2.3. DNA sequencing of gene, acyl-CoA dehydrogenase, medium chain (ACADM)

Genomic DNA was purified from the patients' fibroblasts or blood filter papers using the QIAamp DNA Micro Kit (Qiagen GmbH, Hilden, Germany). Mutation analysis on genomic DNA was performed by PCR for each exon and its intron boundaries followed by direct sequencing [17].

Informed consent to perform DNA analysis was obtained from the parents of the patients. This study was approved by the Ethical Committee of the Shimane University Faculty of Medicine.

3. Results

3.1. Clinical features of patients

The clinical features of 16 Japanese patients with MCADD and 4 carriers (9 males and 11 females) are summarized in Table 1, including previously reported cases [17]. All 7 patients that were diagnosed after metabolic crisis were born before the initiation of newborn screening in their local area. The mean age at onset of the symptomatic cases was 1 y 3 m (range: 8 m to 2 y 2 m). The symptomatic patients were all in good general health with normal development until metabolic crisis. Metabolic crises were triggered by common cold or gastroenteritis in 5 cases. One of them died of SUD. Four cases had mild to severe handicaps, and 2 cases developed normally. The patients who were identified by neonatal screening remain healthy at this time.

3.2. Biochemical results of patients

The results of mass spectrometric analysis are shown in Table 1. Blood acylcarnitine analysis was available in 15 of the 16 patients. Octanoylcarnitine (C8) and octanoyl:decanoylcarnitine (C8/C10) ratio were assessed for detection of MCADD. Marked elevation of C8 and C8/C10 was observed in 14 cases (1.37–7 μmol/L), and slight elevation of C8 and C8/C10 (0.49 μmol/L and 3.77) was found in one case (case 16). The level of C8 was also mildly elevated in 3 (0.44, 0.51 and 0.37 μmol/L, respectively) of the 4 carriers while C8/C10 value was under cut-off (1.02, 0.88 and 1.00). Case 20, who is a mother of case 16, showed no abnormal findings.

Urinary organic acids were analyzed in 11 cases with MCADD and 4 carriers. Both hexanoylglycine and suberylglycine were elevated in 9 patients, and hexanoylglycine or suberylglycine was increased in one case each. However, neither hexanoylglycine nor suberylglycine was identified in the carriers.

3.3. Mutations in acyl-CoA dehydrogenase, medium chain (ACADM) gene

Fourteen types of mutations were identified in 30 independent alleles, 7 of which were novel. These included three types of splice site alterations (IVS3+2T>C, IVS3+5G>A and IVS4+1G>A), and four missense mutations (G46D, Q116L, G337E and K395R). These novel mutations were not detected in 120 alleles from unaffected Japanese individuals. All mutations are summarized in Table 1, together with previously reported cases (cases 2, 3, 5–9, 13 and 16) [17]. A c.449–452delCTGA [20,21] was detected in 10 (33.3%) of 30 independent alleles (2 cases with homozygous and 6 cases with compound heterozygous). A homozygous large deletion including exons 11 and 12 [22] was identified in 4 (13.3%) alleles. R28C (2/30 alleles), R256S (2/30 alleles), P67L (1/30 alleles), M249V (1/30 alleles) and G337E (1/30 alleles) were also observed (Table 1) [9,17,22].

4. Discussion

We investigated the relationship between clinical and molecular spectrums of 16 Japanese patients with MCADD. While symptomatic patients remained undiagnosed until metabolic crisis, asymptomatic patients were identified by neonatal mass screening (8 cases), or by sibling screening (1 case). Most of the symptomatic cases developed metabolic crisis associated with hypoglycemia triggered by common infection and prolonged fasting [3,4]. Those patients had poor outcomes such as mild to severe impairments or SUD. However, expansion of blood acylcarnitine analysis using MS/MS for neonatal mass screening in Japan allowed earlier detection of MCADD in the asymptomatic/presymptomatic stage. Subsequent prophylactic management for those children was conducted in a more appropriate and timely manner during metabolic stress such as fever, viral infection and other medical procedures.

Fourteen mutations were identified in 30 independent alleles including seven novel mutations. The amino acids affected by the novel missense mutations (G46D, Q116L, G337E and K395R) are highly conserved among different species (*Pan Troglodytes, Rattus norvegicus, Xenopus laevis* and *Danio rerio*), suggesting that these amino acids play an important role in medium acyl-CoA dehydrogenase activity. There are also splice site alterations such as IVS3+2T>C, IVS3+5G>A and IVS4+1G>A positioned at a 5′ donor splice site. Shapiro and Senapathy 5′ splice site scores [23] of altered sites changed from 76.4 to 58.6 for IVS3+2T>C, from 76.4 to 62.4 for IVS3+5G>A, and from 86.3 to 68.1 for IVS4+1G>A, respectively, suggesting that these changes are likely responsible for aberrant mRNA splicing. It is reported that point mutations in donor splice site produced exon skipping or aberrant 5′ donor splice site activation [24]. Since these changes likely resulted in aberrant splicing and premature truncation, non-sense mediated mRNA decay [25] or translation into shorter proteins with unlikely residual activity would result.

Most of the mutations detected in Japanese patients were unique, but Q20R, R28C, R256S and c.449–452delCTGA were previously reported in other nationalities [9,22,26,27]. The Japanese patient with compound heterozygous of R28C was one quarter of Caucasian. In contrast, a common missense mutation c.985A>G (80–90%) of Caucasian [8,15,28–30] was not detected in any Japanese patients in this study.

Our study demonstrates that detection in the asymptomatic/presymptomatic stage is essential to achieve favorable outcomes of patients with MCADD. Neonatal mass screening is absolutely a beneficial system to improve the quality of life of patients with MCADD. Genetic background of Japanese patients with MCADD is different from those in Caucasians. It is likely that there is no correlation between genotype and phenotype in Japanese patients with MCADD, and a specific genotype does not predict the clinical outcome.

Acknowledgments

We thank all of the attending physicians for providing clinical information regarding each patient. We are also grateful to Y. Ito, M. Furui, T. Esumi and N. Tomita for their technical assistance. This work was supported by a Grants-in-Aid for scientific research from the Japan Society for the Promotion of Science (J.P., and S.Y.); and a Grant from the Ministry of Education, Science, Technology, Sports and Culture of Japan, and the Ministry of Health, Labour and Welfare of Japan (S.Y.).

References

[1] L. Waddell, V. Wiley, K. Carpenter, B. Bennetts, L. Angel, B.S. Andresen, B. Wilcken, Medium-chain acyl-CoA dehydrogenase deficiency: genotype-biochemical phenotype correlations, Mol. Genet. Metab. 87 (2006) 32–39.
[2] B. Wilcken, M. Haas, P. Joy, V. Wiley, M. Chaplin, C. Black, J. Fletcher, J. McGill, A. Boneh, Outcome of neonatal screening for medium-chain acyl-CoA dehydrogenase deficiency in Australia: a cohort study, Lancet 369 (2007) 37–42.
[3] A.K. Iafolla, R.J. Thompson Jr., C.R. Roe, Medium-chain acyl-coenzyme A dehydrogenase deficiency: clinical course in 120 affected children, J. Pediatr. 124 (1994) 409–415.
[4] R.J. Pollitt, J.V. Leonard, Prospective surveillance study of medium chain acyl-CoA dehydrogenase deficiency in the UK, Arch. Dis. Child. 79 (1998) 116–119.
[5] B. Wilcken, J. Hammond, M. Silink, Morbidity and mortality in medium chain acyl coenzyme A dehydrogenase deficiency, Arch. Dis. Child. 70 (1994) 410–412.

