation confirmation, and *in vitro* bezafibrate responses using *in vitro* probe acylcarnitine (IVP) assay. Patients 1 and 2 are products of nonconsanguineous parents. Both patients developed cardiac arrest at day 3 of life but survived the initial events. Their blood chemistry revealed hypoglycemia and metabolic acidosis. The acylcarnitine profiles in both patients demonstrated increased long-chain acylcarnitines, suggesting CACT or carnitine palmitoyltransferase-2 (CPT2) deficiency.

Results: The mutation analysis identified homozygous IVS2-10T>G in the *SLC25A20* gene in both patients, confirming the diagnosis of CACT deficiency. The IVP assay revealed increased C16, C16:1, but decreased C2 with improvement by bezafibrate in the cultured fibroblasts. The short-term clinical trial of bezafibrate in Patient 1 did not show clinical improvement, and died after starting the trial for 6 months.

Conclusion: This splicing mutation has been identified in other Asian populations indicating a possible founder effect. IVP assay of cultured fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial of more enrolled patients is required for evaluation of this therapy.

Keywords: CACT deficiency; *SLC25A20* mutation; IVP assay; Bezafibrate

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1. Introduction

Mitochondrial fatty acid oxidation (FAO) disorders are among the causes of neuromuscular symptoms as well as acute encephalopathy or even sudden death. In particular, the carnitine cycle is important in energy-producing pathway for cardiac and skeletal muscle and for preventing from hypoglycemia especially during prolonged fasting or increased muscular exercise. Carnitine–acylcarnitine translocase (CACT, EC 2.3.1.21) is one of the enzymes in the carnitine cycle, which catalyzes the transfer of the long-chain fatty acylcarnitines across the inner mitochondrial membrane in exchange of free carnitine. CACT deficiency (OMIM 212138) was first described in 1992 [1]. It is an autosomal-recessive disease caused by mutations of the *SLC25A20* gene located in chromosome 3p21.31 [2]. The gene consists of 9 exons and encodes protein comprising 301 amino acids [3]. CACT deficiency is a very rare disorder with so far as approximately 30 patients have been described, and accounted for 10% of patients with FAO disorders in French population [4]. However, it might be a common FAO disorder in some East Asian countries such as Hong Kong with the estimated incidence of 1 in 60,000 live births, and accounted for 33% of patients with FAO disorders [5]. Most patients develop neonatal-onset encephalopathy with nonketotic hypoglycemia, hyperammonemia, and hypothermia, or sudden death from cardiac arrhythmias. Cardiomyopathy and hepatic dysfunction may be the associated complications. CACT deficiency could be detected by elevations of C16 and C18 acylcarnitines, and low free carnitine in acylcarnitine profiles. However, the same profile could be found in neonatal carnitine palmitoyltransferase-2 (CPT2) deficiency. Therefore, confirmation of diagnosis requires CACT enzyme assay or molecular analysis of the *SLC25A20* gene [6]. Treatment includes intravenous glucose for acute decompensation, and avoidance of long fasting with frequent meals. Long-chain fatty acids may be restricted in diet, but medium-chain triglyceride (MCT) oil is supplemented instead. Carnitine therapy is still controversial. Despite aggressive treatment, most patients still died in infancy [7]. However, there have been some patients who received early treatment with good outcomes [8,9]. Novel therapy for FAOD using bezafibrate, which is a hypolipedimic drug acting as a peroxisome proliferator-activated receptor (PPAR) agonist has been reported. The clinical trials of bezafibrate showed clinical improvement in adult patients with CPT2 deficiency [10], and a child with glutaric acidemia type 2 (GA2) [11]. *In vitro* probe acylcarnitine (IVP) assay can be used to evaluate FAO disorders [12], and determine the effect of bezafibrate [13]. We herein report the clinical findings of two unrelated cases with neonatal-onset CACT deficiency, and *in vitro* bezafibrate response using the IVP assay.

