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Newborn screening for inherited metabolic disorders in central China: a retrospective study of 153,956 infants using non-derivatized tandem mass spectrometry

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Inherited metabolic disorders (IMDs) are genetic conditions characterized by toxic metabolite accumulation or deficiencies in essential products, often leading to severe clinical symptoms. Neonatal screening using advanced methods, such as tandem mass spectrometry (MS/MS), facilitates early detection and intervention, significantly expanding the range of detectable disorders. However, IMD incidence varies considerably across and within different regions globally. This study examined the incidence, spectrum, and genetic features of IMDs detectable by the regional MS/MS screening panel in a cohort of 153,956 newborns in Changzhi, central China, over the period from May 2015 to April 2020. MS/MS served as the primary screening tool, while GC/MS and next-generation sequencing (NGS) provided confirmatory analyses. Of the screened population, 129 infants received a diagnosis of IMDs, resulting in an incidence rate of 1 in 1,193 neonates. Confirmed cases comprised amino acid disorders (87 cases, 1:1,770), organic acidemias (23 cases, 1:6,694), and fatty acid oxidation disorders (18 cases, 1:8,553), with phenylalanine hydroxylase deficiency (PAHD) being the most prevalent disorder (64.4%, 1:1,855). Genetic analysis revealed particular mutations associated with specific IMDs. These findings provide region-specific epidemiological and genetic data on screened IMDs in central China, which may help inform future refinement of newborn screening panels and counselling strategies.

Keywords Inherited metabolic disorders, Neonatal screening, Tandem mass spectrometry, Genetic mutations

Abbreviations

NBS	Newborn screening
IMDs	Inherited metabolic disorders
GC/MS	Gas chromatography-mass spectrometry
NGS	Next-generation sequencing
MS/MS	Tandem mass spectrometry
OAs	Organic acidemias
FAODs	Fatty acid oxidation disorders
AAs	Amino acid disorders
DBS	Dried blood spot
MLPA	Multiplex ligation-dependent probe amplification
PAHD	Phenylalanine hydroxylase deficiency
BH4D	Tetrahydrobiopterin deficiency

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cPKU	Classic phenylketonuria
mPKU	Mild phenylketonuria
MHP	Mild hyperphenylalaninemia
SCADD	Short-chain acyl-CoA dehydrogenase deficiency
PCD	Primary carnitine deficiency
MCADD	Medium-chain acyl-CoA dehydrogenase deficiency
CPT1	Carnitine palmitoyltransferase deficiency I
MADD	Multiple acyl-CoA dehydrogenase deficiency
MMA	Methylmalonic aciduria
IBDD	Isobutyryl-CoA dehydrogenase deficiency
IVA	Isovaleric acidemia
3MCCD	3-methylcrotonyl CoA carboxylase deficiency
EE	Ethylmalonic encephalopathy
CTLN1	Citrullinemia type I

Inherited metabolic disorders (IMDs) represent a heterogeneous group of hereditary conditions that disrupt metabolic processes by impairing the function of enzymes, coenzymes, receptors, or transporters mechanisms. These disruptions result in the accumulation of toxic metabolites or the depletion of essential biochemical products, leading to potentially severe clinical manifestations, such as anemia, shock, acidosis, or hypoxia in some infants. However, most affected neonates are asymptomatic at birth but are at significant risk of rapid physical decline, lifelong disability, or mortality if untreated[1,2]. The primary objective of neonatal screening is the early detection of IMDs, ideally before the onset of clinical symptoms, to enable timely intervention and reduce associated risks while improving long-term health outcomes. Advances in screening technologies, particularly tandem mass spectrometry (MS/MS), have revolutionized neonatal screening by enabling the simultaneous detection of multiple metabolic markers. This innovation has greatly expanded the range of detectable disorders. MS/MS has been widely adopted in newborn screening (NBS) programs worldwide to identify IMDs, including organic acidemias (OAs), fatty acid oxidation disorders (FAODs), amino acid disorders (AAs), and urea cycle disorders[3]. Furthermore, next-generation sequencing (NGS) has emerged as a powerful tool for uncovering the genetic basis of IMDs. It provides genotypic data for studying genotype-phenotype correlations and enhances diagnostic precision, thereby supporting more targeted and effective interventions.

With the widespread implementation of IMD screening, significant regional differences in the incidence and disease spectrum of IMDs have become evident. Previous studies have highlighted substantial variations not only between countries but also within different regions of the same country. For example, in China, pronounced disparities in IMD incidence have been observed between northern and southern regions, likely influenced by regional characteristics and population mobility dynamics[4,5].

Recognizing these regional disparities highlights the critical need for localized data to better understand the epidemiology of IMDs and to develop targeted screening and intervention strategies. This study addresses this gap by examining the incidence, disease spectrum, and genetic characteristics of MS/MS-detected IMDs in Changzhi, a central region of China, over a five-year period involving 153,956 newborns.

Materials and methods

Newborn screening

A total of 153,956 newborns (79,164 males and 74,792 females) were screened for IMDs using MS/MS between May 2015 and April 2020 in Changzhi, a central city in China. The newborn screening coverage rate reached approximately 90% in this population. According to national technical specifications and expert consensus in China, heel-prick capillary blood for newborn screening is recommended to be collected at or after 48 h of life, typically between 48 h and 7 days after birth, once adequate feeding has been established. The samples were spotted onto Whatman S&S Grade 903 blood collection paper, naturally dried, and stored in sealed plastic bags. Transportation to the screening center was carried out under cold-chain logistics at approximately 4 °C within one week.

