Original Article

Application of high-throughput sequencing technologies with target capture/target next-generation sequencing in diagnosis of neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)

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Abstract: Background: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) is an autosomal recessive metabolic disorder, which is caused by pathogenic mutations in the SLC25A13 gene. Due to complicated clinical features, many NICCD cases are difficult to diagnose. In this study, we use high-throughput sequencing technologies with target capture (target NGS), an effective and fast gene testing method, to explore whether it is suitable for NICCD genetic detection. Methods: Peripheral blood was collected from 16 patients with clinically suspected NICCD (diagnosed by clinical manifestations, tandem mass spectrometry (MS/MS) and gas chromatography mass spectrometry (GC/MS)) and their parents. Capture chips were made to detect 149 genes which were responsible for NICCD or disease-causing genes related to similar clinical features as NICCD. Target NGS were performed to detect these 16 patients. Sanger sequencing was performed to confirm the variants of patients identified by capture chips and corresponding loci of their parents. Results: Fourteen kinds of mutation were found, including 11 point mutations, 2 deletion mutations and 1 insertion mutation. The grouping of the molecular diagnosis was as follows: 8 with NICCD, 2 with progressive familial intrahepatic cholestasis type 2 (PFIC2), 1 with galactosemia, 1 with chronic idiopathic jaundice and 4 without pathogenic mutations. All of these positive molecular genetic diagnoses were confirmed by Sanger sequencing. Conclusion: Target NGS can be used for molecular genetic diagnosis in patients with NICCD. It can distinguish NICCD from other diseases which have similar clinical features with NICCD.

Keywords: Next generation sequencing, target sequence capture, SLC25A13, neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), novel mutation

Introduction

Citrin deficiency is an autosomal recessive disease, caused by dysfunction of citrin protein encoded by the SLC25A13 gene [1]. Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) is the major pediatric citrin deficiency phenotype, and its definite diagnosis relies on SLC25A13 genetic analysis [2]. The clinical manifestations of NICCD are complicated and unspecific, including intrahepatic cholestasis, fatty liver, coagulopathy, hypertryosinemia, hypercitrullinemia, galactosemia, hypoprothrombinemia, hypoglycemia, high alpha-fetoprotein (AFP) level and so on. Tandem mass spectrometry (MS/MS) and gas chromatography mass spectrometry (GC/MS) analyses are two valuable tools for the diagnosis of NICCD [3], since they can rapidly and simultaneously detect numerous metabolic compounds with high precision and sensitivity [4]. Besides, SLC25A13 genetic analysis has been also regarded as reliable tool for the definite diagnosis of such patients [5, 6]. However, routine SLC25A13 genetic analysis, such as polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) and Sanger sequencing, have much deficiencies like fussy operation, high cost, low throughput and can’t detect all SLC25A13 mutations [6].
A novel method for diagnosis of NICCD

These technologies fail to distinguish between NICCD and other genetic diseases which have similar clinical features.

High-throughput sequencing based on target gene capture (target NGS) combine next generation sequencing with target sequence capture, is widely applied to screening and diagnosis of some kind of disease or disease with a certain phenotype. The major advantage of this technology is that it allows simultaneous detection of multiple genes. This study was aimed to explore whether this technology can distinguish NICCD from other genetic diseases which with similar clinical features. We constructed 149 gene chips of multiple known causative or associated genes in highly heterogeneous diseases, including NICCD, congenital bile acid synthesis, gallbladder disease, hypermethioninemia, tyrosinemia, citrullinemia, galactosemia, Niemao-Pick disease (NPD), glycogen storage disease (GSD), Gaucher disease, and congenital glycosylation disease. These gene chips were then used to do analysis of suspected NICCD patients by using the target NGS. This technology can effectively distinguish between NICCD and other diseases which have similar clinical features.

Materials and methods

Subjects and ethics

Sixteen patients suspected to have NICCD (diagnosed by clinical manifestation, MS/MS and MS/GS) and their parents were enrolled in this study from May, 2015 to April, 2016 in the genetic metabolic disease laboratory of the Sixth Affiliated Hospital of Sun Yat-sen University. This study was approved by the Medical Ethical Committee of the local university, and informed consent was obtained from each adult and minor’s guardians for the use of their peripheral blood.

