Metabolic findings of amino acids and fatty acids in high-altitude neonates with birth defects

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Abstract

Background

Birth defects (BDs) was a global public health problem, the study aimed to investigate amino acids and fatty acids metabolism in high-altitude BDs.

Methods

296 cases were collected from 2016 to 2018, and 179 Western China neonates birth data was analyzed. Liquid chromatography tandem and gas chromatography mass spectrum were used to analyze blood amino acids and fatty acids. Core family genetic analysis examined the neonate genes with abnormal metabolism outcomes.

Results

Fetal age (less than 37 weeks), low birth weight, mother pregnancy age (more than 30 years-old), and abnormal birth childbearing were the risk factors for BDs. Compared to their high-altitude normal neonates, the levels of methionine, arginine C5OH\C4DC, C6DC, C8, C14, and C14:2 were significantly different from those in high-altitude neonates, and the levels of C0, C6DC, C8:1, C14, C14:2, and C16:1 in the high-altitude normal neonates were significantly different from those in high-altitude BDs neonates. Methylmalonic aciduria, hypermethioninemia and phenylketonuria are genetic metabolic diseases leading to high-altitude BDs, and their pathogenic gene variants were *MAT1A* c. 274 T > C and c. 895 C > T heterozygous mutation, *MMACHC* c. 482G > A and *PAH* c. 728G > A.

Conclusion

Fetal age, low birth weight, mother pregnancy age, and abnormal birth childbearing were the risk factors of BDs. There were different amino acid and fatty acids metabolism between high-altitude normal and BDs neonates. Methylmalonic aciduria, hypermethioninemia and phenylketonuria are genetic metabolic diseases leading to high-altitude BDs.

Introduction

Birth defects (BDs) was defined as congenital abnormalities in neonates' shape, structure, function, metabolism, spirit, and behavior, and it was generally manifested as malformations, disgnosia, or heredity metabolic diseases. BDs was caused by aberrant embryogenesis that may also trigger miscarriage stillbirth, perinatal mortality, and infant mortality. Throughout the world, the incidence rate of BDs has been a relatively constant 3%-6%[1], while in China, there were approximately 900,000 new BDs cases each year, with an incidence rate of 5.6%[2, 3]. BDs not only affected children's health and quality

of life, but also affected the quality of the entire country's population in terms the resultant burden placed on families and society. Therefore, BDs had become a prominent public health issue.

Located in northwestern China, the Qinghai province is characterized by an environment of underdevelopment, high altitude, and oxygen deficiency, which all correspond with the epidemiologic features of BDs incidence. Therefore, in Qinghai, a higher BDs incidence rate than the average national level can be detected, with a total incidence rate of 12.098 per thousand. The mortality rates of neonates and perinatal infants caused by BDs are 54.55% and 17.14%, with higher rates in rural rather than urban regions [4]. Based on the Chinese researchers' discovery that metabolic diseases of blood amino acids, organic acids, and fatty acids were a potential cause of BDs in Beijing, it is speculated that these factors are also probably responsible for BDs in Qinghai. However, the relationship between BDs and the metabolism of amino acid, organic acid, and fatty acid dosage does not draw considerable attention due to underdevelopment in Qinghai as well as current lack of understanding regarding their specific metabolisms. Phenylketonuria, which is caused by the defective metabolism of phenylalanine, screening reveals that the morbidity rate in northwestern China is higher than the average level for the country. Moreover, the attempt of early diagnosis and treatment had great reduced mortality and disability rate of patients with phenylketonuria, which makes it one of the most successful models for preventing and treating BDs caused by genetic metabolic diseases in China. Nevertheless, so far there is still a lack of comprehensive clinical data on most metabolic diseases, such as phenylketonuria, biotinidase deficiency, methylmalonic aciduria, and carnitine deficiency. Only in areas such as Beijing, Shanghai, Guangzhou, and Hubei[5] has the understanding, diagnosis, and treatment of metabolic diseases been developing rapidly. This is primarily in terms of clinical case reporting, while in most other areas, especially in the northwest, there are no reports about these diseases at all, implying their diagnosis and treatment is lacking.

