Expanded Newborn Screening for Inborn Errors of Metabolism and Genetic Characteristics in a Chinese Population

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The incidence of inborn errors of metabolisms (IEMs) varies dramatically in different countries and regions. Expanded newborn screening for IEMs by tandem mass spectrometry (MS/MS) is an efficient approach for early diagnosis and presymptomatic treatment to prevent severe permanent sequelae and death. To determine the characteristics of IEMs and IEMs-associated mutations in newborns in Jining area, China, 48,297 healthy neonates were recruited for expanded newborn screening by MS/MS. The incidence of IEMs was 1/1178 in Jining, while methylmalonic acidemia, phenylketonuria, and primary carnitine deficiency ranked the top 3 of all detected IEMs. Thirty mutations in nine IEMs-associated genes were identified in 28 confirmed cases. As 19 cases with the mutations in phenylalanine hydroxylase (PAH), solute carrier family 22 member 5 (SLC22A5), and methylmalonic aciduria (cobalamin deficiency) cblC type with homocystinuria (MMACHC) genes, respectively, it suggested that mutations in the PAH, SLC22A5, and MMACHC genes are the predominant causes of IEMs, leading to the high incidence of phenylketonuria, primary carnitine deficiency, and methylmalonic acidemia, respectively. Our work indicated that the overall incidence of IEMs is high and the mutations in PAH, SLC22A5, and MMACHC genes are the leading causes of IEMs in Jining area. Therefore, it is critical to increase the coverage of expanded newborn screening by MS/MS and prenatal genetic consulting in Jining area.

Keywords: newborn screening, inborn errors of metabolism, incidence of IEMs, IEMs-associated gene mutation

INTRODUCTION

Inborn errors of metabolisms (IEMs) are a group of genetic diseases leading to severe complications in newborns, infants, children, adolescents, or adults. IEMs are caused by the defect of an enzyme, its cofactor or a transporter resulting in the accumulation of a substrate and/or the deficiency of its downstream products. Up to now, more than 500 IEMs have been detected. Although individual IEM is rare, the incidence of overall IEMs is high and varies dramatically in different countries and regions (Campeau et al., 2008). For example, the incidence of IEMs was reported to be 1/667 in Saudi Arabia (Moammar et al., 2010), 1/784 in United Kingdom (Sanderson et al., 2006), 1/2500 in
Newborn screening for IEMs is an efficient approach for early diagnosis and presymptomatic treatment, which could improve the survival and well-being of affected infants. In addition to the classical newborn screening, expanded newborn screening by tandem mass spectrometry (MS/MS) has been applied widely since last decade. By analyzing amino acids and acylcarnitines in blood, three groups of IEMs could be simultaneously detected, including amino acid disorders, organic acid disorders, and fatty acid oxidation disorders. In China, expanded newborn screening started in 2004 and the incidence of IEMs was 1/3795 in a pilot study (Shi et al., 2012). However, later studies showed that the incidence of IEMs varied dramatically in different cities and regions of China, from 1/1683 to 1/8304 (Huang et al., 2011; Fan et al., 2013; Zhu et al., 2015; Lu et al., 2016). Expanded newborn screening for 25 IEMs is widely used in Jining since late 2014. To determine the incidence and characteristics of IEMs in Jining, 48,297 healthy neonates were recruited for expanded newborn screening of 25 IEMs by MS/MS. In the present study, we showed that the incidence of IEMs was 1/1178 in Jining, which is higher the incidence in China. In addition, 30 mutations in nine IEMs-associated genes were identified in the 28 confirmed cases (Yamaguchi, 2008; Yamaguchi et al., 2013).

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MATERIALS AND METHODS

Subject

A total of 48,297 infants born in Jining, China, between January 2015 and December 2015, were recruited for expanded newborn screening by MS/MS. In all, 15,731 infants were born in the urban area and 32,566 infants were born in the suburban and rural areas. Informed and written consent was obtained from the parents. This study was approved by the Ethical Committee of Jining Medical University.

