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Diversity in the incidence and spectrum of organic acidemias, fatty acid oxidation disorders, and amino acid disorders in Asian countries: Selective screening vs. expanded newborn screening

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ABSTRACT

Background: Expanded newborn screening (ENBS) utilizing tandem mass spectrometry (MS/MS) for inborn metabolic diseases (IMDs), such as organic acidemias (OAs), fatty acid oxidation disorders, (FAODs), and amino acid disorders (AAs), is increasingly popular but has not yet been introduced in many Asian countries. This study aimed to determine the incidence rates of OAs, FAODs, and AAs in Asian countries and Germany using selective screening and ENBS records.

Materials and methods: Selective screening for IMDs using gas chromatography–mass spectrometry and MS/MS was performed among patients suspected to be afflicted in Asian countries (including Japan, Vietnam, China, and India) between 2000 and 2015, and the results from different countries were compared. Similarly, ENBS results from Japan, South Korea, Taiwan, and Germany were compared. Additionally, the results of selective screening and ENBS in Japan were compared.

Results: Among 39,270 patients who underwent selective screening, IMDs were detected in 1170. Methylmalonic acidemia was frequently identified in several countries, including Japan (81/377 diagnosed IMDs), China (94/216 IMDs), and India (72/293 IMDs). In Vietnam, however, β-ketothiolase deficiency was particularly frequent (33/250 IMDs). ENBS yielded differences in overall IMD rates by country: 1:8557 in Japan, 1:7030 in Taiwan, 1:13,205 in South Korea, and 1:2200 in Germany. Frequently discovered diseases included

Abbreviations: GC/MS, gas chromatography–mass spectrometry; MS/MS, tandem mass spectrometry; OA, organic acidemia; FAOD, fatty acid oxidation disorder; AA, amino acid disorder; UCD, urea cycle disorder; IMD, inherited metabolic disease; NBS, newborn screening; ENBS, expanded newborn screening; MMA, methylmalonic acidemia; PPA, propionic acidemia; MCD, multiple carboxylase deficiency; GA1, glutaric acidemia type I; MCCD, 3-methylcrotonyl-CoA carboxylase deficiency; MGA, 3-methylglutaconic aciduria; HMGL, 3-hydroxy-3-methylglutaryl-CoA lyase; 4-OH-BA, 4-hydroxybutyric acidemia; 2-OH-GA, 2-hydroxyglutaric acidemia; BKTD, β-ketothiolase deficiency; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthetase; OXPA, 5-oxoprolinemia; GA2, glutaric acidemia type II; VLCAD, very long-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; PCD, primary carnitine deficiency; CPT1, carnitine palmitoyltransferase I; CPT2, carnitine palmitoyltransferase II; TFP, trifunctional protein; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; CACT, carnitine-acylcarnitine translocase; HAD, 3-hydoxyacyl-CoA dehydrogenase; PKU, phenylketonuria; MSUD, maple syrup urine disease; HCU, homocystinuria; CTLN1, citrullinemia type I; ASA, argininosuccinic aciduria

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propionic acidemia (PPA) and phenylketonuria (PKU) in Japan, 3-methylcrotonyl-CoA carboxylase deficiency (MCCD) and PKU in Taiwan, MCCD and citrullinemia type I in South Korea, and PKU and medium-chain acyl-CoA dehydrogenase deficiency in Germany. Furthermore, in Japan, selective screening and ENBS yielded respective PPA frequencies of 14.7% and 49.4% among all organic acidemias.
Conclusion: The incidence rates of IMDs vary by country. Moreover, the disease spectra of IMDs detected via selective screening differ from those detected via ENBS.