This study was designed to analyze blood amino acids and fatty acids of neonates born in western China and Beijing by liquid chromatography tandem mass spectrometry and gas chromatography mass spectrum analysis, respectively. Core pedigree analysis of disease-associated genes will be conducted for families of neonates with positive diagnoses, and genetic counseling and prenatal diagnosis information will be provided to reduce the risk of BDs. In addition, data regarding the metabolic diseases of amino acids and organic acids of BDs in high-altitude regions can also be preliminarily accumulated, which will contribute to the study of metabolic diseases of amino acids and organic acids in China.

Materials And Methods

Subjects

This study included 179 neonates (30 with BDs, 149 without BDs) in western China (altitude of 2200 meters) and 117 normal neonates in Beijing (altitude of 43 meters) from January 2016 to October 2018. These neonate grandparents were all born, raised, and lived for more than 30 years in western China, which belongs to high-attitude neonates. It has an annual average temperature of 6℃ and 17.59% of

oxygen content in the air compared to sea level. Normal neonates born in Beijing were considered the plain normal neonates. The study complied with the ethical standards of the responsible committee on human experimentation (Xining and Beijing), approved by the Ethical Committee of Affiliated Hospital of Qinghai University and informed consent was obtained for all neonate parents.

Methods

The diagnosis of BDs for the involved neonates was based on the definition, clinical features, and diagnostic criteria of the 23 types of BDs according to the Chinese National Criteria of BDs.

Analysis of blood amino acid and acylcarnitine

Qualified dried blood spot specimen on filter paper (containing about 3 μl of whole blood) were laid out in a 96-microwell plate, and an 100 μl of extract liquor containing internal standard was added. After being sealed, the plate was incubated at 45℃ with 750 rpm rotation for 45 minutes to extract the amino acids and acylcarnitine from the blood. Then, 75 μl of microwell sample was transferred to another 96-microwell plate with a V-shaped bottom to be analyzed using Applied Biosystems API 3.200, a liquid chromatography tandem mass spectrometer (AB SCIEX, Framing- ham, MA, USA) and ChemoView software. 80% acetonitrile was used as the mobile phase for the assay, and the parameter for the automatic sampler was 20 μl each time. Concentrations of amino acids and acylarnitine were automatically calculated based on the detected ion intensities of the isotope internal standard, amino acids, and acylcarnitine in the sample[6].

Analysis of urinary organic acid

First, a concentration of urinary creatinine was assayed using 5–10 ml of fresh urine, and then 20 μl of urease was added to an amount of urine containing 0.2 mg urinary creatinine. After incubation at 37℃ for 30 minutes and the addition of tropic acid, heptadecanoic acid, and lignoceric acid (40 μg of each), distilled water was added to the mixture until it reached an overall volume of 2 ml. Next, the sample was mixed with 500 μl of 5% hydroxylammonium chloride and 400 μl of 5N NaOH and then left to stand at room temperature for 60 minutes. After the addition of 350 μl 6N HCl, the sample was centrifuged and the supernatant was collected and mixed with 5 ml of ethyl acetate, which was then followed by centrifugation and the addition of 5 ml of ethyl acetate to the supernatant once more. Following centrifugation and the addition of 6 g anhydrous sulfuric acid to the supernatant, the sample was centrifuged to discard the supernatant. Subsequently, the sediment was dried with N_2 at 60 and dissolved with 100 μl of BSTFA and TMCS (with a ratio a 10:1). Finally, the sample was laid out at 80℃for 30 minutes and then analyzed via gas chromatography-mass spectrometry (GC-MS) using the Shi-madzu GCMS-QP2010 analyzer (Shimadzu, Kyoto, Japan) and the Inborn Errors of Metabolism Screening System software for the differential diagnosis of organic aciduria[7].

Genetic analysis

Gene sequencing analyses were performed for patients and their parents. The total DNA was extracted from the blood using conventional salting-out protocols[8]. Then, the entire coding sequence and exon-