Procedures of Screening Test and Follow-Up Testing

All the procedures were carried out in accordance with the Jining Newborn Screening Programme (2015) and Technical Guide of Newborn Screening in China (2010). Briefly, blood taken from heelstick was spotted on Whatman 903 filter paper, which was dried and sent by mail to the screening laboratory. The time of sampling was between the 3rd to 10th day of life. Dried blood spots were pre-processed following the instruction of NeoBase™ non-derivatized MS/MS kit (PerkinElmer, MA, United States), and then they were analyzed by using TQD tandem mass spectrometry system (Waters, MA, United States) and NeoBase non-derivatized MS/MS kit (PerkinElmer, United States). The analytes include alanine (ALA), arginine (ARG), citrulline (CIT), glycine (GLY), leucine (LEU), methionine (MET), ornithine (ORN), phenylalanine (PHE), tyrosine (TYR), valine (VAL), free carnitine (C0), acylcarnitines (C2, C3, C5DC, C4, C4DC, COH, C5, C5:1, C5DC, C5OH, C6, C8, C8:1, C10, C10:1, C10:2, C12, C12:1, C14, C14:1, C14:2, C14OH, C16, C16:1, C16OH, C16:1 OH, C18, C18:1, C18:2, C18OH, C18:1OH) and succinylacetone. The normal ranges and cut-off values of analytes in the manual of NeoBase™ non-derivatized MS/MS kit (Cat. 3040-001Z, PerkinElmer, MA, United States) and from the worldwide collaborative project were applied (McHugh et al., 2011). Suspected positive cases were recalled for the repeated test by MS/MS. The follow-up testing commences for the second time positive cases, including biochemical tests or genetic analysis. The recall and follow-up protocol is based on the clinical symptoms, screening test, and biochemical and genetic analysis. The parents of all cases with definitive diagnosis were informed and referred to specialists for the treatment.

Screened Panel of IEMs

Twenty-five IEMs were screened in this study. Nomenclature and abbreviations are adapted from the guidelines “Follow-Up Testing for Metabolic Disease Identified by Expanded Newborn Screening Using Tandem Mass Spectrometry” was applied in our study (Bennett, 2009; Dietzen et al., 2009). Definitive diagnosis is made by specialists based on the clinical symptoms, screening test, and biochemical and genetic analysis. The parents of all cases with definitive diagnosis were informed and referred to specialists for the treatment.

Genetic Analysis

Genetic analysis was performed by BioSan Biochemical Technologies (Hangzhou, Zhejiang, China) and Be Creative Lab (Beijing, China) using the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA, United States), respectively. Briefly, genomic DNA was extracted from whole

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blood using QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). Primers were designed by using Typer 4.0 software (Sequenom, San Diego, CA, United States). A total of 942 sites in IEMs-associated genes, ACADL, ACADM, acyl-CoA dehydrogenase, C-2 to C-3 short chain (ACADS), ACADVL, ACAT1, BTD, CPT2, ETFB, electron transfer flavoprotein dehydrogenase (ETF5DH), glutaryl-CoA dehydrogenase (GCDH), HCLS, HMGCL, IVD, MCC1, MCC2, MMACHC, methylmalonyl-CoA mutase (MUT), PCCA, PCCB, SLC22A5, SLC25A20, ARG1, ASS1, BCKDHA, BCKDHB, CBS, DBT, FAH, MAT1A, MTHFR, OTC, PAH, 6-pyruvoyltetrahydropterin synthase (PTS), SLC25A13, are included based on the mutations in PubMed, OMIM, and HGMD databases. The protocol of Sequenom iPLEX genotyping was strictly followed. One picomole PCR primers, 5 ng genomic DNA, and reaction mix (Sequenom, San Diego, CA, United States) were applied in each PCR. After SAP enzyme treatment and single-base extension, the products were spotted onto a SpectroCHIP with the Sequenom MassARRAY RS100 Nanodispenser (Sequenom, San Diego, CA, United States). Mass determination was done with the Sequenom MassARRAY mass spectrometer (Sequenom, San Diego, CA, United States).

Statistical Analysis
The data was presented descriptively and Chi-square tests were performed to compare the incidences or rates in the urban and suburban/rural areas. Statistical significance is accepted when $P < 0.05$.

