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NEWBORN MASS SCREENING IN JAPAN—**1984**

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Since 1977 nation-wide newborn mass screening test program was developed in Japan for early detection and early treatment of inborn errors of metabolism (phenylketonuria, maple syrup urine disease, homocystinuria, histidinemia and galactosemia) and congenital hypothyroidism (since 1979). Within these 7 years, the mass screening program spread out all over the country, covering more than 98% of total newborn infants born in Japan. Annual report of Japanese Ministry of Health and Welfare informs that 228 patients suffering either of these kinds of metabolic disorder and 181 patients with congenital hypothyroidism were detected from April of 1983 through March of 1984 (Tables 1 & 2). Total number of the accumulated patients already reached to more than 2,000. Most of these patients are now placed under the medical care and are growing without any handicap as well as other healthy infants. Apparently newborn mass screening program has been established.

Incidentally to the accumulation of cases, however, persons being involved in mass screening are aware of new problems appearing. For instance, we now know there are at least 8 kinds of hyperphenylalaninemia in the newborn infants (Table 3). Only one of them (Type 1) is called classical phenylketonuria (phenylalanine hydroxylase deficiency), which was a target of screening test to check blood phenylalanine levels. But we have studied that another group of infants with hyperphenylalaninemia due to inborn errors of pteridine metabolism other than classical phenylketonuria should be detected to prevent developmental delay. Even among the patients of classical phenylketonuria, female infants will be at high risk in future

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Table 1. Number and frequency of patient discovered by newborn screening test for inborn errors of metabolism in Japan (Jpn. Ministry of Health and welfare). Slightly modified by the authors.

	1977	'78	'79	'80	'81	'82	'83	Total	Frequency
Phenylketonuria	6	20	30	23	19	14	19	131	1/70,900
Maple syrup urine disease	0	5	0	6	5	1	6	23	1/403,900
Homocystinuria[a]	7	15	13	16	21	12	12	96	1/96,800
Histidinemia	36	155	224	183	214	178	163	1,153	1/8,100
Galactosemia	3	9	12	19	30	25	28	126	1/73,700

N.B.: [a] "Homocystinuria" also contains "Hypermethioninemia without homocystinuria."

Table 2. Number of frequency of patient discovered by newborn screening test for congenital hypothyroidism in Japan (Jpn. Ministry of Health and Welfare).

	Number of newborn infants tested	Ratio of tested to total infants	Number of patients discovered	Frequency
1979	335,795	20.6%	41	1/8,200
'80	1,206,905	77.6	151	1/8,000
'81	1,441,472	94.8	185	1/7,800
'82	1,489,600	98.1	177	1/8,400
'83	1,487,573	98.4	181	1/8,200
Total	5,961,345	—	735	1/8,100

Table 3. Classification of hyperphenylalaninemia (Tourian and Sidbury, 1983).

I.	Phenylketonuria
II.	Persistent hyperphenylalaninemia
III.	Transient mild hyperphenylalaninemia
IV.	Dihydropteridine reductase deficiency
V.	Abnormal dihydrobiopterin function
VI.	Persistent hyperphenylalaninemia and tyrosinemia
VII.	Transient neonatal tyrosinemia
VIII.	Hereditary tyrosinemia

to have their own babies with severe malformations even if their phenylalanine metabolizing enzyme might be intact (maternal phenylketonuria).

Furthermore it becomes evident that frequency and geographical distribution of those patients with inborn errors of metabolism are characteristic in Japan (Yasuda, 1984). Comparing with data from U.S. and Europe, the Japanese has much higher incidence of histidinemia and, on the other hand, lower incidence of phenylketonuria and homocystinuria (Thalhammer, 1983).

During these 7 years' experiences, we realize to have a plenty of question to be solved in future from the genetical view point.