For the analysis of amino acids and acylcarnitines in dried blood spot (DBS) samples, pre-processing was performed according to the protocol outlined in the Neo-Base™ non-derivatized MS/MS kit (PerkinElmer, USA). Briefly, DBS specimens were placed individually into wells of a 96-well microtiter plate, and 100 µL of extracting solution containing internal standards for amino acids and acylcarnitines was added to each well. The plates were agitated at 700 rpm and incubated at 45 °C for 45 min. Subsequently, 75 µL of the supernatant was transferred to V-bottom plates and allowed to stand at room temperature for 2 h. Finally, 25 µL of the solution was injected into an ACQUITY TQD mass spectrometer (Waters, Milford, MA, USA) for metabolic analysis. The analysis was conducted using electrospray ionization in positive mode (ES+). Operational parameters included a capillary voltage of 3.5 kV, extractor voltage of 3 V, source temperature of 120 °C, and desolvation temperature of 350 °C. The cone gas flow was set at 20 L/hr, and the desolvation gas flow at 650 L/hr. Collision gas flow was maintained at 0.08 mL/min. Detailed detection parameters for specific analytes are provided in Supplemental Table 1. Quality control was ensured using two levels of internal standards representing low and high concentrations. QC was monitored using Levey–Jennings charts with acceptance limits (mean ± 2 SD); runs were accepted only when both QC levels were within limits. Intra- and inter-day CVs for key amino acid and acylcarnitine analytes were generally < 10%. If CVs exceeded predefined thresholds or drift persisted, instrument performance was reviewed and maintenance was performed (ion source and quadrupole cleaning, system recalibration), with repeat testing when indicated. Initial cut-offs were derived from manufacturer guidance, published international data, and our pilot results. Following the 2019 national expert consensus (National Center for Clinical Laboratories), we recalculated laboratory-specific decision limits as local screening data accumulated. We analyzed ~ 10,000

results from apparently healthy neonates sampled at 48 h–7 days, excluding repeat measurements and runs failing internal QC. Decision limits were set at the 0.5th and 99.5th percentiles and were iteratively updated as sample size increased, with the goal of keeping the primary screen-positive rate < 3% in line with national quality indicators.

For infants with MS/MS results suggestive of primary carnitine deficiency (PCD), both infant and maternal samples were simultaneously collected for retesting to determine whether the mother also had PCD. If maternal PCD was confirmed, guidance was provided to ensure timely carnitine supplementation. Final confirmation of maternal PCD was based on genetic testing.

This study was approved by the Clinical Research Ethics Committee of Changzhi Maternal and Child Health Care Hospital. Written informed consent was obtained from the parents/legal guardian of the patients included in this study.

Diagnosis and genetic testing

Newborns with clearly aberrant initial screening results were promptly referred for confirmatory testing, including biochemical and genetic analysis. For newborns with mildly abnormal initial screening results, repeat screening was conducted. If the results of the second screening remained positive, the newborn was referred for confirmatory testing, which included gas chromatography-mass spectrometry (GC/MS) for urine organic acid profiling and genetic analysis. The diagnosis of each IMD was based on biochemical investigations that identified specific abnormal metabolites, including blood amino acid levels, total homocysteine measurements, and urine organic acid profiles, with clinical symptoms taken into account when present.

The analysis of organic acids in urine was performed using a semi-quantitative method with GC/MS. This approach employed a single reference standard to estimate the concentrations of target compounds within the samples. Urine creatinine was first measured, and the volume of urine equivalent to 0.2 mg of creatinine was calculated and used for analysis. The selected urine sample underwent urea removal, followed by an oximation reaction. Organic acids were then extracted using ethyl acetate. The extract was derivatized with a silylation reagent before analysis using the GCMS QP-2010 Plus instrument (Shimadzu, Kyoto, Japan) in accordance with the manufacturer's protocol (Aiwan, Shenzhen, China). Heptadecanoic acid was used as an internal standard, and semi-quantitative results were calculated by determining the ratio of the peak area of the target compound to that of the internal standard.

Genetic analysis was performed by Genuine Diagnostics Company (Hangzhou, Zhejiang, China) using a NGS genetic diagnostic panel for hereditary metabolic diseases. The panel of inherited metabolic disorders consisted of 94 genes (*PAH*, *PTS*, *PCBD1*, *QDPR*, *SPR*, *GCH1*, *BCKDHA*, *BCKDHB*, *DBT*, *DLD*, *AMT*, *GCSH*, *GLDC*, *MAT1A*, *CBS*, *CTH*, *MTHFR*, *SUOX*, *MOCS1*, *MOCS2*, *GPHN*, *FAH*, *TAT*, *HPD*, *HGD*, *MUT*, *MMAA*, *MMAB*, *MMACHC*, *MMADHC*, *LMBRD1*, *ABCD4*, *MCEE*, *CD320*, *MLYCD*, *SUCLA2*, *SUCLG1*, *SUCLG2*, *PCCA*, *PCCB*, *GCDH*, *IVD*, *BTD*, *ACADSB*, *AUH*, *DNAJC19*, *CLPB*, *TMEM70*, *SERAC1*, *HMGCL*, *MCCC1*, *MCCC2*, *HLCS*, *ACADS*, *ACADM*, *ACADVL*, *HADH*, *HADHA*, *HADHB*, *ACAD8*, *ETHE1*, *ETFA*, *ETFB*, *ETFDH*, *ACAT1*, *SLC22A5*, *SLC25A20*, *CPT1A*, *CPT2*, *ARG1*, *ASL*, *ASS1*, *SLC25A13*, *CPS1*, *OAT*, *OTC*, *SLC25A15*, *SLC5A5*, *TPO*, *TG*, *TSHB*, *TSHR*, *PAX8*, *DUOX2*, *CYP11B1*, *CYP11B2*, *HSD3B2*, *STAR*, *CYP17A1*, *CYP11A1*, *POR*, *G6PD*, *ATP7B*, *PC*). Mutation nomenclature was in accordance with the HGVS guidelines (https://www.HGVS.org/varnomen), and the pathogenicity of each variant was assessed following the guidelines of the American College of Medical Genetics and Genomics (ACMG)[6]. Single nucleotide polymorphisms (SNPs) were excluded by cross-referencing the 1,000 Genomes Data (https://www.1000genomes.org) and gnomAD (https://gnomad.broadinstitute.org). The pathogenicity of novel variants was predicted by using respective online tools (e.g. MutationTaster, PolyPhen-2, GeneSplicer, and NetGene2). All candidate variants were subsequently confirmed using Sanger sequencing, which included analysis of samples from infants as well as relevant pedigree analysis. Moreover, for patients strongly suspected of phenylketonuria but presenting with only a single variant or lacking pathogenic mutations, further assessment was performed using multiplex ligation-dependent probe amplification (MLPA) with the SALSA MLPA P055 *PAH* probe mix (MRC Holland, Amsterdam, The Netherlands). Only patients diagnosed through genetic analysis were included in this study.

