Newborn Screening in China: Phenylketonuria, Congenital Hypothyroidism and Expanded Screening

Xuefan Gu,[1]*MD, PhD*, Zhiguo Wang,[2]*MD*, Jun Ye,[1]*MD*, Lianshu Han,[1]*MD*, Wenjuan Qiu,[1]*MD, PhD*

Abstract

This study was to investigate the current status of neonatal screening in China, to further clarify the incidences of hyperphenylalaninemia (HPA) and congenital hypothyroidism (CH). From 2000 to 2007, a total of 17,961,826 newborns had been screened for HPA and 1527 cases were detected, giving a HPA prevalence of 1:11,763. At the same time, 18,284,745 newborns had also been tested for CH, with 8918 cases being detected (1:2050). It is remarkable that the mean number of newborns screened per year had increased 5 times between 2000 and 2007. In Shanghai, 116,000 newborns were screened using tandem mass spectrometry and 6 different were detected. The overall prevalence of an inborn errors of metabolism identified was 1 in 5800 healthy newborns, with hyperphenylalaninemia being the most common. Neonatal screening had developed rapidly in China in recent years, and a pilot study using tandem mass spectrometry has been started. The biggest challenge is still to increase coverage to the entire country, especially in the mid-western area.

Ann Acad Med Singapore 2008;37(Suppl 3):107-10

Keywords Congenital hypothyroidism, Hyperphenylalaninemia, Neonatal screening, Phenylketonuria, Tandem mass spectrometry

Introduction

The diseases on the neonatal screening panel are difficult to diagnose by normal medical examination. Neonatal screening can make diagnosis and treatment possible even before the occurrences of signs or symptoms. Prompt recognition, diagnosis and treatment are important as the patient could benefit from the early, presymptomatic diagnosis. Abnormal amino acids, organic acids, and fatty acids metabolism are the major causes of many inborn errors of metabolism and many other diseases.[1,2] The diseases commonly screened for in the newborn period are principally inborn errors of metabolism which have a diverse spectrum in different countries, with different rates of incidence.

As an important measure for preventive medicine, neonatal screening programme in China was started in 1981, and it has now become one of the most popular measures for control and treatment of congenital problems. The aims of this study were (i) to describe the current status of neonatal screening for hyperphenylalaninemia (HPA)congenital hypothyroidism (CH), and their incidence; (ii) to report the recent development of neonatal screening using tandem mass spectrometry in Shanghai, and to further clarify the incidence of these diseases and their spectrum.

Methods

Dried blood spot (DBS) specimens were collected from 3-day old babies using a heelstick and spotted onto filter paper (S&S 903), and sent to the local neonatal screening laboratory. All screening laboratories participated in the external quality assessment activity organised by National Neonatal Screening Quality Control, National Center for Clinical laboratories (NCCL). The parents were provided information about the screening and they can indicate their choice in written form.

Neonatal Screening for Hyperphenylalaninemia and Congenital Hypothyroidism

The data on neonatal screening for HPA and CH were collected from the neonatal screening centres from the whole country by National Neonatal Screening Quality Control Laboratory, National Center for Clinical Laboratories. Phenylketonuria (PKU) screening was

[1] Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Institute for Pediatric Research, Shanghai, China
[2] National Center for Clinical laboratory, Beijing, China
Address for Correspondence: Professor Xuefan Gu, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Institute for Pediatric Research, Shanghai 200092, China.
Email: guxuefan@online.sh.cn.

achieved by fluorometric method, quantitative enzymatic method, and bacterial inhibition assay. The cut-off value was 120 μmol/L for hyperphenylalaninemia and differentiation between PKU and tetrahydrobiopterin (BH_4) deficiency was performed in majority of patients. Thyroid stimulating hormone (TSH) was tested by time-resolved fluorescence immunoassay (TRFIA), fluorescence enzyme immunoassay (FEIA) and enzyme-linked immunosorbent assay. The cut-off value was 10 IU/L for CH screening.