(A) *Newborn mass screening program*

Newborn infants during 5 to 7 days after the birth are, according to the parents' will, taken a few drops of blood by heel puncture. The blood is put on a filter paper specially manufactured for this purpose, and is dried up through the natural room air. The blood filter paper, with some description necessary for the identification, are sent to a central laboratory located in each prefecture. In each laboratory, blood discs of 3 mm in diameter are punched out for the assay.

Semiquantitative assay of blood phenylalanine (for phenylketonuria), leucine (for maple syrup urine disease), methionine (for homocystinuria) or histidine (for histidinemia) is performed with Guthrie's bacterial inhibition assay method (Guthrie and Susi, 1963). For the detection of galactosemic patients, Beutler's method, and/or Paigen-phage method are generally employed. For congenital hypothyroidism screening, these blood discs are subjected to the radio immuno assay (RIA) method or the enzyme immuno assay (EIA) method to determine blood contents of TSH and/or T_4.

All the costs for the assay are supported by public expenses.

(B) *Newborn mass screening for inborn errors of metabolism*

Table 1 shows the summary of these 7 years' data on the newborn screening for inborn errors of metabolism. It should be noted that these are not necessarily conclusive yet, because these probably include atypical and transient cases of inborn errors of metabolism. Following long term courses of those cases, Tada (1983) reported that final diagnosis was established in 966 cases among 1,071 infants contained in items from 1977 to 1981 of Table 1. Furthermore, the differentiation of hypermethioninemia (not accompanied with homocystinuria) from true homocystinuria is not easy. Therefore the number of hypermethioninemia (without homocystinuria) belongs to the item of homocystinuria in Table 1. This is the reason why a frequency of homocystinuria is markedly higher in Japan comparing with other countries. So far as we know, 7 cases of cystathionine β-synthase deficiency and 2 cases of hepatic methionine adenosyltransferase deficiency are counted in the item of homocystinuria in Table 1.

1) *Maintenance and development of the screening system.* Kitagawa, Aoki and Naruse are now studying on the maintenance and development of genetic screening system. Aoki (1984) is dealing with data on newly discovered patients as well as other patients being followed by each institution with computerized system. Statistic information will be open every three years. Naruse and his colleagues (1984) are making their effort to maintain high quality of mass screening program in each laboratory.

2) *Phenylketonuria.* Frequency of phenylketonuria in Japan is far less than

that in U.S. and in European countries (Bickel, 1973). Only the country having similarly low frequency of phenylketonuria to Japan is Finland. Geographical distribution of patients in Japan is precisely analyzed by Yasuda (1984).

3) *Malignant hyperphenylalaninemia.* Malignant hyperphenylalaninemia, which represents unresponsiveness of clinical features such as mental retardation and so on to the dietary restriction of phenylalanine intake, needs differentiation from other disorders and to be placed under the bioptein supplement. For this purpose Tada *et al.* (1984) developed new screening method using filter paper inpregnated with urine.

4) *Maternal phenylketonuria.* Most of patients with phenylketonuria screened by this program are reported to be growing well without any physical and mental retardation. But if a patient is a female, she will be at high risk in future to bear severely handicapped baby even if the baby has normal phenylalanine hydroxylase activity. This phenomenon is explained from the unusual circumstance, that is abnormally high concentration of phenylalanine in maternal blood, around a fetus.

Yoshino *et al.* (1984) found a fetus of such hyperphenylalaninemic mother had chronic and persistent damage of energy producing system of nervous tissues and visceral organs. To prevent such disaster during prenatal period, it is recommended for phenylketonuric mother to take phenylalanine restricting diet when she expects to have a baby by Okano *et al.* (1984). Shimizu (1984) reported that patients in adolescence who could not have any benefits of dietotherapy are already confronted by various problems around them. He also pointed out those female patients have higher possibilities to be pregnant without any special consideration probably due to their impaired mentality.

Gomibuchi (1984) and Sumiyoshi (1984) studied on medical and social situation around phenylketonuric patients in foreign countries. In the serial study they found that 79% of the institutions had their own registration system of these patients. In Japan, we do not have such registration system yet.

