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Original Research Article

Expanding the screening of newborns for detecting inborn errors in metabolism using next generation sequencing following mass spectrometry/immunoassay

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ABSTRACT

Background: Inborn errors of metabolism are rare inherited disorders which leads to significant morbidity and mortality in patients. Very few studies have been conducted in India to assess prevalence of Inborn Errors of Metabolism (IEM) in newborns. We proposed testing by TMS/TR-FIA followed by NGS. This pilot study would be one of the first expanded NBS studies in India.

Objectives: The aim of this study was to determine the prevalence of IEM in newborns based on the samples received at Metropolis Global Reference Lab, India. Next-generation sequencing (NGS) was done as a confirmational analysis for patients tested presumptive positive on Newborn screening using Tandem Mass spectrometry (TMS) and Time-resolved fluoroimmunoassay (TR-FIA).

Materials and Methods: Two years retrospective study was conducted based on incidences of IEM using TMS and TR-FIA. NGS testing was performed on presumptive positive newborns for cystic fibrosis (CF), galactosemia and urea cycle disorder/ organic academia (UCD /OA) who had undergone NBS by TMS and TR-FIA.

Results: Highest prevalence of 1.98% & 1.58% was detected for G6PD and TSH respectively by TR-FIA. Prevalence of AA disorders (3.20%), OA (1.60%) and UCD (1.43%) was observed to be the highest amongst the diseases detected by TMS. Presumptive positive case of Argininemia and Cystic Fibrosis were found to be concordant with NGS. Out of three presumptive positive cases, one presumptive positive case of CF and two of galactose were found discordant.

Conclusions: Our prevalence study showed similarities to the prevalence reports published by other Asian countries. Expanded NBS program can be improved by including NGS as a first follow-up test after detection of abnormal metabolites in DBS. This approach will help in reducing the encumbrance of false-positive as well as false-negative cases. Our study will be influential in conducting more prospective studies and routine implementation of NGS-based analysis in NBS in India.

1. Introduction

Inborn errors of metabolism (IEM) can cause a major health problem with consequences ranging from minor disabilities to sudden death. With the discovery of many IEMs, a need for a standardized newborn screening procedure arose. NBS is used to detect anomalies early in children with congenital disease so that therapy can be prescribed as soon as feasible to prevent or mitigate the condition's long-term effects. Around 500 inborn errors of metabolism (IEM) are seen in Indian population and it is stated that 1:20 Indian have some form of disorder or birth defects.[1]

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Prevalence of IEMs differs between countries, likely because different IEM classifications and IEM screening methods are used. Pooled birth prevalence of IEM in the Eastern Mediterranean region is about 75.7/100 000 live births.[2] As of yet, there are no global estimates of the burden of morbidity or mortality associated with IEM. (5) About 25 million babies were born per year and infant mortality rate of 35 /1000 live births were reported in India.[3] Total fatality rate in developing countries is 33% accounting for 0.4% of child deaths all over the world.[2]

Variety of diagnostic tests are available for detection of IEM in newborns from DBS, including TR-FIA, Plasma amino acid assay by HPLC, Urine organic acid assay by (GCMS), and Acyl carnitine and amino acid profile by TMS. But each technique comes with the possibility of reporting a False Positive or False Negative.[4]

Next generation sequencing (NGS) is evolving rapidly and is offering the simultaneous analysis of several genes accounting for variety of disorders. Therefore, it is becoming a method of choice for newborn screening. There is strong interest in implementing NGS into NBS programs,[5] but there are still limited reports on its implementation.

Because of the rapid advancement of NGS, the cost of sequencing and genetic analysis has decreased and NGS may now be used in NBS.[6] The addition of NGS to the NBS panel could broaden the range of illnesses that can be screened beyond IEMs. In cases where enzyme activity analysis or the detection of causative genetic variations is required for confirmation, NGS as a follow-up test could allow for faster final confirmation of the disease. NGS can assist to eliminate diagnostic bias in unwell babies, allowing for the most effective early treatment measures for those who are affected, as well as determining carrier status, which could aid future reproductive planning.[7] Only a few studies have shown that NGS can be used as a first-tier or second-tier confirmatory test in newborn screening.[8] Therefore, in our prototype study we proposed that NGS is a possible method for a follow-up test after an abnormal NBS result and compared its effectiveness in our cohort.

