

Newborn Screening in Japan: Restructuring for the New Era

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Abstract

Nationwide neonatal mass screening for inherited metabolic diseases has started in Japan since 1977. At least 8000 children have probably been spared from handicaps resulting from such diseases over the past 30 years. Recently remarkable changes have been made to the evolving neonatal screening system. Declining birth rate and economic problems in Japan have demanded a more effective neonatal screening system. Development of new innovative screening methods and treatment tools, e.g. tandem mass spectrometry (MS/MS) technology and enzyme replacement therapy for mucopolysaccharidosis (MPS), have facilitated expansion of target diseases in neonatal screening. We have carried out pilot screening using MS/MS in 6 laboratories in Japan. The incidence of inherited metabolic diseases was found to be 1 in 9330 (65 cases out of 606,380 babies screened) during the period between 1997 and 2007. The incidence was lower than those of Europe or USA (about 1 in 4000 to 5000). The disease frequency between unscreened symptomatic cases and asymptomatic cases detected through MS/MS screening were also found to be different. In MS/MS screening, the most common organic acidemia was propionic acidemia, whereas in symptomatic cases, methylmalonic acidemia was the most common. Further study of ethnic diversity in severity of propionic acidemia is required. The outcomes of patients detected in the MS/MS screening were significantly favourable. The results showed the benefits of MS/MS screening. The diagnostic support network for gas chromatography-mass spectrometry (GC/MS) analysis and enzyme determination has also been developed. We have developed an automated system of GC/MS data processing and auto-diagnosis which allowed the GC/MS data processing to be extremely fast and simple. Enzyme evaluation for diagnostic support for screening, including a method using peripheral blood and high performance liquid chromatography (HPLC), and another method of in-vitro probe assay using cultured cells and MS/MS. Furthermore, re-location of screening laboratories for a more efficient screening network will be required such that at least 30,000 samples can be analysed in each laboratory.

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Introduction

The nationwide neonatal mass screening for inherited metabolic diseases started 30 years ago in Japan, and now 6 different kinds of diseases are being screened. It is estimated that at least 8000 children could have been spared from being handicapped as a result of these diseases. For the last 30 years, however, the circumstances surrounding neonatal screening have remarkably changed. Our challenges to reform neonatal screening in Japan for the new era will be discussed in this paper.

History of Neonatal Screening in Japan

In the early 1960s, the Guthrie method was developed, and inherited metabolic diseases were given much attention.

In 1964, the Japanese Society of Inborn Metabolic Disease was formed, and in 1973, the Japanese Society of Mass Screening was formed. In 1977, a nationwide neonatal mass screening project which included 5 targeted diseases, was started by the Japanese government. In 1979, congenital hypothyroidism was included in the screening process, and in 1989, congenital adrenal hyperplasia was then added. On the other hand, histidinemia was eliminated from the list of targeted diseases since no clinically significant handicaps were observed. Hence, the current neonatal screening includes 6 targeted diseases.

Although mass screening for neuroblastoma using urine collected from 6-month-old children was carried out between 1984 and 2002, it is now no longer carried out

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except for a few areas in Japan. Recently, studies to expand the targeted diseases, such as organic acidemias, fatty acid disorders, Wilson disease, and so on, have been carried out.

Since the 1990s, a new innovative screening method, tandem mass spectrometry (MS/MS) was developed.^{1,2} In 1997, pilot neonatal screening using MS/MS was initiated by Professor Shigematsu from the Fukui University in Japan.³ Since 2004, an expanded pilot screening using MS/MS has gained support by receiving funds from the Ministry of Health, Labor and Welfare of Japan.

Population Dynamics and Present Situation of Neonatal Screening in Japan

The number of births in Japan for the past 50 years has declined tremendously from 2.33 million in 1950 to 1.76 million in 1977, and to 1.08 million in 2005. The infant mortality rate in 2005 was only 2.8 per 1000 births, compared to 60.1 in 1951 and 8.9 in 1977.

Table 1 shows the results of neonatal screening in Japan for the past 30 years. It shows that (i) congenital hypothyroidism was the most common disease and the most cost-effective to screen for, (ii) the incidence of

phenylketonuria (PKU) was unexpectedly low (1 in 80,000) compared to western Europe (1 in 10,000), (iii) the frequency of maple syrup urine disease (MSUD) and homocystinuria was extremely low.

