

# C4OH is a Potential Screening Marker - A Multicenter Retrospective Study of Patients with Beta-Ketothiolase Deficiency in China

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## Research

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# Abstract

**Background:** Beta-ketothiolase deficiency (BKTD) is an autosomal recessive disorder caused by biallelic mutations in *ACAT1* that affects both isoleucine catabolism and ketolysis. Scant information is available regarding the incidence, newborn screening (NBS), and mutational spectrum in China.

**Methods:** We collected NBS, biochemical, clinical, and *ACAT1* mutation data from 18 provinces or municipalities in China between January 2009 and May 2020, and systematically assessed all available published Chinese BKTD patients data.

**Results:** Totally 16,088,190 newborns were screened and 14 were identified through NBS, with an estimated incidence of 1 per 1 million newborns in China. Twenty-nine patients were genetically diagnosed as BKTD and 12 patients were newly identified. Most patients showed typical blood acylcarnitine and urinary organic acid profiles. In particular, almost all patients (15/16, 94%) showed elevated C4OH levels. Eighteen patients presented acute metabolic decompensations and displayed variable clinical symptoms. The acute episodes of 9 patients were triggered by infections, diarrhea, and vaccination. About two thirds of patients have favorable outcomes, one showed developmental delay, while three had died. Twenty-seven distinct variants were identified in *ACAT1*, among which 5 were found to be novel.

**Conclusion:** This study presented the largest series of BKTD cohort in China. Our results indicated that C4OH is a useful marker for the detection of BKTD. The performance of BKTD NBS could be improved by adding C4OH to the current panel of C5OH and C5:1 markers in NBS. The mutational spectrum and molecular profiles of *ACAT1* in Chinese population were expanded with 5 newly identified variants.

## 1. Introduction

Beta-ketothiolase deficiency (BKTD, OMIM #203750) is an autosomal recessive disorder caused by a defect in mitochondrial acetoacetyl-CoA thiolase (T2, EC 2.3.1.9), affecting both isoleucine catabolism and ketolysis [1–3]. The disease is clinically characterized by intermittent ketoacidotic episodes. The T2 encoding gene, *ACAT1*, is located on chromosome 11q22.3-23.1 and comprises 12 exons spanning approximately 27 kb. Characteristic laboratory findings include marked ketonuria and elevated urinary excretion of isoleucine catabolic intermediates, such as 2-methyl-3-hydroxybutyrate (2M3HB), tiglylglycine (TIG), and 2-methylacetoacetate (2MAA). Notably, 2MMA is unstable and hardly detected by gas chromatography-mass spectrometry, especially in non-fresh urine samples.

BKTD is included in newborn screening (NBS) programs in many countries, and both 3-hydroxyisovalerylcarnitine (C5OH) and tiglylcarnitine (C5:1) are primarily utilized as screening markers [4]. BKTD patients commonly have elevated levels of C5OH and C5:1. However, normal acylcarnitine profiles were reported in some patients even during acute metabolic crises [5, 6]. Therefore, NBS for BKTD can be challenging as some patients may not be identified, indicating that only two markers C5OH and C5:1 are not enough for BKTD NBS.

Since the first description of BKTD in 1971, approximately 250 patients have been detected worldwide [1]. While several retrospective studies investigating BTKD patients in various ethnic backgrounds have been reported [7–10], scant information is available regarding the incidence, NBS, and mutational spectrum in China.

We encountered a case of genetically diagnosed BKTD with increased C4OH only but no abnormal C5OH and C5:1 profile at screening and no increased 2M3HB in urine even during acute metabolic disorder. To further evaluate the significance of C4OH in BKT, we launched a multicenter national cohort study on the platform of Zhejiang Neonatal Disease Screening Center, a unit of China Neonatal Screening Group. The specific objectives of the study were: (a) to investigate the baseline level of amino acids and acylcarnitines in BKT; (b) to evaluate the importance of C4OH as well as C5OH and C5:1 in BKT screening; (c) to further understand the incidence, clinical features, genetic features and prognosis of BKT.