2. Materials and Methods

This retrospective study was performed at global reference laboratory (GRL) in Mumbai and included DBS samples received for initial newborn screening. Two years data was collected from January 2020 to April 2022 to find out the IEM with the highest prevalence. Presumptive positive patients and parents of patients were contacted to become part of the current study. Few agreed for the follow-up NGS testing. The study was approved by independent ethics committee. 3ml EDTA whole blood sample was collected from the patient for NGS testing. Consent was obtained from the parents of presumptive positive patients. Out of 8001 patients tested for Galactosemia, 29 were diagnosed as presumptive positives. Out of 29 cases, parents of affected twins agreed for the follow up test. Out of 3602 patients tested for CF, 88 were diagnosed as presumptive positive. Parents of two patients agreed for the follow up test. Out of 500 patients tested for NBS by TMS, 7 cases were diagnosed as presumptive positive for Organic/Amino aciduria. Parents of two patients agreed for the follow up test.

2.1. Laboratory methodology

2.1.1. Dried blood spot analysis using tandem mass spectrometry and time resolved fimmunofluroassay

Blood spot samples were analyzed on the Waters Xevo TQD LCMS (Waters, USA) system using NeoMass AAAC Plus kit for amino acids and acyl carnitines from dried blood spot (Labsystem, Finland). Non- derivatized method was used for analysis. Blood spot samples were analyzed to find out concentration of total galactose in the sample by using fluorescent galactose oxidase method using 1420 VICTOR 2D flurometer (Perkin-Elmer, USA). Blood spot samples were analyzed to find out levels of IRT (Immuno-reactive Trypsinogen) in the sample by using time resolved fluroimmunoassay method using 1420 VICTOR 2D flurometer (Perkin-Elmer, USA).

2.1.2. Next generation sequencing (NGS) testing

DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. Amplification of the coding region and flanking introns of the CFTR gene was performed with the purified DNA (50 ng/μL) template. The libraries sequenced to mean >80-100X coverage on Illumina sequencing platform. GATK (Genomic Analysis Toolkit) best practices framework used for identification of variants in the sample using Sentieon (v201808.01). Gene annotation of the variants performed using VEP program against the Ensemble release 99 human gene model. In addition to SNVs and small Indels, copy number variants (CNVs) were detected from targeted sequence data using the Exome Depth (v1.1.10) method. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM (updated on 11th May 2020), GWAS, HGMD (v2020.2) and SwissVar.. Only non-synonymous and splice site variants found in the target (CFTR/GALT) gene were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region were excluded from reporting.

2.2. Statistical analysis

For prevalence study all the obtained data was analyzed retrospectively and represented by using Microsoft excel 2019. Prevalence was calculated by using below-mentioned formula.

Prevalence = Total no. of people in sample with characteristic / Total no. of people in sample

Correlation of NGS results with the presumptive positive TMS/TR-FIA results: After the NGS results are obtained, they were correlated with the TMS and the TR-FIA results. Statistical analysis was evaluated as Concordant and Discordant pairs.

3. Results

3.1. Prevalence of 8 IEM by TR-FIA

Out of the 8 IEM, it was found that the prevalence of TSH and Glucose-6-phosphate Dehydrogenase (G6PD) detected by TR-FIA was the highest, which was 1.58% and 1.98%, respectively (Table 1).

3.2. Prevalence of 29 IEM by TMS

500 newborns were tested for diagnosis of 29 IEM by TMS, which were categorized into 5 groups of diseases: AA Disorders, OA, FAOD, Carnitine Cycle deficiency and UCD.

The prevalence of AA disorders (3.20%), OA (1.60%) and UCD (1.40%) was observed to be the highest (Table 2). The total number of presumptive positive patients was 48. Among all the AA disorders diagnosed, MSUD (2.0%) and ALT deficiency (0.8%) was the highest. For OA diseases, the prevalence PA/MMA (0.8%) was found to be highest and among all the UCDs known, the prevalence of HHH (0.8%) was highest. 12 of the 48 patients were diagnosed with elevations of various analytes which could not be correlated with any particular condition.