1. Introduction

In recent years, mass spectrometric techniques, including gas chromatography–mass spectrometry (GC/MS) and tandem mass spectrometry (MS/MS), have been used for the biochemical diagnosis of inherited metabolic diseases (IMDs) such as organic acidemias (OAs), fatty acid oxidation disorders (FAODs), and amino acid disorders (AAs). Newborn screening (NBS) for OAs, FAODs, and AAs utilizing MS/MS is becoming popular worldwide and is referred to as expanded NBS (ENBS) [1]. The prognoses of patients with diseases targeted by ENBS have markedly improved in countries that have implemented [2–4].

Japan introduced nationwide NBS in 1977 and implemented nationwide ENBS in 2014 following a pilot study performed between 1997 and 2012 [5]. The latter is also being implemented in other Asian countries, including Taiwan [6] and South Korea [7]. However, ENBS has yet to be introduced in several other Asian countries in which epidemiological data pertaining to IMDs remain limited [8,9]. Accordingly, Shimane University has provided biochemical IMD diagnostic services using GC/MS and MS/MS for symptomatic patients (defined as selective screening) from several Asian countries, including Vietnam, China, and India, for > 15 years.

To investigate variations in the incidence rates of IMDs by nation, we investigated the frequencies of OAs, FAODs, and AAs among Asian countries using our selective screening and ENBS records. Furthermore, we compared the detection rates using selective screening and ENBS in Japan with the aim of reevaluating the target diseases of ENBS.

2. Materials & methods

2.1. Selective screening

2.1.1. Subjects

Screening for IMDs was performed for symptomatic patients upon request by medical institutes in Japan and other Asian countries, including Vietnam, China, India, Indonesia, Thailand, Mongolia, South Korea, Malaysia, Taiwan, and Turkey. The screening was performed using GC/MS and MS/MS at the Department of Pediatrics, Shimane University Faculty of Medicine, Japan between 2000 and 2015. Samples from patients with clinical findings suspected to indicate IMDs, such as metabolic acidosis, ketosis, hyperammonemia, hypoglycemia, lethargy, hypotonia, myopathy-like symptoms, acute encephalopathy, and sudden infant death of unknown cause, were analyzed. If the request included the above symptoms, blood and/or urine samples with the patient's data (e.g., clinical course and administered medication) were sent to Shimane University. The frequencies of detected IMDs were retrospectively compared between countries. This study was approved by the Shimane University Institutional Committee on Ethics (registration No. 20170920-2).

2.1.2. GC/MS analysis

Urine samples were delivered at room temperature from Asian countries using dried urine filter paper [10], whereas frozen urine samples were sent from Japanese medical institutes to Shimane University. The urinary organic acid analysis was conducted as reported previously [10,11]. A 'GCMS QP-2010 Plus' instrument (Shimadzu, Kyoto, Japan) was used for the analysis.

2.1.3. MS/MS analysis

Dried blood filter papers were shipped from overseas at room temperature to Shimane University, as used for ENBS. Blood serum samples from some Japanese patients were analyzed. Only samples from India were analyzed at Fukui University, Japan. Blood acylcarnitines and amino acids were analyzed with MS/MS using in butyl-derivatized specimens [12] at both Shimane and Fukui Universities. An API-3000 or API-4000 instrument (Applied Biosystems, Foster City, CA, USA) or an LC/MS/MS-8040 instrument (Shimadzu, Kyoto, Japan) was used for MS/MS.

2.1.4. Diagnosis

Biochemical diagnoses were based on the results of GC/MS and/or MS/MS. Data of organic acid analysis were processed using a personal computer-based automated GC/MS data processing and diagnostic system [11]. Whereas the diagnoses of nearly all Japanese patients were finally confirmed based on enzyme activity measurements and/or gene mutations, the diagnoses of foreign patients were based on the results of biochemical analyses indicating obvious specific abnormal metabolites in accordance with clinical data (e.g., age, sex, clinical course, laboratory data, and medication use) by several expert physicians who were familiar with IMDs.

2.2. ENBS

To investigate the detection rate of IMDs using ENBS, we obtained nationwide ENBS data from the principal ENBS investigators in Japan, Taiwan, South Korea, and Germany (the latter is a representative European country).