Follow up

All confirmed cases were enrolled in a follow-up program, including periodic clinical evaluations, biochemical monitoring, and genetic counseling. Follow-up durations ranged from 6 months to 3 years based on the disorder and patient needs.

Results

During the retrospective cohort study spanning a 5-year period, a total of 153,956 neonates underwent screening. The blood collection from the newborns occurred between 2 and 21 days of age, with a median collection age of 4 days. Following primary screening, 3,183 infants (2.07%) were identified as suspected positive cases. Among these, 2,987 (93.8%) were recalled for secondary screening, and 387 (13.0%) subsequently proceeded to confirmatory diagnostic assessment. Comprehensive evaluations ultimately confirmed 129 patients (62 males and 67 females) with inherited metabolic disorders (IMDs). Detailed genetic findings and follow-up outcomes are provided in Supplemental Table 2. This yielded an overall incidence of 1 in 1,193 neonates, with a positive predictive value (PPV) of 4.05%. In retrospective follow-up, we did not ascertain any confirmed false-negative cases; accordingly, the observed sensitivity in this dataset was 100%. Nevertheless, mild, asymptomatic, or late-onset IMDs may not yet have come to clinical attention; thus, sensitivity should be interpreted as an apparent rather than an absolute estimate. Consequently, the programme's true sensitivity and specificity cannot be established with complete certainty using retrospective follow-up alone. The confirmed cases encompassed 18

different species of IMDs (Table 1). Among these, 87 (67.4%) were AAs, 18 (14.0%) were FAODs, 23 (17.8%) were OAs, and 1 (0.8%) was a urea cycle disorder (Fig. 1).

Amino acid disorders

Among the detected AAs, the majority consisted of non-consanguineous patients with phenylalanine hydroxylase deficiency (PAHD). A total of 83 PAHD patients were identified, consisting of 41 males and 42 females. Additionally, four patients (2 males and 2 females) were diagnosed with tetrahydrobiopterin deficiency (BH4D). The overall incidence rate of amino acid disorders was found to be 1 in 1,770 neonates. Notably, PAHD accounted for the largest proportion of screened diseases in Changzhi city, representing more than half of the cases (64.4%), while BH4D was relatively rare (3.1%).

PAHD patients were classified into three groups based on blood phenylalanine (Phe) concentrations: classic phenylketonuria (cPKU, ≥1200 μmol/L), mild phenylketonuria (mPKU, 360–1200 μmol/L), and mild hyperphenylalaninemia (MHP, 120–360 μmol/L) (Table 2). Among the PAHD patients, 26 (31.3%) were classified as cPKU, 17 (20.5%) as mPKU, and 40 (48.2%) as MHP. Patients with cPKU and mPKU who received early treatment, including a low phenylalanine diet, exhibited normal intellectual development during infancy. BH4D patients were treated with BH4 in combination with levodopa and 5-hydroxytryptophan therapy following diagnosis. All the MHP patients did not undergo specific treatment.

A total of 53 variants were identified in the *PAH* gene across 83 PAHD patients. The most common mutation observed was c.728G > A (p.R243Q), accounting for 18.07% of alleles, followed by c.158G > A (p.R53H) with a frequency of 14.46%. Two large scale deletions were detected through MLPA analysis in two patients who lacked two potential disease-causing mutations. One deletion spanned exon 1 and its upstream region, while the other encompassed exons 4–5. In patients with BH4D, five different mutations in the *PTS* gene were detected. The most frequent mutation was c.200 C > T (p.T67M), accounting for 37.5% of alleles.

Fatty acid oxidation disorders

In the study, five types of FAODs were identified among 18 diagnosed patients (Table 3). The most prevalent FAOD was short-chain acyl-CoA dehydrogenase deficiency (SCADD), accounting for 38.9% of cases, followed by PCD at 27.8% and medium-chain acyl-CoA dehydrogenase deficiency (MCADD) at 22.2%. Carnitine palmitoyl transferase I deficiency (CPT1) and multiple acyl-CoA dehydrogenase deficiency (MADD) were each observed in 5.6% of cases. Maternal PCD was identified in five mothers and confirmed through genetic testing (Supplemental Table 3).

Patients diagnosed with PCD, SCADD, and MCADD received different doses of L-carnitine supplements, followed a low-fat diet, and avoided prolonged fasting. The patient with CPT1 experienced growth failure, feeding difficulties, and hepatic failure, ultimately leading to death at 3 months old.