Changes in Neonatal Screening during Past 30 years in Japan

During the last 30 years, remarkable changes in socio-economics affected neonatal screening in Japan (Table 2). (i) The birth rate has rapidly declined from 1.8 million in 1977 to 1.1 million in 2005. (ii) Economic problems have surfaced. These problems demanded efforts for a more effective neonatal screening programme. (iii) Patients or families' awareness of screening and treatment options are also changing. For example, emphasis on better quality of life (QOL). (iv) New innovative screening methods, such as MS/MS or hearing screening, were developed recently. (v) New methods of treatment for inherited metabolic diseases such as enzyme replacement therapy for mucopolysaccharidosis (MPS) were also established. These technological innovations have facilitated expansion of targeted diseases in neonatal screening. (vi) To respect the privacy of patients, there may be problems in the tracking of patients detected in neonatal screening. New information on treatments should be provided to patients as early as possible through continuous exchange of information between government officers, doctors, researchers, technicians, and patients' families.

Reform Project of the Neonatal Screening in Japan

We are now embarking on projects supported by funds from the Japanese government to reform the current neonatal screening system in Japan. These are as follows, (i) Investigation of the natural history of possible new targeted diseases, which may be essential for determining the benefits of neonatal screening. (ii) Development of new screening methods. (iii) Development of diagnostic support system

Table 1. Results of Neonatal Screening in Japan (as of 2007)

Disease	Incidence
1. Phenylketonuria (Biopterin disorder = 1:1,580,00)	1:80,000
2. Maple syrup urine disease	1:500,000
3. Homocystinuria	1:800,000
4. Galactosemia* (type 1 = 1: 800,000) (type 2 = 1: 600,000)	1:30,000
5. Congenital hypothyroidism	1:2200
6. Congenital adrenal hyperplasia	1:20,000

* The incidence includes transient galactosemias

Table 2. Changes in Neonatal Screening in Japan during the Past 30 years

Changes during the past 30 years	Future actions
1. Declining birth rate 1.8 million (1977)→1.1 million (2005)	Improve cost-effectiveness of the screening programme
2. Economic problems	
3. Social stigma for patients and family	Improving quality of life, ethical issues
4. Newer methods of screening – MS/MS, hearing screening	Expansion of targeted diseases? Determination of cost-benefit
5. Newly developed treatment – Enzyme replacement for MPS etc.	
6. Others – Problems in tracking of patients, screening quality	Providing new information Assurance of screening quality

Table 3. Results of Screening in the Pre-symptomatic and Symptomatic stages

Diseases	Japan		Asia
	Screening* (Fukui + pilot)	Symptomatic** (Shimane)	
Organic acidemias			
1. MMA	9	40	142
2. PPA	16	12	22
3. MCD	4	15	26
4. GA1	3-	6	13
5. HMG	3	5	3
6. MCC def	-	4	4
7. 3KT def	1	1	14
8. IVA	-	1	2
9. Other OA		12	16
Fatty acid disorders			
1. VLCAD def	4	12	3
2. GA2 (MAD)	3	9	2
3. MCAD def	3	5	3
4. CPT2 def	2	3	2
5. SC def	2	5	1
6. MTP def	-	3	-
7. SCAD def	1	2	1
8. CPT1 def	3	2	2
9. SCHAD def	1	-	-
10. Other FAOD	-	-	3
Amino acidemias			
1. MSUD	-	(3)	17
2. PKU	8	(1)	10
3. ASA	1	-	-
4. Citrin def	6	-	-

Incidence Ratio 1: 9300

* TMS screening from 1997 to 2007

** metabolic screening on symptomatic patients from 2001 to 2007 at Shimane University

3KT def: 3-ketothiolase deficiency; ASA: argininosuccinic acidemia;

Citrin def: citrin deficiency (including citrulinemia type 2)

CPT1 def and CPT2 def: carnitine palmitoyltransferase-1 and 2 deficiencies, respectively; GA1: glutaric acidemia type 1; GA2: glutaric acidemia type 2;

HMG: 3-OH-3-methylglutaric acidemia; IVA: isovaleric acidemia;

MCC def: methylcrotonyl-CoA carboxylase deficiency;

MCD: multiple carboxylase deficiency;

MMA: methylmalonic acidemia;

MSUD: maple syrup urine disease;

MTP def: mitochondrial trifunctional protein deficiency;

PKU: phenylketonuria; PPA: propionic acidemia;

SC def: systemic carnitine deficiency;

VLCAD def: MCAD def, and SCAD def, very-long-, medium-, and short-chain acyl-CoA dehydrogenases, respectively;

SCHAD def: short-chain 3-OH-acyl-CoA dehydrogenase deficiency;

Other OA means other organic acidemias including 5-oxoprolinemia, alkaptonuria, or glycerolemia.

for screening of new targeted disorders. (iv) Medical support for patients in treatment and availability of new information. (v) Restructuring of the screening system, including regionalisation of screening laboratory.