[6] E.H. Touma, C. Charpentier, Medium chain acyl-CoA dehydrogenase deficiency, Arch. Dis. Child. 67 (1992) 142–145.
[7] T.G. Derks, D.J. Reijngoud, H.R. Waterham, W.J. Gerver, M.P. van den Berg, P.J. Sauer, G.P. Smit, The natural history of medium-chain acyl CoA dehydrogenase deficiency in the Netherlands: clinical presentation and outcome, J. Pediatr. 148 (2006) 665–670.
[8] T.H. Zytkovicz, E.F. Fitzgerald, D. Marsden, C.A. Larson, V.E. Shih, D.M. Johnson, A.W. Strauss, A.M. Comeau, R.B. Eaton, G.F. Grady, Tandem mass spectrometric analysis for amino, organic, and fatty acid disorders in newborn dried blood spots: a two-year summary from the New England Newborn Screening Program, Clin. Chem. 47 (2001) 1945–1955.
[9] K. Tanaka, I. Yokota, P.M. Coates, A.W. Strauss, D.P. Kelly, Z. Zhang, N. Gregersen, B.S. Andresen, Y. Matsubara, D. Curtis, et al., Mutations in the medium chain acyl-CoA dehydrogenase (MCAD) gene, Hum. Mutat. 1 (1992) 271–279.
[10] Y. Matsubara, K. Narisawa, S. Miyabayashi, K. Tada, P.M. Coates, Molecular lesion in patients with medium-chain acyl-CoA dehydrogenase deficiency, Lancet 335 (1990) 1589.
[11] Y. Matsubara, K. Narisawa, S. Miyabayashi, K. Tada, P.M. Coates, C. Bachmann, L.J. Elsas II, R.J. Pollitt, W.J. Rhead, C.R. Roe, Identification of a common mutation in patients with medium-chain acyl-CoA dehydrogenase deficiency, Biochem. Biophys. Res. Commun. 171 (1990) 498–505.
[12] J.H. Ding, C.R. Roe, A.K. Iafolla, Y.T. Chen, Medium-chain acyl-coenzyme A dehydrogenase deficiency and sudden infant death, N. Engl. J. Med. 325 (1991) 61–62.
[13] N. Gregersen, A.I. Blakemore, V. Winter, B. Andresen, S. Kolvraa, L. Bolund, D. Curtis, P.C. Engel, Specific diagnosis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in dried blood spots by a polymerase chain reaction (PCR) assay detecting a point-mutation (G985) in the MCAD gene, Clin. Chim. Acta 203 (1991) 23–34.
[14] S. Giroux, A. Dube-Linteau, G. Cardinal, Y. Labelle, N. Laflamme, Y. Giguere, F. Rousseau, Assessment of the prevalence of the 985A>G MCAD mutation in the French-Canadian population using allele-specific PCR, Clin. Genet. 71 (2007) 569–575.
[15] G.A. Horvath, A.G. Davidson, S.G. Stockler-Ipsiroglu, Y.P. Lillquist, P.J. Waters, S. Olpin, B.S. Andresen, J. Palaty, J. Nelson, H. Vallance, Newborn screening for MCAD deficiency: experience of the first three years in British Columbia, Canada, Can. J. Public Health 99 (2008) 276–280.
[16] I. Yokota, P.M. Coates, D.E. Hale, P. Rinaldo, K. Tanaka, Molecular survey of a prevalent mutation, 985A-to-G transition, and identification of five infrequent mutations in the medium-chain Acyl-CoA dehydrogenase (MCAD) gene in 55 patients with MCAD deficiency, Am. J. Hum. Genet. 49 (1991) 1280–1291.
[17] J. Purevsuren, H. Kobayashi, Y. Hasegawa, Y. Mushimoto, H. Li, S. Fukuda, Y. Shigematsu, T. Fukao, S. Yamaguchi, A novel molecular aspect of Japanese patients with medium-chain acyl-CoA dehydrogenase deficiency (MCADD): c.449-452delCTGA is a common mutation in Japanese patients with MCADD, Mol. Genet. Metab. 96 (2009) 77–79.
[18] B. Wilcken, V. Wiley, J. Hammond, K. Carpenter, Screening newborns for inborn errors of metabolism by tandem mass spectrometry, N. Engl. J. Med. 348 (2003) 2304–2312.
[19] M. Kimura, T. Yamamoto, S. Yamaguchi, Automated metabolic profiling and interpretation of GC/MS data for organic acidemia screening: a personal computer-based system, Tohoku J. Exp. Med. 188 (1999) 317–334.
[20] G. Tajima, N. Sakura, H. Yofune, Y. Nishimura, H. Ono, Y. Hasegawa, I. Hata, M. Kimura, S. Yamaguchi, Y. Shigematsu, M. Kobayashi, Enzymatic diagnosis of medium-chain acyl-CoA dehydrogenase deficiency by detecting 2-octenoyl-CoA production using high-performance liquid chromatography: a practical confirmatory test for tandem mass spectrometry newborn screening in Japan, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 823 (2005) 122–130.
[21] K. Yokoi, T. Ito, Y. Maeda, Y. Nakajima, A. Ueta, T. Nomura, N. Koyama, I. Kato, S. Suzuki, Y. Kurono, N. Sugiyama, H. Togari, Acylcarnitine profiles during carnitine loading and fasting tests in a Japanese patient with medium-chain acyl-CoA dehydrogenase deficiency, Tohoku J. Exp. Med. 213 (2007) 351–359.
[22] R. Ensenauer, J.L. Winters, P.A. Parton, D.F. Kronn, J.W. Kim, D. Matern, P. Rinaldo, S.H. Hahn, Genotypic differences of MCAD deficiency in the Asian population: novel genotype and clinical symptoms preceding newborn screening notification, Genet. Med. 7 (2005) 339–343.
[23] M.B. Shapiro, P. Senapathy, RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression, Nucleic Acids Res. 15 (1987) 7155–7174.
[24] E. Buratti, M. Chivers, J. Kralovicova, M. Romano, M. Baralle, A.R. Krainer, I. Vorechovsky, Aberrant 5′ splice sites in human disease genes: mutation pattern, nucleotide structure and comparison of computational tools that predict their utilization, Nucleic Acids Res. 35 (2007) 4250–4263.
[25] P.A. Frischmeyer, H.C. Dietz, Nonsense-mediated mRNA decay in health and disease, Hum. Mol. Genet. 8 (1999) 1893–1900.
[26] M.J. Nichols, C.A. Saavedra-Matiz, K.A. Pass, M. Caggana, Novel mutations causing medium chain acyl-CoA dehydrogenase deficiency: under-representation of the common c.985 A>G mutation in the New York state population, Am. J. Med. Genet. A 146A (2008) 610–619.
[27] H.I. Woo, H.D. Park, Y.W. Lee, D.H. Lee, C.S. Ki, S.Y. Lee, J.W. Kim, Clinical, biochemical and genetic analyses in two Korean patients with medium-chain acyl-CoA dehydrogenase deficiency, Korean J. Lab. Med. 31 (2011) 54–60.
[28] K. Carpenter, V. Wiley, K.G. Sim, D. Heath, B. Wilcken, Evaluation of newborn screening for medium chain acyl-CoA dehydrogenase deficiency in 275 000 babies, Arch. Dis. Child. Fetal Neonatal Ed. 85 (2001) F105–F109.
[29] H.R. Seddon, A. Green, R.G. Gray, J.V. Leonard, R.J. Pollitt, Regional variations in medium-chain acyl-CoA dehydrogenase deficiency, Lancet 345 (1995) 135–136.
[30] T.G. Derks, T.S. Boer, A. van Assen, T. Bos, J. Ruiter, H.R. Waterham, K.E. Niezen-Koning, R.J. Wanders, J.M. Rondeel, J.G. Loeber, L.P. Ten Kate, G.P. Smit, D.J. Reijngoud, Neonatal screening for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in The Netherlands: the importance of enzyme analysis to ascertain true MCAD deficiency, J. Inherit. Metab. Dis. 31 (2008) 88–96.

Newborn Screening for Inborn Errors of Metabolism in Japan

A History of the Development of Newborn Screening

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Abstract

In 1977, the Ministry of Health and Welfare (MHW) directed prefectural officials in charge of maternal and child health to start publicly funded newborn mass-screening (NBS) for phenylketonuria (PKU), maple syrup urine disease (MSUD), histidinemia, homocystinuria and galactosemia and a study group of MHW formulated the treatment guideline for the target diseases. In 1980, MHW launched the Japan Cooperative Project on Special Formula (JCPSF) to ensure a stable supply of special formula and also organized the committee for JCPSF. From 1977 to 2003, a study group of MHW conducted a follow-up study of the patients detected by the screening. From the follow-up it was concluded that dietary therapy was unnecessary for histidinemia and the screening for the disease was discontinued. In 1995, the guideline for the treatment of PKU was revised to keep lower blood phenylalanine levels. The guideline committee for the treatment of BH_4 deficiency was establish in 1996 to obtain better prognosis. In 2012, the MHW decided to initiate publicly funded NBS using MS/MS for inborn errors of amino acid, organic acid, and fatty acid metabolism. The Japanese nationwide NBS has been performed for 35 years. This paper reviews the Japanese history of the development of NBS which has enabled more IEM patients to lead active and productive lives today.

Ref: *Ped. Endocrinol. Rev. 2012;10(suppl 1):8-25*

Keywords: *Newborn screening, Treatment guidelines, Outcome surveys, Inborn errors of metabolism, Amino acid metabolism, Organic acid metabolism, Fatty acid metabolism, Tandem mass spectrometry*

Introduction

In 1934 Følling(1) in Norway described patients, several of them sibs, who excreted phenylpyruvic acid and were mentally deficient. In 1940 Jervis(2) in the U.S. found that phenylalanine (Phe) concentration in blood and cerebrospinal fluid of these patients was elevated, and in 1953 the same author(3) demonstrated that the phenylalanine hydroxylase of the liver was inactive in these patients. In 1953 and 1954, Bickel *et al.* (4, 5) reported that a diet low in Phe was effective for abnormal behavior and biochemistry in these patients and subsequently, many investigators showed that dietary treatment for phenylketonuria (PKU) was most effective if initiated soon after birth and low blood phenylalanine level was kept for a long period of time.

Robert Guthrie in Cancer Research Institute in Buffalo in the State of New York in U.S. developed a new Bacterial Inhibition Assay (BIA) Method to determine a tiny amount of Phe in the newborn blood taken on a filter paper and demonstrated the usefulness of the early detection of PKU by the screening using this procedure in James Town, Virginia in 1961(6, 7).

Regarding PKU therapy in Japan during this period, the first case where dietary therapy was used was a male infant

born in 1958 and diagnosed with PKU when he was 8 months old at a hospital in Tokyo (8), and whose developmental quotient (DQ) at the time was 53. The patient was treated with low-Phe formula imported from the United States. His facial expression improved, convulsions disappeared, and the DQ at the age of 2.5 years recovered to around 70. Subsequently, the patient left Japan because of difficulties in obtaining a low-Phe diet and went to the United States for treatment up to the age of 6 years. His intelligence quotient (IQ) score improved to almost 80. Then, he returned to Japan because, at the time, it was thought that an IQ score would not reduce at 6 years or older even if treatment were discontinued. The patient remains in Japan, untreated, without having visited a medical institution specializing in PKU. He visited the Department of Pediatrics, Nihon University Hospital, when he was 22 years old. However, his IQ score had already reduced to 25. A low-Phe diet was readministered but had little effect. Thus, he had an unfortunate outcome.

The second case was an infant born in 1962 who had received appropriate dietary therapy at the age of 10 months and later had a favorable outcome because the attending doctor was Dr. Toshiaki Oura (Department of Pediatrics, Osaka City University Medical School), who studied dietary therapy for PKU in the United States (9). The low-Phe formula used at this time was Lofenalac, manufactured by Mead Johnson, Inc., in the United States. The company donated the amount required to treat 2 patients for a year because the patient had a sibling. Professor Toshio Takai of the Department of Pediatrics, Osaka City University Medical School, submitted a request for low-Phe formula production to Snow Brand Milk Products Co. Ltd., while treating the patients with the donated Lofenalac. Simultaneously, he facilitated procedures to obtain approval from the Ministry of Health, Labour and Welfare and for the National Health Insurance (NHI) price listing. Thus, Snow Brand Milk Products Co. Ltd., developed the high-quality, low-Phe Lophemilk, which was registered as a pharmaceutical. PKU dietary therapy has been covered by insurance since 1963.

In the United States, Dr. Guthrie initiated newborn mass-screening for PKU in 1961. In Japan, milk production for PKU therapy began in 1962, and this milk was first used as a pharmaceutical in 1963. Thus, a wide gap exists between Western countries and Japan.

Preparation for nationwide newborn screening (1964-1976)

1) Set-up newborn screening(NBS) system for inborn errors of metabolism (IEM) in Japan

Before Guthrie method was widely accepted to be used in newborns, the diagnostic method for PKU involving a urinary ferric chloride test, employed in Europe and the United States, was found to generate many false-negative results. Thus, the Guthrie test, using blood samples collected from newborns, has been examined in Japan since 1966 by the study group of the Ministry of Health and Welfare (10).

Thereafter, several Japanese researchers including myself visited Dr. Guthrie, who was affiliated with the State University of New York, Buffalo, in the United States to learn the method for measuring small amounts of amino acids using the bacterial inhibition assay (BIA) (11) and screening methods for galactosemia using the Beutler (12) and Paigen-phage tests (13). I visited Dr. Guthrie's laboratory in 1973 to learn screening methods for various diseases and on the way back to Japan, visited Dr. Harvey Levy, Professor of Pediatrics, Harvard University School of Medicine and Chairman of Newborn Screening Center, at the Massachusetts State Laboratory, to study actual screening tests and enquire about system construction (14). Dr. Levy talked about the test performance of the Guthrie test and the screening results of amino acid metabolic disorders using paper chromatography of urine samples at the Massachusetts State Screening Center (15). At the same time, he detailed the revenue imbalance in a year between screening and treatment costs at the center and the expenses for caring for disabled infants who were as-yet unscreened and who had received no appropriate treatment, i.e., the former was much lower than the latter (16). Dr. G. M. Komrower of the United Kingdom reported similar results in 1974 (17). We referred to the literature to calculate the revenue balance if NBS for PKU were conducted in Japan and reported the results in detail (18). According to the literature, the incidence of PKU in Japan at the time was unknown. According to reports from the United States and Europe, 2-3 in 100 children who had developmental delay and were housed in facilities had PKU. We conducted PKU screening using a ferric chloride test of the urine samples from approximately 600 children housed in facilities for children with developmental delay in Kanagawa Prefecture, and found only 2 children with PKU. Thus, PKU incidence in Japan was estimated to be approximately one-fifth of that in Western countries. Thus, newborn mass-screening employed in Japan would have to be more effective than the screening methods employed in Western countries. For example, Guthrie's test (19), which allows simultaneous screening for multiple treatable IEM in a single test, should be employed. The number of researchers who actually used the method was increasing. The method was to be examined from the viewpoints of techniques and maternal and child health by doctors, laboratory technicians, and public health experts. The Japan Society of Screening for

Metabolic Disorders & Related Diseases (today's Japanese Society for Mass-screening) was created in 1973, and the first seminar was held the same year (20). The second workshop was held in March 1974.