3.3. Correlation of NGS results with presumptive positive TR-FIA results

Referring doctors of Presumptive positive patients diagnosed with CF and Galactose detected by TR-FIA, and OA and UCD detected by TMS were contacted for a follow up confirmatory test by NGS (Table 3). Out of which parents of five presumptive positive patients gave consent for the confirmatory test.

1. One patient, for which no clinical history was provided, was diagnosed as presumptive positive for CF. The patient's sample was tested for mutations of the CFTR gene by NGS using the Illumina platform. The patient was found to lack any CFTR mutations, i.e., the patient was found to be normal. Thus, the TR-FIA and NGS results were discordant since it was presumptive positive by TR-FIA and Negative by NGS.
2. Fraternal twin's patients with a clinical history of Gastric discomfort were diagnosed as presumptive positive for Galactosemia. The two patient's sample was tested for mutations of the GALT gene by NGS using the Illumina platform. Both the patients were lacking the GALT mutations, i.e., both were normal. Thus, results of TR-FIA and NGS were discordant, as the twins were diagnosed as presumptive positive by TR-FIA, but results were negative for NGS.
3. Another patient with clinical history, was diagnosed as presumptive positive for Argininemia. The patient's sample was tested for mutations of the Arg1(+) (ENST00000356962.2) gene by NGS using the Illumina platform. Patient was found to be likely pathogenic i.e. positive. Thus, TMS and NGS results were Concordant.
4. One more patient whose mother was hypothyroid, with bad obstetrics history and death of three male children previously, was diagnosed as GA type II. The patient's sample was tested for organic academia panel by NGS using Illumina platform. Patient was found to lack mutations in ETFA, ETFB, and ETFDH genes that cause GA type II. Results were found to be discordant.
5. A patient with history of Acute encephalopathy, Hypoglycemia, and Meconium ileus was diagnosed as Presumptive positive for Cystic Fibrosis. Repeat sample was taken and sent for Gene mutational analysis for CFTR Gene by NGS using Illumina platform. Patient was found to be pathogenic. Results were concordant.

4. Discussion

This is a one-of-a-kind pilot study to estimate the overall incidence of a wide range of inborn metabolic disorders in Indian newborns using patient data from Metropolis global reference laboratory (GRL) in Mumbai. In current Indian scenario there is unyielding requirement for expanded screening program. Limited studies in regards to expanded NBS have been conducted.

In our retrospective newborn screening study carried out among 53823 neonates and each newborn was tested for at least one of the 8 IEM. G6PD disorder was found to be most prevalent with 1.98% (244 cases of 12347 screened). Second most prevalent was TSH with 1.58% (160 cases of 10125) and followed by Congenital Adrenal Hyperplasia (CAH) with 1.22% (152 cases of 12420), CF with 0.94% (32 cases of 3389), Galactose with 0.79% (60 cases of 7576 screened), Biotinidase with 0.19% (5 cases of 2686 screened), Phenylalanine with 0.11% (3 cases of 2735 screened), MSUD 0.04% (1 case in 2545 screened).

Kapoor S et al conducted a study among 125 thousand neonates and reported G6PD deficiency, congenital hypothyroidism, homocysteinaemia, hyperglycinaemia, MSUD, and phenylketonuria (PKU) to be the most common disorders with good prevalence.[9] Similarly, Gopalakrishnan V et al in year 2014 have documented CH, CAH, G6PD, Biotinidase deficiency, galactosaemia and cystic fibrosis as the most prevalent disorders.[10]

Table 1: Prevalence of diseases found by TR-FIA

	Positive (n)	Normal (n)	Total (n)	Prevalence (%)
17 OHP	152	12268	12420	1.22%
Biotinidase	5	2681	2686	0.19%
CF	32	3357	3389	0.94%
G6PD	244	12103	12347	1.98%
Galactose	60	7516	7576	0.79%
MSUD	1	2544	2545	0.04%
Phenylalanine	3	2732	2735	0.11%
TSH	160	9965	10125	1.58%