2. Patients and methods

2.1. Patients

2.1.1. Case 1

This patient was the first child of possibly consanguineous parents from the southern province of Thailand. He was born at 37 weeks of gestation with birth weight of 2460 g (25th percentile), length 48 cm (3rd percentile), and head circumference 30 cm (<3rd percentile). He developed hypothermia at 10 h of age. Sepsis was suspected, but the patient rapidly responded to rewarming treatment. However, after rooming-in with the mother, he developed hypothermia again. At 60 h after birth, he had cardiac arrest. On physical examination, no abnormalities were found. Serum glucose was 1.2 mmol/L and acetoacetate was 0 mmol/L. Venous blood pH was 7.24 and serum bicarbonate was 13 mmol/L with an anion gap of 20. Plasma ammonia was 471 μmol/L (normal, <110 μmol/L). There were mildly elevated liver enzymes aspartate aminotransferase (AST) (97 U/L; normal, 0–32) and alanine aminotransferase (ALT) (78 U/L; normal, 0–33). Serum creatine kinase was 4439 U/L (normal, <190). He had a good response to treatment with intravenous glucose administration. Urine organic acids were unremarkable. A dried blood spot acylcarnitine profile by tandem mass spectrometry (MS/MS) showed free carnitine (C0), 5.26 μM (10–60); C16-acylcarnitine, 14.14 μM (0.6–7); C18-acylcarnitine, 2.71 μM (0.15–2.1); C18:1-acylcarnitine, 4.3 μM (0.3–3.2); and a (C16 + C18)/C0 ratio, 3.21 (0.007–0.5). The profile was consistent with CPT2 or CACT deficiency. The patient has been treated with a modular medical formula, which has been composed of modified fats (long-chain fatty acid restriction along with supplementation of 83% of fat as medium-chain triglyceride oil), protein, maltodextrins, minerals, and fat-, and water-soluble vitamins. L-Carnitine at a daily dosage of 100–150 mg/kg has been supplemented. Thereafter, he has had several episodes of hypoglycemia, hyperammonemia, and metabolic acidosis following infections. At 8 months of age, he developed cholestasis and hepatomegaly. At 9 months of age, an echocardiogram revealed hypertrophic cardiomyopathy. At the age of 15 months, he had mild developmental delay and generalized hypotonia. He could stand with support, put block in cup, and say one word. Then he had a metabolic crisis, and developed generalized weakness. After he recovered from encephalopathy, neurologic examination revealed normal cranial nerves, muscle weakness (grade 3/5), and decreased muscle tone and deep tendon reflexes (1+) in all extremities. A brain computed tomography scan was normal. Serum creatine kinase was elevated (1419 U/L). A nerve conduction study showed no evidence of demyelination. He had been ventilator-dependent since then. At 2½ years of

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age, he had several complications including chronic liver disease, upper gastrointestinal bleeding, and osteoporosis. He died at the age of 2 years and 8 months from upper gastrointestinal bleeding and metabolic decompensation.

2.1.2. Case 2

The patient was the first child of nonconsanguineous parents. She was born at 35 weeks of gestation with a birth weight of 2.3 kg (50th percentile), length 44 cm (25th percentile), and head circumference 30 cm (10th percentile). At 2 days after birth, she developed lethargy, poor feeding, and cardiac arrest. Blood glucose was 0.56 mmol/L. She responded to cardiac resuscitation and intravenous glucose infusion. Serum acetoacetate was 0 mmol/L. Venous blood pH was 7.39 and serum bicarbonate was 13 mmol/L with an anion gap of 20. Plasma ammonia was 157 μmol/L (normal, <110 μmol/L). There were elevated liver enzymes AST (638 U/L; normal, 0–32) and ALT (83 U/L; normal, 0–33). Plasma lactate dehydrogenase (LDH) was 522 U/L (normal, 240–480). An echocardiogram revealed no cardiomyopathy. A dried blood spot acylcarnitine profile by MS/MS analysis showed C0, 13.8 μM (10–60); C16-acylcanitine, 15 μM (0.6–7); C18-acylcarnitine, 4.3 μM (0.15–2.1); C18:1-acylcarnitine, 5.9 μM (0.3–3.2); and a (C16 + C18)/C0 ratio, 1.4 (0.007–0.5). The profile was consistent with either CPT2 or CACT deficiency. The patient had been treated with a high-MCT formula (Portagen®, Mead Johnson Nutritionals), and 100 mg/kg/day of L-carnitine. At 1 month of age, she developed anemia from Hb AE Bart's disease – a thalassemia intermedia resulting from the interaction between α-thalassemia and heterozygous Hb E, which required monthly blood transfusion. At the age of 4 months, she had poor feeding and cardiac arrest. Blood glucose was 0.5 mmol/L. The patient died without any response to resuscitation. An autopsy revealed left ventricular hypertrophy, micro/macrovesicular steatosis of the liver with focal areas of bridging fibrosis, and abnormal lipid accumulation in skeletal muscles and the proximal renal tubules.

2.2. Materials and methods

This study was approved by the Siriraj Institutional Review Board. The written informed consents for the mutation analysis, IVP assay, and bezafibrate trial were obtained from the parents. Genomic DNA was extracted from leukocytes. Mutation analyses of the *CPT2* and *SLC25A20* genes were performed in case 1, and only *SLC25A20* gene in case 2. All coding exons and their flanking intron sequences (up to 20 bases for both sides) of the *CPT2* and *SLC25A20* genes were PCR-amplified and directly sequenced according to the previously described method [14]. The IVP assay was performed using the skin fibroblasts in the absence and presence of bezafibrate according to the previously described method [11].