A study group for screening for IEM (principal investigator: Yutaka Moriyama) was launched in 1974, funded by a grant for the prevention of psychosomatic disorders from the Ministry of Health and Welfare (21). A test on amino acid metabolism using the BIA method was initiated in various regions across Japan for PKU, maple syrup urine disease (MSUD), homocystinuria (HCU), histidinemia, tyrosinemia, and galactosemia. A screening test involving the Paigen and Beutler tests was initiated nationwide for galactosemia and galactokinase deficiencies. A PKU screening test detected PKU in 8 of approximately 410,000 newborns. Thus, the incidence was 1 in almost 50,000 newborns. More newborns should have been tested to determine the incidence of IEM in Japan.

Thus, the study group launched in 1974 by the Ministry of Health and Welfare conducted newborn mass-screening by BIA, resulting in high accuracy for each disease. However, tyrosinemia screening by measuring tyrosine in the blood often generated false-positive results. Additionally, tyrosinemia incidence in Japan was low. Some members of the study group insisted that tyrosinemia should be excluded. Accordingly, only PKU, MSUD, HCU, histidinemia, and galactosemia were included in newborn mass-screening for IEM, covered by public funds.

2) A guideline for treatment of diseases to be screened

In 1977, a study group of the Ministry of Health and Welfare formulated and recommended a guideline for the treatment of diseases to be screened (22). This guideline will be revised because it has been followed in Japan until 1995.

a) PKU

The daily Phe requirement vary with age (**Table 1**); a higher amount is required during the growing period. Caution regarding the amount of Phe administered should be exercised. Thus, if Lophemilk were formulated at a standard concentration, the lack of Phe would cause deficiency symptoms. Therefore, breast milk or formula for infants should be added to compensate for the lack of Phe. The amount of Phe required varies among individuals; therefore, Phe intake should be determined by measuring blood Phe levels as frequently as possible.

However, when this guideline was formulated, Japanese doctors lacked experience in treating PKU in older children. Thus, children aged 3 years or older were treated according to the same guideline.

Age	Phenylalanine intake (mg/kg/day)
0-3 months	70-50
3-6 months	60-40
6-12 months	50-30
1-2 years	45-25
2-3 years	40-20
Over 3 years	35-20

Table 1. *Recommended treatment guidelines (PKU). (Reproduced by permission from Ref. #22)*

The above phenylalanine (Phe) intake was utilized as a tentative indicator to start treatment. Phe intake analysis is aimed at maintaining fasting blood Phe levels at 4-8 mg% during infancy and 4-12 mg% during early childhood. The maintenance intake varies with each individual. Thus, blood Phe levels should be measured as frequently as possible (2-3 times a week), particularly for a month after starting treatment. Caution should be exercised regarding clinical symptoms, weight gain, and serum protein and hemoglobin levels to prevent Phe-deficiency symptoms. For 1 month and later after starting treatment, blood Phe levels should be measured once a week during infancy and once or twice a month during early childhood and later. Particularly, for 1 or 2 months after starting treatment, a patient should be hospitalized at a PKU-specialized hospital to establish the treatment course. Low-Phe formula is used as a major protein source. The lack of Phe should be compensated for with natural protein (milk formula for infants, breast milk, or general diet). Treatment should be continued while physical growth, DQ, electroencephalography (EEG) findings, etc., are observed on a regular basis.

b) MSUD

Delayed diagnoses result in severe symptoms such as feeding difficulties, respiratory failure, and convulsions. Formula for MSUD treatment should be immediately provided to treat patients with high blood leucine levels determined by the Guthrie test.

This formula is free of leucine, isoleucine, and valine. Thus, the diet therapy should be continued to maintain branched-chain amino acid levels at 2-5 mg/dL according to the guideline in **Table 2**, by frequently measuring blood amino acid levels.

	Branched-chain amino acid intake(mg/kg/day)		
	Leucine	Isoleucine	Valine
0-3 months	160-80	70-40	90-40
3-6 months	100-70	70-50	70-50
6-12 months	70-50	50-30	50-30

Table 2. *Recommended treatment guidelines (MSUD). (Reproduced by permission from Ref. #22)*

The branched-chain amino acid intake above was utilized as a tentative indicator for initiating treatment. Determination of the branched-chain amino acid intake was aimed at maintaining fasting blood branched-chain

amino acid levels at 2-5 mg%. The maintenance dose varies with each individual. Thus, blood branched-chain amino acid levels should be measured daily or every 2 days, particularly for a month after starting treatment. Caution should be exercised regarding clinical symptoms, weight gain, and serum protein and hemoglobin levels to prevent branched-chain amino acid deficiency symptoms. For 1 month and later after starting treatment, blood branched-chain amino acid levels should be measured once a week during infancy for continuing treatment. Particularly, for 1 or 2 months after starting treatment, a patient should be hospitalized at a MSUD-specialized hospital to establish the treatment course. Free of branched-chain amino acid formula is used as a major protein source. The lack of branched-chain amino acids should be compensated for with natural protein (formula for infants, breast milk, or general diet). The treatment should be continued while physical growth, DQ, EEG findings, etc., are observed on a regular basis.

c) **HCU (22, 23)**

Dietary therapy with low-methionine and high-cystine diets should be administered for 6 months for infants diagnosed with HCU. Subsequently, the infants should be hospitalized when they are 6 months old and fed general diets, followed by 10-day oral administration of 40 mg pyridoxine/kg/day. If blood methionine and urine homocystine levels are decreased, the infants are considered vitamin B6-responsive and should be treated by continuously administering the minimum required amount of vitamin B6. Pyridoxine should be discontinued for infants who are nonresponsive to vitamin B6. They should be diagnosed as vitamin B6-nonresponsive, and the dietary therapy should be continued.

Dietary therapy for infants who are vitamin B6-nonresponsive is conducted as shown in **Table 3**.

	Methionine (mg/kg/day)	Cystine (mg/kg/day)
9-6 months	40	150
6 months-1 year	20	150
Over 1 year	10-15	150

Table 3. *Recommended treatment guidelines (HCU). (Reproduced by permission from Ref. #22)*

The above methionine-cystine intake was utilized as a tentative indicator for initiating treatment. Determination of methionine intake is aimed at maintaining fasting blood methionine levels at ≤1.0 mg%. The maintenance dose varies with each individual. Thus, blood methionine levels should be measured as frequently as possible, particularly for a month after starting treatment. Caution should be exercised regarding clinical symptoms, weight gain, serum protein levels, and hemoglobin levels. For 1 month and later after starting treatment, blood methionine levels should be measured once a week for continuing treatment. Particularly, for 1 or 2 months after starting treatment, a patient should be hospitalized at an HCU-specialized hospital to establish the treatment course. Low-methionine and high-cystine formula is used as a major protein source. The lack of methionine should be compensated for with natural protein (formula for infants, breast milk, or general diet). The treatment should be continued while physical growth, DQ, liver function, platelet adhesion test, EEG, ophthalmologic findings (ectopia lentis), etc., are observed on a regular basis.

d) **Histidinemia**

In 1977, mandatory newborn mass-screening in Japan was initiated, histidinemia was included as a target disease for screening in some Western countries because this disease was considered to cause speech disturbance and/or mental retardation on a normal diet(24), however, these symptoms could be prevented if patients were placed on low-histidine diet in newborn period. Therefore, histidinemia was included as a target disease for newborn screening in Japan as well (22). So, patients with histidinemia were treated with low-histidine or histidine-free formula to maintain blood histidine levels at ≤10 mg/dL. However, some older children and adults with histidinemia in the patients' families were found to lead a normal life without treatment. And no difference in IQ score was noted between infants who did and did not receive low-histidine diet. Therefore, dietary restrictions were gradually eased. In 1992, it was concluded that dietary therapy was unnecessary for histidinemia, and it was excluded as a target disease for screening(25).

e) **Galactosemia** (22)

Treatment should be initiated immediately if galactosemia is suspected in mass-screening (**Figure 1**). Milk with lactose replaced by another sugar such as soluble oligosaccharide(dextrin) and glucose should be used because lactose contains galactose. Galactose removal from diets does not lead to any issues because it is not an essential sugar. Thus, galactosemia can be treated relatively easily. Soybean protein is used in Bonlact. Therefore, Bonlact contains no lactose and can be used to treat galactosemia. Sugar must be added to sugar-free milk. Caution should be exercised regarding increase in osmotic pressure when a monosaccharide is added to milk formula. Protein and energy levels do not affect galactosemia.

1. Breast milk, etc., should be discontinued immediately after diagnosis. Next, dietary therapy with lactose-free milk should be initiated. Intake of lactose-containing food should be avoided during the weaning period and later.
2. Treatment should be continued while liver function, urinary findings, physical growth, DQ, EEG, ophthalmology findings (cataract), etc., are observed on a regular basis.

Figure 1. *Recommended treatment guidelines (galactosemia). (Reproduced by permission from Ref. #22)*

Thus, newborn mass-screening for IEM was initiated, creating a system for conducting medical examination and treatment immediately after detection. However, some special formula for treatment of IEM was not approved as a pharmaceutical and awaited NHI price listing. As described below, the Ministry of Health and Welfare set up the Special Formula for the Treatment of IEM Office (Special Formula Office) in the Imperial Gift Foundation Boshi-Aiiku-Kai in 1980 to support for the dietary treatment of IEM.

Start of Newborn Mass-Screening for IEM (1977)

1) Establishment of the Japan Cooperative Project on Special Formula (JCPSF)

In October 1977, the Children and Families Bureau of the Ministry of Health and Welfare directed prefectural officials in charge of maternal and child health to start carrying out publicly funded newborn mass-screening for IEM. The officials in charge held a meeting with representatives from medical associations, the Japan Association of Obstetricians and Gynecologists, medical institutions specializing in metabolic disorders, screening centers, etc., to initiate the newborn mass-screening according to the instructions of the Ministry of Health and Welfare. Thus, newborn mass-screening by the Guthrie test was initiated nationwide in October 1977. However, its implementation rate was 80-90% at the end of 1979 because some local governments delayed taking action. However, the implementation rate subsequently increased to 95-105%.

The Maternal and Child Health Divisions of the Ministry of Health, Labour and Welfare and the prefectural governments mainly promoted the implementation of this newborn mass-screening. Additionally, the Japan Medical Association, Japan Pediatric Society, Japan Society of Obstetrics and Gynecology, Japanese Society for Inherited Metabolic Diseases, Japanese Society for Mass-screening, and various academic institutions made contributions. Furthermore, dietary therapy with special formula was critical for preventing disorders caused by IEM in children. As described above, the Ministry of Health, Labour and Welfare launched JCPSF (26) in 1980 to ensure a stable supply of special formula for the treatment of IEM and its quality management and improvement, if necessary. The ministry also organized the Committee of JCPSF, consisting of physicians, special formula manufacturers, and nutritionists, to facilitate their participation in the project.