Neonatal Screening Using Tandem Mass Spectrometry

Neonatal screening using tandem mass spectrometry was actually performed only in Shanghai. It was started in early 2003. The blood samples were drawn from newborns delivered at 50 local maternity and children's health hospitals or general hospitals in Shanghai. The dried blood samples on 3.2 mm filter paper were punched and derived using n-butanol hydrochloride with isotope internal standard, which was purchased from the American Cambridge Isotope Laboratory. The analysis was carry out on the API 2000 tandem mass spectrometer as previously reported.[3] The quality control samples were provided by the Department for Screening Neonates, Centre for Disease Control and Prevention, USA.

The diagnosis of disease was performed according to the tandem mass spectrum profiles, with appropriate confirmatory diagnostic testing, and gas-chromatographic mass spectrometry for organic acids analysis. A few diseases were confirmed by DNA analysis and therapeutic outcome.

Results

Neonatal Screening for Hyperphenylalaninemia and Congenital Hypothyroidism

According to the data collected by the National Center for Clinical Laboratory, China had 143 neonatal screening laboratories in 2007 and 117 centres had reported their screening data. For screening of phenylalanine, the fluorometric method was used in 69.7% of the laboratories, bacterial inhibition assay was used in 21.2%, and quantitative enzymatic method in 9.1%. For screening of TSH, time-resolved fluorescence immunoassay was used in 56.8% of the laboratories, enzyme-linked immunosorbent assay 28.8% and fluorescence enzyme immunoassay method in 14.4%.

From 2000 to 2007, a total of 17,961,826 newborns had been screened for phenylalanine and 1527 cases were detected, giving a hyperphenylalaninemia prevalence of 1:11,763. At the same time, 18,284,745 newborns had also been tested for TSH and 8918 cases were detected. Hence, the prevalence of CH was 1:2050. The annual number of newborns screened and cases identified are shown in Table 1. It is remarkable that the mean number of screened per year had increased 5 times from 2000 to 2007. The national coverage of neonatal screening was almost 33.2% in 2007.

Table 1. Annual Number Screened and Cases Identified in China (2000-2007)

Year	Number screened for HPA	Cases of HPA identified	Number screened for CH	Cases of CH identified
2000	1,112,980	94	1,113,682	469
2001	1,520,750	142	1,520,000	582
2002	1,139,273	117	1,139,054	547
2003	1,140,663	119	1,153,004	614
2004	1,993,782	141	1,992,571	945
2005	2,802,270	224	2,813,883	1 361
2006	2,929,236	219	2,944,022	1 701
2007	5,322,872	471	5,608,529	2 699
Total	17,961,826	1527	18,284,745	8 918

Neonatal Screening Using Tandem Mass Spectrometry

From January 2003 to 2007, 116,000 neonatal samples in Shanghai were analysed using tandem mass spectrometry. Twenty newborns were screened positive and were also confirmed to have inborn errors of metabolism, and these included 6 different kinds of diseases (Table 2). Thus the resulting overall prevalence of an inborn error of metabolism identified in newborn screening using tandem mass spectrometry in Shanghai was 1 in 5800 healthy newborns. Classification of these disorders into different groups revealed 11 patients with amino acidemias (1:10,545), 7 with organic acidemias (1:16,571) and 2 with fatty acid oxidation disorders (1:58,000). The result shows that hyperphenylalaninemia was the most common disease of all inborn of errors of metabolism in this group.

Discussion

Neonatal screening began with the screening of PKU in

Table 2. Results of Expanded Newborn Screening by Tandem Mass Spectrometry for 116,000 Newborns in Shanghai