Genetic analysis identified seven mutations in the *ACADS* gene, with the most common variant being c.1031 A > G (p.E344G), accounting for 3 of 14 alleles (21.4%). The c.164 C > T variant was identified in 2 of 14

Disorders	Number of patients	Incidence of a disease
Amino acid disorders	87	1:1770
1. Classic phenylketonuria (cPKU)	26	1:5921
2. Mild phenylketonuria (mPKU)	17	1:9056
3. Mild hyperphenylalaninemia (MHP)	40	1:3849
4. Tetrahydrobiopterin deficiency (BH4D)	4	1:38489
Fatty acid oxidation disorders	18	1:8553
5. Primary carnitine deficiency (PCD)	5	1:30791
6. Short-chain acyl-CoA dehydrogenase deficiency (SCADD)	7	1:21994
7. Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)	4	1:38489
8. Carnitine palmitoyltransferase deficiency I (CPT1)	1	1:153956
9. Multiple acyl-CoA dehydrogenase deficiency (MADD)	1	1:153956
Organic acidemias	23	1:6694
10. Methylmalonic aciduria cblC type (MMA cblC)	7	1:21994
11. Methylmalonic aciduria mut(0) type (MMA MUT)	3	1:51319
12. Methylmalonic aciduria cblF type (MMA cblF)	1	1:153956
13. Isobutyryl-CoA dehydrogenase deficiency (IBDD)	4	1:38489
14. Isovaleric acidemia (IVA)	3	1:51319
15. Propionic acidemia (PA)	2	1:76978
16. 3-methylcrotonyl CoA carboxylase dedicency (3MCCD)	2	1:76978
17. Ethylmalonic encephalopathy (EE)	1	1:153956
Urea cycle disorders	1	1:153956
18. Citrullinemia type I (CTLN1)	1	1:153956
Total numbers	129	1:1193

Table 1. Positive patients with IMDs detected by MS/MS newborn screening.

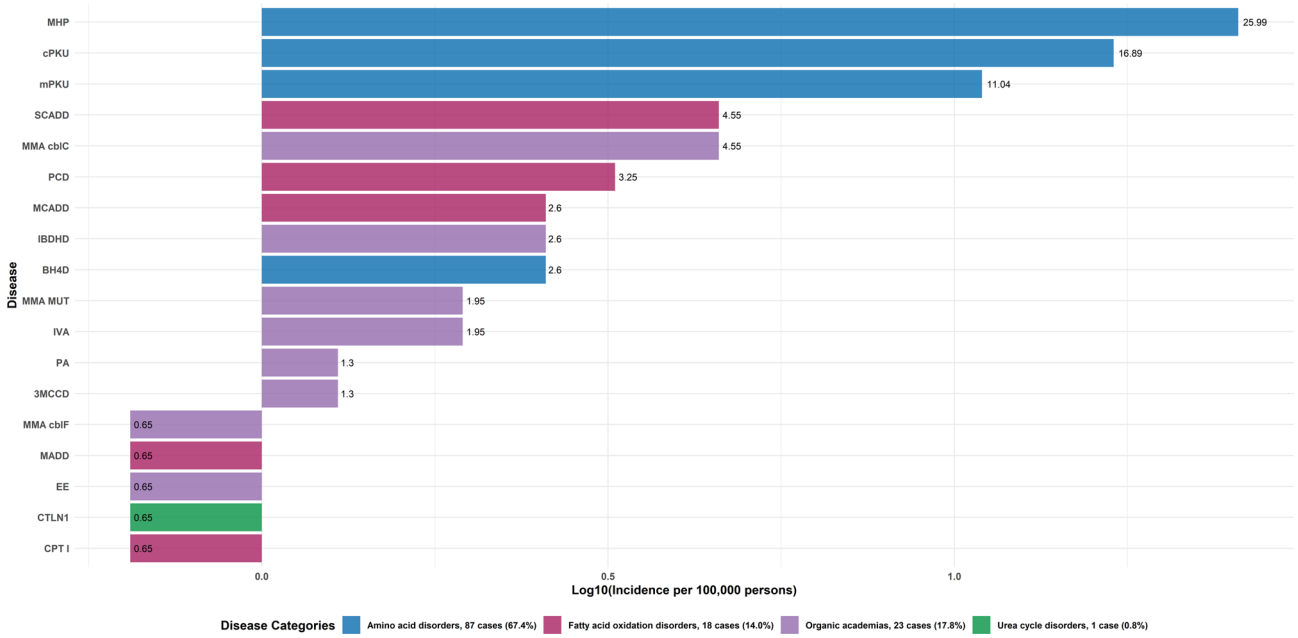

Fig. 1. Incidence rates of different IMDs (Inherited Metabolic Disorders) per 100,000 persons. The incidence rates are log10-transformed on the horizontal axis, while the original values are shown as numeric labels next to the bars. Diseases are categorized by type and color-coded accordingly.

