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Spectrum Analysis of Common Inherited Metabolic Diseases in Chinese Patients Screened and Diagnosed by Tandem Mass Spectrometry

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Background: Information concerning inherited metabolic diseases in China is scarce. We investigated the prevalence and age distributions of amino acid, organic acid, and fatty acid oxidation disorders in Chinese patients. *Methods:* Blood levels of amino acids and acylcarnitines (tandem mass spectrometry) were measured in 18,303 patients with suspected inherited metabolic diseases. Diagnosis was based on clinical features, blood levels of amino acids or acylcarnitines, urinary organic acid levels (gas chromatography–mass spectrometry), and (in some) gene mutation tests. *Results:* Inherited metabolic diseases were confirmed in 1,135 patients (739 males, 396 females). Median age was 12 months (1 day to 59 years). There were 28 diseases: 12 amino acid disorders (580 patients, 51.1%), with hyperphenylalaninemia (HPA) being the most common; nine organic acidemias (408 patients, 35.9%), with methylmalonic acidemia (MMA) as the most common; and seven fatty acid oxidation defects (147 patients, 13.0%), with multiple acyl-coenzyme A dehydrogenase deficiency (MADD) being the most common. Onset was mainly at 1–6 months for citrin deficiency, 0–6 months for MMA, and in newborns for ornithine transcarbamylase deficiency (OTCD). HPA was common in patients aged 1–3 years, and MADD was common in patients >18 years. *Conclusions:* In China, HPA, citrin deficiency, MMA, and MADD are the most common inherited disorders, particularly in newborns/infants. J. Clin. Lab. Anal. 29:162–168, 2015.

Key words: tandem mass spectrometry; inherited metabolic disease; amino acids; acylcarnitine

INTRODUCTION

Inherited metabolic diseases are caused by genetic defects in the biosynthesis of certain enzymes, proteins, or receptors, leading to the accumulation or deficiency of related metabolites in cells or body fluids, and damage to organisms. Based on the biochemical properties of the substances involved, the wide variety of inherited metabolic diseases that exist may be categorized as disorders involving carbohydrates, amino acids, organic acids, fatty acids, hormones, and cholesterol (1).

Many inherited metabolic diseases have no specific clinical features and are difficult to diagnose using clinical manifestations only or conventional laboratory tests (2). Failure to accurately diagnose inherited metabolic diseases can result in delayed treatment, resulting in enormous burdens on affected patients and care providers. Therefore, improving early diagnosis and treatment could profoundly reduce mortality and morbidity associated with these disorders (3).

Using relatively simple tests involving the detection of amino acids and acylcarnitines in dried blood spots on

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filter paper, tandem mass spectrometry (MS/MS) allows for rapid screening and diagnosis of more than 40 metabolic disorders in amino acids, organic acids, and fatty acid oxidation, substantially improving the efficiency and accuracy of early diagnosis (4, 5). Recently, Lee et al. reported that the large spectrum of inherited metabolic diseases in individuals from Hong Kong was detectable using MS/MS techniques (6), and Niu et al. similarly analyzed the occurrence of these disorders in Taiwanese (7). However, no such studies have been conducted in mainland China.

Thus, we began screening for inherited metabolic diseases among patients in mainland China in 2002 using MS/MS techniques and continued our efforts till June 2012. In the present study, we analyzed age distributions, prevalence, and age of onset for each of these disorders in Chinese patients, aiming to improve awareness of these conditions.

SUBJECTS AND METHODS

Subjects

From February 2002 to June 2012, 18,303 patients with suspected inherited metabolic diseases from 99 hospitals distributed across China (scattered throughout all the provinces of mainland China and Macao, with the exception of five provinces (Hainan, Neimenggu, Tibet, Ningxia, and Hong Kong)) were screened using MS/MS techniques. The protocol was approved by the Ethics Committee of the current hospital (XHEC-D-2009–014), and written informed consent was obtained from the patients and/or their parents.

Levels of Amino Acids and Acylcarnitines in Dried Blood Spots

MS/MS was used to detect the levels of amino acids and acylcarnitines in dried blood spots. The method used has been described in previous studies (8, 9). Dried blood spots were extracted with methanol, which contained amino acids and acylcarnitines as internal standards. Following derivatization with *n*-butyl alcohol-hydrochloric acid, samples were tested using MS/MS. The tandem mass spectrometer (API2000/API4000) was purchased from Applied Biosystems (Foster City, CA), and high-performance liquid chromatography system (Agilent 1100) was purchased from Agilent Technologies (Santa Clara, CA).