n = no. of samples, % = Percentage

Table 2: Prevalence study for diseases detected by TMS

Categories	Disorder	Positive (n)	Prevalence (%)	Prevalence of each category
AA Disorders	MSUD	10	2.00%	3.20%
	PKU	1	0.20%	
	Non-ketotic hyperglycinemia	1	0.20%	
	ALT deficiency	4	0.80%	
	PA/MMA	4	0.80%	
OA	IVA	1	0.20%	1.60%
	HMG-CoA lyase deficiency	3	0.60%	
FAOD	SCAD deficiency	2	0.40%	0.80%
	Multiple acyl-CoA dehydrogenase(MAD) deficiency	2	0.40%	
Urine cycledisorder	CUD	1	0.20%	1.40%
	Argininemia	3	0.60%	
	HHH	4	0.80%	
Elevations that do not specify particular conditions		12	2.40%	2.40%
Total		48		

n = no. of samples, % = Percentage

Table 3: Summary of NGS and TMS/TR-FIA results

Sample no.	Target Genes	Variant	Zygosity	Diseases (OMIM)	Inheritance	TMS/ TR-FIA Results	NGS result	Association
1	CFTR	-	-	Cystic Fibrosis	-	Presumptive Positive for Cystic Fibrosis	N	Dis
2	GALT	-	-	Galactosemia	-	Presumptive Positive for Galactosemia	N	Dis
3	GALT	-	-	Galactosemia	-	Presumptive Positive for Galactosemia	N	Dis
4	Arg1 (+) (ENST00000356962.2)	c.319G> A (p.Gly107Arg)-	Homozygous	Argininemia	AR	Presumptive Positive for Argininemia	LP	Con
5	ETFA, ETFB, and ETFDH	-	-	GA Type II	-	Presumptive Positive for GA Type II	US	Dis
6	CFTR (+) (ENST00000003084.11)	c.583del (p.Ala196 HisfsTer19)	Homozygous	Cystic Fibrosis	AR	Presumptive Positive for Cystic Fibrosis	P	Con

Table 4: Prevalence of IEM in different Asian countries

Categories	Prevalence					
	India (n=421)	Oman (n=1100)	Kingdom of Bahrain (n=1986)	Iran (n=13,327)	Saudi Arabia (n=7,75,000)	China (n=70,99,664)
AA Disorder /OA	4.80%	4.68%	1.06%	3.35%	0.04%	0.03%
FAOD	0.95%	0.40%	0.10%	0.24%	0.00%	0.00%
Carnitinecycledeficiency	0.24%	0.25%	0.00%	0.53%	0.00%	0.01%
UCD	1.43%	0.65%	0.10%	0.37%	0.01%	0.00%
Lysosomal Storage Disease	0.00%	0.00%	0.00%	0.86%	0.00%	0.00%
Other Disorders	0.00%	0.00%	0.00%	2.35%	0.04%	0.00%

The total number of newborn DBS samples tested by TMS was 500 among which 36 patients were observed to have abnormal elevations of analytes which could be correlated to one of the 29 IEM. The prevalence of different IEM category was AA disorders (3.20%), OA (1.60%), UCD (1.40%), FAOD (0.80%) and Carnitine Transporter deficiency (0.20%). MSUD (2.0%) and ALT deficiency (0.8%) were found to be the most prevalent IEM in AA disorders. In the OA and UCD category, most prevalent disorders were PA/MMA (0.8%) and HHH (0.8%) respectively. Also, 12 presumptive positive cases were detected which could be because the patient had multiple metabolic deficiencies or because of association with medical intervention, diet, Total parenteral nutrition (TPN), prematurity or drugs. The other reasons could be inappropriate sample collection on the Whatman Filter paper 903, pouring EDTA whole blood on the Whatman filter paper 903, double spotting/caking on the Whatman filter paper and improper storage of DBS samples.