We systematically reviewed the available previous literatures of clinical reports of BKT in Chinese population and retrospectively analyzed the biochemical, clinical, and molecular features of total 29 Chinese BKTD patients from our NBS and selective metabolic screening (SMS). Notably, 3-hydroxybutyrylcarnitine (C4OH) was primarily used to evaluate the metabolic profile of BKTD in previous studies, whereas elevated levels of C4OH can often be observed in our patients during NBS. Thus, we propose that C4OH is a potential marker for BKTD screening. Meanwhile, we identified 5 novel variants among total 27 distinct variants of *ACAT1* in Chinese population.

## 2. Methods

### 2.1. BKTD Special Case Report

A female newborn (patient #4 in Supplemental Table S1), now 23 months old, was found with increased C4OH (2.19  $\mu\text{mol/L}$ , 4 times more than normal) only at NBS with tandem mass spectrometry (MS/MS). Her C4OH level returned to normal after 2 weeks when recalled and thus released with the regular process of NBS. She showed up at 11 months old with fever, severe acidosis, and drowsiness after injection of meningococcal vaccine and admitted to pediatric ICU. Her C4OH level at that time was 1.28  $\mu\text{mol/L}$  (2 times more than normal) and only increased 3-hydroxybutyric acid (ketone body) was found with urine organic acid analysis. Genetic diagnosis revealed that her genotype *ACAT1* variants are compound heterozygous c.163T > A (p. 555L) and c.1119dup (p.V374Sfs\*86), respectively. At 1-year-old, she was hospitalized again due to fever, acidosis, somnolence, and metabolic disorder with increased C4OH level at 1.18  $\mu\text{mol/L}$ . Although her condition became stable after emergency treatment, she had irreversible mental and motor retardation. In this case, regardless at the time of NBS or during acute metabolic crises, only increased C4OH but not characteristic index of BKT (C5OH and C5:1 or corresponding increase of 2M3HB in urine) was found. This special case triggered us to further evaluate whether C4OH is a potential marker to be used like C5OH and C5:1 for BKT screening.

### 2.2. Participating Study Centers

Participating centers from 18 provinces and municipality cities were selected nationally with the strict criteria of the China Neonatal Screening Group. The selected centers cover seven national administrative regions, including Northwest, Northeast, East, Middle, South, Southwest, and North China. Each center had more than 15,000 accumulated newborns screened by MS/MS. Internal quality controls were used in each sample batch. The results were evaluated by adding two in-house quality control specimens per 96-well microplate for the MS/MS analysis. External quality control programs from the China National Center for Clinical Laboratories (NCCL) and Centers for Disease Control and Prevention (CDC) were also included for yearly evaluations. This study was approved by the Ethical Committee of Children's Hospital, Zhejiang University School of Medicine (reference number: 2018-IRB-077) and was performed in accordance with the Declaration of Helsinki. Written informed consent was signed by and obtained from the parents of all infants.

## 2.3. Study Population

The study cohort covered NBS and reports between January 2009 and May 2020 (Fig. 1). Patients genetically diagnosed as BKTD (compound heterozygous or homozygous for *ACAT1* variants) were included. All cases of genetically confirmed Chinese BKTD patients published previously were reviewed and included in the study. Datasets on Chinese BKTD patients were retrieved from PubMed (<https://www.ncbi.nlm.nih.gov/pubmed>) by searching the keywords: beta-ketothiolase deficiency,  $\beta$ -ketothiolase deficiency, T2 deficiency, mitochondrial acetoacetyl-CoA thiolase deficiency, MAT deficiency, or 2-methylacetoacetyl-coenzyme A thiolase deficiency; Chinese or China; and *ACAT1*.