Genomic DNA extraction

Two milliliter of peripheral blood from 16 patients and their parents were collected into EDTA vacuum tubes. Genomic DNA (gDNA) was extracted by the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The purity and concentration of gDNA was assessed by spectrophotometry (NanoDrop 2000c Spectrophotometer, Thermo Scientific, Waltham, MA, USA).

gDNA library construction

The DNA libraries were constructed from 3 µg of extracted gDNA of the patients and their parents followed by Illumina paired-end library construction protocol. Briefly, 30 ng/µL gDNA in 1 × low TE buffer was fragmented to 150 bp using an ultrasonoscope (Covaris S2, Massachusetts, USA). Subsequently, the DNA libraries were constructed by using NEB Next DNA kit (NEB, USA). PCR reaction was performed to purify the amplified products. Quanlity detection of this gDNA libraries were performed by using Nanodrop 2000 (Thermo) with 1 µL constructed gDNA sample, and by using 1% agarose gel electrophoresis with 3 µL constructed DNA sample. Gualified gDNA libraries were stored at -20°C until next step.

Target NGS

A custom designed array which contains 149 common genes related to cholestatic liver disease and patients’ DNA libraries were used in our study. Mix these two subjects by Gencap sequence capture technology so that disease-causing segment can hybridize with designed array. Washing procedure was performed to remove non-target sequences and enrich disease-causing segment. Sequencing was then performed with the HiSeq2000 (Illumina, San Diego, USA) to produce paired-end reads (approximately 90 bp at each end) according to the manufacturer’s instructions.

Data filtering and bioinformatics analysis

Some low-quality reads were removed from the primary data using Trim-Galore progress, and the remaining reads were considered suitable for further analysis (quality above 20, length of sequences above 80 bp). Short-read alignment and mapping (compared with human genome database HG19) were performed using BWA (Burrows Wheeler Aligner) software. Then, point mutations, insertion mutations and deletion mutations were collected by using GATK software. Annotated these mutations by using CCDS, human genome database (HG19), dbSNP (v138) database to determine mutation gene, coordinate, mRNA sites, variations of coding region sequence and influence to amino acid.