Disease Diagnosis

Disorders diagnosis was made based on the presence of specific markers (amino acids or acylcarnitines) detected by MS/MS, together with clinical features, conventional laboratory tests, and the organic acid levels tested in urine by gas chromatography–mass spectrometry (8, 10). For some patients, diagnosis was confirmed by enzyme activity tests and gene mutation tests (11–13).

Grouping According to Age of Onset

Patients were divided into the following groups according to their age at disease onset: newborn, 1–6 months, 6–12 months, 1–3 years, 3–6 years, 6–12 years, 12–18 years, and more than 18 years old.

Statistical Analysis

For non-normally distributed variables, values are expressed as medians. Differences between groups were compared by the rank-sum test, using SPSS v. 18.0 (SPSS Inc., Chicago, IL). *P*-values less than 0.05 were considered statistically significant ($P < 0.05$).

RESULTS

In 18,303 patients with suspected inherited metabolic diseases, 1,135 cases with 28 different diseases were confirmed. Among these cases, 739 patients were male and 396 were female. Median age was 12 months (range, 1 day to 59 years). Clinical features included recurrent vomiting, seizures, coma, growth and mental retardation, abnormal muscular tension, jaundice, and hepatosplenomegaly. Laboratory tests suggested metabolic acidosis, hypoglycemia, hyperammonemia, hyperlactacidemia, and ketone bodies in the urine. Among the 1,135 confirmed cases, amino acid disorders were the most common, including 580 cases (51.1%) and 12 disease types, corresponding to 3.2% of all tested patients. Organic acidemias were found in 408 patients (35.9%) with nine disease types, representing 2.2% of all tested patients. There were seven diseases involving fatty acid oxidation defects found in 147 patients (13.0%), representing 0.8% of all patients tested.

Spectrum Analysis of Amino Acid Disorders

The disease spectrum of amino acid disorders is shown in Table 1. Hyperphenylalaninemia (HPA) was the most common disease (311 cases, 57.1%), followed by citrin deficiency (121 cases, 20.9%), ornithine transcarbamylase deficiency (OTCD; 42 cases, 7.2%), maple syrup urine disease (MSUD; 33 cases, 5.7%), and citrullinemia type I (12 cases, 2.1%). The remaining identified disorders were comparatively rare.

TABLE 1. Disease Spectrum of Amino Acid Disorders

Amino acid metabolic disorder	*n*	Of all cases of amino acid disorders (%)	Of all confirmed cases (%)	Of all tested cases (‰)
HPA	331	57.1	29.2	18.1
Citrin deficiency	121	20.9	10.7	6.6
OTCD	42	7.2	3.7	2.3
MSUD	33	5.7	2.9	1.8
Citrullinemia-I	12	2.1	1.1	0.7
Tyrosinemia-I	11	1.9	1.0	0.6
Tyrosinemia-II or Tyrosinemia-III	10	1.7	0.9	0.5
Argininemia	7	1.2	0.6	0.4
Hypermethioninemia	6	1.0	0.5	0.3
Hyperhomocysteinemia	5	0.9	0.4	0.3
Nonketotic hyperglycinemia	1	0.2	0.1	0.1
Carbamoyl phosphate synthetase deficiency	1	0.2	0.1	0.1
Total	**580**	**100.0**	**51.1**	**31.7**

HPA, hyperphenylalaninemia; OTCD, ornithine transcarbamylase deficiency; MSUD, maple syrup urine disease.

Spectrum Analysis of Organic Acidemias

The results of tests for organic acidemias are shown in Table 2. Methylmalonic acidemia (MMA) was the most common disorder (242 cases, 59.3%), followed by propionic acidemia (PA; 55 cases, 13.5%), glutaric acidemia type I (GA-1; 31 cases, 7.6%), isovaleric acidemia (IVA; 24 cases, 5.9%), and holocarboxylase synthesis deficiency (HLCS; 24 cases, 5.9%). Other identified diseases were comparatively rare.

Spectrum Analysis of Fatty Acid Oxidation Defects

The disease spectrum of fatty acid oxidation defects is shown in Table 3. Multiple acyl-coenzyme A dehydrogenase deficiency (MADD) was the most common disease (79 cases, 53.7%). Additionally, carnitine transport defect (28 cases, 19.0%), very long chain acyl-coenzyme A dehydrogenase deficiency (VLCAD; 18 cases, 12.2%), and short-chain acyl-coenzyme A dehydrogenase deficiency (13 cases, 8.8%) were common.