## 2.4. Genetic Diagnosis and Data Analysis

The incidence was calculated by dividing the number of BKTD patients diagnosed via NBS by the total number of screened newborns. The patients' acylcarnitine profiles, clinical information, biochemical and genetic testing results were collected for analysis. For new identified patients, genetic testing was performed by the Genetic Diagnostic Laboratory at Children's Hospital, Zhejiang University School of Medicine (Hangzhou, Zhejiang, China). Target next-generation sequencing (NGS) was done as previously described [11], all potentially pathogenic variants identified through NGS were validated by Sanger sequencing. One hundred healthy newborns who were screened negative for BKTD were selected to assess variant frequencies in normal controls. The pathogenicity of novel variants was assessed using several *in silico* tools, including SIFT, PolyPhen-2, PROVEAN, and MutationTaster.

## 3. Results

### 3.1. BKTD NBS and acylcarnitine analysis

During the study period, a total of 16,088,190 newborns were screened, six of the 18 provinces or municipalities have detected BKTD, and 14 patients eventually diagnosed with BKTD through NBS. The overall incidence of BKTD was 1 in 1,149,156 births (Table 1). In total, 29 Chinese patients were genetically diagnosed as BKTD in this cohort, 17 had been previously reported [12–16]. The median C4OH concentration was  $1.38 \pm 0.94 \mu\text{mol/L}$  (range 0.26–3.58  $\mu\text{mol/L}$ , reference value 0.02–0.3  $\mu\text{mol/L}$ ), almost all patients (15/16, 94%) showed elevated C4OH levels except for one patient (Supplemental Table S1, No. 10). The median C5OH concentration was  $1.36 \pm 0.87 \mu\text{mol/L}$  (range 0.44–3.4  $\mu\text{mol/L}$ , reference value 0.06–0.4  $\mu\text{mol/L}$ ). The median C5:1 concentration was  $0.37 \pm 0.28 \mu\text{mol/L}$  (range) 0.02–1.22  $\mu\text{mol/L}$ , reference value: 0–0.02  $\mu\text{mol/L}$ ). Almost all patients (23/25, 95%) showed elevated C5OH and C5:1 levels (Supplemental Table S1). Of particular notes, patients No. 4 and 11 had increased C4OH only but not C5OH and C5:1, even at the time of metabolic disorders. However, as described in the above case report, C4OH level in patient No. 4 returned to normal when recalled after 2 weeks of NBS, indicating that C4OH could be normal in the stable phase. In contrast, patient No. 10 had increased C5OH and C5:1 but not C4OH. All others have both increased C4OH as well as C5OH and C5:1 at the same time of NBS in the current available data.

Table 1  
Data of newborn screening for BKTD

Province/municipality	Screened newborns	Confirmed BKTD	Incidences
Zhejiang	3,830,012	4	1:957,503
Shandong	3,060,547	4	1:765,137
Jiangsu	2,240,078	2	1:1,120,039
Hunan	1,400,320	1	1:1,400,320
Shanghai	1,230,125	2	1:615,063
Fujian	977,173	0	0
Henan	879,231	0	0
Guangdong	598,007	0	0
Anhui	560,000	1	1:560,000
Gansu	500,789	0	0
Jiangxi	250,010	0	0
Jilin	156,126	0	0
Shanxi	150,003	0	0
Chongqing	78,096	0	0
Beijing	70,235	0	0
Sichuan	60,170	0	0
Hainan	30,012	0	0
Yunnan	17,256	0	0
Total	16,088,190	14	1:1,149,156

### 3.2. Biochemical and clinical features

Urinary organic acid results were available in 27 patients. Almost all patients exhibited the characteristic increase of urinary 2M3HB and TIG except patient No. 4, who had only increased urinary ketone-3-hydroxybutyric acid but no 2M3HB and TIG when breaking out at 11 months old after injection of meningococcal vaccine. This is consistent with the only increased blood C4OH examined by tandem MS/MS.