The nationwide ENBS data for each country were acquired as follows. The screening data of approximately 3.36 million infants were screened between 1997 and 2015 in Japan; this population included 1.95 million infants screened during the pilot study period between 1997 and 2012 as described in a domestic journal [5] and available data of 1.41 million infants screened from 2013 to 2015. Nationwide ENBS data were not available at least in the period, because ENBS was conducted on a province-by-province basis. Although the annual birth number in Japan was about 1.0 million and the coverage rate was > 99.9%. In Taiwan, approximately 1.39 million babies (coverage rate was > 99.9%; government-funded) participated in ENBS during the period between 2001 and 2014, which included a pilot study conducted from 2001 to 2009 [6]. In South Korea, approximately 3.44 million babies (coverage rate was approximately 40 to 80%; paid screening) were screened between 2000 and 2015 as described in a Korean-language domestic report. In Germany, approximately 7.51 million babies (coverage rate was > 99.9%; government-funded) were screened between 2002 and 2015, as described in a domestic report [13].

In each country, ENBS was performed according to nationally standardized methods as follows. In Japan, the butyl-derivatization method was used during pilot study; subsequently, a non-derivatized method has been used since at least 2014. In other countries, the butyl-derivatization method was used during the study period, as previously described [4,6,7]. Dried blood spots were collected 4–5 days after birth in Japan, 48–72 h after birth in South Korea and Taiwan, and 36–72 h after birth in Germany.

3. Results

3.1. Selective screening

3.1.1. Total number of analyzed cases

Among the 39,270 patients analyzed during the study period, 30,625 and 8645 were from Japan and other Asian countries, respectively (Table 1). A total of 58,463 patient samples were analyzed, including 28,469 and 29,994 samples examined using GC/MS and MS/MS, respectively. Of these samples, 43,983 were obtained from Japanese patients and 14,480 were from patients in other Asian countries. Although similar numbers of GC/MS and MS/MS examinations were performed, only the latter type of analysis was requested for patients in whom FAOD was strongly suspected.

Selective screening identified 1170 patients with IMDs, of which 377 and 793 were from Japan and other Asian countries, respectively (Table 1). The overall detection frequency was 3.0% (1.2% in Japan and 9.2% in other Asian countries). The detection frequencies were 8.2%, 8.6%, 13.9%, 4.4%, 4.8%, 1.2%, and 1.6% in Vietnam, China, India, Indonesia, Thailand, Mongolia, and other countries, respectively.

3.1.2. Diseases detected using selective screening

The selective screening results are shown in Table 2. Among 377 identified patients with IMDs in Japan, methylmalonic acidemia (MMA) was detected in 81, urea cycle disorders (UCDs) in 60, and glutaric acidemia type II (GA2) in 30. Propionic acidemia (PPA) and multiple carboxylase deficiency (MCD) were each identified in 24 patients. In Vietnam, IMDs were discovered in 250 of the 3054 screened patients; here, maple syrup urine disease (MSUD) was identified in 36, UCDs in 34, β-ketothiolase deficiency (BKTD) in 33, PPA in 22, and 5-oxoprolinemia (OXPA) in 19 patients. In China, IMDs were detected in 216 of the 2519 examined patients; MMA was identified in 94, UCDs in 20, PKU in 18, PPA in 12, and glutaric acidemia type I (GA1) in 10 patients. In India, 293 of the 2105 examined patients were diagnosed with an IMD; the most prevalent diseases were MMA in 72, UCDs in 48, PPA in 26, MSUD in 24, and PKU in 23 patients. In other Asian countries, 34 of the 967 examined patients were diagnosed with IMDs, although no particular diseases were prevalent.