RESULTS

Incidence and Characteristics of IEMs
To investigate the incidence of IEMs in Jining, 48,297 infants were recruited for expanded newborn screening by MS/MS in Jining. Of 769, 725 positive cases were successfully recalled for the second examination by MS/MS. The follow-up testing immediately commences for the positive cases in the second MS/MS test, including plasma amino acid analysis, urine amino acid analysis, urine pterin metabolite analysis, enzyme activity analysis, and/or genetic analysis (Bennett, 2009; Dietzen et al., 2009). The parents of all cases with definitive diagnosis were informed and referred to specialists for the treatment. Forty-one cases with IEMs were confirmed by follow-up testing and clinical investigation. The incidence of IEMs was 1/1178 and the corrected incidence was 1/1110 (Table 1).

A total of 15,731 neonates in the urban area and 32,566 neonates in the suburban or rural area were screened. Six and 35 confirmed cases were detected in the urban area and the suburban or rural area, respectively. The incidence of IEMs in the suburban or rural area was significantly higher than that in the urban area, 1/930 vs. 1/2622 ($P < 0.05$; Table 1). Based on the positive predictive values, 2.41 and 7.35% ($P < 0.01$), the corrected incidence was 1/862 and 1/2560 ($P < 0.05$) in the suburban or rural area and urban area, respectively (Table 1).

Nine types of IEMs were diagnosed in 41 cases. The incidences of amino acid disorders, fatty acid disorders, and organic acid disorders were 1/2841, 1/5366, and 1/3019, respectively. Methylmalonic acidemia, phenylketonuria, and primary carnitine deficiency ranked the top 3 of all detected IEMs with the incidence of 1/3220, 1/4391, and 1/9659, respectively. The incidences of maple syrup urine disease, argininemia, ornithine transcarbamylase deficiency, short-chain acyl-CoA dehydrogenase deficiency, glutaric acidemia type II, and glutaric acidemia type I were 1/24,149, 1/16,099, 1/48,297, 1/12,074, 1/48,297, and 1/48,297, respectively.

**TABLE 1 | Incidence and characteristics of IEMs.**

|                | Urban  | Suburban and rural |
|----------------|-------|-------------------|
| Recruited      | 15,731| 32,566            |
| Presumptive positive (positive in first screening) | 255   | 514               |
| Positive rate (%) | 1.62  | 1.58              |
| Recalled       | 249   | 476               |
| Recall rate (%) | 1.58  | 1.46              |
| Definitive diagnosis | 6     | 35                |
| Positive predictive value (%) | 2.41  | 7.35, 7.49        |
| Incidence (1 in) | 2622  | 930               |
| Corrected incidence (1 in) | 2560  | 882               |

**DISCUSSION**

A total of 48,297 neonates were recruited for expanded newborn screening by MS/MS and the corrected incidence of IEMs is 1/1110, which was higher than the average incidences of IEMs in China (1/3795) (Shi et al., 2012) and in many other
### TABLE 2 | Spectrum of variants in confirmed cases.