2) Follow-up study to examine the effectiveness of NBS

The Special Formula Office took charge of follow-up for approximately 20 years between 1983 and 2003, funded by a grant for psychosomatic disorder research from the Ministry of Health and Welfare (27).

Additionally, the Special Formula Office issued a handbook for treatment, Food Exchange List for Phenylketonuria - Dietary Therapy in 1989 (28), and Diet Therapy Guidebook - for inborn errors of amino acid metabolism in 1998 (29), revising them in 2004 to improve outcomes (30).

In 1992, Aoki *et al.* (31) investigated the IQ score and education status of schoolchildren with IEM identified in mass-screening. According to the results, the IQ scores of PKU patients aged 6-10 years and treated according to the criteria of 1977 were ≥85 (about 75%), 71-84 (about 15%), and 51-70 (about 10%). As shown in **Table 4**, the IQ scores of 36 patients diagnosed as classical PKU (serum Phe level: ≥16.5 mg/dL) were 72-136 (average: 103 ± 13) after treatment.

Disease	IQ (M±SD)	Max.	Min.
PKU	103±13 (n=36)	136	72
HPA	111±10 (n=10)	125	89
BH_4 deficiency	81±27 (n=4)	124	50
MSUD	71±23 (n=10)	103	35
HCU	72±29 (n=8)	101	20

Table 4. Median IQ by disease. (Reproduced by permission from Ref. #31)

The IQ scores of 10 patients with hyperphenylalaninemia (HPA) (serum Phe level at diagnosis: <5.0-16.5 mg/dL) were 89-125 (average: 111 ± 10) after treatment, slightly higher than that for those with classical PKU. Academic performance and problems with school life for the target diseases of newborn mass-screening are detailed in **Table 5**.

The academic performance of children with HPA (low blood Phe levels) was better than that of those with classical PKU (high blood Phe levels). School life was more problematic for those with classical PKU than for those with HPA. In 1992, the survey of Owada, *et al.*(32) demonstrated that infants with serum Phe levels lower (maintained at 2-8 mg/dL) than that indicated by the guideline had an IQ score of ≥ 100 and those with higher Phe levels had a lower IQ score at 4-6 yeas of age. The follow-up survey conducted by the Committee of JCPSF, the Maternal and

Child Health Center, Boshi-Aiiku-Kai, demonstrated that of 115 patients aged ≥3 years, 21 (18.3%) had a final IQ score of <90. The relationship between serum Phe levels and IQ scores at the time of IQ scoring is shown in **Figure 2** (33, 34), suggesting a significant negative correlation (-0.419). Patients with higher serum Phe levels had significantly lower IQ scores. Thus, maintaining low serum Phe levels was considered critical for improving the IQ scores of PKU patients.

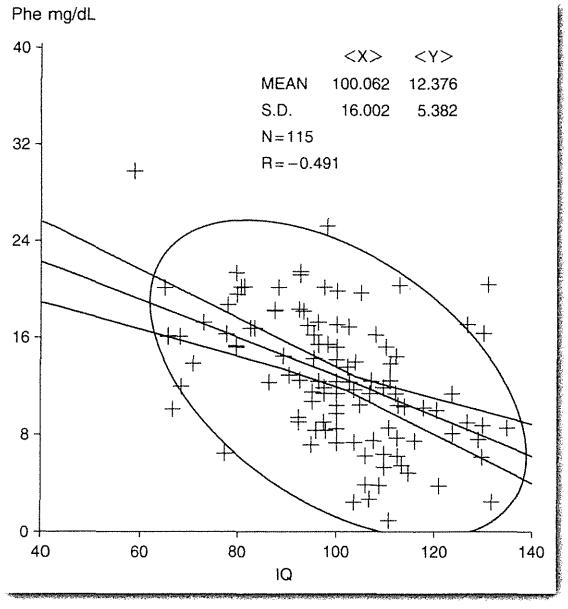

Figure 2. Relationship between final IQ scores and blood Phe levels. (Reproduced by permission from Ref. #34)

In Europe and the United States, special education was required for 12-34% of patients with PKU who were treated early. It is notable that the patients often made errors in spelling and calculation (35). Thus, the treatment guidelines in various countries were revised. The IQ scores of 10 MSUD patients were 35-103 (average: 71 ± 23), lower than the scores of those with classical PKU. The IQ scores of 8 HCU patients were as low as those of the MSUD patients (**Table 4**). Many students with MSUD demonstrated poor academic performance and encountered problems with school life; students with HCU demonstrated slightly better academic performance than those with MSUD (31). Neither patients with galactosemia type I nor those with galactokinase deficiency faced problems with school life if treated early (**Table 5**).

Revision of PKU Treatment Guideline (33, 34) (1995)

JCPSF referred to the results of the PKU survey above to organize the PKU Treatment Guideline Revision Committee in 1995 to collect literature from Japan and abroad and to evaluate the follow-up survey results. The blood Phe maintenance ranges and intakes by age (**Figure 3[A][B]**) were revised, and recommended protein and energy intakes in the guideline were described with consideration to the affected infants' growth (33, 34).

This was because serum Phe levels are abnormally increased in PKU patients with markedly-lowed protein intake and insufficient energy intake in spite of the appropriate restrictions on Phe intake.

Disease	Problem		Grades			Change in grades			Difference in grades between siblings		
	Yes	No	Good	Fair	Poor	Improved	No change	Deteriorated	No difference	Better	Worse
PKU	4	68	19	43	10	12	39	2	26	7	15
HPA	0	13	4	9	0	0	13	0	6	0	0
BH_4 deficiency	2	4	1	3	2	0	6	0	1	0	3
MSUD	5	11	0	6	9	1	9	1	1	0	7
HCU	1	8	1	5	3	1	7	1	2	2	5
Galactosemia	0	6	0	5	1	1	4	0	3	0	1

Table 5. Problems and academic performance in school. (Reproduced by permission from Ref. #31)

1) The presence of abnormal biopterin metabolism should be examined for newborns with HPA identified in newborn mass-screening, and if no abnormality is found, protein intake is within the normal range (2-3 g/kg/day), and blood Phe level exceeds 10 mg/dL, dietary therapy should be started by day 20 after birth. If the blood Phe level is below 10 mg/dL, the patient should be followed up for several days. If the blood Phe level continues to exceed 7 mg/dL, dietary therapy should be started.

2) Newborns should be treated with blood Phe levels maintained at ≤ 10 mg/dL through appropriate restrictions on Phe intake. Next, the Phe intake should be adjusted to reduce blood Phe levels to 2-4 mg/dL. The intake should be determined by measuring blood Phe levels daily because the tolerability of Phe varies among cases. Such initial treatment requires hospitalization.

3) The maintenance ranges of blood Phe levels are shown in **A**.

4) Recommended Phe intakes by age are shown in **B**, although the tolerability of Phe varies among patients. At 1 month and later after starting treatment, the Phe intake should be adjusted by measuring blood Phe levels once a week during infancy and once or twice a month during early childhood. The Phe levels of blood samples collected on filter paper are determined by the Guthrie test or high-performance liquid chromatography (HPLC) method, and serum Phe levels are measured with an amino acid analyzer.

A Maintenance range of blood Phe levels

Infancy – Early childhood:	2-4 mg/dL
Latter half of early childhood – Early half of elementary school:	3-6 mg/dL
Latter half of elementary school:	3-8 mg/dL
Junior high school:	3-10 mg/dL
Afterward:	3-15 mg/dL

B Recommended Phe intakes by age

Age	Phe intake (mg/kg/day)
0-3 months	70-50
3-6 months	60-40
6-12 months	50-30
1-2 years	40-20
2-3 years	35-20
3 years and up	35-15

5) The daily energy intakes of affected infants should be the same as those of healthy children of the same age. The protein distribution ratios of the affected infants are slightly lower than that of healthy children. Thus, carbohydrate should be sufficiently supplemented to compensate for energy deficiency.

6) Protein (nitrogen source) intake should be adjusted to maintain 2 g/kg/day during infancy, 1.5-1.8 g/kg/day during early childhood, and 1.0-1.2 g/kg/day during the schooling period and later (if protein intake is ≤ 0.5 g/kg, caution should be exercised for serum Phe levels that increase in spite of restrictions on Phe intake). Most proteins are to be ingested from formula for treatment to provide Phe as a natural protein within a range that maintains the blood Phe levels shown in **B**. The recommended intakes of formula for treatment are as follows: 60-150 g/day during infancy, 150–200 g/day during early childhood, and 200-300 g/day during the schooling period and later.

7) As a rule, a patient should visit a hospital every 4 weeks until entry to elementary school to conduct blood Phe level and physical measurements. General blood tests and blood biochemical analyses should be conducted every 3 months. Intelligence development tests (Tsumori-Inage Developmental Quotient Test for patients up to 3 years old and IQ test for those 3 years and older) are to be performed on a regular basis. Additionally, EEG and cerebral imaging should be conducted as needed.

8) The above dietary therapies should be continued until adulthood, or for the rest of the patient's life, if possible.

Figure 3. Recommended treatment guidelines (1995 revision). (Reproduced by permission from Ref. #34)

Age	Country	Japan 1995–	Britain 1993–	Germany 1997–1999
Infancy (age 0)		2-4 mg/dL	2-6 mg/dL	0.7-4.0 mg/dL
Early childhood (age 1-5 years)	First half			
	Latter half	3-6 mg/dL		
Elementary school (age 6-12 years)	First half		2-8 mg/dL	
	Latter half	3-8 mg/dL		0.7-15.0 mg/dL
Junior high school (age 13-15 years)		3-10 mg/dL		
≥15 years		3-15 mg/dL	2.0-11.7 mg/dL	0.7-20.0 mg/dL

Table 6. Comparison of blood Phe level maintenance ranges in PKU treatment guidelines among Japan, Britain, and Germany. (Adapted by permission from Ref. #36)

The Japanese guideline for PKU treatment, revised in 1995 (33, 34), was compared with those formulated in Western countries in 1993 and later (36). The Japanese guideline was similar to those of the United Kingdom and Germany (**Table 6**). In 2000, Aoki *et al.* (37) compared the blood Phe levels of patients born before or after the PKU treatment guideline revision to demonstrate that the Phe levels of affected infants aged 2 months to 4 years were significantly lower after the revision, rather than before it (**Table 7**).

Thus, the prognoses of affected infants in Japan were comparable with those of PKU infants in Western countries.