3.2. ENBS

3.2.1. The incidence of IMDs detected through ENBS

As shown in Table 3, the overall IMD detection incidences with ENBS were 1:8557 in Japan, 1:7030 in Taiwan, 1:13,205 in South Korea, and 1:2200 in Germany. In Japan, diseases with higher incidences included PPA (1:41,000), PKU (1:46,000), very-long chain acyl-CoA dehydrogenase (VLCAD) deficiency (1:93,000), citrin deficiency (1:96,000), MMA (1:120,000), and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency (1:129000). In Taiwan, 3-methylcrotonyl-CoA carboxylase deficiency (MCCD) was most frequently detected (1:41,000), followed by PKU (1:58,000), citrin deficiency (1:61,000), primary carnitine deficiency (PCD) (1:70,000), and MMA, GA1, and

Table 2
Results of selective screening in Japan and other Asian countries.

Country	Japan	Vietnam	China	India	Others[a]
Number of patients	377	250	216	293	34
OA	184	98	140	166	8
Methylmalonic acidemia	81	12	94	72	2
Propionic acidemia	24	22	12	26	1
MCD	24	2	8	6	0
Glutaric acidemia type I	17	1	10	16	0
MCCD	8	2	0	4	0
3-methylglutaconic aciduria	5	2	1	3	1
HMGL deficiency	5	0	3	2	0
Alkaptonuria	5	0	1	9	0
4-hydroxybutyric acidemia	4	1	2	0	0
2-hydroxyglutaric acidemia	4	0	1	4	1
Isovaleric acidemia	2	6	2	4	2
β-ketothiolase deficiency	2	33	4	14	1
HMGS deficiency	2	0	0	0	0
5-oxoprolinemia	1	19	2	6	0
FAOD	88	29	16	10	5
Glutaric acidemia type II	30	10	8	2	2
VLCAD deficiency	23	5	3	3	0
MCAD deficiency	14	1	3	2	0
Primary carnitine deficiency	13	8	1	1	1
CPT2 deficiency	6	4	1	1	1
TFP/LCHAD deficiency	2	1	0	1	1
AA and UCD	74	101	46	108	11
Phenylketonuria	4	10	18	23	3
Maple syrup urine disease	2	36	5	24	3
Homocystinuria	2	12	3	4	3
Urea cycle disorder	60	34	20	48	1
Citrin deficiency	6	9	0	9	1
Other diseases	31[b]	20[c]	14[d]	9[e]	10

OA, organic acidemia; MCD, multiple carboxylase deficiency; MCCD, 3-methylcrotonyl-CoA carboxylase deficiency; HMGL, 3-hydroxy-3-methylglutaryl-CoA lyase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthetase; FAOD, fatty acid oxidation disorder; VLCAD, very long-chain acyl-CoA dehydrogenase, MCAD, medium-chain acyl-CoA dehydrogenase; CPT2, carnitine palmitoyltransferase II; TFP, trifunctional protein; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; AA, amino acid disorder; UCD, urea cycle disorder.

[a] "Other countries" includes 18 patients from Indonesia, 12 from Thailand, 3 from Mongolia, and 1 from Malaysia.

[b] Thirty-one patients in Japan with other diseases included 16 strongly suspected of FAOD with non-ketotic dicarboxylic aciduria, 10 with peroxisomal diseases, and 4 with other diseases.

[c] Twenty patients with other diseases in Vietnam included 16 strongly suspected of having FAOD with non-ketotic dicarboxylic aciduria, 2 with peroxisomal diseases, and 1 each with carnitine palmitoyltransferase I (CPT1) deficiency and short-chain acyl-CoA dehydrogenase deficiency (SCAD) deficiency.

[d] Fourteen patients with other diseases in China included 9 suspected of having FAOD with non-ketotic dicarboxylic aciduria, 2 with SCAD deficiency, and 1 each with CPT1 deficiency, mevalonic acidemia, and tyrosinemia type I.

[e] Nine patients from India with other diseases included 3 strongly suspected of having FAOD with non-ketotic dicarboxylic aciduria, 4 with tyrosinemia type I, and 1 each with ethylmalonic encephalopathy and 3-hydoxyacyl-CoA dehydrogenase deficiency.