Results of Pilot Screening using MS/MS

We have carried out pilot screening using MS/MS in 6 laboratories, which are located in Sapporo, Tokyo, Fukui, Osaka, Shimane, and Kumamoto. A total number of 606,380 babies between 1997 and 2007 were screened, and 65 cases were detected: 33 cases of organic acidemias, 18 fatty acid oxidation disorders, and 14 amino acidemias (Table 3). The overall incidence in Japan was thus calculated to be 1 in 9330 which is lower than that of Europe or USA (about 1 in 4000 to 5000).

Comparison of Disease Proportions between Symptomatic and Asymptomatic Cases in Japan and other Asian Countries

We have had collaborative studies with some hospitals in other Asian countries. We are working closely with Dr Yang YL from Peking University and Dr Gu XF from Shanghai Xiao Tong University in China, Dr Nguyen Thu Nhan from Hanoi Medical University in Vietnam, Dr Wasant P from Mahidol University in Thailand, and Dr Verma IC from Sur Ganguaram Hospital in India. We analysed cases of organic acidemias, acylcarnitine deficiencies or amino acidemias who became “symptomatic” using GC/MS or MS/MS. The results are listed in Table 3. In “symptomatic” cases detected in the laboratory at Shimane University, some differences in the disease incidence were observed between Japan and other Asian countries. 3-Ketothiolase deficiency, 5-oxoprolinemia (data not shown), PKU, or MSUD were more commonly detected in other Asian countries than in Japan. On the other hand, urea cycle defects and multiple carboxylase deficiency were more likely to be detected in Japan.

It was also found that the disease incidence between symptomatic and asymptomatic cases in MS/MS screening were different. In MS/MS screening, the most common organic acidemia was propionic acidemia, followed by methylmalonic acidemia, whereas in symptomatic cases, methylmalonic acidemia is the most common, followed by multiple carboxylase deficiency and propionic acidemia. Using MS/MS screening, it was found that the incidence of Japanese patients with propionic acidemia was at least 10 times higher than previously reported, and it was likely that many Japanese children with propionic acidemia detected in MS/MS screening had no or mild symptoms (the latter needing some intervention at least in childhood).⁴ Further study of ethnic diversity in the severity of propionic acidemia should be carried out.

Table 4. Effectiveness of MS/MS Screening

Disease	Pre-symptomatic (Pilot screening)	Symptomatic cases (Shimane)
Number of cases	54	153
Organic acidemia	39	120
Normal	31 (79%)	3832%
Handicapped	6 (15%)	5445%
Death	2 (5%)	2823%
Fatty acid disorder	15	33
Normal	15 (100%)	1752%
Handicapped	-	618%
Death	-	1030%

Beneficial Effect of MS/MS Screening

The outcomes of Japanese patients detected in the symptomatic and pre-symptomatic (MS/MS screening) stages were compared. As shown in Table 4, in the case of organic acidemias, favourable outcomes were achieved in 83% of the symptomatic and 33% of the MS/MS screening groups respectively. In the case of fatty acid disorders, all 15 cases detected in MS/MS screening had no impairment, whereas in the “symptomatic” group, only 52% of cases had normal intellectual developments. It is encouraging that the outcomes of children with certain disorders detected through newborn screening may be significantly more favourable. On the other hand, we should note that the MS/MS screening detects not only typical cases but also milder cases. Hence, the clinical benefits of screening should be further determined carefully, with more patients studied.

Diagnostic Supports for MS/MS Screening

Basically, analysis of acylcarnitines and amino acids in blood filter paper is just “screening”. There may be a considerable number of false positive or false negative cases. This implies a reliable diagnostic support system is needed. Therefore, we are developing a support network of GC/MS analysis and enzyme evaluation.

For example, an elevation of C3 (propionylcarnitine) suggests not only propionic acidemia but also methylmalonic acidemia. Urinary organic acid analysis is indispensable for differential diagnosis. In Shimane University, an original system of automated GC/MS data processing and diagnosis was established in collaboration with Shimadzu Cooperation, Kyoto, Japan.⁵ In this system, metabolic profiles from the GC/MS data, and “suspected” or “suspicious” disease names were listed automatically in a minute. GC/MS data processing became extremely fast and simple with this system.