In this cohort of total 29 patients, there are 4 pairs of siblings, 15 males and 9 females, and the gender of the remaining 5 patients was not described. Eighteen patients (18/29, 62%) presented clinical symptoms, including hypotonia, fever, vomiting, tachypnea, seizures, neurological impairment, and metabolic acidosis. Of these, 2 patients presented with acute metabolic decompensations during the neonatal period, 13 displayed clinical symptoms beyond the neonatal period (mean 10.5 months), and the remaining 3 had no reported onset time. The acute episodes of 9 patients were triggered by infections, diarrhea, and vaccination. About two thirds of patients (19/29, 66%) have a favorable outcome, one showed developmental delay, while three had died. No information

on the remaining 6 patients was available. Detailed information on biochemical and clinical manifestations of the 29 BKTD patients are summarized in Supplemental Table S1.

### 3.3. Molecular findings

All 29 patients harbored compound heterozygous or homozygous *ACAT1* variants. Twenty-seven distinct variants were identified, among which 51.9% (14/27) were missense variants, 22.2% (6/27) were frameshift variants, 14.8% (4/27) affected splicing, 7.4% (2/27) were nonsense variants, and 3.7% (1/27) was large deletion. Twenty-two of these *ACAT1* variants have been previously described, the other 5 were found to be novel (Table 2). They are c.1119dup (p.V374Sfs\*86), c.631C > A (p.Q211K), c.1154A > T (p.H385L), c.401T > C (p.M134T), and c.481T > C (p.Y161H). All novel variants have not been recorded in disease databases such as ClinVar and HGMD, and were not detected in the control group. All variants were not present or have extremely low allelic frequencies in the dbSNP, ExAC, 1000 Genome, and GnomeAD databases. *In silico* analysis suggested that all novel variants were potentially pathogenic (Supplemental Table S2). The most common variant in this cohort was c.622C > T (p.R208\*) with a frequency of 17.2%, followed by c.1006-1G > C (8.6%) and c.1124A > G (p.N375S) (8.6%). In addition, c.419T > G (p.L140R) and c.997G > C (p.A333P) were relatively common (Table 2).

Table 2  
The detected *ACAT1* variants and their frequencies in Chinese patients

No.	Variants	Locations	Mutant allele (No.)	Frequencies (%)	ClinVar (Clinical significance)	HGMD	References
1	c.622C > T (p.R208*)	Exon 7	10	17.2	P	CM102337	Fukao et al. (1992), Nguyen et al. (2017)
2	c.1006-1G > C	Intron 10	5	8.6	P	CS920725	Fukao et al. (1992), Nguyen et al. (2017)
3	c.1124A > G (p.N375S)	Exon 11	5	8.6	P	CS083860	Fukao et al. (2008)
4	c.419T > G (p.L140R)	Exon 5	4	6.9	NF	NF	Xu et al. (2019)
5	c.997G > C (p.A333P)	Exon 10	4	6.9	P/LP	CM950009	Su et al. (2017)
6	c.121-3C > G	Intron 2	3	5.2	VUS	NF	Su et al. (2017)
7	c.653C > T (p.S218F)	Exon 7	3	5.2	LP	NF	Wen et al. (2016)
8	c.72 + 1G > A	Intron 1	2	3.4	NF	NF	Xu et al. (2019)
9	c.373G > T (p.V125F)	Exon 5	2	3.4	NF	NF	Xu et al. (2019)
10	exon 6-12del	Exon 6	2	3.4	NF	NF	Xu et al. (2019)
11	<b>c.631C &gt; A (p.Q211K)</b>	Exon 7	2	3.4	NF	NF	This study
12	c.83_84del (p.Y28Cfs*38)	Exon 2	1	1.7	P	CD971964	Paquay et al. (2017), Su et al. (2017)
13	c.163T > A (p.F55I)	Exon 3	1	1.7	NF	CX102338	NR
14	c.229del (p.E77Kfs*10)	Exon 3	1	1.7	NF	NF	Xu et al. (2019)

NF: Not found, NR: Not reported, VUS: Variants of uncertain clinical significance, P: Pathogenic, LP: Likely pathogenic.

<sup>a</sup>The previously unreported novel variants of this study are in boldface type.