3. Results

3.1. Mutation analysis and IVP assay

Mutation analysis of the *SLC25A20* gene identified homozygous c.199-10T>G (IVS2-10T>G) mutation in both patients, and heterozygous mutation in their parents (Fig. 1). Mutation analysis of the *CPT2* gene revealed no pathogenic mutation in Case 1. The IVP assay profiles revealed increased C16, C16:1 acylcarnitines, and decreased C2 (acetylcarnitine) indicating a typical pattern of CPT2 or CACT deficiency, with substantial reduction of long-chain acylcarnitines by the presence of bezafibrate in the cultured fibroblasts from both patients (Fig. 2). However, C2 acylcarnitine did not increase as expected.

3.2. Clinical trial of bezafibrate

We started a clinical trial of bezafibrate in case 1 at age of 2 years and 2 months, after the IVP assay which

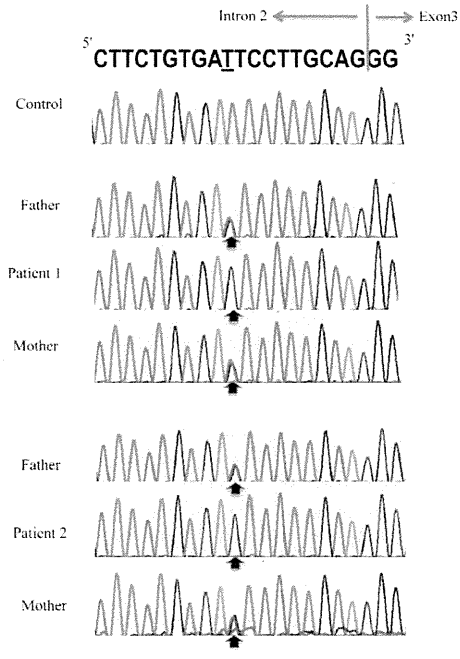

Fig. 1. The reference DNA sequence of an intron 2/exon 3 boundary of the *SLC25A20* gene, and the IVS2-10T>G mutation identified in both patients and their parents denoted by black arrows and the underlined letter.

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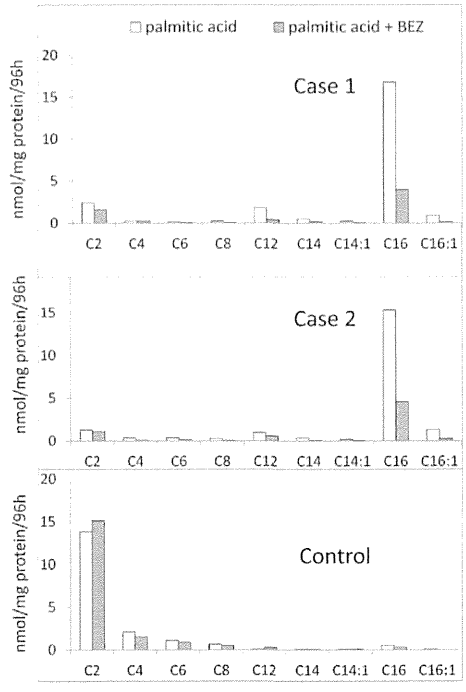

Fig. 2. Acylcarnitine profiles of IVP assay in the presence and absence of bezafibrate (BEZ) of cases 1, 2, and normal control respectively. Unit of vertical lines, nmol/mg protein of acylcarnitines (ACs); the horizontal lines represent acylcarnitines from C2, C4, C6, C8, C12, C14, C14:1, C16, and C16:1. The experiments for each were performed in triplicate, and the mean values of ACs are illustrated with bars.

showed some improvement in acylcarnitine profiles with bezafibrate. We used a dosage of 17–25 mg/kg/day as previously described [11]. Monitoring of liver functions, lactate dehydrogenase (LDH), creatine kinase (CK), and lipid profiles showed no adverse effects of bezafibrate. A short-term evaluation, after 6 months of the trial, did not show clinical improvement except for slightly increased back muscle strength noted by the mother. An echocardiography showed stable but no improvement in a left ventricular mass index. Acylcarnitine profiles in dried blood spots and other biochemical parameters did not show improvement (data not shown). Case 2 died before a clinical trial was considered.

4. Discussion

We report 2 unrelated cases of CACT deficiency with molecular confirmation first identified in Thailand. The c.199-10T>G (IVS2-10T>G) nucleotide change was the most prevalent mutation and identified in 14 out of 76 mutant alleles [15]. This mutation was homozygously identified in three Vietnamese and three Chinese patients. In the present study, in spite that two families had no consanguineous history, both patients were also a homozygotes of the c.199-10T>G mutation. In Japan, three CACT deficient patients have been described. Among them the same mutation was identified heterozygously in only one patient [14]. We propose that this mutation is a founder mutation in Asian populations. Clinical history of the three Chinese patients with homozygous c.199-10T>G mutation were reported [16]. All of them developed cardiac arrest within two days of age, as well as our two patients. Hence the phenotype of homozygotes of c.199-10T>G mutation is severe. This mutation was suggested to reside at a consensus lariat branch point sequence resulting in skipping of exons 3 and 4 or exon 3 alone, which leads to truncation of the protein [17].