Age Distributions for Common Diseases

Age distributions of common inherited metabolic diseases are shown in Table 4. In patients with HPA, the youngest and oldest ages were 1 month and 29 years, respectively, with a median age of 2 years. Onset age was 1–3 years, and no newborns were identified. The youngest patient with citrin deficiency was aged 9 days, and oldest was aged 10 years, with a median age of 3 months. Onset age was predominantly between 1 and 6 months,

TABLE 2. Disease Spectrum of Organic Acidemias

Organic acidemia	*n*	Of all cases of organic acidemia (%)	Of all confirmed cases (%)	Of all tested cases (‰)
MMA	242	59.3	21.3	13.2
PA	55	13.5	4.8	3.0
GA-1	31	7.6	2.7	1.7
IVA	24	5.9	2.1	1.3
HLCS	24	5.9	2.1	1.3
BTD	16	3.9	1.4	0.9
BKT	8	2.0	0.7	0.4
MCC	3	0.7	0.3	0.2
HMG	3	0.7	0.3	0.2
Malonic acidemia	2	0.5	0.2	0.1
Total	**408**	**100.0**	**35.9**	**22.3**

MMA, methylmalonic acidemia; PA, propionic acidemia; GA-1, glutaric acidemia type I; IVA, isovaleric acidemia; HLCS, holocarboxylase synthesis deficiency; BTD, biotinidase deficiency; BKT, beta-ketothiolase deficiency; MCC, 3-methylcrotonyl-CoA carboxylase deficiency; HMG, 3-hydroxy-3-methylglutaryl-CoA lyase deficiency.

TABLE 3. Disease Spectrum of Fatty Acid Oxidation Defects

FAOD	*n*	Of all cases of FAOD (%)	Of all confirmed cases (%)	Of all tested cases (‰)
MADD	79	53.7	7.0	4.3
CTD	28	19.0	2.5	1.5
VLCAD	18	12.2	1.6	1.0
SCAD	13	8.8	1.1	0.7
MCAD	3	2.0	0.3	0.2
CPT-I	3	2.0	0.3	0.2
CPT-II	3	2.0	0.3	0.2
Total	**147**	**100.0**	**13.0**	**8.0**

FAOD, fatty acid oxidation defect; MADD, multiple acyl-coenzyme A dehydrogenase deficiency; CTD, carnitine transport defect; VLCAD, very long chain acyl-coenzyme A dehydrogenase deficiency; SCAD, short-chain acyl-coenzyme A dehydrogenase deficiency; MCAD, medium-chain acyl-coenzyme A dehydrogenase deficiency; CPT-I, carnitine palmitoyl transferase I deficiency; CPT-II, carnitine palmitoyl transferase II deficiency.

TABLE 4. Age Distributions for the Common Inherited Metabolic Diseases

	Citrin deficiency		MSUD		OTCD		MMA		PA		MADD		CUD		HPA	
Time period	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Newborn	12	10	15	45	18	43	71	30	20	36	6	8	3	11	0	0
1–6 months	86	71	3	9	2	5	50	21	7	13	3	4	4	14	18	5
6 months–1 year	18	15	6	18	4	10	45	19	10	18	2	3	7	25	68	21
1–3 years	3	2	5	15	11	26	32	14	13	24	4	5	6	21	108	33
3–6 years	1	1	1	3	4	10	14	6	3	5	2	3	4	14	58	18
6–12 years	1	1	0	0	2	5	8	3	2	4	9	11	2	7	55	17
12–18 years	0	0	0	0	1	2	4	2	0	0	13	16	1	4	15	5
>18 years	0	0	3	9	0	0	9	4	0	0	40	51	1	4	9	3
Total	**121**	**100**	**33**	**100**	**42**	**100**	**233**	**100**	**55**	**100**	**79**	**100**	**28**	**100**	**331**	**100**

MSUD, maple syrup urine disease; OTCD, ornithine transcarbamylase deficiency; MMA, methylmalonic acidemia; PA, propionic acidemia; MADD, multiple acyl-coenzyme A dehydrogenase deficiency; CTD, carnitine transport defect; HPA, hyperphenylalaninemia.

accounting for 86 cases (71%). Among patients with OTCD, the youngest and oldest were aged 2 days and 18 years, respectively. Newborns comprised the majority of patients with this disorder, accounting for 18 cases (43%). For MSUD, the youngest and oldest patients were aged 6 days and 30 years, respectively, and there were 15 cases (45%) in newborns. The youngest and oldest patients with MMA were aged 1 day and 34 years, respectively. Patients aged 0–6 months made up the majority (51%) of cases. The youngest and oldest patients with PA were aged 4 days and 10 years, respectively. Onset age was most commonly under 6 months, and no patients aged greater than 18 years were identified. The youngest and oldest patients with MADD were aged 36 hr and 59 years, respectively. There were 40 cases (51%) aged more than 18 years. The earliest onset age for primary carnitine deficiency (also known as carnitine uptake defect, CUD) was 10 days old, most often occurring in ages between 6 months and 3 years, with only one case observed in an individual aged more than 18 years (42 years old).