Amino acid disorders ($n = 87$)	n	Abnormal parameter and concentration mean (range) (μmol/L)	Reference range (μmol/L)
Classic phenylketonuria (cPKU)	26	Phe: 1642.84 (1342.6–2581.73)	18–110
		Phe/Tyr: 31.09 (27.89–34.55)	0.1–1.5
Mild phenylketonuria (mPKU)	17	Phe: 651.55 (408.34–1162.96)	18–110
		Phe/Tyr: 10.48 (6.59–15.15)	0.1–1.5
Mild hyperphenylalaninemia (MHP)	40	Phe: 156.11 (123.14–263.69)	18–110
		Phe/Tyr: 2.25 (1.63–3.06)	0.1–1.5
Tetrahydrobiopterin deficiency (BH4D)	4	Phe: 240 (153.52–383.38)	18–110
		Phe/Tyr: 3.62 (2.31–7.32)	0.1–1.5

Table 2. Abnormal parameter and results statistics of amino acid disorders.

alleles (14.3%). Four patients were found to carry only a single detectable mutation. Among the five patients with PCD, the c.1400 C > G variant in the *SLC22A5* gene was detected in all cases, representing 80% of the alleles analyzed (8/10). In patients with MCADD, six distinct mutations in the *ACADM* gene were identified. The most prevalent mutation, c.449_452delCTGA (p.T150Rfs*4), was observed in 3 of 8 alleles (37.5%).

Organic acidemias

Among the OAs identified in the study, seven distinct types were observed in 23 cases (Table 4). The most common disorder was methylmalonic acidemia cblC type (MMA cblC), which accounted for 30.4% of cases. Isobutyryl-CoA dehydrogenase deficiency (IBDD) was the second most prevalent disorder (17.4%), followed by methylmalonic acidemia mut type (MMA mut, 13.0%), isovaleric acidemia (IVA, 13.0%), propionic acidemia (PA, 8.7%), 3-methylcrotonyl CoA carboxylase deficiency (3MCCD, 8.7%), methylmalonic acidemia cblF type (MMA cblF, 4.3%), and ethylmalonic encephalopathy (EE, 4.3%). Patients with MMA cblC demonstrated elevated homocysteine concentrations ranging from 38 to 230 μmol/L, while the patient with MMA cblF showed an elevated blood homocysteine concentration of 78 μmol/L. Among the 23 patients, 10 exhibited clinical manifestations shortly after birth, including growth retardation, feeding difficulties, vomiting, seizures, and hypotonia, and showed poor treatment outcomes. In contrast, the remaining patients demonstrated favorable responses to treatment.

Among patients diagnosed with methylmalonic acidemia (MMA), mutations were identified in three genes: *MMACHC*, *MMUT*, and *LMBRD1*. In patients with MMA cblC, five distinct mutations in the *MMACHC* gene were detected among seven cases. The c.609G > A (p.W203X) mutation was the most prevalent, accounting for 50% of the detected alleles (7/14). Other mutations included c.658_660del (p.K220del), c.482G > A (p.R161Q), and c.567dup (p.I190Yfs*13), each identified in two alleles, and c.440_441del (p.E147fs), which was detected in one allele. In MMA mut cases, five distinct mutations in the *MMUT* gene were identified. The most frequent

Fatty acid oxidation disorders ($n=18$)	n	Abnormal parameter and concentration mean (range) (μmol/L)	Reference range (μmol/L)
Primary carnitine deficiency (PCD)	5	C0: 6.20 (2.40–7.78)	8–60
Short-chain acyl-CoA dehydrogenase deficiency (SCADD)	7	C4: 1.27 (0.50–2.44)	0–0.45
		C4/C2: 0.09 (0.05–0.26)	0–0.04
		C4/C3: 0.87 (0.40–2.32)	0–0.35
Medium chain acyl-CoA dehydrogenase deficiency (MCADD)	4	C8: 2.32 (0.39–5.91)	0–0.15
		C8/C2: 0.16 (0.04–0.40)	0–0.02
		C8/C10: 8.17 (2.71–19.62)	0.3–1.4
Carnitine palmitoyltransferase deficiency I (CPT1)	1	C0: 166.04	8–60
		C16: 0.11	0.5–6.5
		C18: 0.09	0.25–1.6
		C0/(C16 + C18): 830.20	2–50
Multiple acyl-CoA dehydrogenase deficiency (MADD)	1	C6: 0.44	0–0.1
		C8: 0.81	0–0.15
		C10: 1.06	0–0.25
		C12: 1.44	0–0.25
		C14: 0.93	0–0.35
		C14:1: 0.87	0–0.22

Table 3. Abnormal parameter and results statistics of fatty acid oxidation disorders.

Organic acidemias ($n=23$)	n	Abnormal parameter of MS/MS and concentration mean (range) (μmol/L)	Reference range (μmol/L)	Abnormal parameter of GC/MS and measured value mean (range)*	Normal range of urine organic acids
Methylmalonic aciduria cblC type (MMA cblC)	7	C3: 8.05 (6.32–10.73)	0.3–5	Methylmalonic acid: 100.33 (49.37–159.54)	0.2–3.6
		C3/C0: 0.54 (0.31–0.63)	0–0.25		
		C3/C2: 0.63(0.28–1.16)	0–0.25		
		Met: 9.74 (4.04–20.31)	6–45		
Methylmalonic aciduria mut(0) type (MMA MUT)	3	C3: 7.96 (6.56–9.55)	0.3–5	Methylmalonic acid: 70.91 (33.84–120.51)	0.2–3.6
		C3/C0: 0.64 (0.31–1.20)	0–0.25		
		C3/C2: 0.59 (0.42–0.70)	0–0.25		
Methylmalonic aciduria cblF type (MMA cblF)	1	C3: 6.22	0.3–5	Methylmalonic acid: 272.32	0.2–3.6
		C3/C0: 0.19	0–0.25		
		C3/C2: 0.42	0–0.25		
Isobutyryl-CoA dehydrogenase deficiency (IBDD)	4	C4: 1.33 (0.77–2.32)	0–0.45	Isobutyryl glycine: 0.80 (0.42–1.70)	0–0.4
		C4/C2: 0.10 (0.04–0.19)	0–0.04		
		C4/C3: 0.82 (0.38–1.54)	0–0.35		
Isovaleric acidemia (IVA)	3	C5: 2.54 (0.68–5.85)	0–0.35	Isovalerylglycine: 144.98 (0.67–432.88)	0–0.4
		C5/C2: 0.25 (0.05–0.63)	0–0.03		
		C5/C3: 8.95 (0.42–25.99)	0–0.3		
Propionic acidemia (PA)	2	C3: 10.97 (9.43–12.50)	0.3–5	3-hydroxypropionate: 798.12 (432.88–1163.35)	0–1.1
		C3/C0: 0.86 (0.56–1.15)	0–0.25	Methylcitrate: 18.52(17.86–19.17)	0–1.1
		C3/C2: 0.96 (0.77–1.14)	0–0.25	Propionylglycine: 147.75 (32.45–263.05)	0
3-methylcrotonyl CoA carboxylase deficiency (3MCCD)	2	C4DC + C5OH: 5.08 (4.94–5.21)	0–0.4	3-methylcrotonylglycine: 7.79 (0.40–15.17)	0
		(C4DC + C5OH)/C0: 0.33 (0.17–0.49)	0–0.03		
		(C4DC + C5OH)/C8: 89.36 (48.46-130.25)	1–15		
Ethylmalonic encephalopathy (EE)	1	C4: 2.28	0–0.45	Ethylmalonic acids: 34.60	0.0–6.2