Although our cases 1 and 2 were homozygotes of the same mutation, Case 1 survived until 2 years and 8 months and Case 2 died at 4 months of age. Several factors might attribute to their different clinical outcomes: (1) Thalassemia disease in case 2 which required repeated blood transfusions might affect cardiac functions by chronic hypoxia, iron overload, or decreased carnitine [18]; (2) differences in possible modifier genes such as *SLC25A29* gene (CACT-like, CACL) which has palmitoyl-carnitine transporting activity [19]; and (3) different formulas using in our cases, one is a synthetic modular formula and the other is a commercial formula. However, the rationale of both special formulas for diet therapy is a reduction in long-chain fatty acids together with supplementation of medium-chain triglyceride oil to be a caloric source shunting an obstruction of long-chain fatty acid β-oxidation.

Although increased FAO flux induced by bezafibrate was clearly shown in fibroblasts only from patients with mild phenotypes of FAO disorders, increased mRNA expression after bezafibrate exposure also occurred in cell lines from patients with severe phenotypes [20]. This could explain *in vitro* response to bezafibrate observed in fibroblasts of patient 1 and 2. Despite the severe genotype leading to barely detectable enzyme activity [21], we believe that there should be some FAO flux which could be enhanced by bezafibrate in these patients. Our hypothesis is if there is entirely absent FAO flux in these patients, they should have anomalies like those found in a lethal neonatal form of CPT2 deficiency or GA2 [22], even though there has been no report of such findings in CACT deficiency. To our knowledge, patient 1 is the first case of neonatal-onset CACT deficiency who underwent a clinical trial of bezafibrate after showing an *in vitro* response by IVP assay. However, no beneficial short-term effect was shown. This might indicate the irreversible damage of the affected organs esp. the cardiac and skeletal muscles, and liver. Moreover, the difference between the *in vitro* and *in vivo* responses is

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probably due to the difference of bezafibrate concentration used in the IVP assay (400 μmol/L) and typical concentrations obtained in patients on bezafibrate therapy (50–200 μmol/L) [23]. Another possible reason is inadequate acetyl-CoA production despite bezafibrate treatment. This hypothesis is supported by persistently low C2 acylcarnitines in IVP assays of our cases and a previous case with CACT deficiency [11]. Moreover, C16 acylcarnitine did not decrease to the control level after bezafibrate treatment. Overall, although some improvement of acylcarnitine profile was shown in the patient 1 and 2's fibroblasts in IVP assay with bezafibrate, the effect of bezafibrate was less than those in fibroblasts from patients with mild forms of FAO disorders [11,24]. Hence clinical improvement in this patient was thought to be limited. Since CACT-deficient patients who developed metabolic decompensation in early neonatal period had poor prognosis with routine management [7], we decided to use bezafibrate treatment in patient 1. He survived until two years of age with bezafibrate treatment. However, it is uncertain whether this longer survival owed to the effect of bezafibrate treatment or not, since no apparent improvement of clinical laboratory data was obtained.

In conclusion, CACT deficiency may be a common FAO disorder in East Asian populations probably from a founder effect. IVP assay of fibroblasts could determine a response to bezafibrate treatment. A long-term clinical trial and more enrolled patients are required for evaluation of this therapy.

