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Case report

NMR-based urinalysis for rapid diagnosis of β-ureidopropionase deficiency in a patient with Dravet syndrome

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ABSTRACT

Background: Beta-ureidopropionase deficiency is a rare inborn error of metabolism (IEM) affecting pyrimidine metabolism. To-date, about 30 genetically confirmed cases had been reported. The clinical phenotypes of this condition are variable; some patients were asymptomatic while some may present with developmental delay or autistic features. In severe cases, patients may present with profound neurological deficit including hypotonia, seizures and mental retardation. Using NMR-based urinalysis, this condition can be rapidly diagnosed within 15 min.

Case: An 11-month-old Chinese boy had dual molecular diagnoses, β-ureidopropionase deficiency and Dravet syndrome. He presented with intractable and recurrent convulsions, global developmental delay and microcephaly. Urine organic acid analysis using GC–MS and NMR-based urinalysis showed excessive amount of β-ureidopropionic acid and β-ureidoisobutyric acid, the two disease-specific markers for β-ureidopropionase deficiency. Genetic analysis confirmed homozygous known disease-causing mutation *UPB1* NM_016327.2: c.977G>A; NP_057411.1:p.R326Q. In addition, genetic analysis for Dravet syndrome showed the presence of heterozygous disease-causing mutation *SCN1A* NM_001165963.1:c.4494delC; NP_001159435.1:p.F1499Lfs*2.

Conclusions: The differentiation between Dravet syndrome and β-ureidopropionase deficiency is clinically challenging since both conditions share overlapping clinical features. The detection of urine β-ureidoisobutyric and β-ureidopropionic acids using NMR or GC–MS is helpful in laboratory diagnosis of β-ureidopropionase deficiency. The disease-causing mutation, c.977G>A of β-ureidopropionase deficiency, is highly prevalent in Chinese population (allele frequency = 1.7%); β-ureidopropionase deficiency screening test should be performed for any patients with unexplained neurological deficit, developmental delay or autism.

1. Introduction

Beta-ureidopropionase deficiency is a rare inborn error of metabolism first described in 2001 using structural-based nuclear magnetic resonance (NMR) spectroscopy by Moolenaar et al. [1] In their study, the affected patient was a 22-month-old girl who presented with hypotonia and microcephaly. MRI brain showed cerebral atrophy and delayed myelination. NMR-based urinalysis persistently showed excessive amount of unknown metabolite in multiple samples. The molecular structure of this unprecedented compound was determined to be β-ureidoisobutyric acid. Together with the excessive amount of urine β-ureidopropionic acid, β-ureidopropionase (EC 3.5.1.6) was determined to be the enzymatic block in the affected patient biochemically (Fig. 1).

Since then, about 30 genetically confirmed cases had been reported in the literature. [2]. Recently, the molecular analysis of *UPB1* gene in affected Japanese patients identified p.R326Q to be a common mutation in patients with β-ureidopropionase deficiency. Intriguingly, the same mutation was also highly prevalent in unaffected healthy Japanese subjects with an allele frequency of 0.9% [2] which was consistent with the high incidence rate of the disease (1 in 6000) in a screening of 24,000 Japanese newborns using GC-MS [3]. Thus, this apparently "rare" condition is likely to be underdiagnosed. In particular the clinical presentation of this condition is variable which could pose a further diagnostic challenge to the clinician. Indeed, some patients were asymptomatic while others could present with hypotonia, seizure, microcephaly, mental retardation, developmental delay and autism [2]. These neurological presentations were thought to be associated with intracerebral or neuronal deficiency of β-alanine, the end product of uracil after the three-step pyrimidine metabolism [4].

Beta-ureidopropionase deficiency has never been reported in Hong Kong Chinese. Here, we report a case of β-ureidopropionase deficiency

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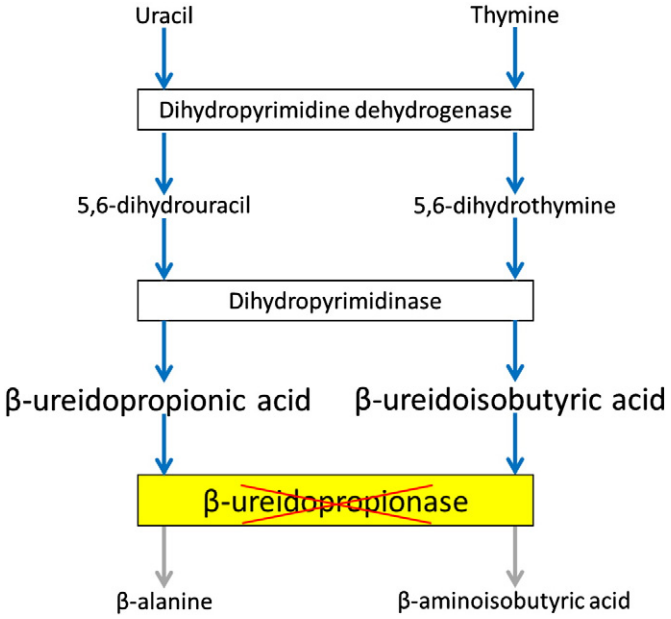

Fig. 1. Pyrimidine metabolism and the metabolic block at β-ureidopropionase.

in a Chinese patient with Dravet Syndrome associated mutation. The patient presented with recurrent and intractable convulsion since 4 months old. Molecular analysis of *SCN1A* confirmed possibly de novo mutation of Dravet syndrome. Urine biochemical analysis by gas chromatography–mass spectrometry (GC–MS) and 2-dimension (1H-13C Heteronuclear Single Quantum Correlation, HSQC) NMR spectroscopy suggested a biochemical diagnosis of β-ureidopropionase deficiency. The dual molecular diagnoses were further confirmed by molecular analysis of *UPB1* and *SCN1A* genes. We envisage that β-ureidopropionase deficiency is not an uncommon condition and should be considered in patients with autism, mental retardation and seizure. Dual molecular diagnoses should also be considered if the clinical presentations and biochemical phenotypes cannot be fully explained by a single gene disorder.