	Before guideline revision (1977–1995)		After guideline revision (1996–2000)	
Age	Mean	SD	Mean	SD
0-1 month	23.40	8.32	23.95	7.92
1-2 months	13.60	8.70	13.32	10.62
2-6 months*	8.19	3.87	5.40	5.13
6-12 months**	7.09	3.62	4.96	2.73
1-2 years	7.97	3.83	4.28	2.49
2-3 years	8.92	4.42	5.41	3.77
3-4 years	9.63	5.12	6.30	4.08

*$p<0.01$, **$p<0.001$

Table 7. Changes in blood Phe levels (mg/dL) after guideline revision. (Reproduced by permission from Ref. #37)

Diagnosis and Treatment of HPA Caused By Tetrahydrobiopterin (BH_4) Deficiency and BH_4-Responsive HPA

1) BH_4 deficiency

Newborn mass-screening for IEM had been successfully conducted nationwide, to identify patients with high blood Phe levels. Dietary therapy with low-Phe formula had been properly carried out to ensure more PKU patients with normal intelligence. However, a small number of patients with HPA exhibited lower intelligence even if they had received early treatment with low-Phe formula (38, 39). The HPA was caused by re-cycling or defective synthesis of BH_4 (coenzyme of Phe hydroxylase), suggesting that BH_4 should be administered early for treatment. Patients found with high blood Phe levels in screening should be immediately differentiated in terms of their causes: defect of BH_4 regenerating or synthetic systems or genetic abnormalities in Phe hydroxylase (enzyme that converts Phe into tyrosine). Next, the patients should be appropriately treated. In 1996, a review committee of an interim recommended guideline for the treatment of BH_4 deficiency was established in the Special Formula Office (40).

a) Diagnosis of BH_4 deficiency

Urine samples should be collected from newborns with HPA before starting treatment, to which 1 mg of ascorbic acid is added per 1 mL of sample for cryopreservation. Subsequently, neopterin, biopterin, and BH_4 are immediately measured at a specialized laboratory or one's own facility.

Instead of low-Phe formula, an aqueous solution of BH_4 is orally administered with a feeding bottle (10 mg per kg of body weight). Blood samples are collected on filter paper every hour for 4 hours and subsequently at 8, 12, and 24 hours to measure blood Phe levels by BIA or high-performance liquid chromatography (HPLC). The newborn is fasted for 30 minutes after the oral administration of BH_4 and then fed with formula for infants. Along with blood Phe measurement, urine samples at 12 and 12-24 hours after BH_4 administration are transferred into containers and are cryopreserved for the measurement of a pterin compound in urine. Changes in the measurements before and after BH_4 administration are observed to determine a disease type. For patients with low blood Phe levels, Phe (100 mg/kg) should be orally administered along with BH_4 to examine the changes in the blood Phe levels. For differential diagnosis, please refer to **Table 8** to determine a disease type. Patients with 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency can be treated without administering low-Phe formula because BH_4 can maintain serum Phe levels within a normal range. In patients with dihydropteridine reductase (DHPR) deficiency, serum Phe levels may not be significantly lowered by the

oral administration of BH_4 (10 mg/kg). Thus, BH_4 should be increased to 20 mg/kg and the responses observed. The methods developed by Fukushima and Nixonn (41) and Suzuki and Owada (42) have been widely used to measure biopterin in the urine and cerebrospinal fluid. In patients for whom it is difficult to make a differential diagnosis between DHPR and PTPS deficiency, erythrocyte DHPR enzyme activity should be measured to confirm the diagnosis (43). We measured changes in blood Phe levels before and after BH_4 administration and urinary pterin levels in one of GTPCH deficiency, two of DHPR deficiency and 14 cases of PTPS deficiency. The results were the same as the data presented to **Table 8**.

b) Treatment of BH_4 deficiency (39)

Patients with BH_4 deficiency should receive oral administration of BH_4 with reference to the BH_4 doses listed in **Table 9**. In patients with DHPR deficiency, the BH_4 doses should be increased to reduce serum Phe levels. Low-Phe formula should be included if increased doses are ineffective.

Please refer to **Table 9** for the recommended doses of neurotransmitter precursor(40). Continued administration of insufficient doses causes side effects such as hypotonia, wheezing, opisthotonus, acampsia, spasm, and developmental delay at 2-3 months of age. On the contrary, excessive doses cause side effects such as bad temper, insomnia, poor appetite, agitation, and diarrhea. Thus, patients should be closely observed to achieve an appropriate dose.

Similar recommended doses of neurotransmitter precursor for the treatment of BH_4 deficiency are described in the literature(44).

For patients with DHPR deficiency, in addition to the above treatment, 5-15 mg of folinic acid should be administered daily because of the decreased amounts of folate in the serum and brain (40) and in cerebrospinal fluid(44) are described. In Japan, 5-10 mg of leucovorin is administered to some patients with DHPR deficiency.

	Urinary pterin (pteridine) analysis				Changes in blood Phe levels after oral administration of BH_4 (10 mg/kg)*	DHPR activity in blood (filter paper)
	Neopterin (Ne)	Biopterin (TB)	Ne/TB	% BH_4		
<BH_4 deficiency>						
1. GTPCH deficiency	Markedly low	Markedly low	Normal	Markedly low	Sharp decline	Normal
2. PTPS deficiency	Markedly high	Markedly low	Markedly high	Markedly low	Sharp decline	Normal
3. DHPR deficiency	High	High	Low-normal	Markedly low	Decline	Low
<Phenylalanine hydroxylase deficiency>						
1. Classical PKU	High	High	Low-normal	High	Unchanged	Normal
2. Persistent HPA	High	High	Low-normal	High	Unchanged	Normal

*BH_4 should be combined with Phe (100 mg/kg) if blood Phe levels are ≤6 mg/dL

Table 8. Differential diagnosis of HPA caused by impaired BH_4 synthesis. (Reproduced by permission from Ref. #40)

	6R- BH_4	L-Dopa (Carbidopa)	5-HTP*
Daily dose	2-10 mg/kg/day**	5-15 (0.5-1.5) mg/kg/day	3-13 mg/kg/day
Frequency of administration	Divided doses (3-4 times, at least 3 times)		

*5-hydroxytryptophan

**If 6R-BH4 is ineffective at 2-10 mg/kg/day in patients with DHPR deficiency, it should be increased to 10-20 mg/kg/day and a low-Phe diet included if needed. In patients with DHPR deficiency, folinic acid (5-15 mg/day) should be included.

Table 9. Doses according to the interim recommended guideline for treatment. (Reproduced by permission from Ref. #40)

Treatment with single-dose administration of BH_4 should be avoided. As a rule, 4 agents, that is, BH_4, L-dopa, carbidopa, and 5-hydroxytryptophan (5-HTP), should be administered. Administering the appropriate dose of L-dopa is critical for treatment.

2) BH_4-responsive HPA

BH_4-responsive HPA is caused by Phe hydroxylase gene mutations. In 1999, Kure *et al.* (45) reported BH_4-responsive phenylalanine hydroxylase (PAH) deficiency, in which blood Phe levels are decreased by BH_4. Their report attracted wide attention in Japan and from abroad. In 2002, an expert committee for the treatment criteria of BH_4-responsive HPA (46) was established in Japan. BH_4 was approved for insurance coverage to be used for the treatment of BH_4-responsive HPA as well as BH_4 deficiency. If the administration of BH_4 alone normalizes blood Phe levels in patients with BH_4-responsive HPA as it does in those with PTPS deficiency, thereby eliminating the need for a low-Phe diet, patients' quality of life (QOL) can be markedly improved.

However, patients with BH_4-responsive HPA who require the combined use of a low-Phe diet exhibit various profiles. For example, tolerability of Phe and responsiveness to BH_4 varies among patients. Thus, detailed instructions regarding BH_4 dose and dietary therapy should be provided.

a) Diagnosis of BH_4-responsive HPA

As described in the section on BH_4 deficiency, a single-dose administration test of BH_4 should be conducted. Patients with blood Phe levels that decrease by ≥20% at 4-24 hours after administration are tentatively considered to have BH_4-responsive HPA (47). A BH_4 administration test can be omitted for classical PKU patients with blood Phe levels ≥20 mg/dL. Additionally, BH_4 administration is less effective in patients with blood Phe levels ≤6 mg/dL. Hence, 100 mg/kg of body weight Phe is orally administered, followed by 10 mg/kg BH_4. Then, as in the single-dose administration test of BH_4, blood Phe levels are measured over time to make a diagnosis (47). A single-dose administration test of BH_4 was conducted for patients with typical BH_4 deficiency (DHPR or PTPS deficiency), classical PKU, or BH_4-responsive HPA whom we encountered. The time-dependent changes in blood Phe levels are shown in **Figure 4**.

For undiagnosable cases, as described in the section on BH_4 deficiency, pterin compounds in the urine and DHPR activity in the blood on filter paper are measured for diagnosis. Simultaneously, BH_4 is administered thrice a day for a week (total 20 mg/kg of body weight). Then, blood Phe levels and urine pterin compounds are measured for diagnosis (47, 48).

b) Treatment of BH_4-responsive HPA

Please refer to **Figure 5** for the treatment rendered.

The mechanisms of the blood Phe-lowering effects of BH_4 on BH_4-responsive HPA are also being investigated in Japan (49). We await the results.

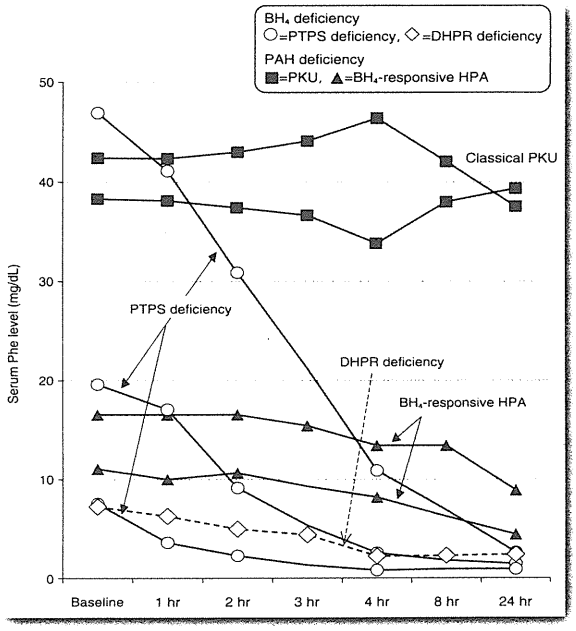

Figure 4. Changes in serum Phe levels in an oral BH_4 loading test.

A normal diet is provided, and as a rule, BH_4 is administered 3 times daily (total 10 mg/kg/day). Subsequent treatment methods are evaluated according to the following treatment criteria based on blood Phe levels while clinical symptoms are observed. Typically, the target blood Phe level during infancy is 2-4 mg/dL, while those of other patients are determined based on the target Phe levels by age, as defined by the PKU Treatment Guideline Revision Committee in 1995.

1. If a serum Phe level reaches the target value and the level is maintained,
   Drug therapy with BH_4 administration should be continued. The BH_4 dose should be decreased as needed depending on changes in blood Phe levels, tolerability of Phe, and clinical symptoms to determine the optimal dose.

2. If a serum Phe level does not reach the target value or the level is not maintained,
   1) The BH_4 dose should be increased as needed depending on changes in blood Phe levels, tolerability of Phe, and clinical symptoms. However, the daily dose should not exceed 20 mg/kg/day, and if adverse effects occur, the BH_4 dose should be decreased or BH_4 administration discontinued.
   2) If a blood Phe level does not reach the target value after 20 mg/kg/day BH_4 administration, a Phe-restricted diet should be included. If BH_4 appears to be ineffective, its administration should be discontinued.
   3) If BH_4 administration becomes difficult because of adverse effects, the dose should be decreased and a Phe-restricted diet should be included, or BH_4 administration discontinued and diet therapy with a low-Phe diet should be conducted.