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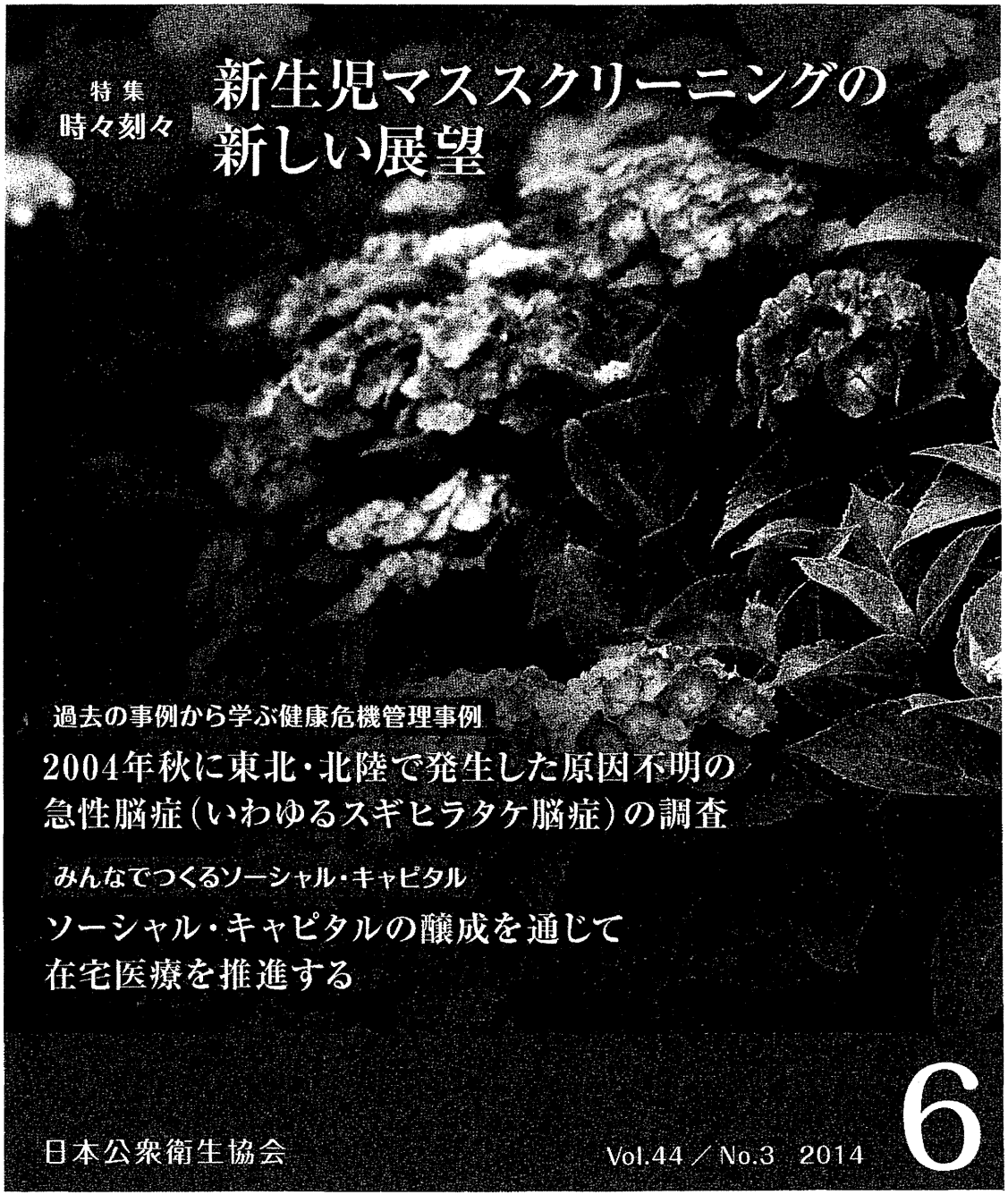


特集
時々刻々

新生児マススクリーニングの新しい展望

過去の事例から学ぶ健康危機管理事例

2004年秋に東北・北陸で発生した原因不明の急性脳症（いわゆるスギヒラタケ脳症）の調査

みんなでつくるソーシャル・キャピタル

ソーシャル・キャピタルの醸成を通じて在宅医療を推進する

日本公衆衛生協会　Vol.44／No.3　2014　6

用される予防医学の典型例であり、子どもたちが健全に成長している姿を社会にアピールできる調査分析をぜひ進めてほしいものです。そのためには、すでにしっかりした組織活動を進めている先進県のケースを参考に、各都道府県におけるしっかりした運営管理のための組織（多分野の複雑な機能を統合するため）を確立することが第一歩になると思います。

13トライバシー問題を何とか克服して（その方法はあると思います）、患児（者）ならびに保護者、研究者（主治医）を中心に行政もその特性を発揮して協力体制を改めて構築することができないものかと切に願うものです。

タンデムマスを導入した新生児マススクリーニングの社会的意義と課題

日本マス・スクリーニング学会 理事長
島根大学医学部小児科 教授
山口 清次

はじめに

新生児マススクリーニング（以下、「新生児MS」という）は、知らずに放置するとやがて障害が発生するような代謝異常症を、発症前の新生児期に発見して、障害発生を防止する公的事業である。1963年に米国で「ガスリーテスト」として、世界で初めて行われ、先進国を中心に普及した。

わが国でも1960年代からパイロット研究が始まり、1977年から全国で実施され最近まで6種類の疾患を対象にした新生児MSが行われてきた。その結果を表1に示している。対象6疾患のうち、もっとも発見頻度の高い疾患は先天性甲状腺機能低下症で、生後1か月以内から治療されると知能予後もよい。治療薬として使われるチラーヂンSも安価な薬であり、対象疾患の中ではもっとも費用便益のよい疾患である。