intron junctions of the SLC3A1 and SLC7A9 genes were amplified using polymerase chain reaction (PCR). The PCR was performed in a 25 μl of reaction system containing 50 ng of DNA, PCR buffer, 1.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.3 mM of each primer, and 1.25 U of the Taq DNA polymerase. The reaction was carried out with an initial denaturation step at 95 °C for five minutes, followed by 30 cycles at 95 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 45 seconds, and a final elongation step at 72 °C for 4 minutes. The mutation analysis was performed using SeqScapeTM software version 2.5 (Applied Biosystems, Foster City, CA, USA). The results were compared with the SLC3-A1 and SLC7A9 reference sequences deposited in the University of California, Santa Cruz UCSC Genome Browser (http://genome.ucsc.edu/). The sequencing data was compared with an integrated set of variants (http://www.hgmd.cf.ac.uk), genotypes, and haplotypes from the 1000 Genomes Project (www.1000genomes. org) in order to identify mutations. Genomic DNA (gDNA) from the patients, their relatives, and the controls was extracted from peripheral lymphocytes using standard protocol. The entire coding sequence and exon-intron junctions of the SLC3A1 and SLC7A9 genes (10 and 13 exons, respectively) were amplified using newly designed primers (primers and conditions available upon request).

Statistical analysis

Statistical analyses were calculated using Stata 19.0. The data used mean ± standard. The inter-group comparison was performed using a one-way analysis of variance. A Pearson chi-square test was used to test the eligibility of the four-grid table counting data. Pearson Continuous Correction Chi-square Test or Fisher Exact Probability Method for Non-conforming Conditions were used.

Results

The clinical characteristics of BDs

The basic characterization of BDs

30 (14 male and 16 female) BDs were found in the study. The main BDs types (as shown in Table 1) included cleft lip and palate; polydactyly; foot deformity; vegetation on vulva, left ear, right ear, right breast, or lower gingiva; congenital heart disease, hydrocephalus; continuous fracture of cerebral cartilage; single umbilical artery; spina bifida; left cervical cyst, bilateral hydronephrosis; congenital dysplasia; lymphocele; pericardial effusion; and scoliosis.

Table 1
The types of BDs in a high-altitude area

Type	Male (n = 14)	Female (n = 16)	Total (n = 30)
Cleft lip and palate	3	4	7
Polydactyly	3	1	4
Foot deformity	0	5	5
Vegetation	3	3	6
Multiple abnormalities	2	3	5

Single factor analysis of BDs

The related factors were analyzed in both the normal neonate and BDSs groups from western China, including birth sex and weight, embryonic age, delivery mode, pregnancy complications, abnormal childbearing, education degree of mother, maternal childbearing age, gravidity and parity history, and abnormal family history. The results in Table 2 show significant differences between the BDs and high-altitude neonates in terms of embryonic age, birth weight, maternal childbearing age, and abnormal birth childbearing ($P < 0.05$).

Table 2
Single factor analysis of BDs

Factor		Normal neonates (%) (n = 149)	BDs (%) (n = 30)	χ^2	P
Gender	Males	83(55.7%)	14(46.7%)	0.822	0.365
	Females	66(44.3%)	16(53.3%)		
Birth weight *	Normal	97(65.1%)	9(30%)	12.74	**0.001**
	Low weight	52(34.9%)	21(70%)		
Fetal age *	< 37 weeks	4(2.7%)	13(43.3%)	43.393	**0.001**
	37 weeks	145(97.3%)	17(56.7%)		
Delivery mode	Vaginal delivery	106(56.7%)	18(56.7%)	1.456	0.228
	Abdominal delivery	43(56.7%)	12(56.7%)		
Pregnancy complications	Yes	30(20.1%)	6(20%)	0.001	0.987
	No	119(79.9%)	24(80%)		
Abnormal childbearing *	Yes	14(9.4%)	11(36.7%)	13.27	**0.001**
	No	135(90.6%)	19(63.3%)		
Education degree of mother (Bachelor's degree or higher)	Yes	47(31.5%)	12(40%)	0.808	0.369
	No	102(68.5%)	18(60%)		
Pregnancy age *	≤ 30 years	116(77.9%)	18(60%)	4.229	**0.04**
	30 years	33(22.1%)	12(40%)		
First pregnancy	Yes	82(55.0%)	11(36.7%)	3.375	0.066
	No	67(45.0%)	19(63.3%)		
Abnormal family history	Yes	4(2.7%)	3(10%)	1.876	0.171
	No	145(97.3%)	27(90%)		
*the factor that shows significant differences between BDs and normal neonates.					

Analysis of amino acids and esteryl carnitine in neonates.

Essential amino acids metabolism

Leucine (Leu)/isoleucine (Ile), methionine (Met), phenylalanine (Phe), tyrosine (Tyr) and valine (Val) were analyzed in 81 high-altitude normal neonates, 117 plain normal neonates, and 14 high-altitude BDs neonates. The levels of Met in the plain normal neonates were significantly higher than those in the high-altitude normal and BDs neonates (P<0.05)(Table 3).