5) *Maple syrup urine disease.* It is well-known that patients with maple syrup urine disease can be divided into 4 groups (Table 4). Jinno *et al.* (1984) investigated on genetic heterogeneity of maple syrup urine disease patients found in Japan. They concluded that cultured lymphocytes for complementation study contributed very well to their differentiation.

6) *Homocystinuria.* One of the characteristics of the findings in Japanese newborn screening is remarkably low frequency of homocystinuria ($1 : 105 \times 10^4$).

Table 4. Classification of maple syrup urine disease.

I.	Classical MSUD
II.	Intermittent MSUD
III.	Intermediate MSUD
IV.	Vitamin B_1 responsive MSUD

Tada (1983) reported two of seven patients diagnosed by the end of 1982 deceased unfortunately during early infantile period. Their causes of death were pulmonary hemorrhage and pulmonary thrombosis.

Another characteristic is that considerable number of hypermethioninemia is summing up as homocystinuria (above mentioned, cf. Table 1). At the beginning of newborn screening test, there was a conceptional confusion of "hypermethioninemia without homocystinuria" with true homocystinuria due to cystathionine β-synthase deficiency. Anyway, it is quite obvious that other kinds of disorder (Table 5) should be differentiated from homocystinuria (Suzuki, 1982).

7) *Histidinemia.* The first case of histidinemia was reported by Ghadimi *et al.* in 1961. Auerbach *et al.* and Kihara *et al.* followed it. All of these cases reported early in 1960's were severely handicapped. Most of the patients detected by newborn mass screening test, however, are growing up well. Only 15 cases out of 588 histidinemic patients revealed below 85 of IQ score, and other 15 cases were elicited to be electroencephalographically abnormal.

Although the efficacy of histidinemia screening will be determined later, at least by 1977, no one knew such high frequency the Japanese had. The frequency (1 : 8,000) was estimated only in Quebec (Auray-Blais *et al.*, 1983) and in Japan. And a question "What is histidinemia in adulthood?" remains still unsolved.

8) *Galactosemia.* Beulter's method based upon NADPH production from galactose-1-phosphate sometimes gives false-positive response for detection of galactose-1-phosphate uridyltransferase deficiency. Hot and wet weather in Japan may be responsible. Seasonal and regional variation of the response is in good accordance with this explanation. In addition, to detect other kinds of galactosemia (galactokinase deficiency and 4-epimerase deficiency), this method is not effective.

For the reasons mentioned above, each laboratory is moving to use Paigen-phage method additionally to Beutler's method. Kawamura (1983) emphasized to take further three methods (fluorometrical assay of galactose, that of galactose-1-phosphate, and epimerase test) for the screening.

9) *Other metabolic disorders.* Isshiki *et al.* (1984) are focussing their efforts on early detection of adenine phosphoribosyltransferase deficiency. In such pati-

Table 5. Classification of hypermethioninemia.

I.	Hepatic methionine adenosyltransferase deficiency
II.	Cystathionine β-synthase deficiency
III.	Vitamin B_6 dependent homocystinuria
IV.	Hypermethioninemia with hepatic injury
V.	Hereditary tyrosinemia
VI.	Transient hypermethioninemia of newborn infant
VII.	Hypermethioninemia with myopathy
VIII.	Persistent hypermethioninemia without myopathy

ents 2,8-dihydroxyadenine, which is an oxidative product of adenine, accumulates and is excreted into urine. As 2,8-dihydroxyadenine is highly insoluble, they are susceptible to form stones. They demonstrated that early detection of the patients is possible using blood filter paper and that they can be treated with allopurinol (xanthine oxidase inhibitor) to avoid renal stones and renal failure.

Arashima (1984) is developing new screening method for Wilson's disease. He proposed simple and sensitive method to detect urinary excretion of copper by means of atomic absorption spectrometer. Actually, he succeeded to find one school boy with this disorder through 9,000 specimens.