Prevalence study from other countries were shown in Table 4. In a similar IEM prevalence study carried out in Oman, 1100 newborns were examined and 119 among them were diagnosed with IEM. The prevalence of AA disorders/OA (4.68%) and UCD (1.43%) was reported as the highest among the IEM categories. Out of the AA disorders/OA diagnosed, the prevalence of MMA/PA (1.72%), MSUD (1.54%) ALT deficiency (1%) and HMG-CoA lyase deficiency (1%) was reported the highest. Al Riyami et al in his study amongst the diagnosed UCD reported highest prevalence of Argininosuccinic aciduria (0.54%).[11] This was in concordance with our study except the UCD findings. In another IEM prevalence study carried out by Golbahar et al. in the Kingdom of Bahrain, 1986 newborns were tested. 25 newborns were diagnosed with IEM. The highest prevalence was reported for OA (1.06%) in which the most prevalent disease was MMA/PA (0.35%).[12] The study findings bear a resemblance to our MMA/PA findings. In a study completed in northwestern Iran, among 13,327 infants examined, 60 different IEMs were diagnosed in 1,118 children. Keyfi et al. stated MMA/PA (0.77%) as the highest prevalent OA.[13] In a prevalence study in Saudi Arabia, 7,75,000 newborns were tested for IEM, out of which 743 newborns were diagnosed with IEM. The authors reported the highest prevalence for OA (0.04%) and other disorders (0.13%). Alfadhel et al. published MMA/PA (0.014%) as the most prevalent disease in OA in their study.[14] In one of the studies carried out in China in the year 2016 by Deng et al, over 70 lakh newborns were screened and 2747 were diagnosed with IEM, yielding an overall incidence of 38.69 per 1,00,000 births. The most common disorders were OA (0.027%) and Carnitine Cycle Deficiency (0.005%). The highest prevalence was for PKU (0.013%) and MMA/PA (0.009%) for OA and Carnitine deficiency or insufficiency (0.005%) for Carnitine Cycle Deficiency.[15]

This study was carried out to understand the prevalence of IEM in India. The other countries studied for comparison, which were Oman, the Kingdom of Bahrain, Iran, Saudi Arabia, and China, are part of Asia which would ensure that the population under study had as much as possible genetic and hereditary similarity. Also, similar climatic conditions are experienced by most of these countries. Thus, enabling to lessen as much of the variation in data as possible. IEM is a rare disorder and thus, even though we observe high percentage of prevalence for each disease category, the prevalence of each condition is much lower. Also, it should be noted that as the population size under study expands, the percentage prevalence decreases, as distinctly apparent from the prevalence study in Saudi Arabia and China. In this study, the highest prevalence was found for MSUD (AA disorder) by TMS. Such a similar pattern is observed in all the countries since the highest prevalence was observed for AA disorder/OA. The second highest prevalence was observed for MMA/PA(OA) and HHH (UCD) by TMS. In Oman and the Kingdom of Bahrain, the second most prevalent IEM was reported as UCD, whereas in Saudi, other disorders and in China it was Carnitine cycle disorder. Thus, this study could be undertaken as a prospective study to get more exhaustive data for the interpretation of the prevalence of IEM in India.

TR-FIA/TMS are only screening tests and there may be a chance that false- positive cases may be reported. It has

been observed that the increase in false positive rate is may be due to unacceptable specimen quality. Also newborns with positive screen do not necessarily have cystic fibrosis. Mostly (~90%), the result is a false positive i.e the screening was abnormal with neoborns being the carrier of cystic fibrosis.. Carriers do not have the condition themselves but are at increased risk to have a child with cystic fibrosis.

5. Conclusion

In our study, prevalence of G6PD and TSH detected by TR-FIA was found to be the highest, which was 1.98% and 1.58%, respectively. Among the diseases detected by TMS, prevalence of AA disorders (3.20%), OA (1.60%) and UCD (1.43%) were found to be the highest. Concordance was observed in One presumptive positive case of Argininemia as well as Cystic Fibrosis when confirmed with NGS for Arg1 gene and CFTR gene respectively. Discordance was observed in one Cystic Fibrosis and two Galactosemia cases when confirmed with NGS for CFTR gene and GALT gene respectively. Extended NBS program will surely lead to a better quality of life for the affected child as well as the parents. Our study establishes that implementation of NGS as a first follow-up test after the discovery of elevated metabolites in DBSs would improve the NBS programs and reduces the burden of false positive results and lower the number of false negative results at recall. An initiative should be taken by Government of India by inclusion of NBS as a routine programme such as the immunization programme to reduce the cost of NBS by large scale diagnosis so that it can be affordable to population at large.