Mutations confirmed by Sanger sequencing

To confirm the variants identified using target NGS, Sanger sequencing was performed to
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Table 1. Clinically suspected NICCD diagnosed by clinical manifestations, tandem mass spectrometry (MS/MS) and gas chromatography mass spectrometry (GC/MS) of 16 patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Clinical manifestation</th>
<th>MS/MS</th>
<th>GC/MS</th>
<th>Suspected diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>5 months</td>
<td>Jaundice, coagulopathy</td>
<td>High values of Cit, Cys, Gin, Gly, Trp, Tyr; High value of C14DC.</td>
<td>High levels of galactose, galactitol, galactonic acid, 4-hydroxy benzene lactic acid and phenyl lactic acid; Abnormal proportions of serine and threonine.</td>
<td>Galactosemia, tyrosinemia or citrin deficiency.</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>7 months</td>
<td>Chronic jaundice, cholestasis, TBA rising, high AFP value</td>
<td>High values of Gly, Met and Ser.</td>
<td>High levels of N-acetyl tyrosine and 4-hydroxy benzene lactic acid; Abnormal proportions of serine and threonine.</td>
<td>Neonatal intrahepatic cholestasis or citrin deficiency.</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>4 months</td>
<td>Jaundice, hepatosplenomegaly</td>
<td>High values of Gly, Met, Orn, Pro, Ser and Tyr; High values of C14, C16 and C18.</td>
<td>High levels of galactose and 4-hydroxy benzene lactic acid; Abnormal proportions of serine and threonine.</td>
<td>Galactosemia, high methionine disease or citrin deficiency.</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>1 months</td>
<td>Jaundice, coagulopathy</td>
<td>High values of Ala, Cit, Gly, Hcy and Orn; High value of C14DC.</td>
<td>High level of galactose.</td>
<td>Galactosemia or citrin deficiency.</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>10 months</td>
<td>Jaundice, hepatomegaly, coagulopathy</td>
<td>High values of Ala, Cit, Gly and Ser.</td>
<td>High level of 4-hydroxy benzene lactic acid; Abnormal proportions of serine and threonine.</td>
<td>Progressive familial intrahepatic cholestasis type III or citrin deficiency.</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>5 months</td>
<td>Jaundice, developmental delay</td>
<td>High values of Hcy, Met, Orn, Ser and Tyr; High values of C14, C16 and C18:2.</td>
<td>High level of galactose; Abnormal proportions of serine and threonine.</td>
<td>Galactosemia or citrin deficiency.</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>3 months</td>
<td>Jaundice, hepatosplenomegaly, anemia</td>
<td>High values of Cit, Met, Tyr, Orn and Ser; High values of C0 and C16.</td>
<td>High level of 4-hydroxy benzene lactic acid; Low ratio of serine/threonine.</td>
<td>Citrin deficiency.</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>4 months</td>
<td>Jaundice, cholestasis</td>
<td>High values of His, Met and Trp; High value of C2.</td>
<td>High levels of galactose, galactitol and galactonic acid.</td>
<td>Galactosemia or citrin deficiency.</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>2 months</td>
<td>Jaundice, coagulopathy, round bunchy face, cholestasis</td>
<td>High values of Cit, Orn and Ser; High values of C14 and C18:2.</td>
<td>High levels of galactose and 4-hydroxy benzene lactic acid.</td>
<td>Galactosemia or citrin deficiency.</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>3 months</td>
<td>Jaundice, cholestasis, coagulopathy</td>
<td>High values of Arg, Cit, Met, Orn, Thr and Tyr; High values of C0, C14, C16, C18 and C20.</td>
<td>High levels of galactose, galactitol, galactonic acid, 4-hydroxy benzene lactic acid and 4-hydroxy benzene pyruvic acid; Abnormal proportions of serine and threonine.</td>
<td>Galactosemia, tyrosinemia or citrin deficiency.</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>10 months</td>
<td>Jaundice, hepatosplenomegaly, cirrhosis, ascites</td>
<td>High values of Cit, Met, Orn and Ser; High values of C14 and C16.</td>
<td>High levels of galactose, galactitol, galactonic acid and 4-hydroxy benzene lactic acid.</td>
<td>Galactosemia, tyrosinemia or citrin deficiency.</td>
</tr>
<tr>
<td>12</td>
<td>Male</td>
<td>17 days</td>
<td>Jaundice, hypoglycemia</td>
<td>High values of Cit, Met, Phe and Tyr; High value of C4.</td>
<td>High levels of phenyl lactic acid, 4-hydroxy benzene lactic acid and N-acetyl tyrosine.</td>
<td>Tyrosinemia, hyperlactacidemia or citrin deficiency.</td>
</tr>
<tr>
<td>13</td>
<td>Female</td>
<td>2 months</td>
<td>Jaundice, hepatosplenomegaly, high AFP value, hepatitis, hepatic fibrosis</td>
<td>High values of Ala, Gly, Met and Ser.</td>
<td>High levels of N-acetyl tyrosine and 4-hydroxy benzene lactic acid.</td>
<td>Neonatal intrahepatic cholestasis or citrin deficiency.</td>
</tr>
<tr>
<td>14</td>
<td>Female</td>
<td>1 months</td>
<td>Jaundice, hepatosplenomegaly, hyperammonemia</td>
<td>High values of Cit, Met, Gly and Tyr.</td>
<td>High levels of galactose, galactitol, galactonic acid and 4-hydroxy benzene lactic acid.</td>
<td>Galactosemia or citrin deficiency.</td>
</tr>
<tr>
<td>15</td>
<td>Female</td>
<td>4 months</td>
<td>Jaundice, coagulopathy</td>
<td>High value of Tyr; High value of C16.</td>
<td>High levels of galactose, 4-hydroxy benzene lactic acid, galactonic acid and 4-hydroxy benzene pyruvic acid; Abnormal proportions of serine and threonine.</td>
<td>Galactosemia, tyrosinemia or citrin deficiency.</td>
</tr>
<tr>
<td>16</td>
<td>Male</td>
<td>3 months</td>
<td>Jaundice, hepatitis, hyperammonemia</td>
<td>High values of Cit, Met, Ser and Tyr; High values of C14 and C16.</td>
<td>High levels of galactose, galactitol, galactonic acid, 4-hydroxy benzene lactic acid; Abnormal proportions of serine and threonine.</td>
<td>Galactosemia or citrin deficiency.</td>
</tr>
</tbody>
</table>
analyze the DNA sequences which include any nucleotide variant and related gene locus of their parents. Primers were designed by Primer 5.0 and the PCR products were sequenced by Life Technologies (Shanghai, China).