Table 1
Profiles of patients subjected to selective screening.

Country	Total	Japan	Asia[a]	Vietnam	China	India	Indonesia	Thailand	Mongolia	Others[b]
Patients analyzed	39,270	30,625	8645	3054	2519	2105	413	251	241	62
Samples analyzed	58,463	43,983	14,480	6004	2912	4137	795	257	302	73
GC/MS	28,496	21,288	7181	2960	1529	2045	385	9	196	57
MS/MS	29,994	22,695	7299	3044	1383	2092	410	248	106	16
Cases detected	1170	377	793	250	216	293	18	12	3	1
Detection frequency (%)	3.0	1.2	9.2	8.2	8.6	13.9	4.4	4.8	1.2	1.6

GC/MS, gas chromatography–mass spectrometry; MS/MS, tandem mass spectrometry.

[a] "Asia" indicates Asian countries other than Japan.

[b] "Others" includes 38 patients from South Korea, 16 from Malaysia, 6 from Taiwan, and 2 from Turkey.

Table 3
Comparison of expanded newborn screening detection incidences of IMDs per country.

Country	Japan*[a]	Taiwan*[b]	Korea*[c]	Germany*[d]
Total No. of newborns screened	3.36 million	1.39 million	3.44 million	7.51 million
Total detection incidence	1:8557	1:7030	1:13,205	1:2200
OA	1:22,000	1:18,000	1:31,000	1:10,000
Methylmalonic acidemia	1:120,000	1:107,000	1:246,000	1:125000
Propionic acidemia	1:41,000	1:464,000	1:313,000	1:250,000
Isovaleric acidemia	1:672,000	1:696,000	1:138,000	1:96,000
MCD	1:1,121,000	1:464,000	n.a.	0
MCCD	1:153,000	1:41,000	1:111,000	1:73,000
HMGL deficiency	0	n.a.	1:861,000	1:550,000
Glutaric acidemia type I	1:280,000	1:107,000	1:492,000	1:127,000
β-ketothiolase deficiency	0	n.a.	n.a.	0
FAOD	1:30,000	1:34,000	1:111,000	1:9000
CPT1 deficiency	1:420,000	1:696,000	n.a.	1:1,020,000
VLCAD deficiency	1:93,000	1:1,392,000	1:383,000	1:76,000
MCAD deficiency	1:129000	1:350,000	1:492,000	1:10,000
TFP/LCHAD deficiency	1:840,000	n.a.	1:1,148,000	1:176,000
CPT2 deficiency	1:257,000	1:696,000	n.a.	1:3,060,000
CACT deficiency	0	n.a.	n.a.	1:7,510,000
GA2	1:480,000	1:696,000	n.a.	1:195000
Primary carnitine deficiency	1:199000	1:70,000	1:345000	1:250,000
HAD deficiency	1:3,363,000	n.a.	1:1,723,000	0
AA and UCD	1:26,000	1:17,000	1:29000	1:5000
Phenylketonuria	1:46,000	1:58,000	1:138,000	1:5000
Maple syrup urine disease	1:841,000	1:107,000	1:1,148,000	1:164,000
Homocystinuria	1:1120,000	n.a.	1:492,000	1:132,000
Tyrosinemia type I	0	n.a.	1:123,000	1:150,000
Citrullinemia type I	1:306,000	1:199000	1:115000	1:60,000
Argininosuccinic aciduria	1:1121,000	1:593,000	1:1,148,000	1:292,000
Citrin deficiency	1:96,000	1:61,000	1:3,445,000	0

OA, organic acidemia; MCD, multiple carboxylase deficiency; MCCD, 3-methylcrotonyl-CoA carboxylase deficiency; HMGL, 3-hydroxy-3-methylglutaryl-CoA lyase; FAOD, fatty acid oxidation disorder; CPT1, carnitine palmitoyltransferase I; VLCAD, very long-chain acyl-CoA dehydrogenase, MCAD, medium-chain acyl-CoA dehydrogenase; TFP, trifunctional protein; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; CPT2, carnitine palmitoyltransferase II; CACT, carnitine-acylcarnitine translocase; GA2, glutaric acidemia type II; HAD, 3-hydoxyacyl-CoA dehydrogenase; AA, amino acid disorder; UCD, urea cycle disorder; n.a., not available.