Disease	n
PKU/hyperphenylalaninemia	10
Maple syrup urine disease	1
Methylmalonic acidemia	3
Propionic acidemia	1
3-methylcrotonyl-CoA carboxylase defection	3
Short chain acyl-CoA dehydrogenase deficiency	2
Total	20

the early 1960s. It was soon followed by multiple screening for many other diseases and DBS on filter paper continue to be the preferred method used for population-based newborn screening. So far, mass screening of newborns for inborn errors metabolism is a tremendous achievement in the field of preventive medicine. In China, neonatal screening first started in 1981. The first paper on the incidence of IEM in Shanghai in 1984 reported a figure of 1 in 15,930 for PKU, and 1in 6309 for CH.[4] However, based on the results of 5.8 million neonatal screening samples collected from the main screening centres, the incidence of hyperphenylalaninemia and CH were 1 in 11,144 and 1 in 3009 respectively.[5] The results of this paper with almost 18 million newborns screened again showed that the prevalence of hyperphenylalaninemia was 1 in 11,763, similar to that reported previously. However, the prevalence of CH was 1 in 2050 which was higher than that previously reported. In fact, the most common screening diseases in most neonatal screening centres in China were HPA and CH.

PKU was the first treatable inherited metabolic disease. HPA might be caused by a deficiency of phenylalanine hydroxylase, the classical type, or by tetrahydrobiopterin (BH_4) deficiency. BH_4 is an essential cofactor for the aromatic amino acid hydroxylases. Deficiency of BH_4 may lead to phenylketonuria phenotype, with mental retardation as well as other severe neurological disorders.[6,7] With neonatal screening for HPA gradually spreading across the entire China, many patients who were detected and treated early had a good physical and nearly normal mental development. However, some patients with HPA caused by either a deficiency of BH_4, a cofactor of phenylalanine, tyrosine and tryptophan hydroxylase, or a deficiency of 6-pyruvoyl-tetrahydropterin synthase (PTPS) or dihydropteridine reductase (DHPR) have clinical symptoms that are confusingly similar to that of classic PKU and are often misdiagnosed as the classical PKU. However, their prognosis are much worse than classical PKU and cannot be solely treated with the PKU regime.[8,9] Thus, to distinguish these cases from those suffering from classical PKU is not only important for appropriate, prompt and specific treatment but also it is also beneficial for genetic counseling.

Patient with BH_4 deficiency have specific urinary pterin profiles that can be indicative of a specific enzyme defect. Therefore, urinary pterin analysis is utilised to rule out BH_4 deficiencies among HPA patients. The incidence of BH_4 deficiency in Caucasian newborns was approximately 1% to 3% of those with HPA.[6] According to a study of our 341 HPA patients from the neonatal screening centre in Shanghai, 44 confirmed cases of BH_4 deficient patients resulted in an incidence of BH_4 deficiency of 12.9% among those with HPA, which was much higher than the Caucasian counterparts. Hence, it is crucial to set up screening for BH_4 deficiency among all HPA patients diagnosed with HPA.

CH is another congenital disorder with a prevalence of 1:2050 in China. This high prevalence warrants our special attention. We believe that the high prevalence is related to 3 factors. First is the evolution of methodology for TSH measurement. At the beginning of the neonatal screening period, the radioimmunoassay (RIA) method used to be popular and the prevalence was low because of the lack of sensitivity of this test. In recent years, time-resolved fluorescence immunoassay, enzyme-linked immunosorbent assay and fluorescence enzyme immunoassay method were used in all screening laboratories and the prevalence has been increased. A second factor is the revised cut-off value. Ten years ago, the cut-off value of TSH for neonatal screening was 15 IU/L to 20 IU/L in most neonatal screening laboratories. Now the cut-off value is set up at 10 IU/L so that milder cases of CH could also be detected. A third factor is the deficiency of iodine. Although nationwide iodinification of salt was implemented in the early 1990s, mild cases of iodine deficiency still exist in some mountainous or remote areas. Hyperthyrotropinemia or mild hypothyroidism is still on the rise.