Table 3
Metabolism of essential amino acids and non-essential amino acids

Analyte	High-altitude normal neonates (n = 81)	Plain normal neonates (n = 117)	High-altitude BD neonates (n = 14)
Leu/Ile	153.94 ± 38.61	156.05 ± 39.22	144.93 ± 34.31
Val	133.27 ± 38.28	130.71 ± 29.45	129.22 ± 27.94
Met	24.54 ± 4.63*	28.71 ± 5.74	23.07 ± 4.61*
Phe	50.73 ± 10.42	52.30 ± 10.04	46.27 ± 8.91
Tyr	102.13 ± 31.06	109.51 ± 37.26	95.58 ± 35.60
Ala	275.76 ± 63.35	272.04 ± 60.18	391.47 ± 68.69
Arg	9.49 ± 4.39*	11.48 ± 5.41	11.80 ± 5.01
Cit	14.15 ± 4.44	13.84 ± 4.32	16.13 ± 4.01
Gly	463.30 ± 142.17	451.24 ± 121.03	391.47 ± 68.69
Orn	111.72 ± 32.97	114.48 ± 37.56	117.99 ± 19.98
Pro	194.86 ± 45.19	188.35 ± 51.57	195.22 ± 49.16
*p < 0.05, significantly different compared to plain normal neonates.			

Non-essential amino acids metabolism

Non-essential amino acids, including alanine (Ala), arginine (Arg), citrulline (Cit), glycine (Gly), ornithine (Orn) and proline (Pro), were analyzed by HPLC-MS/MS. Arg levels in the plain normal neonates were significantly higher than those in the high-altitude normal neonates (P<0.05) (Table 3).

Free carnitine (C_0) metabolism and Acetylcarnitine (C_2) metabolism

C_0 and $C_2$was analyzed by HPLC-MS/MS. The levels of C_0 in the high-altitude and plain normal neonates were significantly higher than those in the high-altitude BDs neonates (P<0.05), and there were no differences levels of C_2 among the three groups (Table 4).

Table 4
The metabolism of free carnitine and acetylcarnitine

Analyte	High-altitude normal neonates (n = 81)	Plain normal neonates (n = 117)	High-altitude BD neonates (n = 14)
Free carnitine	20.64 ± 5.69[a]	22.28 ± 7.25[a]	15.62 ± 4.36
Acetylcarnitine	19.69 ± 7.47	18.91 ± 6.91	16.27 ± 6.93

[a] $P < 0.05$, significantly different compared to high-altitude BD neonates.

The acyl carnitine (C_3-C_{18}) metabolism

Short-chain (C_3-C_6), medium-chain (C_8-C_{14}), and long-chain (C_{16}-C_{18}) acyl carnitine were analyzed in high-altitude normal neonates, plain normal neonates. and high-altitude BDs neonates. C5OH\C4DC in high-altitude normal neonates was higher than in plain normal neonates (P 0.05), while C6DC in high-altitude normal neonates was lower than in plain normal neonates and high-altitude BDs neonates (P 0.05) (Table 5.1). Additionally, C8, C14 in high-altitude normal neonates was lower than in plain normal neonates (P 0.05), and C14:2 in high-altitude normal neonates was higher than in plain normal neonates and high-altitude BDs neonates (P 0.05) (Table 5.2). C16:1 in high-altitude normal neonates was higher than in high-altitude BDs neonates (P 0.05) (Table 5.3).

Table 5.1
Metabolism of short-chain acyl carnitine

Name of short-chain acyl carnitine	High-altitude normal neonates	Plain normal neonates	High-altitude BD neonates
C3	1.5 6 ± 0.74	1.47 ± 0.67	1.27 ± 0.68
C5OH\C4DC	0.19 ± 0.07	0.15 ± 0.04[a]	0.16 ± 0.03
C4	0.18 ± 0.05	0.18 ± 0.05	0.17 ± 0.04
C4OH\C3DC	0.09 ± 0.04	0.08 ± 0.04	0.07 ± 0.02
C5	0.09 ± 0.03	0.09 ± 0.03	0.08 ± 0.01
C5:1	0.0077 ± 0.0023	0.0076 ± 0.0016	0.0077 ± 0.0014
C5DC\C6OH	0.14 ± 0.04	0.14 ± 0.04	0.14 ± 0.03
C6DC	0.0249 ± 0.0416	0.0035 ± 0.0008[a]	0.0046 ± 0.0008[a]
C6	0.036 ± 0.013	0.038 ± 0.013	0.031 ± 0.008

[a] $P < 0.05$, significantly different compared to plain normal neonates.