[a] Data from Japan are from 1997 to 2015.
[b] Data from Taiwan are from 2001 to 2014.
[c] Data from Korea are from 2000 to 2015.
[d] Data from Germany are from 2002 to 2015.

MSUD (1:107,000 each). In South Korea, MCCD was the most commonly identified disorder (1:111,000), followed by citrulinemia type I (CTLN1) (1:115000), tyrosinemia type I (1:123,000), isovaleric acidemia and PKU (1:138,000 each), and MMA (1:246,000). In Germany, PKU (1:5000) was most frequently detected, followed by MCAD deficiency (1:10,000), CTLN1 (1:60,000), MCCD (1:73,000), and VLCAD deficiency (1:76,000).

3.2.2. Comparison of selective screening and ENBS in Japan

According to selective screening, MMA was the most frequently identified disorder in Japan, occurring in 81 (50.9%) of all 163 patients with OA, followed by PPA and MCD (14.7% each) (Table 4). In contrast, ENBS identified PPA as the most frequently diagnosed disorder, occurring in 82 (53.9%) of all 152 patients with OAs, followed by MMA (18.4%), and MCCD (14.5%). MCD was identified in only 2.0% of patients.

Among patients with FAODs, GA2 was the most frequently identified by selective screening (30 of all 88 patients with FAODs, or 34.1%), followed by VLCAD deficiency (26.1%), MCAD deficiency (15.9%), and PCD (14.8%). In contrast, ENBS most frequently detected VLCAD deficiency (36 of 112 patients with FAODs, 32.1%), followed by MCAD deficiency (23.2%), PCD (15.2%), and carnitine palmitoyltransferase II deficiency (11.6%).

Among patients with AA, CTLN1 and citrin deficiency were most frequently discovered via selective screening (27.3% of all AA patients each). Although PKU was identified in only 4 patients upon symptomatic screening, it was the most commonly detected disorder by ENBS (73 of 129 patients, 56.5%).

4. Discussion

Our study revealed differences in the incidence of IMDs among Asian countries; these differences were observed when using selective screening and ENBS (including when compared with a European country– Germany – using the latter). Furthermore, the IMD frequencies and spectra revealed by selective screening differed from those identified by ENBS.

Notably, selective screening and ENBS demonstrated unique characteristics regarding the incidence of OAs in each country. Although MMA was frequently detected in several countries, a high number of patients with PPA was detected in Japan; this can be attributed to a common Japanese-specific mutation (p.Y435C) of *PCCB* [14,15], which

Table 4
Comparison of the results of selective screening and expanded newborn screening in Japan.

	Selective screening	ENBS
Cases diagnosed	377	393
OA	163 (100%)	152 (100%)
Methylmalonic acidemia	81 (50.9)	28 (18.4)
Propionic acidemia	24 (14.7)	82 (53.9)
Isovaleric acidemia	2 (1.2)	5 (3.3)
MCD	24 (14.7)	3 (2.0)
MCCD	8 (4.9)	22 (14.5)
HMGL deficiency	5 (3.1)	0
Glutaric acidemia type I	17 (10.4)	12 (3.1)
β-ketothiolase deficiency	2 (1.2)	0
FAOD	88 (100%)	112 (100%)
CPT1 deficiency	0	8 (7.1)
VLCAD deficiency	23 (26.1)	36 (32.1)
MCAD deficiency	14 (15.9)	26 (23.2)
TFP/LCHAD deficiency	2 (2.3)	4 (3.6)
CPT2 deficiency	6 (6.8)	13 (11.6)
GA2	30 (34.1)	7 (6.3)
Primary carnitine deficiency	13 (14.8)	17 (15.2)
HAD deficiency	0	1 (0,9)
AA	22 (100%)	129 (100%)
Phenylketonuria	4 (18.8)	73 (56.5)
Maple syrup urine disease	2 (9.1)	4 (3.1)
Homocystinuria	2 (9.1)	3 (2.3)
Citrullinemia type I	6 (27.3)	11 (8.5)
Argininosuccinic aciduria	2 (9.1)	3 (2.3)
Citrin deficiency	6 (27.3)	35 (27.1)