Table 4. Abnormal parameter and results statistics of organic acidemias. *The values are derived from a semi-quantitative GC/MS analysis. As the method does not provide absolute concentrations, the measurements are presented as relative values without specific units.

mutation was c.1663G > A (p.A555T), observed in two alleles. One case of MMA cblF was identified, with a single heterozygous c.70–23 C > T variant in the *LMBRD1* gene, while the second allele was not detected. No high-frequency mutations were observed in the cohort of patients affected by other organic acid disorders.

Urea circulatory disorders

Only one case with citrullinemia type I (CTLN1) was detected in this group. The patient was asymptomatic and was managed through a low protein diet along with treatment involving arginine, L-carnitine, and lactulose to control blood ammonia levels.

Discussion

Although certain forms of IMDs may be exceedingly rare, the overall incidence of these disorders remains reasonably high. In this study, we present the five-year incidence (1:1193) of those IMDs that are currently included in the regional MS/MS screening panel in the central region of China. This incidence rate was slightly lower than that reported in Saudi Arabia (1:591) but higher than the rates observed in Denmark (1:4,279), Spain (1:2,960), Portugal (1:2,396), Australia (1:2855), and Singapore (1:3159)[4,7–11]. The overall incidence rate of IMDs in China is 1:3,595[12]; however, significant variations exist between different provinces and cities. For instance, the local incidence rate in our region is considerably higher than those reported in other parts of China, such as Hangzhou (1:5,625), Shanghai (1:4,869), Jiangsu (1:2,763), Guangxi (1:10,165), Jining (1:1,941), and Taiwan (1:5,882)[12–14]. These discrepancies may be attributed to regional cultural and economic differences, which influence population mobility, particularly with regards to the lower mobility observed in northern cities.

The PPV of our study (4.05%) is notably lower compared to other NBS programs, such as those in Portugal (26%), Australia (12.6%), and Singapore (18%). However, it is higher than the PPV reported in Quanzhou (2.7%), Taiwan (1.3%), and Jiangsu (1.1%), but slightly lower than that observed in Jining (5.5%). Overall, relatively low PPVs reported across many regions of China may reflect features of the national screening strategy, particularly the deliberate use of lower cut-off values to minimize missed diagnoses. While this approach maximises sensitivity, it can increase false-positive referrals and may contribute to higher reported IMD incidence. In our cohort, the modest PPV likely reflects sensitivity-oriented thresholds together with a broad, heterogeneous sampling window, which may produce transient or non-specific metabolite elevations in the initial dried blood spot. These findings support incorporating targeted second-tier assays for selected abnormal profiles and optimizing cut-off thresholds to improve PPV while preserving the high sensitivity prioritized in current practice. Although no false-negative cases have been identified to date, indicating high apparent sensitivity of the current MS/MS panel, this estimate should be interpreted cautiously. Mild, attenuated, or late-onset IMDs may remain asymptomatic in early childhood and may not come to clinical attention within the current follow-up window; therefore, missed cases cannot be excluded. Accordingly, sensitivity should be regarded as an apparent estimate based on available follow-up; longer-term surveillance and active case ascertainment are required to refine this metric.