<sup>b</sup>HGMD: <http://www.hgmd.cf.ac.uk/ac/index.php>, <sup>c</sup>ClinVar: <https://www.ncbi.nlm.nih.gov/clinvar/>.

No.	Variants	Locations	Mutant allele (No.)	Frequencies (%)	ClinVar (Clinical significance)	HGMD	References
15	c.238 + 1G > A	Intron 3	1	1.7	NF	NF	Yang et al. (2019)
16	c.354_355delinsG (p.C119Vfs*4)	Exon 5	1	1.7	P	NF	Law et al. (2015)
17	<b>c.401T &gt; C (p.M134T)</b>	Exon 5	1	1.7	NF	NF	This study
18	<b>c.481T &gt; C (p.Y161H)</b>	Exon 6	1	1.7	NF	NF	This study
19	c.642T > G (p.Y214*)	Exon 7	1	1.7	P	NF	NR
20	c.721dup (p.T241Nfs*14)	Exon 7	1	1.7	NF	NF	Yang et al. (2019)
21	c.756_758del (p.E252del)	Exon 8	1	1.7	LP	CD076722	Sakurai et al. (2007)
22	c.829A > C (p.T277P)	Exon 9	1	1.7	VUS	NF	Su et al. (2017)
23	c.890C > A (p.T297K)	Exon 9	1	1.7	VUS/LP	CM950007	Su et al. (2017)
24	c.928G > C (p.A310P)	Exon 9	1	1.7	NF	NF	Yang et al. (2019)
25	<b>c.1119dup (p.V374Sfs*86)</b>	Exon 11	1	1.7	NF	NF	This study
26	<b>c.1154A &gt; T (p.H385L)</b>	Exon 11	1	1.7	NF	NF	This study
27	c.1163G > T (p.G388V)	Exon 11	1	1.7	P	NF	Paquay et al. (2017)
NF: Not found, NR: Not reported, VUS: Variants of uncertain clinical significance, P: Pathogenic, LP: Likely pathogenic.							
<sup>a</sup> The previously unreported novel variants of this study are in boldface type.							
<sup>b</sup> HGMD: <a href="http://www.hgmd.cf.ac.uk/ac/index.php">http://www.hgmd.cf.ac.uk/ac/index.php</a> , <sup>c</sup> ClinVar: <a href="https://www.ncbi.nlm.nih.gov/clinvar/">https://www.ncbi.nlm.nih.gov/clinvar/</a> .							

## 4. Discussion

To our knowledge, so far this is the largest BKTD cohort studied in China. The large number of screened newborns in this study provided a more comprehensive perspective on the incidence of BKTD detected via NBS in China. Biochemical, clinical, and molecular features of Chinese BKTD patients were summarized, contributing to NBS, early diagnosis and timely treatment of this rare disease.

Few studies regarding the incidence of BKTD have been reported. The incidence is estimated at 1:190,000 in northern Vietnam [10], 1:313,000 in North Carolina and 1:232,000 in Minnesota in the United States [5, 17]. Our study investigated for the first time more than 16 million newborns with 14 identified BKTD patients for an incidence of approximately 1:1,000,000 in China. The incidence is lower than those reported in other studies [5, 10, 17], possibly due to differences in ethnic backgrounds, screening experience and awareness of the disease. It is noteworthy that the actual incidence of BKTD may be higher in China because some early neonatal deaths and patients who suffered mild episodes may not be identified.