DISCUSSION

Inherited metabolic diseases are caused by genetic defects in metabolic pathways, leading to the accumulation of abnormal metabolites or deficiency of vital bioactive compounds that cause corresponding clinical symptoms (14). At present, the diagnosis of inherited metabolic diseases mainly aims at detecting specific biochemical changes for many diseases or, alternatively, specific enzyme activities or mutations of pathogenic genes. The establishment of MS/MS methods and their wide primary use in clinical tests has provided an important advancement in clinical diagnosis, and has promoted screening and detection of inherited metabolic diseases (15–17).

By detecting the mass-to-charge ratio (relative molecular mass) of compounds in a sample, MS/MS is able to rapidly analyze substances, both qualitatively and quantitatively. This method can simultaneously detect more than 50 amino acids and acylcarnitines in a single drop of blood, and thereby carry out a rapid screening and diagnosis of more than 40 metabolic disorders in amino acids, organic acids, and fatty acid oxidation (18, 19). Spectrum analysis indicated that in China, amino acidopathy had a higher prevalence than organic acidemias and fatty acid oxidation defects. The comparatively common diseases were HPA, citrin deficiency, OTCD, MSUD, MMA, PA, GA-1, IVA, HLCS, and MADD. These diseases are worthy of further investigation, and may be suggested as preferred diseases for screening in newborns.

Amino acid metabolic disorders are mainly caused by abnormal internal levels of relevant amino acids, often due to dysfunction of amino acid metabolism and corresponding increase in organic acids from the bypass metabolic pathway. MS/MS can achieve diagnosis of amino acid disorders through testing of amino acid levels and ratios of relevant amino acids in dried blood spots (20). The current study found that in China, HPA occurred most frequently, followed by citrin deficiency. In contrast, Lee et al. reported that the most common disease in Hong Kong was citrin deficiency, followed by HPA (6). In Germany, the prevalence of HPA is 1/4,500, much greater than that of MSUD (1/125,000) (21). In Japan, Shigematsua et al. carried out a screening of 102,200 newborns, and found only one case of HPA, corresponding to a prevalence of 1/102,200 (22). Notably, MSUD and citrin deficiency were not identified in the Shigematsua et al. screening (22). In South Korea, Yoon et al. reported the prevalence of HPA to be 1/19,795 and MSUD to be 1/39,590 (23).

Acylcarnitines are intermediates in organic acid and fatty acid oxidation. MS/MS can detect organic acidemia and fatty acid oxidation defects through measurement of blood levels of acylcarnitines (3). The present study

revealed that the most common organic acidemia in mainland China was MMA, followed by PA. These findings differ from the observations by Niu et al. in Taiwan showing that the most common organic acidemia was 3-MCC (3-methylcrotonyl-CoA carboxylase deficiency; a prevalence of 1/32,929), followed by GA-1 and MMA (a prevalence of 1/101,625 for both) (7). Furthermore, Frazier et al. identified 3-MCC as the most common organic acidemia in North Carolina (USA), with a prevalence of 1/36,000, much higher than that of MMA (1/90,000) (24). However, Wajner et al. pointed out that among clinical cases in Brazil, MMA comprised a higher proportion (15.6%) of cases of organic acidemia than 3-MCC (0.92%), indicating that most MCC cases were benign in nature (25). Indeed, 3-MCC is most often a nondisease that is only detected in a newborn screening context. Therefore, since we investigated patients in metabolic decompensation, we observed fewer cases of 3-MCC compared with these previous studies (7,24,25). In the present study, all three 3-MCC cases were confirmed by gene mutation test. Fatty acid oxidation defects can occur at every step following the entry of fatty acids into mitochondria and throughout oxidation process. As in organic acidemia, acylcarnitines are also intermediates of fatty acid oxidation. Screening and diagnosis of metabolic disorders in fatty acid oxidation may be achieved through determination of blood levels of free carnitine and acylcarnitines of different carbon-chain lengths (26, 27). In many countries, MS/MS has been used to detect blood levels of acylcarnitines for diagnosis of fatty acid oxidation disorders, and it has been reported that the prevalence of fatty acid oxidation defects increased over time (28). Furthermore, the spectrum of fatty acid oxidation defects varies between ethnicities. In China, fatty acid oxidation defects had a lower prevalence than those of amino acid disorders and organic acidemias. MADD was the most common disorder, followed by CUD. Among 147 cases of fatty acid oxidation defects confirmed in this study, there were only three cases of medium-chain acyl-coenzyme A dehydrogenase deficiency (MCAD), suggesting that this disease is rare in China. However, there were 18 cases of VLCAD, indicating its commonplace nature. In European and American countries, metabolic disorders in fatty acid oxidation have a higher prevalence, with the most common being MCAD, but VLCAD is rare (29). According to Lindner et al., the prevalence of VLCAD was 1/94,568 in the United States, much lower than that of MCAD (1/10,508) (30). Similarly, in Germany, Schulze et al. found that the prevalence of MCAD was 1/15,600, while that of VLCAD was 1/250,000 (21). Moreover, in Australia, Kasper et al. found the prevalence of MCAD to be 1/24,900, while that of VLCAD was 1/88,927 (31).