Clinical symptoms should be closely examined after starting treatment. As a rule, examination should be conducted once a week during infancy and once or twice a month during early childhood and later. Additionally, developmental tests, EEG, and clinical examination should be conducted as needed.

Figure 5. *Interim treatment guideline for BH_4-responsive HPA in 2002. (Reproduced by permission from Ref. #46)*

Situation of Treatment In IEM Patients Found In Newborn Mass-Screening

In 2002, Aoki *et al.* (50) investigated the treatment situation of patients who were born before 1991 and identified in mass-screening. The results are shown in **Table 10.**

PAH deficiency patients with blood Phe levels ≥1000 μM (16.5 mg/dL) were diagnosed as classical PKU, and those with blood Phe levels <1000 μM were diagnosed as non-PKU HPA between 1977 and 1991. Less than 70% of patients with classical PKU were continuously treated with a low-Phe diet until puberty, while less than 11% of HPA patients with blood Phe levels <1000 μM continued dietary therapy after puberty. Thus, patients of this disease type should be instructed to continue treatment with a low-Phe diet in the long term. PTPS deficiency requires no dietary therapy because BH_4 decreases blood Phe levels in patients. Thus, BH_4 deficiency can be treated by administering BH_4 and a neurotransmitter precursor. Therefore, the treatment can easily be continued. However, according to the above survey(49), only 69% of the patients with BH_4 deficiency continued the treatment. There may have been some problems related to the instructions of the attending physicians before 1991. It was very dangerous if they stopped the dietary treatment for both MSUD and HCU. Regrettably, 12-20% of patients with HCU and MSUD died after they discontinued the treatment. Unfortunately, in 15.4% of patients with BH_4 deficiency, an appropriate therapy was discontinued and similar number of patients died.

However, the Special Formula Office reported in 1993 that the average IQ scores of patients with BH_4 deficiency between 1977 and 1990 were markedly improved from 71.0 ± 15.7 in those born between 1977 and 1980 to 97.3 ± 11.8 in those born between 1981 and 1988 (**Table 11**) (51). Among the MSUD patients detected in the screening, the death rate in those born before 1985 was high, and low in those born after 1985. Most HCU patients who died of pulmonary hemorrhage or suffered from mental retardation were identified at the initial stage of the screening. Few patients who died or suffered from mental retardation were identified in the subsequent stages of screening (51).

Disease (No. of cases)	Treatment continued	Treatment stopped or under observation	Death
PKU (serum Phe level ≥ 1000 μM) (176)	68.2% (120)	31.8% (56)	0%
Non-PKU HPA (serum Phe level <1000 μM) (92)	10.9% (10)	89.1% (82)	0%
BH_4 deficiency (13)	69.2% (9)	15.4% (2)	15.4% (2)
MSUD (36)	52.8% (19)	27.8% (10)	19.4% (7)
HCU (25)	48.0% (12)	40.0% (10)	12.0% (3)
Galactosemia type I (20)	10.0% (2)	80.0% (16)	10.0% (2)
Galactokinase deficiency (33)	6.1% (2)	93.9% (31)	0%

Table 10. Patients (≥10 years) who were detected by newborn mass-screening (1977-1991) and their status (Reproduced by permission from Ref. #50)

Case (birth year, gender)	Enzyme disorder	Age when drug therapy started	DQ/IQ	Average IQ
1. 1977, M	PTPS	14 months	IQ 71 (11 years 8 months)	71.0 ± 15.7
2. 1978, M	PTPS	7 months	IQ 95 (13 years)	
3. 1980, M	PTPS	13 months	DQ 50 (8 years)	
4. 1980, F	DHPR	29 months	IQ 80 (6 years)	
5. 1980, F	PTPS	8 months	DQ 59 (7 months) Died at 1 year 4 months old (pulmonary edema)	
6. 1981, F	PTPS	10 months	IQ 120 (5 years 2 months)	97.3 ± 11.8
7. 1983, M	PTPS	1 month	DQ 81 (7 years 9 months)	
8. 1984, M	PTPS		Premature Died at 25 days (necrotizing enterocolitis)	
9. 1985, M	PTPS	7 weeks	IQ 90 (5 years 6 months)	
10. 1986, M	PTPS	1 month	IQ 98 (5 years 6 months)	
11. 1986, M	PTPS	14 days	IQ 100 (5 years 4 months)	
12. 1988, M	PTPS	21 days	DQ 95 (2 years 4 months)	

Table 11. Patients with BH_4 deficiency identified from screening (1977-1990) (Reproduced by permission from Ref. #51)

Incidence Rate of the Target Disease and Cost/Benefit Balance of NBS For IEM

The incidences of the target diseases of newborn mass-screening, surveyed in March 1999, are listed in **Table 12** (51). All incidences in Japan were lower than that of Western countries.

Referring to these incidences and outcomes of the diseases, Hisashige *et al.* examined the cost/benefits of newborn mass-screening in the evaluation methods for mass-screening study group established by the Ministry of Health and Welfare. They reported that the screening, including that for congenital hypothyroidism and congenital adrenal hyperplasia, brought in an annual benefit of >3.1 billion yen. The balance of revenue was favorable because the incidence of congenital hypothyroidism was as high as approximately 1/4,000 persons. As shown in **Table 13**, only the screening for IEM caused a loss of 146,150,000 yen a year (52, 53). In 2007, Ohkusa *et al.* (54) revealed the revenue balance for the newborn mass-screening for inborn errors of amino acid, organic acid, and fatty acid metabolism using tandem mass spectrometry (MS/MS). Their

IEM	No. of cases detected	Incidence rate
Classical PKU	174	1/114,379
HPA	88	1/226,159
(Hyperphenylalaninemias: Total)	(262)	(1/75,962)
BH_4 deficiency	13	1/1,530,926
MSUD	33	1/603,092
HCU	20	1/995,101
Galactosemia type 1	19	1/1,047,475
Galactosemia type 2 (galactokinase deficiency)	29	1/686,277

Classical PKU = Serum Phe level ≥ 1000 μM
HPA = Serum Phe level < 1000 μM

Table 12. Incidence rate of patients with IEM detected by newborn mass-screening (Reproduced by permission from Ref. #51)

Disease	Cost	Benefit	Net benefit	Effect
PKU + HPA*	333,240	838,390	505,150	Effective
MSUD	256,820	61,460	−195,360	Marginally effective
HCU	264,090	19,190	−244,900	Marginally effective
Galactosemia	220,330	9,290	−211,040	Marginally effective
(Subtotal)	1,074,480	928,330	−146,150	
Cretinism	771,270	3,827,900	3,056,630	Effective
Congenital adrenal hyperplasia	1,018,850	1,241,900	223,050	Effective
Total	2,864,600	5,998,130	3,133,530	

*PKU = Serum Phe level ≥ 1000 μM, HPA = Serum Phe level < 1000μM

Table 13. Cost/benefit of newborn mass-screening (1,000 yen) (Reproduced from Ref. #52)

report found that there were almost 150 patients with IEM in a cohort of 1.2 million persons. A cost-benefit analysis demonstrated an incremental benefit-cost ratio of 1.91. Specifically, mass-screening provided benefits that were 1.91 times greater than the increased cost. The authors reported that newborn mass-screening by MS/MS yielded an incremental net benefit of 8.9 billion yen. Thus, the negative net benefit of screening for inborn errors of amino acid metabolism was negated. The screening for IEM produced a net benefit of 8.7 billion yen even if there had been compensation for the negative net benefit from screening for galactosemia.

However, as described by Aoki *et al.* in 1996 (55), the follow-up survey could be conducted with a response rate of almost 100% between 1977 (when the screening was started) and 1990. Subsequently, it has become difficult to conduct a questionnaire survey regarding patients' conditions because the Personal Information Protection Law was enacted. In 2002, the Committee of JCPSF encountered difficulties continuing the survey because the response rate to the questionnaire was as low as ≤50% (56). In the future, the prognoses of patients identified in the mass-screening by MS/MS should be continuously surveyed.

Adults with PKU

As described above, it has become difficult to follow up patients identified from mass-screening. Thus, unfortunately, except for a survey of patients' prognoses based on clinical reports by the attending physicians, no method is available to conduct screening and examine the effectiveness of early treatment. Currently, what those responsible for the screening project want to know most is PKU patients' statuses during adulthood. According to "Phenylketonuria during adulthood", one of the clinical reports published by 9 facilities in the *Bulletin on Special Formula* in 2011, Owada *et al.* (57) reported that, of 20 PKU patients aged 22-32 years, 14 visited medical institutions specializing in PKU on a regular basis. Of the 14 patients, 12 had blood Phe levels <10 mg/dL and 2 patients had blood Phe levels of approximately 15 mg/dL. On brain MRI scans, 1 patient with blood Phe levels >15 mg/dL had mild abnormalities, whereas another 5 patients had normal results. However, one 31-year-old patient who discontinued dietary therapy at age 12 presented with decreased cognitive ability and abnormalities in a brain MRI. The follow-up of 6 patients who discontinued hospital visits

was difficult. Subsequently, the authors reported that the most important point to consider is how to ensure patients continue their hospital visits.

Yoshino *et al.* (58) reported that 23 of 26 patients with classical PKU during adulthood who visited the Kurume University School of Medicine responded to a questionnaire; the respondents comprised 11 patients born before the start of newborn mass-screening (group A) (age 33-57 years; average 41.9 years) and 12 patients identified in the mass-screening (group B) (age 22-31 years; average 27.1 years). Subsequently, 8 of the group A patients (72%) and 4 of the group B patients (33%) presented with upper limb tremors, depression, and impaired concentration. Seven group A patients (64%) and 6 group B patients (50%) visited a hospital at least once a year and received dietary therapy. Of the group A patients aged <40 years, 64% were employed. All of the patients in group B, except for 1 full-time housewife, were employed. Patients who were identified in the screening and received early treatment led a relatively stable life.

Furthermore, Mochizuki and Yamaguchi (59) of Saitama Children's Medical Center reported a 25-year-old male patient identified in the mass-screening who has led a completely normal life, i.e., liked biology during high school, majored in biology in college, investigated marine species at the Graduate School of Agriculture, and spends his free time viewing Western art. Another patient was a soccer player in junior and high school, graduating from the Faculty of Engineering, University of N, becoming a civil engineer, and working as a construction site supervisor. Both patients graduated from university and received dietary therapy independently, maintaining their blood Phe levels at around 15 mg/dL.

Thus, if a patient can receive dietary therapy on his or her own, the QOL of PKU becomes favorable. However, if one discontinues the dietary therapy, various neurological symptoms develop to preclude routine daily life. The happiness of PKU patients identified in screening appears to depend on whether a patient can continue dietary therapy with a low-Phe diet for the rest of their life with the support of the attending physician and family members.