表1 わが国で行われてきた従来のマススクリーニングの対象疾患と発見頻度

疾患	頻度	費用便益
1）フェニルケトン尿症	1：7万	○
2）メープルシロップ尿症	1：50万	△
3）ホモシスチン尿症	1：80万	△
4）ガラクトース血症（全体）	1：3万*	△
（1型）	（1：80万）	
（2型）	（1：60万）	
5）先天性甲状腺機能低下症	1：3,000	◎
6）先天性副腎過形成症	1：2万	○

* ガラクトース高値症例の大部分は酵素欠損による真の先天性ガラクトース血症ではなく、門脈奇形やシトリン欠損症等の2次性のものである。費用便益：◎＝きわめて良好：○＝良好：△＝あまりよくない

新生児マススクリーニングの社会的意義

病気を対象にする「小児内科」に対し、健康な小児を対象にする予防医学を「小児保健」という。小児保健は小児の健全育成を目的とするもので、小児科医や看護師等のみならず、保健師、保育士、養護教諭、児童相談所や行政などの多くの職種がかかわる。

小児の障害発生を予防する代表的な事業として、①乳幼児健診 ②予防接種 ③新生児MSが挙げられよう。この中で、新生児MSの一般社会での認知度は、いちばん低いかもしれない。しかし、新生児MSを導入している先進諸国では「新生児MSは非常に優良な公衆衛生事業」と位置づけられている。なぜなら、知らずに放置されたために障害児として福祉の助けによって生きることを余儀なくされるのに対し、新生児MSによって障害から免れて成人し社会参加することを考えれば当事者の人生は大きく異なる。

米国などでは、社会経済学的観点から「tax eater（税金の助けによって暮らす人）をtax payer（税金を納める人）に変える事業」だとして認知されている。

タンデムマス法による拡大スクリーニングとは

従来の新生児MSは、ガスリー法などの方法で6疾患を対象として行われてきた。1990年代に

開発されたタンデムマスは、正式には「タンデム型質量分析計」という高感度分析機器である。従来と同じ血液ろ紙で行うことができ、1回の分析で多種類のアミノ酸とアシルカルニチンを同時測定することができる。アミノ酸の測定によってアミノ酸代謝異常症がスクリーニングされ、アシルカルニチン分析によって、有機酸および脂肪酸代謝異常症がスクリーニングされる。1回の分析で20～30種類が一斉にスクリーニングできるため、しばしば「拡大スクリーニング」と呼ばれている。ただガラクトース血症、先天性副腎過形成症、先天性甲状腺機能低下症の3つの疾患はタンデムマス法で検査できないので、これまでと同様の方法で検査する必要がある。

タンデムマス法の対象疾患とわが国の発見頻度

タンデムマス法で約195万人の新生児を対象としたパイロット研究の結果を表2に示している。アミノ酸代謝異常症全体で発見頻度は1:2・7万人、有機酸代謝異常症は1:2・2万人、脂肪酸代謝異常症は1:3・4万人であった。これらは全体では、1:9千人の頻度と計算された[1)2)]。

またパイロット研究の中で「見逃しが少なく、発見されたとき治療効果が期待できる」と判断した16疾患を第1次対象疾患としている(表2の疾患1～16)。一方「現時点では見逃しが多く検討の余地がある」と判断された疾患を2次対象疾患としている(表2の▲印の疾患)。2次対象疾患の扱いについては、原則的に対象疾患とする必要はなく、パイロット研究に位置づけられている。

対象疾患の臨床症状は表2に示すように、発達遅滞のほか、高アンモニア血症、ケトアシドーシス、骨格筋症状(けん怠感、筋力低下など)、急性脳症、突然死などがある。

新生児マススクリーニングの検討すべき課題

わが国で1977年から新生児MSが全国で実施されてから37年が経過した。初期に発見された患

表2　タンデムマス導入後の新生児マススクリーニング対象疾患と頻度

No	疾患	頻度	主な症状
タンデムマス検査(アミノ酸、有機酸、脂肪酸代謝異常症)			
A. アミノ酸代謝異常症(全体で1：2.7万)			発達遅滞 ショック 骨格異常、血管異常 高アンモニア血症 高アンモニア血症 肝炎症状、低血糖
1	フェニルケトン尿症	1/5.3万	
2	メープルシロップ尿症	1/195万	
3	ホモシスチン尿症	1/65万	
4	シトルリン血症1型	1/33万	
5	アルギニコハク酸尿症	1/98万	
▲	シトリン欠損症	1/8万	
B. 有機酸代謝異常症(全体で1：2.2万)			多呼吸、意識障害 ケトアシドーシス 高アンモニア血症 間欠的発作 神経退行など
6	メチルマロン酸血症	1/11万	
7	プロピオン酸血症	1/4.5万	
8	イソ吉草酸血症	1/65万	
9	複合カルボキシ欠損症	1/65万	
10	MCC欠損症	1/15万	
11	HMG血症	—	
12	グルタル酸尿症1型	1/28万	
▲	βケトチオラーゼ欠損症	—	
C. 脂肪酸代謝異常症(全体で1：3.4万)			全身けん怠 肝機能障害 高アンモニア血症 低血糖 急性脳症 突然死 骨格筋症状
13	CPT 1欠損症	1/39万	
14	VLCAD欠損症	1/16万	
15	MCAD欠損症	1/11万	
16	TFP欠損症	1/98万	
▲	CPT 2欠損症	1/28万	
▲	グルタル酸尿症2型	1/33万	
▲	全身性カルニチン欠乏症	1/28万	
タンデムマス検査以外(内分泌疾患、糖代謝異常症)			
17	先天性甲状腺機能低下症(CH)	1: 3,000	発達遅滞
18	先天性副腎過形成症(CAH)	1: 1.7万	電解質異常、色素沈着
19	ガラクトース血症Ⅰ型 ガラクトース血症Ⅱ型	1: 80万 1: 50万	肝障害、白内障 白内障