Enzyme evaluation for diagnostic support for screening has also been developed. The group in Hiroshima University

developed a simple enzyme assay system, using peripheral lymphocytes and high performance liquid chromatography (HPLC) to determine enzyme activity.⁶ Sample to be collected requires only 5 ml heparinised blood. For example, in very-long-chain-acyl-CoA dehydrogenase (VLCAD) deficiency, after palmitate (C16) is used for sample incubation as substrate, its product, C16:1, is measured by HPLC. The amount of C16:1 produced represents the activity of VLCAD.

We are also introducing another method of enzyme evaluation for beta-oxidation, named “in vitro probe assay”.⁷⁻⁹ In this method, palmitate (C16) or octanoate (C8) is added to the culture medium as substrate, and the cells are incubated for 72 or 96 hours. After that, acylcarnitines in the medium are analysed using MS/MS. If long-chain fatty acid oxidation is blocked, C16 acylcarnitine is increased after palmitate loading as shown in Figure 1. If medium-chain fatty acid oxidation is blocked, C8- and longer chain acylcarnitines are increased after loading with both palmitate and octanoate. Hence, fatty acid oxidation defects are clearly detected with cultured cells.

Relocation of Screening Laboratories in Japan

Currently, the population of Japan is 127 million, and the number of births per year is about 1.1 million as mentioned above. There are 47 provinces, and screening laboratories are now located in each province. There are over 50

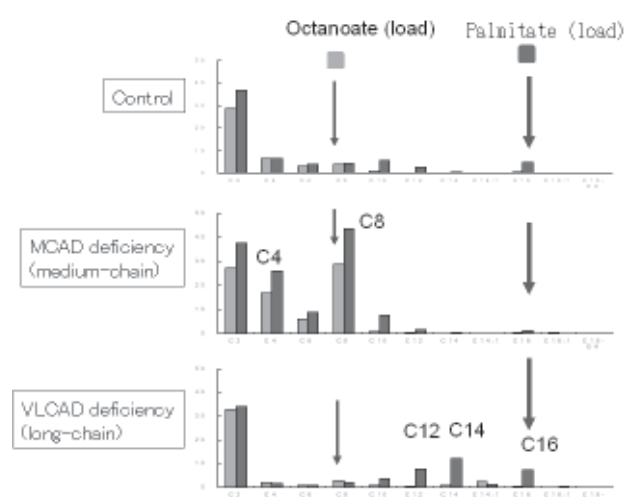


Fig. 1. Results of in vitro probe assay for evaluation of fatty acid oxidation. A: acylcarnitine profile of normal control. B: medium-chain acyl-CoA dehydrogenase (MCAD) deficiency. C: very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency. In MCAD deficiency, C8, C6 and C4 acylcarnitines are increased with both palmitate and octanoate loading. In VLCAD deficiency, C12, C14, and C16-acylcarnitines are increased with palmitate loading, although no abnormality is seen with octanoate loading. Hence, beta-oxidation capacity can be evaluated sensitively.

screening laboratories, and the number of screening in each province varies roughly from 5000 to 100,000 births per year. Furthermore, the birth rate is constantly declining. Relocating the screening laboratories should be considered with the introduction of MS/MS for neonatal screening to be cost-effective.

The rough estimated cost of the MS/MS screening, which includes the costs of reagents, amortised cost of instruments, and labour charge is as follows. If 50,000 samples a year are analysed in a laboratory, the core cost of each sample is calculated to be about 6 dollars. If 30,000 samples are analysed, the cost is about 9 dollars, whereas if only 10,000 samples are analysed, that will be over 25 dollars. Hence, we should consider the economies of scale in use of MS/MS screening. We should also consider that in the future, screening laboratories should be relocated and merged for a more efficient screening network. The number of screening laboratories should be reduced from 50 to 20 or 25 such that at least 30,000 samples are analysed in each laboratory.

Conclusions

Our current activity for restructuring neonatal screening after MS/MS was introduced are summarised as follows: (i) Medical support system: we recently published a guidebook for new targeted diseases detectable in MS/MS screening, and considered the setting up of consultation centres; (ii) Diagnostic support system: a network of several laboratories in which GC/MS, enzyme determination or DNA tests are available, is being considered; (iii) Reliable and quick result reporting system: Screening laboratories should be relocated to analyse at least 30,000 samples per year in each laboratory; (iv) Continuing system for clinical follow-up and feedback to patients: We are consolidating the particulars of the patients detected through neonatal screening in the National Centre for Child Health and Development of Japan, with all their information treated with strict confidentiality. Periodical publications are used as a means of communication between doctors, researchers and patients, and to provide more appropriate and up-to-date information for patients; (v) Others: we should ensure the quality of measurements and intensify efforts to solve ethical issues related to neonatal screening.

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