Newborn Mass-Screening for Inborn Errors of Amino Acid, Organic Acid, and Fatty Acid Metabolism by MS/MS

In other countries, screening for inborn errors of amino acid, organic acid, and fatty acid metabolism has been investigated since the late 1980s using MS/MS. In Japan, Professor Yosuke Shigematsu of the Department of Pediatrics, Fukui Medical University, reported a test result of newborn mass-screening for inborn errors of organic acid metabolism using MS/MS in 1998 (60). He was a part of the screening study group (principal investigator: Professor Tsugutoshi Aoki, Department of Pediatrics, Toho University) funded by a grant for psychosomatic disorders from the Ministry of Health and Welfare to initiate the newborn mass-screening. Subsequently, many reports have been published in other countries regarding the results of newborn mass-screening using MS/MS. Additionally, the usefulness of newborn mass-screening using MS/MS has been recognized in Japan through the steady efforts of Professor Shigematsu. Thus, newborn mass-screening should be examined in various ways. In 2004, a "newborn mass-screening in the 21st century in Japan" study group (principal investigator: Professor Seiji Yamaguchi, Department of Pediatrics, Shimane University) was launched by the Child and Family Research Project, supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare (61).

Currently, the number of live births in Tokyo is approximately 100,000 a year. The Tokyo Health Service Association conducted test MS/MS screening in approximately 13,000-19,000 newborns/year for 6 years (total 973,000 newborns) between 2005 and 2011. Samples were butylated in advance for amino acid and acyl carnitine analyses using Quattro micro API (Waters). Using MS/MS, the test screening identified classical PKU (1 case), BH_4 deficiency (1 case), medium-chain acyl-CoA dehydrogenase deficiency (MCAD) (3 cases), very long-chain acyl-CoA dehydrogenase deficiency (VLCAD) (1 case), glutaric acidemia type II (1 case), 3-methylcrotonylglycinuria (2 cases), and propionic acidemia (1 case). All 10 patients received treatment early and have matured and developed normally. Thus, the newborn mass-screening using MS/MS identified approximately 1 patient with inborn errors of amino acid, organic acid, or fatty acid metabolism in approximately 10,000 newborns (62).

Eight patients identified were born around the same time the test mass-screening study was conducted, but did not undergo MS/MS screening because they were born in hospitals that did not participate in the test screening study group. They presented with symptoms such as less vigorous sucking, vomiting, moodiness, and convulsions, and therefore were suspected to have inborn errors of amino acid, organic acid, or fatty acid metabolism. Thus, they were considered a high-risk group and underwent MS/MS examination. Subsequently, the following conditions were identified in the 8 patients: methylmalonic acidemia (2 cases), MCAD deficiency (2 cases), isovaleric acidemia (1 case), carnitine transporter disorder (1 case), glutaric acidemia type II (1 case) and 3-methylglutaric aciduria type I (1 case). Of these cases, 1 patient with MCAD deficiency and in whom MCAD was suspected by a blood test conducted at a

hospital, to which the patient was transferred, died before diagnosis. Hence, blood sample collected on filter paper during the newborn period was tested by MS/MS to identify MCAD deficiency. Of the other cases, 1 patient with MCAD deficiency and 1 patient with carnitine transporter disorder developed normal intelligence because the emergency hospitals to which they were transferred provided adequate treatment, but the other patients suffered developmental disorders (62).

In 2010, a study group funded by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare reported that MS/MS screening, implemented in 1,277,670 newborns until 2010, identified a total of 141 patients with the following conditions: amino acid metabolism disorder (41 cases), organic acid metabolism disorder (61 cases), and fatty acid metabolism disorder (39 cases) (63). In response to the above report, the Ministry of Health, Labour, and Welfare decided to initiate publicly funded newborn mass-screening using MS/MS in October 2012. The 16 diseases listed in **Table 14** were the primary screening targets (64). For measures and treatment for patients identified in the MS/MS screening, please refer to references 64 and 65.

		Target diseases	Criteria for requesting blood collection for positive cases
Target diseases of primary screening*	Inborn errors of amino acid metabolism 5 diseases	PKU HPA	Phe > 120 μmol/L and Phe > 2.5 mg/dL (HPLC)
		MSUD	Leu + Ile > 350 and Val > 250 μmol/L (or Leu + Ile > 400) and Leu > 3.5 mg/dL (HPLC)
		HCU	Met > 70 μmol/L and Met > 1.5 mg/dl (HPLC)
		Citrullinemia type I	Cit > 100 μmol/L
		Argininosuccinic aciduria (ASA)	Cit > 100 μmol/L and increased ASA
	Inborn errors of organic acid metabolism 7 diseases	Propionic acidemia (PA) Methylmalonic acidemia (MMA)	C3/C2 > 0.25 and C3 > 5.00 nmol/mL
		Isovaleric acidemia (IVA)	C5 > 1.00 nmol/mL
		Glutaric acidemia type I (GA I)	C5DC > 0.25 nmol/mL
		Multiple carboxylase deficiency (MCD) 3-Methylcrotonylglycinuria (3-MCC) 3-hydroxy-3-methylglutaric aciduria (3-HMG)	C5OH > 1.00 nmol/mL
	Inborn errors of fatty acid metabolism 4 diseases	Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	C8 > 0.300 and C8/C10 > 1.00 nmol/mL
		Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	C14:1 > 0.400 and C14:1/C2 > 0.013 nmol/mL
		Carnitine palmitoyltransferase I (CPT-I) deficiency	C0/(C16 + C18) > 100 nmol/mL
		Tri-enzyme/long-chain 3-hydroxy acyl-CoA dehydrogenase (TFP/LCHAD) deficiency	C16OH > 0.050 and C18:1OH > 0.050 nmol/mL
Target disease of secondary screening**	Inborn errors of organic acid metabolism	β-ketothiolase deficiency (3-KT)	C5OH > 0.60 and C5:1 > 0.09 nmol/mL
	Inborn errors of fatty acid metabolism 4 diseases	Carnitine transporter disorder (CTD)	C0 < 9.00 nmol/mL
		Glutaric acidemia type II (GA-II)	C8 > 0.300 nmol/mL and C10 > 0.50 nmol/mL
		CPT-II deficiency	(C16 + C18:1)/C2 > 0.40 and C16 > 3.00 nmol/mL
		Carnitine acyl carnitine translocase (CACT) deficiency	

*Diseases effectively screened
**Diseases ineffectively screened

Table 14. Criteria for MS/MS NBS in Tokyo (Reproduced from Ref. #62)

Conclusions

Thirty-five years have passed since publicly funded newborn mass-screening was initiated in Japan to detect treatable IEM. The incidence of PKU in Japan is approximately one-tenth that of Germany and Scotland and approximately a quarter of that of the United States. Fortunately, the incidence of PKU in Japan is low. However, PKU has attracted little attention from the general public precisely because of its low incidence. Thus, it has not been investigated in detail. The screenings for PKU and organic acid and fatty acid metabolic disorders in Japan were initiated almost 10 years after that of Western countries. If the incidence of PKU is 1/70,000 and 1 million people are born in a year in Japan, it means that 14 persons would develop PKU in a year (140 persons in 10 years) in Japan. The delayed start of newborn mass-screening in Japan, 10 years after that of Western countries, resulted in the 140 patients with PKU. The incidences of MSUD, HCU, and galactosemia are much lower than that of PKU. Thus, the balance of revenue of the screening project was unfavorable. However, the balance of revenue of the screening for amino acid, organic acid, and fatty acid metabolism disorders using MS/MS was favorable. Therefore, it is possible to implement the screening project smoothly.

Fortunately, the Japanese government established a nationwide publicly funded screening system. The system has been followed for 35 years. Therefore, more adult patients with PKU are active in society today, in the same manner as those with other diseases. One patient with MSUD who graduated from a science-oriented university was frequently hospitalized during infancy and childhood because of acute exacerbation, and was saved by dialysis therapy. Thereafter, the patient learned to independently control his blood branched-chain amino acid levels, graduated from School of Agriculture at a certain university, and became employed at a food company; she is now leading a happy life.

In Japan, all citizens can receive medical treatment because of the medical insurance system. Additionally, patients aged <20 years may receive financial support for the treatment of IEM from public funds. Therefore, fortunately, the self-pay ratio is low. Additionally, the association of parents of PKU patients includes MSUD patients, encouraging efforts to lead a happy life. Furthermore, food companies such as dairy food companies manufacturing foods for the treatment of IEM also support the association of parents. These parents are grateful for their support.

The newborn mass-screening project in Japan is characterized by the fact that the administrative authorities, obstetricians, pediatricians, and physicians specializing in metabolic disorders, screening laboratories, patient groups, dairy food companies, etc., are working together to improve the prognoses of patients with IEM.

Acknowledgments

A research grant for newborn mass-screening from the Ministry of Health, Labour and Welfare was awarded to the genetic study group for the prevention of psychosomatic disorders in 1974 (group leader: Eiji Inoue, Faculty of Medicine, the University of Tokyo) in the psychosomatic disorder research project by the Ministry of Health and Welfare. Although research targets changed from screening for IEM (principal investigator: Yutaka Moriyama) to the establishment and improvement of the newborn mass-screening system by the introduction of tandem mass spectrometry (principal investigator: Professor Seiji Yamaguchi), supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare in 2011, we have received a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare for more than 38 years. We are grateful for its support.

The Special Formula Office of JCPSF was established in the Imperial Gift Foundation Maternal and Child Health Center, Boshi-Aiiku-Kai, under the guidance of the Children and Families Bureau of the Ministry of Health and Welfare, to direct and support the research, development, and steady supply of special formula to treat IEM. We thank the following members of the Committee of JCPSF and the Special Formula Office, particularly Dr. Kikumaro Aoki, for surveying the prognoses of the patients identified in the mass-screening, whose results we referenced in this paper. We greatly appreciate their cooperation.

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Disclosure

The author declares no conflict of interest.