PAHD emerges as the predominant disorder detected through MS/MS in the Chinese population, accounting for 64.3% of all confirmed IMD cases in this study. In our region, the incidence of PAHD is 1:1885, which is considerably higher than overall incidence in China (1:11614)[15]. Among the 83 PAHD patients, most genotypes (47/83) were identified only once, potentially due to the relatively small sample size or the genetic heterogeneity of the region. Among the genotypes detected more than once, only c.611 A > G & c.728G > A (observed in 4 cases) were associated with multiple phenotypes, including cPKU (3 cases) and mPKU (1 case), whereas other genotypes were consistently linked to a single phenotype. Variants in the *PAH* gene such as c.611 A > G (13/15, 86.67%), c.728G > A (19/30, 63.33%), c.331 C > T (4/5, 80.00%), c.1197 A > T (6/9, 66.67%), and c.833 C > T (5/5, 100.00%) were predominantly identified in cPKU and mPKU patients. In contrast, the variant c.158G > A was exclusively observed in MHP patients, with 60% of MHP patients carrying this mutation. These findings align with previous reports from Quanzhou, Suzhou, Gansu, and Japan[16–18]. Notably, the variant c.833 C > T (p.T278I), which is reported exclusively in individuals of East Asian ancestry in the gnomAD database (accessed 06 Dec 2024), accounted for 3.01% of alleles and exhibited a higher frequency compared to the global average[19]. This observation likely reflects the genetic background of the study population and broader East Asian genetic characteristics. Furthermore, five cases with only one detected variant (c.722G > A, c.1301 C > A, c.509 + 1G > A, c.134T > A) exhibited MHP phenotypes. This may reflect higher baseline Phe levels in carriers, incomplete detection of pathogenic mutations, or the contribution of yet unidentified genetic modifiers. Additional studies with larger sample sizes and functional in vitro validation are necessary to confirm these findings and clarify the underlying mechanisms. In addition to PAHD, four cases of BH4D were identified, all attributed to 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency. The incidence of BH4D in the study population was 1:38,489, higher than rates reported in Singapore (1:177,000), Taiwan (1:1,495,132), Jining (1:128,559), and Quanzhou (1:91,136), but lower than that reported in Zhejiang province (1:18,147)[20]. No significant regional disparities in incidence between southern and northern China were evident based on these findings. The proportion of cases initially diagnosed as PAHD but later confirmed as BH4D was 4.60%, consistent with prior studies conducted in central China[20]. Among the BH4D-associated mutations, the c.272 A > G variant in the *PTS* gene has been reported exclusively in Northeast Asian populations[21], while the c.200 C > T mutation has been observed in northern China and Thailand[22,23]. Neither mutation, however, is considered a common mutation in China, suggesting that these findings may reflect regional characteristics specific to this area.

Remarkably, all cases of SCADD had remained asymptomatic, even in the absence of preventive interventions, highlighting the benign nature of the biochemical phenotype associated with this disorder. SCADD is not included in the expanded newborn screening panels of several countries, such as Egypt, Netherlands, and Denmark[24], due to its relatively mild clinical presentation. To date, approximately 70 mutations have been reported in SCADD, with the majority being missense mutations. Specific common mutations, such as c.310_312delGAG and c.1138

C > T, have been observed in the Roma ethnic group of Slovakia, while c.511 C > T and c.625G > A are frequently seen in American or European SCADD patients[25,26]. In our study, the most frequent mutation in SCADD patients was c.1031 A > G, consistent with findings from other cities in China, including Suzhou, Zhejiang, and Jining[13,17,24]. Therefore, c.1031 A > G is speculated to be a common mutation in the Chinese population.

PCD is the second most prevalent FAOD in our city, with an incidence rate lower than that observed in certain areas of southern China, such as Quanzhou (1:11,189), Suzhou (1:26,777), Zhejiang (1:25,131), and Liuzhou (1:9,332)[1,12,17]. However, the incidence in our region is higher than that reported in some areas of northern China, including Beijing (1:58,651), Jining (1:39,556), and Henan[13,27,28]. Our findings align with previous studies that identify c.1400 C > G (p.S467C) as a prevalent mutation in northern China; conversely, variants commonly observed in southern regions, such as c.760 C > T (p.R254), were absent in our cohort[29,30]. These regional differences in genetic variation and disease incidence are likely influenced by geographic factors and the limited population mobility characteristic of northern cities.

The third most frequently observed FAOD, MCADD, was identified with an incidence rate of 1 in 9,036 individuals, which is notably higher than those reported in other regions of China, such as Jining (1:171,411), Liuzhou (1:111,986), and Suzhou (1:80,086)[13,17,31]. The c.449_452del (p.T150Rfs*4) mutation in the *ACADM* gene is likely a common mutation, consistent with the findings of previous Chinese studies[32]. In addition, we identified a relatively rare mutation, c.1085G > A (p.G362E), which appears to be specific to our region.

MMA is the most prevalent form of OA in this study. The observed incidence rate exceeds those reported in southern regions of China, such as Quanzhou (1:121,515), Liuzhou (1:111,986), and Taiwan (1:101,625)[4,12,31]. However, it is lower than the rates documented in northern areas, including Jining (1 in 5,590) and Henan (1 in 4,673)[13,33]. This trend highlights a gradual increase in MMA incidence from north to south across China, with the central region's incidence situated between these extremes. The predominant mutation detected in patients with MMA cblC was c.609G > A (p.W203*) in the *MMACHC* gene, identified in 5 of 7 cases. This finding is consistent with prior studies on methylmalonic acidemia conducted across various regions of China. Notably, the mutations c.658_660delAAG (p.K220del) and c.482G > A (p.R161Q), also identified in this study, have not been reported in other regions of China. Two patients carrying the c.482G > A mutation (c.482G > A & c.609G > A and c.482G > A & c.658_660del) demonstrated favorable treatment outcomes, as indicated by normalized biochemical markers following intervention, despite their differing genotypes. In contrast, the other five patients, including four with the c.609G > A mutation (in homozygous or compound heterozygous cases), exhibited poor prognoses despite receiving timely treatment with hydroxocobalamin injections and betaine. Among these, two patients discontinued treatment, and one passed away. This observation contrasts with findings in the literature, where early treatment of patients with the c.609G > A mutation has been associated with favorable outcomes when initiated pre-symptomatically[34]. The discrepancy may be attributed to the relatively small sample size in this study, differences in patient compliance, or the early onset of symptoms (present at birth), which may have caused irreversible effects before treatment initiation. In the three cases of MMA mut identified, two patients carrying the c.1663G > A (p.A555T) mutation in the *MMUT* gene (c.729_730insTT&c.1663G > A and c.428 A > G&c.1663G > A) presented with asymptomatic clinical outcomes post-treatment. In contrast, the third patient (c.323G > A&c.626dup) exhibited a poorer prognosis. These findings suggest that specific mutations, such as c.482G > A in *MMACHC* and c.1663G > A in *MMUT*, may correlate with favorable biochemical and clinical outcomes. However, further studies are required to validate these observations and elucidate the underlying mechanisms.