| IEM                                      | Gene Accession No. | Variant                                      | Case |
|------------------------------------------|-------------------|----------------------------------------------|------|
| Phenylketonuria                          | PAH               | c. 728G>A (p. R243Q)                        | 1    |
|                                        | NG_008690        | c. 1315+6T>A (splicing error)               | 2    |
|                                        |                   | c. 611A>G (p. Y204C)                        | 3    |
|                                        |                   | c. 721C>T (p. R241C)                        | 4    |
|                                        |                   | c. 1252A>C (p. T418P)                       | 5    |
|                                        |                   | c. 971T>A (p. I324N)                        | 6    |
|                                        |                   | c. 1193G>T (T380M)                         |      |
|                                        |                   | c. 1197A>T (V399V)                         |      |
|                                        |                   | c. 166G>A (p. V56M)                        | 7    |
|                                        |                   | c. 296G>A (p. D96N)                        | 8    |
| Omithine transcarbamylase deficiency     | OTC               | c. A916G (p. R306G)                        | 9    |
|                                        | NG_008471        | c. 1400C>G (p. R306G)                       | 10   |
| Primary carnitine deficiency            | SLC22A5          | c. 1400C>G (p. S467C)                       | 11   |
|                                        | NG_008982        | c. 428C>T (p. P143L)                        |      |
|                                        |                   | c. 680G>A (R227H)                          |      |
| Short-chain acyl-CoA dehydrogenase       | ACADS            | c. 164C>T (p. P55L)                         | 12   |
| deficiency                               | NG_007991        | c. 1031A>G (p. E344Q)                       | 13   |
| Short-chain acyl-CoA dehydrogenase       | ACADS/ETF DH     | c. 164C>T (p. P55L)                         | 14   |
| deficiency/glutaric acidemia type II     | ACADS/GCDH       | c. 164C>T (p. P55L/c. 1064G>A (p. R355H))  | 15   |
| Short-chain acyl-CoA dehydrogenase       | MMACHC           | c. 567dupT (p. I190Yfs*13)                 | 16   |
| deficiency/glutaric acidemia type I      | NG_013378        | c. 485_446insA (p. C149*)                   | 17   |
|                                        |                   | /c. 609G>A (p. W203*)                       |      |
|                                        |                   | c. 217C>T (p. R73*)                        | 18   |
|                                        |                   | c. 609G>A (p. W203*)                        |      |
|                                        |                   | c. 1A>G (p. M1V)                           | 19   |
|                                        |                   | c. 1820G>A (p. R616Q)                      | 20   |
|                                        |                   | c. 609G>A (p. W203*)                        |      |
|                                        |                   | c. 567dupT (p. I190Yfs*13)                 |      |
|                                        |                   | c. 609G>A (p. W203*)                        |      |
|                                        |                   | c. 626dupT (p. T210Dfs*3S)                 |      |
|                                        |                   | c. 394C>T (p. R132*)                       |      |
|                                        |                   | c. 658deAAAG (p. K220del)                  |      |
|                                        |                   | c. 315G>G (p. Y105*)                       | 21   |
|                                        |                   | c. 609G>A (p. W203*)                        |      |
|                                        |                   | c. 609G>A (p. W203*)                        |      |
|                                        |                   | c. 609G>A (p. W203*)                        |      |
|                                        |                   | c. 567dupT (p. I190Yfs*13)                 |      |
|                                        |                   | c. 323G>A (R108H)                          | 22   |
|                                        |                   | c. 494A>G (D165Q)                          | 23   |
|                                        |                   | c. 1106G>A (R369H)                         | 24   |
|                                        | MUT               | c. 323G>A (R108H)                          | 25   |
|                                        | NG_007100        | c. 494A>G (D165Q)                          |      |
|                                        |                   | c. 1106G>A (R369H)                         |      |

The symbol “#” in superscript represents homozygous and “/” represents no mutation.

countries, such as 1/2500 in Canada (Applegarth et al., 2000), 1/2900 in Germany (Lindner et al., 2011), 1/1944 in Egypt (Hassan et al., 2016), 1/2916 in Malaysia (Yunus et al., 2016), 1/2800 in South Korea (Yoon et al., 2005), and 1/3165 in Singapore (Lim et al., 2014). Moreover, the incidence of IEMs in Jining was higher than that in many areas or cities of China, e.g., 1/1683 in Zibo, 1/4384 in Yancheng, 1/5626 in Zhejiang province, and 1/8304 in Nanning (Huang et al., 2011; Fan et al., 2013; Zhu et al., 2015; Lu et al., 2016). It indicated that the incidence of IEMs was greater in Jining area than in many regions.

It is known that the genetic variants related to IEMs are mainly detected by Sanger sequencing and MassARRAY (Wright et al., 2008; Zhu et al., 2010; Shibbani et al., 2014; Mutlu-Albayrak et al., 2015). Recently, next generation sequencing (NGS) started to be applied for the clinical genetic analysis of
IEMs (Qian et al., 2017; Smon et al., 2018). Sanger sequencing is the golden standard to determine the DNA sequence, which is applied for the known gene locus in genetic analysis. Both MassARRAY and NGS are high throughput sequencing methods, which can be used for genetic analysis of a spectrum of genes. Compared with NGS, MassARRAY has been used more widely and longer to detect known mutations. In addition, it is reliable and the cost is less. Therefore, 942 sites in IEMs-associated genes were detected by MassARRAY in this study. However, NGS has the advantage to detect the novel mutations. Thus, it may be a good option for the case 11 to discover unknown mutations.