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References

1. Følling A. Über Ausscheidung von Phenylbrenztraubensäure in den Harn als Stoffwechselanomalie in Verbindung mit Imbezillität. Hoppe-Seylers Z Physiol. Chem. 1934;277:169-176 (in German)
2. Jervis GA, Block RJ, Bolling D, Kanze E. Chemical and metabolic studies on phenylalanine: II. The phenylalanine content of the blood and spinal fluid in phenylpyruvic oligophrenia. J Biol Chem 1940;134:105-113
3. Jervis GA. Phenylpyruvic oligophrenia: Deficiency of phenylalanine-oxidizing system. Proc Soc Exp Biol Med 1953;82:514-515
4. Bickel H, Gerrard J, Hickmans EM. Influence of phenylalanine intake on phenylketonuria. Lancet. 1953;2:812-813
5. Bickel H, Gerrard J, Hickmans EM. The influence of phenylalanine intake on the chemistry and behaviour of a phenylketonuric child. Acta Paediatr Scand 1954;43:64-77
6. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 1963;32:338-343.
7. Guthrie R. The introduction of newborn screening for phenylketonuria. A personal history. Eur J Pediatr 1996;155(Suppl 1):S4-5
8. Kitagawa T. People who created and developed diet therapy for phenylketonuria. Bulletin on Special Formula 2003; 39:3-6 (in Japanese)
9. Oura T. The time when domestic low-phenylalanine milk was produced. Bulletin on Special Formula 1981; 2:39-41 (in Japanese)
10. Naruse H. National screening in Japan. Past and present. Jap Soc Mass Scr 1998;8(Suppl 1):17-27
11. Kitagawa T, Masuko N, Ishihara S. Mass-screening for inherited metabolic diseases: Methods and some comment. Shonika Rinsho 1974;27:1276-1287 (in Japanese)
12. Beutler E, Baluda MC. A simple spot screening test for galactosemia. J Lab Clin Med 1966;68:137-141
13. Paigen K, Pacholec F, Levy HL. A new method of screening for inherited disorders of galactose metabolism. J Lab Clin Med 1982;99:895-907
14. Kitagawa T. The system of newborn mass-screening in Massachusetts in the United States. Sanfujinka no Sekai 1974; 26:1251-1258 (in Japanese)
15. Levy HL, Madigan PM, Shih VE. Massachusetts metabolic disorders screening program. I. Technics and results of urine screening. Pediatrics. 1972;49:825-836
16. Massachusetts Department of Public Health. Cost-benefit analysis of newborn screening for metabolic disorders. N Engl J Med 1974; 291:1414-1416
17. Komrower GM. The philosophy and practice of screening for inherited diseases. Pediatrics. 1974;53:182-188
18. Kitagawa T. Cost-benefit balance of newborn mass-screening for inherited metabolic diseases in Japan. No to Hattatsu 1975;7:235-240 (in Japanese)
19. Guthrie R. Screening for "inborn errors of metabolism" in the newborn infant-a multiple test program. Birth Defects Orig Artic Ser 1968;4:92-98
20. Moriyama Y. Research group of screening for metabolic diseases: New beginning. Taishaijo Screening Kenkyukai Kaiho 1976;1:1 (in Japanese)
21. Moriyama Y. Ministry of Health and Welfare Genetic Research Group of Physical and Mental Disabilities. Genetic research of physical and mental disability prevention (Lead researcher: Eiji Inoue). Kenkyu Hokoku 1975 (in Japanese)
22. Tada K, Oura T, Kitagawa T, Matsuda I, Kawamura M, Wada Y. Treatment guidelines for inherited metabolic disease--Target diseases in newborn mass-screening. J Jpn Ped Soc 1977; 81:840-845 (in Japanese)
23. Committee for Homocystinuria Treatment Guideline Revision. Partial revision of treatment guideline for homocystinuria. Bulletin on Special Formula 1996;32:67-68 (in Japanese)
24. Ghadimi H, Partington MW, Hunter A. A familial disturbance of histidine metabolism. N Engl J Med 1961;265:221-224
25. Kitagawa T. Announcement of the Ministry of Health and Welfare about screening for inborn errors of metabolism. Deletion of histidinemia from the target disease list of specific pediatric chronic diseases. Bulletin on Special Formula 1992;25:72-76 (in Japanese)
26. Moriyama Y. Announcement of the establishment of the Japan Cooperative Project on Special Formula and request for collaboration. Bulletin on Special Formula1980;1:4 (in Japanese)
27. Aoki K, Ogushi Y. Follow-up survey of outcome of the patients detected by newborn screening and treated with special formula registered in 1983(exclusive of five diseases). Bulletin on Special Formula 1984;9:40-47 (in Japanese)
28. Kitagawa T. Food Exchange List for Phenylketonuria - Dietary Therapy. Imperial Gift Foundation Maternal/Child health center Special Formula Office 1989 (in Japanese)

29. Committee of the Japan Cooperative Project on Special Formula. Diet therapy guide book for inborn errors of amino acid metabolism. Boshi Aiikukai Maternal/Child health center Special Formula Office 1998 (in Japanese)
30. Committee of the Japan Cooperative Project on Special Formula. Revised diet therapy guide book for inborn errors of amino acid metabolism. Imperial Gift Foundation Boshi Aiikukai 2004 (in Japanese)
31. Aoki K, Ito K. Report of follow-up survey of five diseases—School attendance of patients detected by screening. Bulletin on Special Formula 1992;24:25-28 (in Japanese)
32. Owada M, Abe N, Watanabe S, Suzuki N. Research on treatment for phenylketonuria and benign hyperphenylalaninemia. Shonika 1992;33:867-875 (in Japanese)
33. Committee for Revision of PKU Treatment Guideline. Revision of treatment guideline for phenylketonuria (including a part of hyperphenylalaninemia) and 1995 revision of treatment recommendation. Bulletin on Special Formula 1995;30:43-49 (in Japanese)
34. Committee for Revision of PKU Treatment Guideline. A report of revised treatment guideline for phenylketonuria (including a part of hyperphenylalaninemia). J Jpn Ped Soc 1995;99:1535-1539 (in Japanese)
35. Burgard P. Development of intelligence in early treated phenylketonuria. Eur J Pediatr 2000;159 (Suppl 2): S74-S79
36. Schweitzer-Krantz S, Burgard P. Survey of national guidelines for the treatment of phenylketonuria. Eur J Pediatr 2000;159(Suppl 2):S70-S73
37. Aoki K, Kinoshita K, Hayashi K. Research on follow-up survey of newborn mass-screening. Bulletin on Special Formula 2000;36:50-60 (in Japanese)
38. Smith I, Clayton BE, Walff OH. New variation of phenylketonuria with progressive neurological illness unresponsive to phenylalanine restriction. Lancet 1976;1:1108-1111
39. Kaufman S, Holtzman NA, Milstien S, Butler LJ, Krumholz A. Phenylketonuria due to a deficiency of dihydropteridine reductase. New Engl J Med 1975;293:785-790
40. Review committee of an interim recommended guideline for the treatment of BH4 deficiency. Interim recommended guideline for treatment of tetrahydrobiopterin deficiency. Bulletin on Special Formula 1996;32:53-66 (in Japanese)
41. Fukushima T, Nixson JC. Analysis of reduced forms of biopterin in biological tissues and fluids. Anal Biochem 1980;102:176-188
42. Suzuki K, Owada M. A simple and sensitive method for the determination of pterins in cerebrospinal fluid. Clinical usefulness for management of tetrahydrobiopterin deficiency. J Inher Metab Dis 1991;14:825-830
43. Arai N, Narisawa K, Hayakawa H, Tada K. Hyperphenylalaninemia due to dihydropteridine reductase deficiency: diagnosis by enzyme assay on dried blood spots. Pediatrics 1982;70:426-430
44. Blau N, Thöny B, Cotton RGH, Hyland K. Disorder of tetrahydrobiopterin and related biogenic amines. In: The Metabolic and Molecular Basis of Inherited Disease Vol 2, 8th ed. Editors Scriver CR *et al*. New York: McGrow Hill Inc., 2001;1725-1776
45. Kure S, Hou DC, Ohura T, Iwamoto H, Suzuki S, Sugiyama N, Sakamoto O, Fujii K, Matsubara Y, Narisawa K. Tetrahydrobiopterin-responsive phenylalanine hydrosxylase deficiency. J Pediatr 1999;135:375-378
46. Matsubara Y, Kure S, Ohura T. Second research report from the committee for treatment guideline for BH4-responsive hyperphenylalaninemia. Bulletin on Special Formula 2002;38:44-59 (in Japanese)
47. Shintaku H. Single-dose administration test of BH4. Shoninaika 2006;38:1326-1332 (in Japanese)
48. Shintaku H. Diagnosis and treatment of BH4-responsive mild phenylketonuria. Bulletin on Special Formula 2007;43:11-16 (in Japanese)
49. Kure S. BH4-responsive phenylalanine hydroxylase deficiency: From the establishment of disease concept to treatment. Bulletin on Special Formula 2007;43:8-10 (in Japanese)
50. Aoki K, Kinoshita K. Four diseases identified by newborn mass-screening: Adolescent patients and their current status. Bulletin on Special Formula 2002;38:24-28 (in Japanese)
51. Maternal/Child Healthcare Center, Imperial Gift Foundation Boshi-Aiiku-Kai. Chapter 3: Follow-up survey of patients identified by newborn mass-screening in Japan. In: History of the Japan Cooperative Project on Special Formula, Tokyo: Special Formula Office, 1993; pp24-34 (in Japanese)
52. Hisashige A. Research on technology assessment of mass-screening system. In: 1993 Ministry of Health and Welfare research of physical and mental disabilities: Research on evaluation method of mass-screening system (Lead researcher: Yasuhiro Kuroda). Tokyo: Ministry of Health and Welfare, 1994; 63-67 (in Japanese)
53. Hisashige A. Health economic analysis of the neonatal screening program in Japan. Int J Technol Assess Health Care 1994;10:382-391
54. Okusa Y. Sugawara T, Yamaguchi S. Cost-benefit analysis of newborn mass-screening by tandem mass spectrometry. J Jpn Soc for Mass-Screening 2007;17:35 (in Japanese)
55. Aoki K. Current status of the follow-up survey of newborn mass-screening. Bulletin on Special Formula 1996;32:42-52 (in Japanese)
56. Aoki K, Matsuda I, Tada K, Oura T, Kitagawa T, Maruyama H, Owada M. Reassessment of follow-up system for the patients detected by newborn mass-screening. Bulletin on Special Formula 2002;38:21-23 (in Japanese)
57. Owada M, Abe N, Yokoyama M, Usui H. Treatment for adult patients with phenylketonuria: Experience of 20 patients. Bulletin on Special Formula 2011;47:25-30 (in Japanese)
58. Yoshino N, Watanabe J, Okada J. Medical and social outcomes of adult phenylketonuria patients. Bulletin on Special Formula 2011;47:6-7 (in Japanese)
59. Mochizuki H, Yamaguchi S. Adult male phenylketonuria patients leading productive social lives. Bulletin on Special Formula 2011;47:35-37 (in Japanese)
60. Shigematsu Y, Hata I, Tanaka Y, Sudo M. Fundamental investigation of newborn mass-screening by electrospray tandem mass spectrometry: Diagnosis of inborn error of organic acid metabolism, Ministry of Health and Welfare research of physical and mental disabilities: Research on effective mass-screening practice (Lead researcher: Tsugutoshi Aoki). 1996 Kenkyu Hokokusho 1996;197-200 (in Japanese)
61. Yamaguchi S. Research on 21st-century newborn mass-screening in Japan. Child and family research project by the Welfare, Labour and Science Research Subsidy 2004 (Lead researcher: Seiji Yamaguchi) Kenkyu Hokokusho. 2004;3-10 (in Japanese)
62. Kitagawa T. Newborn mass-screening by tandem mass spectrometry: Preparation and results of trial run. Annual Report of Tokyo Health Service Association 2012;41:137-144 (in Japanese)
63. Shigematsu Y. Accuracy improvement of tandem mass spectrometry and its maintenance. Research on target diseases for mass-screening. Research project by the Welfare, Labour and Science Research Subsidy. Research on preparation for and quality improvement of newborn mass-screening by tandem mass spectrometry (Lead researcher: Seiji Yamaguchi). 2010 Kenkyu Hokokusho 2011;49-60 (in Japanese)
64. Yamaguchi S. New neonatal mass-screening by tandem mass spectrometry Q and A 2012. Tokyo: Welfare, Labour and Science Research. Newborn mass-screening by tandem mass spectrometry research: Preparation and quality improvement in 2012 (in Japanese)
65. Kitagawa T. 2011 Meeting for special formula for treatment of IEM: Development and improvement, First session of joint research report. How to treat to the patients detected by newborn mass-screening using tandem mass spectrometry. Bulletin on Special Formula 2011;47:40-48 (in Japanese)