BKTD is an ideal disease for NBS from a clinical perspective. However, false-negatives (FN) has been reported in several NBS programs [5, 6, 18]. Although both C5OH and C5:1 acylcarnitines are well-known markers for BKTD screening, these two markers are not necessary high during NBS and even at acute metabolic crisis. In our study, two patients (No. 4 and 11) had increased C4OH only but not C5OH and C5:1, even in the period of acute decompensation. However, we also found one patient (No. 10) showed increased levels of C5OH and C5:1 but no increase in C4OH. Notably, C4OH level in patient No. 4 was significantly high during NBS but returned to normal when recalled, then C4OH increased again at 11 months old after injection of meningococcal vaccine, indicating that C4OH is sometimes variable and could be normal in a stable condition. Beta-ketothiolase not only acts in ketone body utilization (ketolysis) by catalyzing thiolytic cleavage of acetoacetyl-coenzyme A to produce 2 molecules of acetyl CoA in extrahepatic tissues, but also catalyzes conversion of 2-methylacetoacetyl-coenzyme A in isoleucine catabolism. D-3-hydroxybutyrate ketone body can be converted into D-3-hydroxybutyrylcarnitine (C4OH) in vivo and in vitro [19]. It is therefore reasonable that there is not only an increase in C5OH and C5:1 but also an increase in C4OH in BKTD patients. Taking together, we speculate that some BKTD patients may be missed if either one of the three acylcarnitines (C5OH, C5:1 and C4OH) is used independently as a screening marker. Conversely, the FN results can be reduced by using several markers and/or their combinations. Our study therefore strongly suggests that C4OH is a very useful and powerful marker for the detection of BKTD. The performance of BKTD NBS may be improved by adding C4OH to C5OH and C5:1 combination in NBS.

In this study, despite almost all patients (except patient No. 4) exhibited the characteristic increase of urinary 2M3HB and TIG, both urinary markers were slightly elevated or undetectable even during acute crisis. Sometimes the analysis of urinary organic acid profiles is not typical, as observed in patient No. 4. Thus the diagnosis of BKTD should be confirmed by genetic test. In addition, elevated excretion of urinary 2M3HB and TIG indicates not only BKTD but also HSD10 mitochondrial disease (HSD10MD, OMIM #300438), which is a rare X-linked recessive disorder caused by a hemizygous or heterozygous mutation in the *HSD17B10* gene [20]. Fukao et al. reported a 6-year-old Japanese boy who was initially diagnosed with BKTD based on metabolic profiles, however, enzyme activity assays and mutation analysis finally confirmed the patient had HSD10MD [21]. Grunert et al. recently described two patients who may actually have HSD10MD but were misdiagnosed as BKTD in earlier reports [1]. Thus the diagnosis of BKTD cannot be based solely on metabolite data. Given the confusing blood acylcarnitine and urinary organic acid profiles between the two disorders, enzyme activity assays or mutation analysis are essential for differential diagnosis.

Most symptomatic patients in this cohort presented with acute metabolic decompensations or displayed neurologic impairment. Similar to previous study, neonatal presentation was very rare in this cohort and only two patients with neonatal onset [1]. About two thirds of patients have a favorable outcome. However, three clinical patients who did not undergo NBS presented with acute metabolic decompensations and died early, highlighting the importance of NBS for BKTD. NBS may be the only method for early detection of BKTD, and severe metabolic

crises could be avoided if patients are properly managed. It is well known that acute episodes of most BKTD patients were associated with infections. Consistent with previous studies, six of our patients were triggered by respiration tract infections or diarrhea [4]. Notably, this study reported two patients developed severe metabolic crises triggered by vaccination and one had died. The patient who died experienced acute metabolic crises at 8 months that were triggered by febrile reaction to inactivated Japanese encephalitis vaccine, indicating that the risk of metabolic decompensation should be considered and special caution should be taken for BKTD patients before and after the injection of the vaccine.