Although the corrected incidence was significantly higher in the suburban and rural area compared with that in the urban area, 1/862 vs. 1/2560, it is unlikely that the high incidence of IEMs in the suburban and rural area is caused by consanguineous marriage and close genetic relationship. First, consanguineous marriage including cousin marriage is prohibited by Chinese Marital Law, which is widely accepted and strictly abided by the residence. Moreover, genetic analysis showed that only one case was homozygous in 22 cases in the suburban area, while one in six cases was homozygous in the urban area. The aforementioned evidence indicated that the close genetic relationship is not the cause of high incidence of IEMs in the suburban/rural area. However, the underlying reasons of the difference need to be further investigated, such as environmental factors and lifestyle.

The incidence of phenylketonuria varies in countries and regions (Williams et al., 2008). For example, the incidence of phenylketonuria varies from 1/7325 to 1/39,338 in countries of southeastern Europe, while the incidences are 1/11,400 in the United States and 1/4172 in Turkey (Hertzberg et al., 2011; Zerjav Tansek et al., 2015). The incidence of phenylketonuria is 1/11,614 in China based on the newborn screening data of past 30 years (Shi et al., 2012). However, we found that the incidence of phenylketonuria was 1/4391 in Jining, which was much higher than the incidence of phenylketonuria in China. Genetic analysis showed that six cases carried PAH mutations and one case carried PTS mutant in patients with phenylketonuria, suggesting that mutations in PAH gene are the predominant cause of phenylketonuria in Jining.

The incidence of methylmalonic acidemia was 1/3220 in the suburban area, 1/862 vs. 1/2560. The incidence of methylmalonic acidemia, phenylketonuria, and primary carnitine deficiency ranked the top 3 of all detected IEMs. In addition, 30 mutations in nine IEMs-associated genes were identified in the 28 confirmed cases and 19 cases with the mutations in nine IEMs, leading causes of IEMs resulting in phenylketonuria, primary carnitine deficiency, and methylmalonic acidemia, respectively, in Jining area. Therefore, it is critical to increase the coverage of expanded newborn screening by MS/MS and prenatal genetic consulting in Jining area. Furthermore, the underlying reasons of higher incidence of IEMs in Jining and particularly higher incidence of IEMs in the suburban area need to be investigated to achieve the decrease of the incidence of IEMs in the future.

**AUTHOR CONTRIBUTIONS**

KG and XC performed screening by MS/MS and collected the data. YW and XZ formulated the study, analyzed the data, wrote the manuscript, and designed the tables. CL and QK provided intellectual thoughts, revised the manuscript, and led the project.
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REFERENCES
Applegarth, D. A., Toone, J. R., and Lowry, R. B. (2000). Incidence of inborn errors of metabolism in British Columbia, 1969-1996. Pediatr. 105:e10. doi: 10.1542/peds.105.1.e10

Bennett, M. J. (2009). Follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry. Clin. Biochem. 35, 1615–1626. doi: 10.1016/j.clinbiochem.2009.131300

Campeau, P. M., Scriver, C. R., and Mitchell, J. J. (2008). A 25-year longitudinal analysis of treatment efficacy in inborn errors of metabolism. Mol. Genet. Metab. 95, 11–16. doi: 10.1016/j.ymgme.2008.07.001

Dietzen, D. J., Rinaldo, P., Whitley, R. J., Rhead, W. J., Hannon, W. H., Garg, U. C., et al. (2009). National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary. Clin. Chem. 55, 1615–1626. doi: 10.1373/clinchem.2009.131300