6. Abbreviations

IEM, Inborn error of metabolism; TSH, Thyroid-stimulating hormone; UCD, Urea cycle disorder; OA, Organic academia; G6PD, glucose-6-phosphate dehydrogenase deficiency; CH, Congenital Hypothyroidism; CAH, Congenital adrenal hyperplasia; TR-FIA, Time-resolved fluoroimmunoassay; DBS, Dried blood spot; TMS, Tandem Mass spectrometry;

FAOD, Fatty Acid Oxidation Defects; HPLC, High-Performance Liquid Chromatography; GCMS, Gas chromatography–mass spectrometry; NGS, Next Generation Sequencing; MSUD, Maple Syrup Urine Disease; CF, Cystic Fibrosis; PKU, Phenylketonuria; ALT deficiency, Alanine transaminase deficiency; PA/MMA-Propionic academia/ Methyl malonic academia; IVA, Isovaleric academia; AA disorders, Amino acid disorders; SCAD, Short-chain acyl-CoA dehydrogenase deficiency; CUD, Carnitine uptake defect; HHH, Hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome

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None.

8. Conflict of Interest

Authors declare no conflict of interest.

References

1. Narayanan MP. Inborn Errors of Metabolism: Indian Scenario. *Acta Sci Paediatr*. 2019;2:50–2.
2. Waters D, Adeloye D, Woolham D, Wastnedge E, Patel S, Rudan I. Global birth prevalence and mortality from inborn errors of metabolism: a systematic analysis of the evidence. *J Glob Health*. 2018;8(2):021102.
3. Kamath S. Reducing the infant mortality rate of India to 20 by 2020: together we can do. *Indian Pediatr*. 2015;52(3):193–4.
4. Balakrishnan U. Inborn Errors of Metabolism-Approach to Diagnosis and Management in Neonates. *Indian J Pediatr*. 2021;88(7):679–89.
5. Landau YE, Lichter-Konecki U, Levy HL. Genomics in newborn screening. *J Pediatr*. 2014;164(1):14–9.
6. Luo X, Sun Y, Xu F, Guo J, Li L, Lin Z, et al. A pilot study of expanded newborn screening for 573 genes related to severe inherited disorders in China: results from 1,127 newborns. *Ann Transl Med*. 2020;8(17):1058.
7. Smon A, Lampret BR, Groselj U, Tansek MZ, Kovac J, Perko D, et al. Next generation sequencing as a follow-up test in an expanded newborn screening programme. *Clin Biochem*. 2018;52:48–55.
8. Bodian DL, Klein E, Iyer RK, Wong WS, Kothiyal P, Stauffer D, et al. Utility of whole-genome sequencing for detection of newborn screening disorders in a population cohort of 1,696 neonates. *Genet Med*. 2016;18(3):221–30.
9. Kapoor S, Kabra M. Newborn screening in India: current perspectives. *Indian Pediatr*. 2010;47(3):219–24.
10. Gopalakrishnan V, Joshi K, Phadke S, Dabadghao P, Agarwal M, Das V, et al. Newborn screening for congenital hypothyroidism, galactosemia and biotinidase deficiency in Uttar Pradesh, India. *Indian Pediatr*. 2014;51(9):701–5.
11. Riyami SA, Maney MA, Joshi SN, Bayoumi R. Detection of inborn errors of metabolism using Tandem mass spectrometry among high-risk Omani patients. *Oman Med J*. 2012;27(6):482–5.
12. Golbahar J, Al-Jishi EA, Altayab DD, Carreon E, Bakhiet M, Alkhayyat H, et al. Selective newborn screening of inborn errors of amino acids, organic acids and fatty acids metabolism in the Kingdom of Bahrain. *Mol Genet Metab*. 2013;110(1-2):98–101.
13. Keyfi F, Nasseri M, Nayerabadi S, Alaei A, Mokhtariye A, Varasteh A, et al. Frequency of inborn errors of metabolism in a Northeastern Iranian sample with high consanguinity rates. *Hum Hered*. 2018;83(2):71–8.
14. Alfadhel M, Othaim AA, Saif S, Mutairi FA, Alsayed M, Rahbeeni Z, et al. Expanded Newborn Screening Program in Saudi Arabia: Incidence of screened disorders. *J Paediatr Child Health*. 2017;53(6):585–91.
15. Deng K, Zhu J, Xiang YE, Yuan L, Yao X, Li Y, et al. Incidence of inborn errors of metabolism detected by tandem mass spectrometry in China: A census of over seven million newborns between 2016 and 2017. *J Med Screen*. 2016;28(3):223–9.

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