2. Case report

The proband is a five-year-old Chinese boy born to non-consanguineous parents with unremarkable birth history. He presented with recurrent afebrile convulsion since 4-month-old and electroencephalogram (EEG) revealed abnormal left temporal spike waves. No structural abnormality was identified by computed tomography (CT) of the brain. Piracetam, gabapentin and amino acid supplements were given to the patient but they all failed to control the seizure. The patient was admitted at 6-month-old for further management. The epileptic attacks were usually preceded by sudden eye staring, followed by generalized tonic–clonic convulsion (GTC) around 10 min. The parents found the frequency of the attacks increased from once weekly to several times weekly and the attacks were precipitated by febrile illnesses. Video EEG performed showed one epileptic attack after photic stimulation with associated ictal discharges. Physical examination showed normal neurological examination and developmental milestones. His head circumference was 47 cm (97th percentile), body weight was 7.1 kg (2nd–10th percentile) and body height was 61 cm (<3rd percentile). Laboratory investigations for complete blood count, liver and renal function tests, blood random glucose, ammonia and uric acid levels were all unremarkable. There was no metabolic acidosis. He was given phenobarbitone. However, in view of the mixed seizure pattern including generalized tonic, tonic–clonic and myoclonic convulsions, sodium valproate (80 mg thrice daily) and topiramate (12.5 mg daily) were given to the patient.

Despite anti-epileptic drug treatment and good compliance, the parents reported increase frequency of attacks. The frequency of GTC could reach 6 times *per* month and the seizure attack was not precipitated by any febrile illness. The dosage of topiramate was stepped up to 37.5 mg/37.5 mg/50 mg but it still failed to control the attack. Sodium valproate was further stepped up to 100 mg thrice daily since the last follow-up at 4 years old. Assessment of developmental milestone at 14 months old revealed delay of motor, cognitive and speech developments. Growth chart recorded from 14 months to 3 y showed head circumference and body weight below the 3rd percentile and body height at the 50th percentile. MRI brain was arranged when the patient was at 13-months old and showed no abnormality.

3. Materials and methods

3.1. GC–MS-based and NMR-based urinalysis

Details of GC–MS based urine organic acid analysis and ^{1}H NMR based urinalysis had been described in [5–7]. For 2D heteronuclear single-quantum correlation (HSQC), the pH of the urine sample was further adjusted to 2.0 and the NMR spectra were acquired using Bruker pulse sequence, "hsqcetgpprsisp2.2" on a BrukerAvance 600 MHz NMR spectrometer at 298 K. Spectra were referenced to the internal standard, 3-(trimethylsilyl)propionic-2,2,3,3-d4 (TSP) at 0.00 ppm. The overall analytical time of both 1H and 2D HSQC NMR experiment was about 30 min. Peak annotation of the NMR spectra was performed using Chenomx (NMR suite 7.0, Chenomx), BrukerBiofluid Reference Compound Database (BBIOREFCODE 2.0.0, Bruker) and published literature [1].

3.2. Mutational analysis of the UPB1 and SCN1A genes

Blood samples were collected from the proband and his parents after informed consent. Extraction of genomic DNA was performed using Qiagen DNA extraction kit (Qiagen). Methods for polymerase chain reaction (PCR) and sequencing are described in [8]. Primer sequences are available upon request. Sequencing results of all coding exons and the flanking intronic regions of the *UPB1* and *SCN1A* genes were compared with the National Center for Biotechnology Information (NCBI). Confirmation of indel and sequence alignment was performed using PrimeIndel (ver 2.0) (available online — http://hpcf.cgs.hku.hk/primeindel/indelchecker2) [9].

4. Results

Urine metabolic screening using GC–MS detected excessive pyrimidine metabolites, including marked increase of β-ureidopropionic and β-ureidoisobutyric acid, moderate increase of dihydrouracil and dihydrothymine, and mild increase of uracil and thymine. Using 1H and 2D HSQC NMR spectroscopy, excessive β-ureidoisobutyric acid and β-ureidopropionic acid were found in urine sample of the proband and their chemical identities were confirmed by their proton and carbon resonances (Fig. 2a). The excessive amount of urine β-ureidoisobutyric acid and β-ureidopropionic acid suggested the enzymatic block at β-ureidopropionase. In contrast, β-ureidoisobutyric acid and β-ureidopropionic acid were undetected in our control samples using 1H NMR spectroscopy. We had also performed 2D HSQC NMR spectroscopy using urine sample from control (Fig. 2b), both β-ureidoisobutyric acid and β-ureidopropionic acid were undetected in HSQC spectra after signal enhancement.

Mutation analysis for *UPB1* gene of the proband showed a homozygous mutation NM_016327.2: c.977G>A; NP_057411.1:p.R326Q and a heterozygous mutation NM_016327.2: c.91G>A; NP_057411.1:p.G31S. Both mutations were known to be pathogenic and cause β-ureidopropionase deficiency [2]. Genetic analysis of the parents revealed heterozygous c.977G>A in the mother while the father harbored