Regrettably, all 10 patients with OAs who exhibited poor prognoses presented with clinical symptoms shortly after birth. Among these, six patients succumbed to their conditions, while the remaining four experienced persistent global developmental delays and recurrent episodes of metabolic acidosis despite receiving treatment. This finding underscores the critical importance of prenatal diagnosis and monitoring for these disorders. Furthermore, it highlights the need to explore feasible in utero therapeutic interventions as a potential strategy to improve clinical outcomes and reduce the burden of early-onset complications associated with OAs. In addition to these unfavorable outcomes, our data indicate a key bottleneck: actionable results for suspected organic acidemias may not reach clinicians rapidly enough. In the present programme, an abnormal MS/MS profile typically triggers urine organic acid profiling by GC–MS and often subsequent molecular testing; together, these steps lengthen the time to confirmation and can postpone clinical decision-making. Restructuring the confirmation process—triaging high-risk screens and, where feasible, adding rapid second-tier assays on dried blood spots—may shorten turnaround and facilitate earlier treatment initiation. Notably, although specimens were collected at a median of day 4 in our cohort (aligned with national practice), some very early-onset disorders can deteriorate within the first 48–72 h. Accordingly, regional pilot work could assess earlier sampling (e.g., 24–48 h after birth) in prespecified high-risk neonatal groups.

This study identified 12 patients with only a single detected mutation, despite the recessive inheritance pattern typically associated with IMDs. These included 5 cases of MHP, 4 cases of SCADD, 2 cases of IVA, and 1 case of MMA cblF. This observation raises several possibilities: (i) a second pathogenic variant on the other allele may exist but was not detected by the targeted NGS approach, potentially due to limitations in identifying large fragment deletions/duplications or deep intronic mutations; and (ii) the observed biochemical phenotype may result from partial enzymatic impairment caused by the single detected mutation, or from specific mutations affecting different components of enzyme interactions, such as dimerization, leading to a milder phenotype. For these specific disorders, biochemical testing of the parents revealed no abnormalities, suggesting that adult carriers may have fully compensated enzymatic activity due to organ development and metabolic adaptations over time. It is essential to emphasize that the diagnosis of these disorders should be based on a combination of biochemical indicators and clinical manifestations, rather than relying solely on genetic testing results. Further investigations, including comprehensive genomic approaches such as whole-genome sequencing and functional

studies, are necessary to explore these hypotheses and provide a more nuanced understanding of the genetic architecture of these cases.

This study has several limitations. First, post-diagnostic follow-up was relatively short because expanded MS/MS screening was implemented only recently in our region. Consequently, longer follow-up is needed to quantify the impact of early diagnosis on neurocognitive development, educational attainment, growth trajectories, and late-onset complications in many IMDs. Second, age at sample collection varied substantially. National expert consensus in China recommends collection at 48 h–7 days after birth; however, in our catchment area, sampling is performed by a decentralized network of county-level institutions, and timely collection is challenging without a strictly enforced administrative requirement, particularly given early discharge and limited parental awareness. Accordingly, sampling occurred at 2–21 days of age (median, 4 days) in our cohort. This heterogeneity may affect metabolite concentrations and, consequently, screening sensitivity and PPV for very early-onset IMDs. Future optimization should focus on strengthening training for county-level staff, improving parent-facing education, and advocating for policy measures that support collection within a narrower window (ideally ~ 48–72 h after birth). Third, as our hospital primarily serves as a screening and referral center, most children with confirmed IMDs receive long-term care at provincial or national tertiary centers. Consequently, we lack a unified longitudinal dataset capturing treatment adherence, biochemical control, and standardized neurocognitive outcomes across the full cohort; therefore, we cannot provide a structured long-term outcome analysis comparable with dedicated clinical cohort studies. Finally, incidence and performance estimates apply only to IMDs included in the current MS/MS panel and should not be extrapolated to the full spectrum of inherited metabolic disorders in the population.

In conclusion, our findings support the integration of MS/MS-based newborn screening with targeted genetic testing to facilitate early detection and clinical management of IMDs included in the current regional panel in Changzhi. We delineate regional incidence patterns, genetic features, and recurrent variants among these screened IMDs, providing evidence to inform refinement of screening panels and counselling strategies in comparable settings. Future work should prioritise functional assays for variant interpretation, analyses in larger and more diverse cohorts, and integration of emerging genomic technologies to improve diagnostic accuracy and optimize intervention pathways. Addressing key barriers—particularly modest PPV and unresolved genotype–phenotype relationships—will be essential to further strengthen the public health impact of newborn screening programmes.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author contributions

DH conducted the experimental work, supervised patient follow-up, reviewed previously published cases, and drafted the manuscript. YT developed the experimental design, carried out data analysis, prepared figures, and revised the manuscript. LW and JL were responsible for patient management and gathered clinical data. WS performed data analysis. XL supervised the study. All authors reviewed and approved the final manuscript.

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Declarations

Consent for publication

The authors affirm that human research participants provided informed consent for publication of research paper.

Ethics approval and consent to participate

Informed consent was obtained from the patients for drafting the manuscript and the research follows ethical guidelines.

Competing interests

The authors declare no competing interests.

Additional information

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