Table 5.2
Metabolism of medium-chain acyl carnitine

Name of medium-chain acyl carnitine	High-altitude normal neonates	Plain normal neonates	High-altitude BD neonates
C8:1	0.10 ± 0.04	0.11 ± 0.04	0.14 ± 0.06[a]
C8	0.04 ± 0.01	0.05 ± 0.02[a]	0.04 ± 0.01
C10:2	0.014 ± 0.004	0.015 ± 0.003	0.016 ± 0.004
C10:1	0.057 ± 0.021	0.063 ± 0.020	0.060 ± 0.022
C10	0.064 ± 0.027	0.072 ± 0.036	0.061 ± 0.030
C12:1	0.033 ± 0.022	0.034 ± 0.025	0.027 ± 0.021
C12	0.072 ± 0.030	0.070 ± 0.035	0.067 ± 0.027
C14:2	0.015 ± 0.003	0.013 ± 0.003[a]	0.019 ± 0.005[ab]
C14:1	0.083 ± 0.029	0.081 ± 0.032	0.078 ± 0.026
C14	0.134 ± 0.055	0.152 ± 0.058[a]	0.098 ± 0.037[ab]
C14OH	0.009 ± 0.003	0.010 ± 0.003	0.010 ± 0.003

[a] $P < 0.05$, significantly different compared to high-altitude normal neonates.

[b] $P < 0.05$, significantly different compared to plain normal neonates.

Table 5.3
The metabolism of long-chain acyl carnitine

Name of long-chain acyl carnitine	High-altitude normal neonates	Plain normal neonates	High-altitude BD neonates
C16:1	0.118 ± 0.067	0.102 ± 0.063	0.076 ± 0.047[a]
C16	2.3 6 ± 1.141	2.24 ± 1.07	1.63 ± 0.67
C16:1OH	0.02 5 ± 0.009	0.026 ± 0.011	0.023 ± 0.008
C18:2	0.275 ± 0.087	0.290 ± 0.110	0.320 ± 0.092
C18:1	1.348 ± 0.442	1.269 ± 0.405	1.240 ± 0.543
C18	0.800 ± 0.303	0.731 ± 0.280	0.656 ± 0.250
C16OH	0.011 ± 0.004	0.011 ± 0.004	0.011 ± 0.003
C18:1OH	0.021 ± 0.011	0.020 ± 0.008	0.026 ± 0.011
C18OH	0.007 ± 0.003	0.007 ± 0.003	0.007 ± 0.002

[a] $P < 0.05$, significantly different compared to high-altitude normal neonates.

The gene analysis of genetic metabolic diseases

3 cases of inherited metabolic diseases were found from 149 neonates, and there were hyperphenylalaninemia, hypermethioninemia, and methylmalonic aciduria.

Hyperphenylalaninemia

The analysis of amino acids and acyl carnitine revealed that the level of inherited metabolic disorder phenylalanine was 274.23 μmol/L, which is higher than the normal level of 23.3–100 μmol/L); and the experimental results reveal phenylalanine levels between 270.34 and 301.52 μmol/L. An analysis of urine organic acid showed that the levels of phenyl-pyruvic acid and phenyllactic acid had also increased. Additionally, the results of gene diagnosis revealed the exon 7 of an inherited pathogenic *PAH* variant, which was (*PAH*)c.728 G > A(p.R243Q), which caused arginine at position 243 to be replaced by glutamine. Core pedigree analysis showed that the parents carried this pathogenic variation.

Hypermethioninemia

The analysis of amino acid and acyl carnitine showed that methionine (MAT) levels were 619.52 ~ 778.37 μmol/L, which is higher than the normal level (3.68 ~ 41.35 μmol/L). The analysis of urine organic acid was normal. Mutation in exon 3 and 7 of an inherited pathogenic *MAT1* variant was discovered during gene analysis. There were c.274 T > C(p.Y92H)and c.895 C > T□p.R299C□, which resulted in the replacement of tyrosine at position 92 and arginine at position 299 by histidine and cysteine, respectively.