Neonatal screening has evolved conceptually from a laboratory test for a disorder into a public health system. Previously, many children were not screened for inborn errors of metabolism as these were considered rare diseases and thus were not treated in the early stages. This led to delayed mental and motor development, and sometimes even death. The introduction of the analysis of amino acids and acylcarnitines by tandem mass spectrometry to population-based neonatal screening has tremendously increased the number of treated cases of detectable forms of inborn errors of metabolism. Advancement in newborn screening technology, coupled with recent advancement in the diagnosis and treatment of rare but serious neonatal congenital conditions provide increased opportunities for effective management of patients and their families.[10-12] According to the American College of Medical Genetics (ACMG), a uniform neonatal screening panel of 29 disorders including 6 amino acid disorders, 9 organic acidemias, 5 fatty acid oxidative disorders, 3 hemoglobinopathies and 6 others was advocated in recent years.[13]

China is a fast developing country. Development in managing the morbidity and mortality of inborn errors of metabolism should take precedence over socio-economic and technology development. Among all the neonatal diseases, more than 500 inborn errors of metabolism are especially important firstly, because of their relative frequency, and secondly, because rational therapy has been or will be available in the near future. Our pilot study of neonatal screening using tandem mass spectrometry in

Shanghai had revealed that the prevalence of detectable disorders was 1 in 5800, and those affected patients can have substantial improvements in mortality and morbidity. We expect that newborn screening using tandem mass spectrometry could be further expanded, and more experience in neonatal screening, diagnosis and treatment of IEMs can be accumulated and shared among Chinese scientists and physicians.

Acknowledgement

This work was supported by Hi-Tech Research and Development Program (2007AA02Z447), National Science and Technology Program (2006BAI05A05, 2006BAI05A07) and Shanghai Bureau of health (2008ZD001).

REFERENCES

1. Schulze A, Lindner M, Kohlmuller D, Olgemöller K, Mayatepek E, Hoffmann GF. Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications. Pediatrics 2003; 111:1399-406.
2. Chace DH, Kalas TA. A biochemical perspective on the use of tandem mass spectrometry for newborn screening and clinical testing. Clin Biochem 2005; 38:296-309.
3. Han LS, Ye J, Qiu WJ, Gao XL, Wang Y, Gu XF. Selective screening for inborn errors of metabolism on clinical patients using tandem mass spectrometry in China: a four-year report. J Inherit Metab Dis 2007; 30:507-14.
4. Chen RG, Sun M, Ni YY. Neonatal hypothyroidism, phenylketonuria and galactosemia screening in metropolitan Shanghai. Chin Med J 1984;97:61-5.
5. Gu XF, Wang ZG. Screening for phenylketonuria and congenital hypothyroidism in 5.8 million neonates in China. Zhonghua Yu Fang Yi Xue Za Zhi 2004;38:99-102.
6. Scriver CR, Kaufman S, Eisensmith RC. The hyperphenylalaninemias. In: Scriver CR, Beauder AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. 7th ed. New York: McGraw-Hill, 1995:1015-75.
7. Ye J, Liu X, Ma X, Zhang Y, Huang X, Chen R, et al. Screening for tetrahydrobiopterin deficiency among hyperphenylalaninemia patients in Southern China. Chin Med J 2002;115:217-21.
8. Blau N, Barnes I, Dhondt JL. International database of tetrahydrobiopterin deficiencies. J Inherit Metab Dis 1996:19:8-14.
9. Jäggi L, Zurflüh MR, Schuler A, Ponzone A, Porta F, Fio L, et al. Outcome and long-term follow-up of 36 patients with tetrahydrobiopterin deficiency. Mol Genet Metab 2008;93:295-305.
10. Hoffmann GF, von Kries R, Klose D, Lindner M, Schulze A, Muntau AC, et al. Frequencies of inherited organic acidurias and disorders of mitochondrial fatty acid transport and oxidation in Germany. Eur J Pediatr 2004;163:76-80.
11. Rinaldo P, Tortorelli S, Matern D. Recent developments and new applications of tandem mass spectrometry in newborn screening. Curr Opin Pediatr 2004;16:427-33.
12. Frazier DM, Millington DS, McCandless SE, Koeber DD, Weavil SD, Chaing SH, et al. The tandem mass spectrometry newborn screening experience in North Carolina: 1997-2005. J Inherit Metab Dis 2006;29:76-85.
13. American College of Medical Genetics Newborn Screening Expert Group. Newborn screening: toward a uniform screening panel and system – executive summary. Pediatrics 2006;117:S296-S307.