(C) *Newborn mass screening for congenital hypothyroidism*

Japanese newborn mass screening program for congenital hypothyroidism started in 1979. Accumulated data of these 5 years are shown in Table 2. It should be noted, however, as was the case of metabolic disorders written in (B), that these figures are not always confirmative.

Nakajima and his group (1984) followed each case described in Table 2 (1979–1982), and reported that 403 cases was congenital hypothyroidism, 57 cases transient hypothyroidism, 55 cases transient hyperthyrotropinemia and 31 cases undetermined. According to Nakajima and Irie's statistical analyses in 1984, situations are improved for early diagnosis and early treatment. Screened infants are placed under the precise examination on 25 days (mean) of age. Male to female ratio found in patients is one to two. In Japan only 3 cases of hypothyroidism having normal TSH were detected by T_4 studies through 1982. Approximately 25% of patients had normal (>7 μg/100 ml) T_4 levels.

For many years TSH estimation has been emphasized especially in Japan. But recently each laboratory has tendency to estimate not only TSH but also T_4 combinedly.

1) *Classification.* Out of 254 cases with congenital hypothyroidism, 139 (54.3%) had ectopic thyroid, 74 (29.1%) had aplastic thyroid gland, 40 (15.7%) had synthetic defect of the hormone, and 1 (0.4%) was due to hamartoma of the pituitary gland.

2) *Complication.* 44 cases (10.9%) had, to some extent, any complications such as congenital heart disease (14 cases) and minor anomalies (12 cases).

3) *Intellectual and physical development.* As is shown in Table 6, IQ or DQ of 215 cases (95.6%) were above 80. Out of 4 cases whose DQ was below 70, 2 were with Down syndrome, 1 was with Cornelia de Lange syndrome and the last was with PDA.

4) *Transient hypothyroidism.* According to long-term follow-up study, 57 cases were concluded as transient hypothyroidism. Possible causes for transient hypofunction of the thyroid gland were fetography, prematurity and transport of maternal TSH-binding inhibitor immunoglobulin. But causes of 26 cases are still unknown.

5) *Transient infantile hyperthyrotropinemia.* Miyai *et al.* firstly reported the

Table 6. IQ or DQ after the treatment (Nakajima *et al.*).

	Mean SD (n)	≧90	80-89	70-79	<70
12M DQ	105±11 (150)	n=143	n= 6	n=0	n=1
18M DQ	103±17 (63)	49	11	1	2
2Y DQ	109±18 (110)	97	7	2	4
3Y DQ	105±19 (50)	45	2	2	1
4Y DQ	99±13 (7)	5	2	0	0
IQ	106±16 (10)	9	1	0	0
5Y DQ	116± 6 (2)	2	0	0	0
IQ	127±15 (3)	3	0	0	0
6Y IQ	116± 8 (2)	2	0	0	0
The last IQ or DQ at this study	105±15 (225)	203 (90.2%)	12 (5.3%)	6 (2.7%)	4 (1.8%)

entity. In 1982 study, 55 cases were found to be in this category. Further study will make it clearer to differentiate disorders in other categories.

6) *Methodological studies.* Naruse (1984) and Nakajima (1984) found 30 cases of congenital hypothyroidism with parallel assays of TSH and T_4. As 5 of them had normal T_4 level, they were in favor of TSH assay except cases of premature infants.

Katsumata and Suwa (1984) independently studied on 33 cases of congenital hypothyroidism and found a peculiar case with panhypofunction of pituitary gland. They emphasized merit of parallel assay of TSH and T_4.

Concerning enzyme immuno assay (EIA), Katsumata and Suwa (1984) are involved in sandwich method, which was found to be applicable to newborn mass screening. Murata *et al.* (1984) investigated to assay free T_4 by radio immuno assay (RIA). They concluded estimation of free T_4 had advantages to T_4 estimation.

7) *Maintenance and development of the hypothyroidism screening system.* Saito (1984) pointed out the importance of quality control of each laboratory.