Core gene analysis showed that the father carried an inherited pathogenic C. 895C > T variant and that the mother carried an inherited pathogenic C. 274T > C variant.

Methylmalonic aciduria

The analysis of amino acid and acyl carnitine showed that C3 levels were 5.4 μmol/L, which was higher than normal levels (0.24 ~ 3.8 μmol/L). The analysis of urine organic acid showed that methylmalonic acid levels were 743.6 μmol/L, which was higher than the normal level (0.2 ~ 3.6 μmol/L). Gene analysis revealed an inherited pathogenic *MMACHC* variant: MMACHC c.482G > A(p.R161Q), which resulted in arginine at position 161 being replaced by glutamine. Core gene analysis revealed that the parents carried an inherited pathogenic *MMACHC* c.482G > A variant.

Discussion

According to the survey on BDs in China, it has become the second highest cause of infant death and a major cause of child disability. Congenital heart disease, polydactyly, cleft lip and palate, neural tube defects, congenital hydrocephalus, limb shortening, equinovarus foot, hypospadias, syndactyly, and microtia being the 10 most high-risk BDs in Chinese neonates[9]. BDs in high-altitude areas revealed that the three main types of BDs were cleft lip and palate, polydactyly, and deformed feet, which are consistent with BDs prevalence patterns reported in China. The incidence rate of polydactyly was similar to that reported by other Chinese researchers[10, 11], but it had higher rate than the studies by Tomoyuki[12] and Boris[13]. The incidence rate of cleft lip and palate we found was different from some domestic studies[14, 15] and abroad studies [12, 13], and the incidence rate of deformed feet was similar to that reported by Tomoyuki[12]. Previous researches had shown that about 25% of BDs are caused by genetic factors, about 10% are due to environmental factors, and the remaining 65% may be caused by a combined effect of genetic and environmental factors or other unknown reasons[16]. The study found that fetal age (less than 37 weeks), low birth weight, mother pregnancy age (more than 30 years old), and abnormal birth childbearing were risk factors for BDs.

Heredity metabolic diseases, as a main manifestation of BDs, were often overlooked or misdiagnosed as miscellaneous diseases due to their complexity. Nevertheless, the diseases, caused by aberrant metabolism, have drawn considerable attention due their severity and high incidence rate among neonates. Furthermore, amino acids not only play a central role in building proteins, but also participate in metabolism functioning as an intermediate. The corresponding disorders can lead to clinical signs and symptoms, even serious diseases, in metabolism, immunity and the cardiovascular and nervous systems. It was reported that the concentration of amino acid in children's blood varies with BD-associated factors, such as birth weight and embryonic age, as revealed in the study. Domestic studies have shown that there are differences in the distribution of amino acids and ester carnitine in newborns from different regions. This study found there was a significant difference in the levels of both Met and Arg between the plain normal neonates and the high-altitude normal neonates, and the levels of these two types of amino acids were similar between the high-altitude normal and BDs neonates. It suggests that a higher level in plain areas is essential but not insufficient for normal neonates in high-altitude. There were significant

different levels of C5OH\C4DC, C6DC, C8, C14, and C14:2 in the plain normal neonates and the high-altitude normal neonates, and the levels of C0, C6DC, C8:1, C14, C14:2, and C16:1 were significantly different between the high-altitude normal and BDs neonates. These results reveal a difference in multiple ester carnitines between the normal neonates in the plain and the high-altitude normal and BDs neonates. It was speculated that the metabolic status of short, medium, and long chain fatty acids might be related to disease state and high-altitude environment factors such as high altitude and oxygen deficit.

Inherited metabolic diseases are a type of disease that can cause BDs, with an overall incidence about 1/3000-1/5000. In China, the increasing incidence of phenylketonuria, which caused 1200 to 1500 new cases each year, has become a main cause of fetal and neonatal mortality. This study found two amino acid metabolic diseases (hyperphenylalaninemia and hypermethioninemia) and one organic aciduria (methylmalonic aciduria with homocysteinemia). There were no fatty acid metabolic diseases, which may be related to the low incidence rate of fatty acid oxidative metabolic disorders and few screening cases. Two amino acid metabolic disease were observed during the neonatal period and did not produce clinical symptoms.