Fan, X., Chen, S. B., Luo, J., Li, W., Lin, F., Luo, C., et al. (2013). Application of tandem mass spectrometry in genetic metabolism screening in Nanning, Guangxi. J. Guangxi Med. Univ. 30, 756–758. doi: 10.16190/j.cnki.45-1211/t.2013.05.055

Han, B., Cao, Z., Tian, L., Zou, H., Yang, L., Zhu, W., et al. (2016). Clinical presentation, gene analysis and outcomes in young patients with early-treated combined methylmalonic academia and homocysteinemia (cblC type) in Shandong province. China. Brain Dev. 38, 491–497. doi: 10.1016/j.braindev.2015.10.016

Hassan, F. A., El-Mougy, F., Sharaf, S. A., Mandon, I., Morgan, M. F., Selim, L. A., et al. (2016). Inborn errors of metabolism detectable by tandem mass spectrometry in Egypt: the first newborn screening pilot study. J. Med. Screen. 23, 124–129. doi: 10.1177/0969141315618229

Hertzberg, V. S., Hinton, C. F., Thrrell, B. L., and Shapiro, S. K. (2011). Birth prevalence rates of newborn screening disorders in relation to screening practices in the United States. J. Pediatr. 159, 555–560. doi: 10.1016/j.jpeds.2011.04.011

Huang, X. W., Yang, J. B., Tong, F., Yang, R. L., Mao, H. Q., Zhou, X. L., et al. (2013). Application of tandem mass spectrometry screening for neonatal genetic metabolic diseases in Yancheng area. J. Birth Health Hered. 24, 83–85. doi: 10.13400/j.cnki.cjbjh.2016.03.036

Lu, H., Zheng, J., Yao, Y., Yang, M., Yao, S., Shi, N., et al. (2016). A retrospective analysis of tandem mass spectrometry screening for neonatal genetic metabolic diseases in Yancheng area. J. Birth Health Hered. 24, 83–85. doi: 10.13400/j.cnki.cjbjh.2016.03.036

Magoulas, P. L., and El-Hattab, A. W. (2012). Systemic primary carnitine deficiency: an overview of clinical manifestations, diagnosis, and management. Orphanet J. Rare Dis. 7:68. doi: 10.1186/1750-1172-7-68

McHugh, D., Cameron, C. A., Abdurahman, J. E., Abdulrahman, M., Adair, O., Al Nuaimi, S. A., et al. (2011). Clinical validation of cutoff target ranges in newborn screening of metabolic disorders by tandem mass spectrometry: a worldwide collaborative project. Genet. Med. 13, 230–254. doi: 10.1097/GIM.0b013e318256e767

Moammar, H., Cheriyan, G., Mathew, R., and Al-Sannaa, N. (2010). Incidence and patterns of inborn errors of metabolism in the Eastern Province of Saudi Arabia, 1983-2008. Ann. Saudi Med. 30, 271–277. doi: 10.1016/j.amssec.2009.12.001

Mutfu-Albayrak, H., Bene, J., Oltaz, M. B., Tanyalcin, T., Caksen, H., and Melegh, B. (2015). Identification of SLC22A5 gene mutation in a family with carnitine uptake defect. Case Rep. Genet. 2015:259627. doi: 10.1155/2015/259627

Qian, J., Wang, X., Liu, J., Zhong, J., Le, Y., Melchior Tellier, L. C. A., et al. (2017). Applying targeted next generation sequencing to dried blood spot specimens from suspicious cases identified by tandem mass spectrometry-based newborn screening. J. Pediatr. Endocrinol. Metabol. 30, 979–988. doi: 10.1515/jpep-2017-0003

Sanderson, S., Green, A., Preece, M. A., and Burton, H. (2006). The incidence of inherited metabolic disorders in the West Midlands, UK. Arch. Dis. Child. 91, 896–899. doi: 10.1136/adc.2005.091637

Scolamiero, E., Cozzolino, C., Albano, L., Ansalone, A., Caterino, M., Corbo, G., et al. (2015). Targeted metabolomics in the expanded newborn screening for inborn errors of metabolism. Mol. Biosyst. 11, 1525–1535. doi: 10.1039/c4mb00729h