タンデムマス対象疾患の頻度は、1997～2012年にわが国で行われたパイロット研究(約195万人対象)の結果。タンデムマス法による発見頻度は全体で約9千人に1人。疾患1～19はタンデムマス導入後の新生児マススクリーニング対象疾患。▲：タンデムマス2次対象疾患(現時点では見逃しが多いなどの理由で1次対象となってないもの)。疾患17～19は、タンデムマス法で検査できないため、従来と同様の方法で検査される。すなわちCH(TSH)とCAH(17OHP)ではELISA法：ガラクトース血症ではガラクトース濃度を酵素法、またはELISA法で測定し、Ⅰ型の酵素活性はボイトラー法で検査される。

表3　タンデムマス導入後の新生児マススクリーニングの主な課題

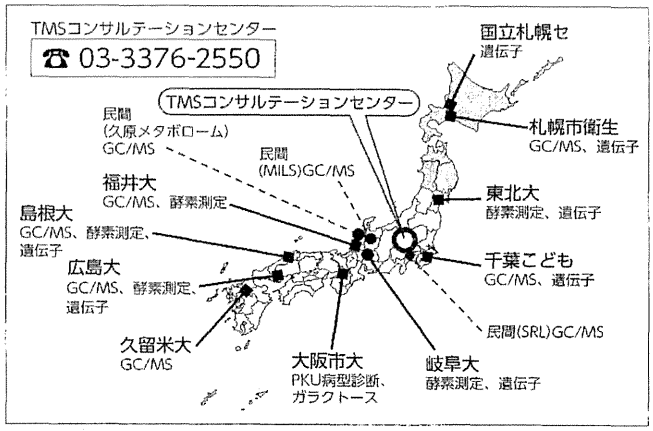
課題	対応
1. 稀少疾患の診療支援体制	TMS コンサルテーションセンター 全国ネットワーク構築
2. 精度管理体制	定期的な精度管理 検査機関連携による診断精度の向上
3. 患者コホート体制	発見された患者登録・追跡体制の構築
4. 患者 QOL の向上	患者家族会と行政・専門家等との連携
5. 治療用食品、特殊ミルクの安定供給	海外事例の調査検討 財政援助のしくみを検討
6. カウンセリング体制	陽性者に対応できる専門家の養成 研修会等
7. 関連部署の連携、社会啓発	自治体新生児 MS 連絡協議会の設置 中核医療機関（中核医師）の指定 定期的情報交換、情報誌の発刊 研修会、市民講座開催等

者はすでに成人し、新生児ＭＳのいくつかの課題が明らかになってきた。表３に主な課題を挙げ、タンデムマス導入を機に検討すべき対応を列挙した。

1. 稀少疾患の診療支援体制

タンデムマス法で発見される疾患は稀少疾患であり、小児科専門医でもなじみのない疾患が多く、診療にあたって指針が必要である[3]。また確定診断のためにしばしば特殊検査が必要となるが[1]、それが提供できる施設は現在限られている（図１）。これらの特殊検査のできる施設を自治体ごとに設置することは現実的に不可能であり、むだが多い。そこで、中央にコーディネート機関をおいて、専門施設の全国ネットワークを作り、全国どこからでもアクセス可能な体制が効率的である。２０１４年４月より「ＴＭＳコンサルテーションセンター」が設置された（☎03-3376-2550；http://tandem-ms.or.jp/）。

図１　確定診断のための特殊検査をしている施設の例（2012年時点）



2. 精度管理体制

国立成育医療研究センターのマススクリーニング研究室に委託して、精度管理を行う体制となっている。原則として、検査機関を対象に年１回の精度試験（ＱＣテスト）、年３回の技能試験（ＰＴテスト）を行う。そして検査機関が連携して、測定値の偏り、見逃し例のチェックおよび精度の高い診断指標の検討を行う。