8) *Thyroid function of premature infants.* Many investigators are interested in the assessment of thyroid function especially in the premature baby. In general, premature infants reveal, more or less, lower values of T_3, T_4, free T_3, free T_4, comparing with maturated infants. But even in those premature babies, TSH, TBG and reverse T_3 are all within normal range or sometimes higher than in normal infants.

(D) *Forthcoming items of the screening*

Following the success of the newborn mass screening for these six disorders, Hirayama and his colleagues (1984) are developing another newly devised screening system to detect neuroblastoma. Methods are based upon the chemical reaction of 4-hydroxy-3-methoxy-mandelic acid (vanillinemandelic acid, VMA), which is con-

tained abundantly in the urine of most of patients with neuroblastoma. For this purpose they are setting up the system to collect filter papers inpregnated with urine from infants at the age of 6 months. The Ministry of Health and Welfare of Japan is planning to spread their methods all over Japan at the beginning of 1985. Seven patients were detected through the regional pilot study in 1983. Its frequency of newborn baby is estimated at 1 to 7,335.

Suwa *et al.* (1984) emphasized the importance of newborn mass screening for adrenogenital syndrome (21-hydroxylase deficiency), hyperammonemia due to urea cycle dysfunction and congenital biliary atresia. A part of the fundamental data were already published by them.

(E) *Attitudes to the genetic screening*

All the babies born to the human society must be expecting to enjoy healthy and secure lives. However, their genetic backgrounds are, indeed, various and complicated. Approximately 5% of newborn infants will manifest genetic problems, minor or major, throughout their whole lives. Surprisingly, only 7% of physicians perceive this matter. To save such unhappy infants' lives and to promote their development, preventive medicine should have responsibility rather than traditional therapeutic medicine.

From this point of view, genetic screening and surveillance for genetic diseases are moving into the limelight. "Surveillance" and "screening" are sometimes used analogically. But the meanings are quite different. Surveillance needs long-term and wide enough study on outbreak of diseases. In contrast to it, genetic screening is usually a short-term, cross-sectional activity to determine if a subject, at that time, suffers from the disease or not. It is not an indulgence to disorders. It is not almighty.

Therefore the authors should start our career again to make the definition and the objectives of screening clear here. AAP Task Force on Genetic Screening (1976) wrote the formal definition of screening as "the presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures which can be applied rapidly. Screening tests sort out apparently healthy persons who probably have a disease from those who probably do not." Of course, the screening may be large-scale, and encompass a whole population (mass screening); or it may be selective and directed at a high-risk subgroup.

The objectives of genetic screening are (1) to provide opportunities for medical intervention to prevent genetic disease or minimize its effect, (2) to provide opportunities for counseling about reproductive options, and (3) to collect data pertinent to public health policy and basic knowledge (research). In Japan, most people concerned in genetic screening are undoubted on that the main objective is medical intervention to the patients. That is why the motto for genetic screening is always hackneyed "early detection, early treatment" as the authors even themselves used above.

By up-to-the-minute informations from abroad, abrupt progress in genetical

field is going on. By means of restriction fragment length polymorphisms (RFLPs), diagnosis (prenatal or postnatal) and carrier detection of Duchenne muscular dystrophy (Harper *et al.*, 1983; Pembrey *et al.*, 1984), phenylketonuria (Woo *et al.*, 1983) and Huntington's disease (Gusella *et al.*, 1983) are now possible. In this situation, Japanese genetic screening also cannot stay put. Extending the field for counseling, collection and administration of information and progress of genetical research, all of these matters should be of our interests.

Since Bickel *et al.* wrote a brief but an impressive report on dietotherapy of phenylketonuria in 1953, general perceptions for genetic disorders are rapidly changing. Numerous articles on gene technology are published every month. Gene technologists look like omnipotent beings. But now the authors are confident it is the time to consider to what extent preventive devices or technical treatments for genetic disorders can be possible and also permissible.

Similarly to the Task Force's comment, the authors would like to close this article as "every screener a geneticist."

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