At least 105 *ACAT1* variants associated with BKTD have been described so far [22]. Most are private variants, only four variants have been identified in more than six families. The most frequent variant, c.622C > T (p.R208\*), was found in 28 families and most of them are Vietnamese origin [22–24]. This variant was detected in 6 families and 10 individuals in our patient cohort, and counted for 17.2% of all the mutant alleles identified in Chinese patients. This is consistent with previous studies that it is the most common variant. The second most common variant was c.1006-1G > C, a splice site variant associated with exon 11 skipping, that was detected in 13 families and most of them are also Vietnamese [22, 25]. This variant was identified in 4 families in this cohort and as reported in previous literature was the second common variant in China. Two other common variants, c.578T > G (p.M193R) and c.455G > C (p.G152A), however, were not observed in Chinese patients [8, 26]. Notably, another second common variant in our cohort was c.1124A > G (p.N375S), but it was rarely identified in other populations. This variant has been proven to activate a cryptic splice donor site and cause aberrant splicing [8, 22, 27]. Thus the *ACAT1* mutational spectrum appears to vary among different ethnic groups. Our study identified five previously unreported variants. They are c.1119dup (p.V374Sfs\*86), c.631C > A (p.Q211K), c.1154A > T (p.H385L), c.401T > C (p.M134T), and c.481T > C (p.Y161H). And all novel variants were predicted to be pathogenic by *in silico* analysis, expanding the molecular profiles of *ACAT1*.

To summarize, this study for the first time revealed that the incidence of BKTD in China was about 1 per 1 million newborns. Most patients have a favorable outcome, but severe metabolic decompensation and even death may occur. NBS is an effective method to identify BKTD early and prevent severe metabolic crises. C4OH is a potential screening marker, the performance of BKTD NBS may be improved and FN results can be reduced by adding C4OH to C5OH and C5:1 for the combination in NBS. The mutational spectrum of *ACAT1* in Chinese population was established. Previously unreported five novel variants were identified, expanding the molecular profiles of *ACAT1*.

## Abbreviations

BKTD: beta-ketothiolase deficiency; T2: mitochondrial acetoacetyl-CoA thiolase; 2M3HB: 2-methyl-3-hydroxybutyrate; TIG: tiglylglycine; 2MAA: 2-methylacetoacetate; NBS: newborn screening; C5OH: 3-hydroxyisovalerylcarnitine; C5:1: tiglylcarnitine; SMS: selective metabolic screening; NGS: next-generation sequencing; C4OH: 3-hydroxybutyrylcarnitine; FN: false-negatives; HSD10MD: HSD10 mitochondrial disease.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Ethical Committee of Children's Hospital, Zhejiang University School of Medicine (reference number: 2018-IRB-077) and was performed in accordance with the Declaration of Helsinki. Written informed consent was signed by and obtained from the parents of all infants for collection of samples and publication of medical data.

### **Consent for publication**

We confirm that the family has signed a written informed consent for publication of their own and their children's genetic data, clinical details, and/or any accompanying images.

### **Availability of data and materials**

The datasets used and/or analysed during the current study can be obtained from the corresponding author upon a reasonable request.

### **Conflict of Interest**

The authors declare no conflicts of interest.

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### **Authors' contributions**

YM Lin performed the data analysis, drafted and revised the manuscript; ZT Yang analyzed and interpreted data, revised the manuscript; CJ Yang, HL Hu, HY He, TT Niu, MF Liu, DJ Wang, Y Sun, YY Shen, XL Li, HM Yan, and YY Kong followed the patients and collected the clinical data; XW Huang designed and supervised the research study. All authors contributed to the data analysis, revising and approving the final manuscript to be published.