(%) percentage of each group disease.
Selective screening was performed at Shimane University. Newborn screening was performed across Japan during the period from 1997 to 2015, as described in the text. Underlined values indicate the diseases in which large differences in incidence were observed between symptomatic screening and ENBS.
OA, organic acidemia; MCD, multiple carboxylase deficiency; MCCD, 3-methylcrotonyl-CoA carboxylase deficiency; HMGL, 3-hydroxy-3-methylglutaryl-CoA lyase; FAOD, fatty acid oxidation disorder; CPT1, carnitine palmitoyltransferase I; VLCAD, very long-chain acyl-CoA dehydrogenase, MCAD, medium-chain acyl-CoA dehydrogenase; TFP, trifunctional protein; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; CPT2, carnitine palmitoyltransferase II; GA2, glutaric acidemia type II; HAD, 3-hydoxyacyl-CoA dehydrogenase; AA, amino acid disorder.

is generally associated with mild phenotypes and unlikely to be detected during selective screening. Compared to other Asian countries, BKTD and OXPA were more frequent in Vietnam, while MMA was more frequent in China. The high incidence of BKTD in the Vietnamese population can be attributed to a common Vietnamese-specific *ACAT1* mutation (p.R208X) [16]. OXPA was also frequently detected in this study, and many patients presented with typical symptoms of glutathione synthetase deficiency, such as chronic metabolic acidosis and hemolytic anemia. However, data on the genetic etiology of OXPA are presently unavailable, and further consideration will be needed.

The high incidence of MMA in China as detected via ENBS is consistent with previous reports [17–19] and might be attributable to a common Chinese population-specific *MMACHC* mutation (p.W203X) [20,21]. Patients with this type of MMA develop homocystinuria (HCU) consequent to a *Cbl* C defect. Although MMA-like disease caused by vitamin B12 deficiency is well-known in South Asian countries such as Nepal and India, no Chinese patients with MMA caused by dietary vitamin B12 deficiency were identified in our study, possibly because of the lack of cases involving episodes and histories indicating a dietary vitamin B12 deficiency (e.g., megaloblastic anemia and gastro resection). Although MMA, PPA, and BKTD were also frequently detected in India, the reasons for these relatively higher frequencies are currently unknown. Several Indian research institutes are now conducting pilot studies on ENBS [22,23], which may clarify the genetic backgrounds of these OAs.

Regarding FAODs, GA2, VLCAD deficiency, MCAD deficiency, and PCD were frequently detected during the selective screening of Japanese children, whereas GA2 was relatively common in other Asian countries. Using ENBS, the incidence rate of MCAD deficiency detected in Germany was 1:10,000, which is over 10-fold higher than that of Japanese patients. Approximately 90% of the mutant alleles of *ACADM* in Caucasian patients with MCAD deficiency are known to harbor a common variant (p.K329E) [24], and the p.Y67H mutation of *ACADM* has been identified in asymptomatic European patients [25]. In Asian countries, MCAD deficiency was more frequent in Japan than in Taiwan and South Korea and was associated with the common *ACADM* mutation p.T150Rfs (c.449-452delCTGA), which is found in 30–40% of mutant alleles in Japanese patients with MCAD deficiency [26,27]. This mutation was also reported in some patients from South Korea [28]. Hence, further studies in other Asian countries and beyond should clarify the genetic background of MCAD deficiency.