Phenylalanine is one of the essential amino acids. Phenylalanine hydroxylase (PAH) and tetrahydrobiopterin take part in the conversion of phenylalanine into tyrosine. Mutations in the gene encoding of phenylalanine hydroxylase may cause defects in enzyme activity, leading to hyperphenylalaninemia, which is also known as phenylketonuria. It has been reported that 44 pathogenic genotypes of phenylketonuria in Shenzhen were detected, and the mutation rate was 81.48%, which was lower than the 92.65% in the Tai'an area[17] and the same as the 80.8% in the Qinghai area[18]. This study found *PAH* c.728 G > A (p.r243q), which is a common pathogenic variant genotype.

Hypermethioninemia is a genetic metabolic disease, and the deficiency of methionine adenosine transferase activity is one of the main etiologies. The genetic pattern of methionine adenosyltransferase deficiency is usually autosomal recessive inheritance, but few studies showed autosomal dominant inheritance[19, 20]. In this study, methionine was abnormally elevated in children with hypermethioninemia by LC-MS/MS without any symptoms. Gene analysis confirmed the mutation of exon 3 C. 274T > C and exon 7 C. 895C > T of a complex inherited pathogenic *MAT1A* variant. Core family gene analysis showed that parents carried the two pathogenic variants, which corresponded to autosomal recessive inheritance.

Methylmalonic aciduria is the most common organic aciduria in China, and 10 kinds of gene defects have been found to cause hereditary methylmalonic aciduria. Specifically, they are single-gene hereditary diseases and most are autosomal recessive heredity. Methylmalonate aciduria with homocysteinemia may be the main clinical phenotype in Chinese patients with methylmalonate aciduria. Previous studies have found that *cblC* and *MMACHC* are common enzyme deficiencies in Chinese patients[21, 22]. The clinical manifestations of methylmalonate aciduria with homocysteinemia are complex and varied, including psychomotor retardation, anemia, visual impairment, skin lesions, and the liver and kidneys damagements. A case of methylmalonate aciduria with homocysteinemia was found to present with low

response, poor appetite, and aggravated jaundice after birth. Biochemical examination indicated hypoglycemia, metabolic acidosis, and hyperammonemia. Blood amino acid ester acyl carnitine spectrum analysis showed that C3 and C3/C2 levels increased, suggesting that it may be methylmalonic acidemia or propionic acidemia. Urine organic acid analysis showed that methylmalonic acid levels also increased significantly, while gene analysis revealed an *MMACHC* c.482G > A (p.R161Q) pathogenic variant. Core pedigree analysis showed that the parents were carriers of the pathogenic variant. Finally, the neonates were diagnosed with methylmalonate aciduria.

Testing of amino acids, fatty acids and gene analysis in blood is helpful for finding amino acids and fatty acids metabolism disorders, clinical diagnosis and treatment.

Conclusions

Fetal age (less than 37 weeks), low birth weight, mother pregnancy age (more than 30 years-old), and abnormal birth childbearing were the risk factors for BDs. Compared to their high-altitude normal neonates, the levels of methionine, arginine C5OH\C4DC, C6DC, C8, C14, and C14:2 were significantly different from those in high-altitude neonates, and the levels of C0, C6DC, C8:1, C14, C14:2, and C16:1 in the high-altitude normal neonates were significantly different from those in high-altitude BDs neonates. Methylmalonic aciduria, hypermethioninemia and phenylketonuria are genetic metabolic diseases leading to high-altitude BDs, and their pathogenic gene variants were *MAT1A* c. 274 T > C and c. 895 C > T heterozygous mutation, *MMACHC* c. 482G > A and *PAH* c. 728G > A.

Declarations

Conflict of interest:

The authors have no conflicts of interest to declare.

Funding source:

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Ethical approval:

The study complied with the ethical standards of the responsible committee on human experimentation (Xining and Beijing), approved by the Ethical Committee of Affiliated Hospital of Qinghai University, Xining, Qinghai, China and informed consent was obtained for all neonate parents.

Conflict of interest:

The authors have no conflicts of interest to declare.

Author contributions:

Hou Jing and Yan-Yan Ma designed the manuscript, Tao Zhang and Jian-Hua Li drafted and revised the final manuscript. Zhi-Qin Li and Mei-Yuan Tian collected and analyzed data. All authors have seen and approved the final version submitted for publication and take full responsibility for the manuscript.

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