**Results**

**Clinical data analysis**

This study included 16 patients, among which there are 11 males and 5 females, aged from 17 days to 10 months. As the data given in Table 1, all the 16 patients were presented with jaundice. Patient No. 11 and patient No. 13 have severe liver disease like hepatosplenomegaly, hepatocirrhosis, ascites, hepatitis and hepatic fibrosis; no similar severe liver complaint were observed in other patients. Urine GC/MS detection showed galactosemia indicators (galactose, galactitol, galactonic acid, etc.) and tyrosinemia indicators (4-hydroxy benzene lactic acid, 4-hydroxy benzene pyruvic acid, etc.) appeared at the same time. Blood pot MS/MS detection showed increased concentration of citrulline, tyrosine, methionine and threonine. MS/MS analysis showed an increased concentration of long chain fatty acids in some patients. These results indicate clinical manifestations, GC/MS and MS/MS cannot distinguish citrin deficiency from cholestasis, galactosemia and tyrosinemia.

**Result based on target NGS**

All 16 samples were sequenced through target NGS, target area coverage rate was 99.7% and average sequencing depth was above 200 × (Table 2). Among these 16 patients, 8 patients were diagnosed with NICCD through target NGS, 3 were homozygous mutation and 5 were compound heterozygous mutations. There were 7 kinds of mutations: c.852_855del (p.R284fsX286), c.128_134del (p.R43RfsX17), etc.

### Table 2. Target NGS results of 16 patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>Coordinate</th>
<th>Genotype</th>
<th>Amino acid change</th>
<th>Homozygous/Heterozygous</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SLC25A13</td>
<td>Chr7:95906586-95906592</td>
<td>c.128_134del</td>
<td>p.R43RfsX17</td>
<td>Heterozygous</td>
<td>Citrin deficiency</td>
</tr>
<tr>
<td>2</td>
<td>ABCB11</td>
<td>Chr2:169842621</td>
<td>c.1082T&gt;C</td>
<td>p.3610&gt;R2</td>
<td>Heterozygous</td>
<td>Progressive familial intrahepatic cholestasis disease type II</td>
</tr>
<tr>
<td>3</td>
<td>SLC25A13</td>
<td>Chr7:95813610</td>
<td>c.1156G&gt;A</td>
<td>p.G3865</td>
<td>Heterozygous</td>
<td>Citrin deficiency</td>
</tr>
<tr>
<td>4</td>
<td>ABCB11</td>
<td>Chr2:169787318</td>
<td>c.3268G&gt;A</td>
<td>p.1090R&gt;X1</td>
<td>Heterozygous</td>
<td>Progressive familial intrahepatic cholestasis disease type II</td>
</tr>
<tr>
<td>5</td>
<td>SLC25A13</td>
<td>Chr7:95818685-95818688</td>
<td>c.851_854del</td>
<td>p.R284fsX286</td>
<td>Heterozygous</td>
<td>Citrin deficiency</td>
</tr>
<tr>
<td>6</td>
<td>ABCB11</td>
<td>Chr2:169870818</td>
<td>c.1450G&gt;A</td>
<td>p.49Q&gt;X1.2</td>
<td>Heterozygous</td>
<td>Progressive familial intrahepatic cholestasis disease type II</td>
</tr>
<tr>
<td>14</td>
<td>GALT</td>
<td>Chr9:34647847</td>
<td>c.69C&gt;G</td>
<td>p.H23Q2</td>
<td>Heterozygous</td>
<td>Galactosemia</td>
</tr>
<tr>
<td>15</td>
<td>SLC25A13</td>
<td>Chr7:95818685-95818688</td>
<td>c.851_854del</td>
<td>p.R284fsX286</td>
<td>Heterozygous</td>
<td>Citrin deficiency</td>
</tr>
<tr>
<td>16</td>
<td>SLC25A13</td>
<td>Chr7:95751240-95751240</td>
<td>c.1663_1664ins</td>
<td>p.A555fs</td>
<td>Heterozygous</td>
<td>Citrin deficiency</td>
</tr>
</tbody>
</table>