Shi, X. T., Cai, J., Wang, Y. Y., Tu, W. J., Wang, W. P., Gong, L. M., et al. (2012). Newborn screening for inborn errors of metabolism in mainland china: 30 years of experience. JIMD Rep. 6, 79–83. doi: 10.1007/978-90-447-65254

Shibbani, K., Fahed, A. C., Al-Shaar, L., Arabi, M., Nemer, G., Bitar, F., et al. (2014). Primary carnitine deficiency: novel mutations and insights into the cardiac phenotype. Clin. Genet. 85, 127–137. doi: 10.1111/cge.12112

Smon, A., Repic Lampret, B., Groselj, U., Zerjav Tansek, M., Kovac, J., Perko, D., et al. (2018). Next generation sequencing as a follow-up test in an expanded newborn screening programme. Clin. Biochem. 52, 48–55. doi: 10.1016/j.clinbiochem.2017.10.016

Tu, W. J. (2011). Methylmalonic academia in mainland China. Ann. Nutr. Metab. 58:281. doi: 10.1159/000331469

Williams, R. A., Mamotte, C. D., and Burnett, J. R. (2008). Phenyketonuria: an inborn error of phenylalanine metabolism. Clin. Biochem. Rev. 29, 31–41.

Wright, W. T., Heggarty, S. V., Young, I. S., Nicholls, D. P., Whittall, R., Humphries, S. E., et al. (2008). Multiplex MassARRAY spectrometry (iPLEX) produces a fast and economical test for 56 familial hypercholesterolaemia-causing mutations. Clin. Genet. 74, 463–468. doi: 10.1111/j.1399-0004.2008.01071.x

Yamaguchi, S. (2008). Newborn screening in Japan: restructuring for the new era. Ann. Acad. Med. Singapore 37, 13–15.

Yamaguchi, S., Purevasuren, J., Hasegawa, Y., Kobayashi, H., Mushimoto, Y., Yamada, K., et al. (2013). “Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency and expanded newborn screening in Japan,” in Proceedings of the Genetic Testing Symposium and the International Society for Neonatal Screening and Featured presentation at Newborn Screening Atlanta.

Yoon, H. R., Lee, K. R., Kang, S., Lee, D. H., Yoo, H. W., Min, W. K., et al. (2015). Screening of newborns and high-risk group of children for inborn
metabolic disorders using tandem mass spectrometry in South Korea: a three-year report. *Clin. Chim. Acta* 354, 167–180. doi: 10.1016/j.cccn.2004.11.032

Yunus, Z. M., Rahman, S. A., Choy, Y. S., Keng, W. T., and Ngu, L. H. (2016). Pilot study of newborn screening of inborn error of metabolism using tandem mass spectrometry in Malaysia: outcome and challenges. *J. Pediatr. Endocrinol. Metab.* 29, 1031–1039. doi: 10.1515/jpem-2016-0028

Zerjav Tansek, M., Groselj, U., Angelkova, N., Anton, D., Baric, I., Djordjevic, M., et al. (2015). Phenylketonuria screening and management in southeastern Europe - survey results from 11 countries. *Orphanet J. Rare Dis.* 10:68. doi: 10.1186/s13023-015-0283-0

Zhang, X., Wu, Y., Cai, F., Liu, S., Bromley-Brits, K., Xia, K., et al. (2018). A novel Alzheimer-associated SNP in Tmp21 increases amyloidogenesis. *Mol. Neurobiol.* 55, 1862–1870. doi: 10.1007/s12035-017-0459-9

Zhu, F., Xu, J., Dong, L., and Mou, K. (2015). Analysis of 25243 cases of neonatal genetic metabolic disease screening by tandem mass spectrometry. *J. Binzhou Med. Univ.* 38, 300–302.

Zhu, T., Qin, S., Ye, J., Qiu, W., Han, L., Zhang, Y., et al. (2010). Mutational spectrum of phenylketonuria in the Chinese Han population: a novel insight into the geographic distribution of the common mutations. *Pediatr. Res.* 67, 280–285. doi: 10.1203/PDR.0b013e3181c9fb85

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