The present study of onset age of inherited metabolic diseases indicated that onset occurs predominantly in newborns and infants. The onset of MSUD, OTCD, MMA, and PA most commonly occurred in the newborn period, while that of citrin deficiency and HLCS tended to occur in infancy. However, HPA, GA-1, VLCAD, and CUD more often occurred in childhood. These diseases also exhibited a progressively worsening trend if not diagnosed or treated during the early period, with untreated patients being at high risk of early mortality or permanent neurological disabilities (3). Patients with MADD fall into two categories: neonatal-onset and late-onset. In this study, late-onset was most common, though six patients presented symptoms in the neonatal period and two patients died, consistent with a previous report (32). Therefore, it is important that the possibility of inherited metabolic disease be considered in young patients with unidentified clinical symptoms, such as repeated emesis, seizures, hypotonia, and intellectual disability. Also, these conditions may occur in young patients with metabolic acidosis, hypoglycemia, hyperlactacidemia, and hyperammonemia identified by laboratory investigations. Techniques such as MS/MS can be used promptly as specific tests to ensure that these diseases are diagnosed and treated in time, thereby greatly reducing mortality and disability of affected infants (33, 34).

The samples in this study were from 99 hospitals in mainland China, predominantly in the southeastern regions. Thus, it is important to consider that different ethnicities may have different gene mutations, leading to variation in the spectrum of inherited diseases. Furthermore, screening of newborn diseases is quite widely performed in the southeastern area of China, and the environment of the southeastern area of China may have a profound influence on study results, necessitating further confirmation within individual regions. In addition, three of the most common disorders observed in the present study, OTCD, Carbamyl Phosphate Synthase deficiency, and citrin deficiency, are difficult to diagnose only using MS/MS, and we had to rely on clinical symptoms, biochemical assays, and the organic acid levels in urine tested by gas chromatography–mass spectrometry. Thus, the real prevalence of these three disorders could be more important than the prevalence reported in the present study.

Widely applicable MS/MS techniques play an important role in screening and diagnosis of diseases in newborns. Furthermore, MS/MS may be helpful in facilitating early diagnosis and timely treatment of inherited metabolic disorders. Identifying common inherited metabolic diseases in Chinese patients, as well as their features and onset ages, may provide clinicians with a deeper understanding of these conditions, allowing for improved early diagnosis and treatment of these diseases.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

CUD = carnitine uptake defect
FAOD = fatty acid oxidation defect
GA-1 = glutaric acidemia type I
HLCS = holocarboxylase synthesis deficiency
HMG = 3-hydroxy-3-methylglutaryl-CoA lyase deficiency
HPA = hyperphenylalaninemia
IVA = isovaleric acidemia
MADD = multiple acyl-coenzyme A dehydrogenase deficiency
MCAD = medium-chain acyl-coenzyme A dehydrogenase deficiency
MCC = 3-methylcrotonyl-CoA carboxylase deficiency
MMA = methylmalonic acidemia
MS/MS = tandem mass spectrometry
MSUD = maple syrup urine disease
OTCD = ornithine transcarbamylase deficiency
PA = propionic acidemia
VLCAD = very long chain acyl-coenzyme A dehydrogenase deficiency

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