Note: 0 showed spontaneous mutation; 1 showed mutation from father; 2 showed mutation from mother; "-" showed no mutations in relative genes.

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(p.A555fs), c.713G>A (p.S238N), c.493C>T (p.Q165X), c.1156G>A (p.G386S) and IVS6+5G>A. Mutation c.128_134del and c.493C>T were novel mutations. Two patients were diagnosed with progressive familiar intrahepatic cholestasis type II, whose causal gene was ABCB11. Mutation types were homozygous mutation and compound heterozygous mutation and there were 3 kinds of mutations: c.145G>A (p.49Q>X), c.1082T>C (p.361Q>R) and c.3268G>A (p.1090R>X). One patient was diagnosed with galactosemia, whose causal gene was GALT, the mutation type was compound heterozygous mutation (c.69C>G (p.H23Q) and c.647C>T (p.P216L)). One patient were diagnosed with chronic idiopathic jaundice, whose causal gene was ABCC2, the mutation type was compound heterozygous mutation (c.3292T>G (p.S1098A) and c.2366C>T (p.S789F)). Taken together, fourteen kinds of mutations in 12 samples were analyzed by biological method, 3 of which are clinical significance unclear (c.69C>G, c.3292T>G and c.2366C>T), and other 11 are innocuousness. Otherwise, 4 samples were not found any pathologic mutations.

Sanger sequencing results of patients and their parents

Verify positive mutation sites of 16 samples through Sanger sequencing and the coincidence rate respectively is 100%, taking the common mutation in SLC25A13 as an example showed in Figure 1. In addition, relative gene sites of patients' parents were detected by Sanger sequencing, the result was that only one mutation (c.713G>A) was spontaneous mutation while other mutations of patients were all inherit from their parents which were shown in Table 2.

Discussion

In this study, 16 patients with suspected citrin deficiency were diagnosed by clinical manifestation, GC/MS and MS/MS detection. However, the diagnosis results also indicated the suspected galactosemia, tyrosinemia and citrulminemia. Existing biochemical and clinical results can't make a definite diagnosis of citrin deficiency because of its complicated pathogenesis. Although its pathogenesis is not very clear, part of the detection results can be explained through known knowledge. Citrin is a mitochondrial inner membrane aspartate-glutamate carrier that takes part in urea cycle as the substrate of argininosuccinate synthetase (ASS). Patient with citrin deficiency or citrin function defect always have clinical manifestations such as citrullinemia, hyperammonemia and hypoproteinemia; while these clinical manifestations are also indicated urea cycle disorders and protein synthesis disorder. Besides, citrin also transfers cytosolic NADH-reducing equivalent into the mitochondria as part of the malate-aspartate shuttle in the liver. Patients with citrin deficiency likely have an impairment of gluconeogenesis due to an increase in the cytosolic NADH and/or NADH/NAD ratio in the liver; therefore patients with citrin deficiency also have clinical manifestations such as hypoglycemia and hyperlactacidemia. Moreover, citrin deficiency can inhibit the activity of UDP-glucose 4-epimerase so that cause galactosemia as well. Consequently, on account of its particular pathogenesis and metabolic characters, citrin deficiency should be diagnosed definitely by genetic detection.