Regarding AAs, the frequencies of PKU, MSUD, and UCDs identified by selective screening were greater in other Asian countries than in Japan. MSUD was particularly common in Vietnam and India, suggesting that this condition may be prevalent in southeastern Asian countries. In contrast, the numbers of Japanese patients with PKU, MSUD, and HCU detected via selective screening were very small. These diseases are already included in Japanese NBS panels, and therefore are not normally requested during selective screening. PKU and citrin deficiency were detected relatively frequently during ENBS in Japan and Taiwan. Citrin deficiency is considered more prevalent in East Asian countries [29]. Notably, the incidence of PKU in Germany was 10-fold higher than that in Japan and Taiwan. These findings suggest that the incidence rates of AAs differ between European and Asian populations.

A comparison of IMD detection rates in Japan via selective screening versus ENBS revealed different disease frequencies per screening method. PPA, MCCD, VLCAD deficiency, MCAD deficiency, PKU, and citrin deficiency were discovered more frequently with ENBS than with selective screening. In particular, PPA was less frequently identified by selective screening than by ENBS. On the other hand, a larger number of patients with MCD were identified via selective screening, whereas very few were discovered with ENBS. Of the 24 Japanese patients with MCD, at least 7 were diagnosed with MCD secondary to dietary biotin insufficiency, which might have contributed to the large number of MCDs identified via selective screening. Moreover, a Japanese group first reported the cloning of the *HCS* gene, which encodes holocarboxylase synthetase, and the discovery of a mutation contributing to the underlying etiology of MCD [30,31]. This discovery may have raised awareness of MCD along with its characteristic clinical features among practicing physicians in Japan. However, the actual MCD detection rate with ENBS was likely very low. Moreover, biotinidase deficiency has not been observed in the Japanese population.

This study had several limitations of note. During selective screening, the diagnoses of most patients were based mainly on the results of biochemical analysis. Because we did not examine genetic factors or enzyme activities, we could not conclude whether the incidence of each IMD was influenced by the genetic background or by other causes (e.g., dietary vitamin B12 and biotin deficiencies or the intakes of some drugs). Indeed, it may be difficult to prove that the high prevalence rates of some diseases could be attributed solely to population-specific common mutations. Furthermore, it might be difficult to completely compare the results of selective screening with those of ENBS. Nevertheless, the observed differences in incidence rates from before to after the implementation of ENBS will help other Asian countries to determine which diseases should be included in ENBS panels.

During the last 40 years, NBS has played an important role in preventing neurological impairment by detecting diseases such as PKU in the pre-symptomatic phase. Although this is also true for ENBS [1,2,4], ENBS can also detect diseases that may not require treatment, such as mild PPA or asymptomatic VLCAD deficiency, which are relatively common in Japan and Europe [3]. Although no previous report has described symptoms among patients with mild PPA, the evidence that these patients remain asymptomatic throughout their lives has not yet reported. Therefore, we cannot conclude whether mild PA should or should not be excluded from screening at the present point. Our results, however, suggest that such diseases could potentially be excluded in the future after determining the natural disease history. Additionally, the diseases targeted by ENBS may be amended to reflect the individual country. Finally, the target diseases identified by ENBS are generally extremely rare. Therefore, international collaborative activities such as the current study are important to the clarification of the natural histories of these diseases, development of diagnostic methods and therapies, and elucidation of genetic backgrounds.

5. Conclusion

Our study identified diverse IMD incidence rates and disease spectra among Asian countries. The IMD disease spectra determined by selective screening differed from those detected by ENBS. These data may facilitate improvements in ENBS implementation, the development of diagnostic and therapeutic groundwork, and the enhancement of welfare policies (including reductions in screening costs).

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Conflicts of interest

The authors have no conflicts of interest to declare regarding the publication of this manuscript.

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