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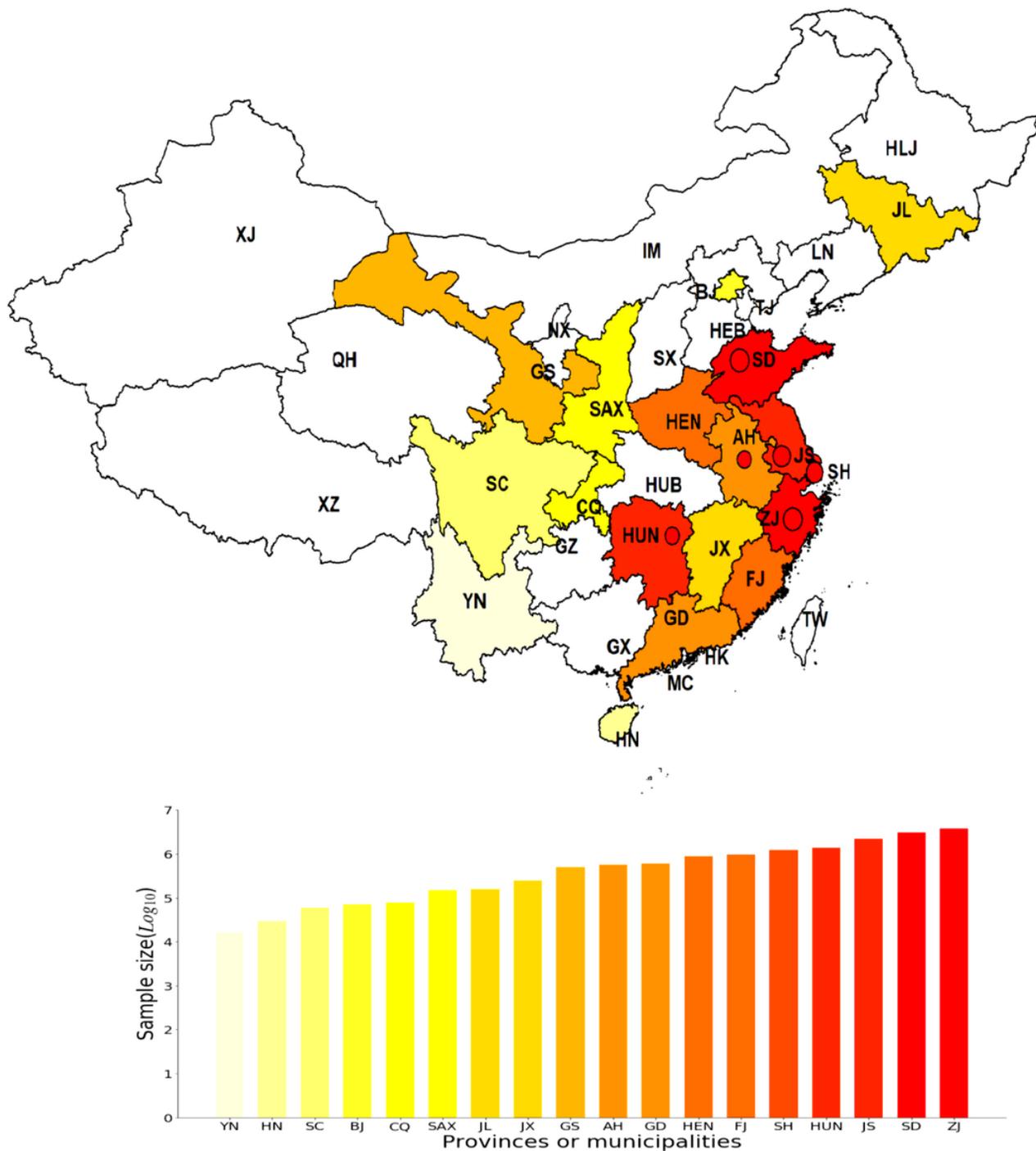
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## Figures



**Figure 1**

The distribution and sample size of study population at each province/municipality in China. HLJ, Heilongjiang; JL, Jilin; LN, Liaoning; XJ, Xinjiang; IM, Inner Mongolia; BJ, Beijing; TJ, Tianjin, HEB, Hebei; SX, Shanxi; SAX, Shaanxi; NX, Ningxia; GS, Gansu; QH, Qinghai; SD, Shandong; JS, Jiangsu; AH, Anhui; HEN, Henan; SH, Shanghai; HUB, Hubei; CQ, Chongqing; SC, Sichuan; ZJ, Zhejiang; JX, Jiangxi; HUN, Hunan; GZ, Guizhou; YN, Yunnan; FJ, Fujian; TW, Taiwan; GD, Guangdong; GX, Guangxi; HN, Hainan, HK, Hongkong; MC, Macau. Gray areas mean no screening data included. The darker in color, the larger in sample size. The red circles represent the detection of positive BKTD cases with genetic confirmation. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square

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