Target NGS combined the second generation sequencing technology with the target sequence capture technology. Recently, this technology has been studied in several specific phenotype diseases. Ma et al., described that target NGS is a rapid, high-throughput and cost-efficient method for routine gene diagnosis of congenital cataract [7]. Liu et al., showed target NGS could be used for genetic diagnosis of neuro-degenerative diseases [8]. Yubero et al., used target NGS to diagnose metabolic genetic disorders [9]. Chen et al., applied this technology to detect bad mutations in genes related to ossification of the posterior longitudinal ligament of the spine [10]. In addition, some studies illustrated that target NGS could take place of Sanger sequencing in clinical diagnosis of some specific genetic diseases [11, 12]. Thus, compared with Sanger sequencing and exome sequencing, target NGS is a flexible, high-throughput and cost-efficient method, and is more suitable for screening and clinical diagnosis of specific genetic disease.

Our study made gene capture chips of 149 known pathogenic genes related to NICCD and other genetic diseases showed similar clinical manifestation, detected 16 suspected NICCD patients with target NGS. As a result, 8 patients
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Figure 1. Sanger sequencing for confirming the result of target NGS (mutation c.851_854delGTAT). A. Target NGS result. B. Sanger sequencing result.
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diagnosed definitely with NICCD, 3 of which were homozygous mutation (c.852_855del), and other 5 were compound heterozygous mutation and had mutation site c.852_855del as well. c.852_855del mutation is a high frequency mutation site in Chinese population and about 70% SLC25A13 gene mutation carriers have this mutation site. In our study, the ratio of c.852_855del mutation carriers was about 68.7%. Meanwhile, our study discovered 2 candidate disease-causing mutations (c.128_134del and c.493C>T), which showed harmful in biological analysis. Combined with clinical diagnosis of patients and genetic detection result of their parents, we speculated these mutation sites were pathogenic mutations. Two patients were diagnosed with progressive familiar intrahepatic cholestasis type II, whose causal gene ABCB11 is inherited in an autosomal recessive manner. The sequence analysis also indicated three unknown mutations (c.145G>A, c.1082T>C, c.3268G>A) which confirmed harmful through biological analysis. Combined with clinical diagnosis of patients and genetic detection result of their parents, we deduced these three mutation sites were pathogenic mutations. One patient was diagnosed with galactosemia, whose pathogenic gene is GALT. Detection result showed compound heterozygous mutations (c.69C>G and c.647C>T) in this gene. The mutation (c.647C>T) was reported as disease-causing mutation while the mutation (c.69C>G) has not reported yet. The biological analysis indicated the mutation (c.69C>G) was a mutation of unclear clinical significance. However, combined genetic detection result of patient’s parents and autosomal recessive heredity rule of GALT gene, thus the mutation (c.69C>G) had been considered as a candidate mutation. One patient was diagnosed with chronic idiopathic jaundice, whose pathogenic gene is ABCC2, autosomal recessive inheritance. We also found two unknown mutations (c.3292T>G and c.2366C>T), which are significance unclear till now. But combined with clinical diagnosis of patients and genetic detection result of their parents, we considered the two mutations as candidate mutations.

Based on the target NGS, we found 14 kind of mutations in 16 patients. 11 were single-nucleotide mutations, one was a 4 bp deletion mutation (c.852_855del), one was a 7 bp deletion mutation (c.128_134del) and the other was a 23 bp insertion mutation (c.1663_1664ins). These mutation sites were confirmed by Sanger sequencing, the coincidence rate respectively is 100%. This research suggested that target NGS has high accuracy. However, 4 samples were not found any pathologic mutations, we deduced that these 4 patients may have similar clinical manifestation to NICCD but they haven’t got inherited diseases. Another possible reason was the disease-causing gene wasn’t among the selected genes of our research. Because the coverage rate was 99.7% and average sequencing depth was above 200 ×, we excluded missing possibility. Furthermore, MS/MS and GC/MS detection results would be influenced by drugs, food and other diseases so that showed false positive outcome. This also prompted that we must be more scientific and religious in selecting objects of study to provide guarantee for reliability of our research. We will do in-depth interview to these 4 patients to clarify the etiology later on.

In conclusion, clinical suspected citrin deficiency or other inherited diseases of similar clinical manifestation could get definite genetic diagnosis through targeting NGS. This technology could precisely analyze relative genes of various inherited diseases through a single detection. Therefore, target NGS is of great clinical value that is feasible for the genetic diagnosis of diseases as well as for time-saving of the clinical diagnosis.

Disclosure of conflict of interest

None.

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