3. 患者コホート体制

これまで新生児ＭＳで発見された患者数が年１回厚労省母子保健課に報告されるものの、その後のコホートについては、追跡するしくみがないのが現状である。個人情報に配慮した全国規模の患者登録、コホート体制の構築に関する研究を開始した。患者登録がされていれば、新しい治療法が開発されたときなどにより早く患者にフィードバックでき、また行政レベルでは事業評価、行政サービスの向上、新生児ＭＳ事業の社会啓発にも役立つ。

4. 患者ＱＯＬの向上

新生児ＭＳで発見された患者家族は、周囲に同様の患者がいないため、想像以上に孤独感を感じ、不安な生活を強いられるかもしれない。最近は患者家族会ができつつあるが、遠方からも参加しやすいしくみをつくり、定期的に情報誌などを発刊するなどして専門家からの新しい情報を得たり、情報交換できれば不安の解消につながり、ＱＯＬ向上に役立つ。

5. 治療用食品、特殊ミルクの安定供給

治療用特殊ミルクは新生児ＭＳの開始以来、メーカーのボランティに頼ってきた面が多い。年々患者が増え年齢が長じるにつれ

て、メーカーの厚意だけに頼ることは難しくなりつつある。さらに20歳を過ぎると、小児慢性特定疾患から外れるために医療費の自己負担が多くなるという問題が顕在化している。成人後に中途で治療をやめたために健康障害が再発するという症例も報告されつつある。この問題については海外のしくみを研究するなどして早急に検討される必要がある。

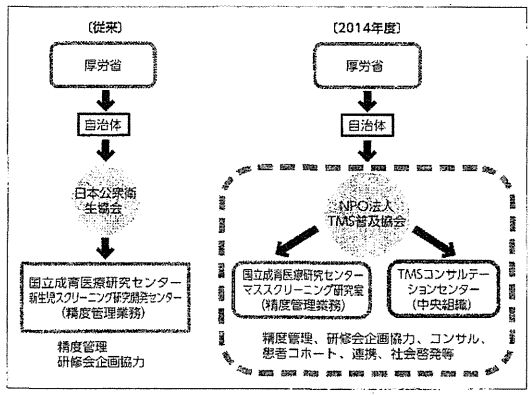
6. カウンセリング体制

新生児ＭＳで陽性と診断されても、多くの場合症状はない。偽陽性も少なくない。また診断が確定しても、無治療でいいこともあるし、ただちに治療を始める場合もある。すでに発症していることもある。患者家族はいつまで治療するのか、予後はどうなのか不安でいっぱいである。「偽陽性だったのでよかったですね」といっても、家族にとっては出産の祝賀気分に冷や水をかけられたような気持ちかもしれない。新生児ＭＳの社会的意義、対象疾患の特性、タンデムマス検査陽性の意味等について教育された人材によるカウンセリングが必要になる。

7. 関連部署の連携、社会啓発

新生児ＭＳ事業が始まった当初に比べると、マンネリ化しているためか、行政の担当部署の人でさえ「新生児ＭＳがなぜ行われるのか」という意味が十分に理解されていないケースもある。新生児ＭＳの対象疾患が、もしも不作為のためにみすみす小児が障害を残したら、その時初めて新生児ＭＳのありがたさがわかる「空気」のような存在なのかもしれない。自治体ごとに「新生児ＭＳ連絡協議会」や、相談窓口となる「中核医療機関（中核医師）」を設置して、発見された患者に対する診療の向上に努めるべきである。このために情報誌等による情報交換、研修会などによる生涯教育の場を活用して質を維持する必要がある。

図2　新生児マススクリーニング体制の変更(2014年4月より)



新生児マススクリーニング体制の変更

タンデムマス法導入を機に、新生児ＭＳ体制の変更が行われている。図2に示すように、従来は日本公衆衛生協会が窓口となって、自治体から検査精度管理を委託され、協会は国立成育医療研究センター内で新生児スクリーニング研究開発センターとして業務を行っていた。２０１４年度から、ＮＰＯ法人タンデムマス・スクリーニング普及協会（ＴＭＳ普及協会）が自治体からの委託を受けて精度管理業務とコンサルテーションセンター業務を行う形態となった。さらに関連学会と協力して情報誌発刊やホームページ運営、研修会企画などへも参画する。

おわりに

最近、予防接種の拡大によって「髄膜炎が減った」「ロタウイルス乳児下痢症の入院患者が減った」など小児医療の現場の声が聞かれる。タンデムマス法を導入した新生児ＭＳの普及によって、急性脳症や突然死様症状の救急患者に対する対応も変化することが予想される。小児保健事業の拡大は小児医療現場に変化をもたらす。これから長い人生を歩む新生児や乳幼児にとって「小児の病気は治療よりも予防」が重要である。障害発生防止のためのセーフティーネットを強化することは、少子化対策にも貢献すると思われる。

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