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Research paper

# Succinic semialdehyde dehydrogenase deficiency of four Chinese patients and prenatal diagnosis for three fetuses

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ABSTRACT

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder that affects the degradation of γ-aminobutyric acid (GABA). Only a few cases of SSADH deficiency have been documented in mainland China and prenatal diagnosis has not been performed. SSADH deficiency in four patients (three girls and one boy) from four unrelated Chinese families was detected by selective screening at the age of 50 days to 1 year. Four patients were admitted due to intractable seizures and psychomotor retardation. Their urine 4-hydroxybutyric acid was significantly elevated. Seven mutations in their *ALDH5A1* gene were identified, of which the following six were novel: c.127-128insGGCCC (p.L31Pfs*62), c.615delT (p.F206Sfs*5), c.1313T>C (p.L438P), c.1568C>T (p.S523F), 1383-2delA and a 0.15-Mb deletion harboring *ALDH5A1*. Only one mutation, c.820C>T, had been previously reported. Three mothers of Patients 1–3 underwent amniocentesis during their third pregnancy and the fetuses were not affected by SSADH deficiency. Normal development and urine organic acid levels of the infants confirmed the prenatal diagnosis after birth.



## 1. Introduction

Succinic semialdehyde dehydrogenase (SSADH, OMIM: 271980) deficiency, also known as 4-hydroxybutyric aciduria or gamma-hydroxybutyric aciduria, is a rare autosomal recessive disorder involving the degradation pathway of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). The disorder was first reported in 1981 (Jakobs et al., 1981). Thus far it has been identified in approximately 450 patients worldwide (Pearl et al., 2011), with a significant proportion of the affected population from consanguineous families. Fifty-two mutations of aldehyde dehydrogenase 5 family, member A1 (ALDH5A1, OMIM: 610045) gene have been identified in patients with SSADH deficiency (http://www.hgmd.cf.ac.uk). However, there is no previous report of a large deletion involving whole *ALDH5A1* in patient with this disorder.

SSADH deficiency is caused by accumulation of two neuromodulators, GABA and 4-hydroxybutyric acid (or γ-hydroxybutyric acid). In normal condition, GABA is converted to succinic semialdehyde, which in turn is oxidized to succinate by SSADH. Succinic semialdehyde can also be converted to 4-hydroxybutyric acid by succinic semialdehyde reductase. In patients with SSADH deficiency, instead of being oxidized to succinate, the remaining succinic semialdehyde is converted into 4-hydroxybutyric acid (Maitre, 1997; Gibson et al., 2003), resulting in accumulation of GABA and 4-hydroxybutyric acid in brain, blood, urine, and cerebral spinal fluid. Generally, the increase in the 4-hydroxybutyric acid is much higher than the increase in the GABA in SSADH deficiency (Kim et al., 2011).

Only a few cases of SSADH deficiency have been documented in mainland China (Lin et al., 2014; Jiang et al., 2013). Here, we described four cases with SSADH deficiency in four families, prenatal diagnoses for three fetuses in three families were carried out.

## 2. Materials and methods

### 2.1. Patients

Informed consents were obtained from the parents. Four patients (three girls and one boy) from four unrelated Chinese families were diagnosed as having SSADH deficiency in the Department of Pediatrics, Peking University First Hospital. Clinical onset of the symptoms occurred between 1 day and 6 months in the four patients. They were admitted with suspected diagnoses of hypoxic–ischemic encephalopathy, intractable seizures, anemia, developmental retardation,

*Abbreviations:* EEG, electroencephalography; GABA, γ-aminobutyric acid; SSADH, succinic semialdehyde dehydrogenase.

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and psychomotor retardation (Table 1). Secondary causes of 4-hydroxybutyric aciduria, such as presence of the 4-hydroxybutyric acid prodrug gamma-butyrolactone residue in the catheter coating, were excluded (Wamelink et al., 2011). Two patients (Patients 2 and 3) had abnormal family histories as both the siblings of the two probands exhibited similar phenotypes. All the parents were healthy and non-consanguineous. This study was approved by the Institutional Review Board (IRB) IRB of the hospital in accordance with the Declaration of Helsinki.

The mothers of Patients 1–3 subsequently visited us at 13–15 weeks of pregnancy seeking genetic counseling and prenatal diagnoses.

### 2.2. Routine tests and metabolic studies

Routine blood, urine, liver, and renal function, serum electrolytes, glucose, ammonia, ketones, creatine kinase, creatine kinase isoenzymes, and electroencephalogram tests were conducted.

Urinary organic acids were analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 analyzer and the Inborn Errors of Metabolism Screening System software for the differential diagnosis of organic acidurias (Kimura et al., 1999; Fu et al., 2000; Gibson et al., 1989).

Blood amino acids, free carnitine and acylcarnitines were analyzed by liquid chromatography tandem mass spectrometry (LC-MSMS) using an Applied Biosystems API 3200 analyzer and ChemoView software for the differential diagnosis of aminoacidopathies, fatty acid metabolic disorders, and other organic acidurias (Fernandez-Lainez et al., 2012).

### 2.3. ALDH5A1 gene analysis

Genomic DNA was extracted from the peripheral blood lymphocytes of the patients, their parents and 100 healthy controls using the TIANamp Blood DNA Kit (Tiangen Biotech Co. Ltd., Beijing, China). The exons and flanking intronic regions of the *ALDH5A1* gene were amplified using PCR and sequenced. The results were compared with the reference sequence of *ALDH5A1* (NM_170740). Sequence data were compared with an integrated set of variants (http://www.hgmd.cf.ac.uk), genotypes, and haplotypes from the 1000 Genomes Project (www.1000genomes.org) to identify mutations.

### 2.4. Prediction of the effects of novel ALDH5A1 mutations and conservation analysis

Multiple sequence alignments were performed to verify the degree of conservation. The PolyPhen program was used to predict the impact of missense alterations on protein function (http://genetics.bwh.harvard.edu/pph/). Multiple sequence alignments were performed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

### 2.5. Single nucleotide polymorphism (SNP) array analysis

Microdeletion screening was performed by an SNP-array platform (HumanCytoSNP-12; Illumina, San Diego, CA, USA). Array data were analyzed using the GenomeStudio version 2010.1, KaryoStudio version 1.2 (Illumina, standard settings) and Nexus Copy Number 5.0 (BioDiscovery, El Segundo, CA, USA) (Srebniak et al., 2011).

### 2.6. Analysis of mitochondrial respiratory chain complex enzyme activities

The activities of enzymes in the peripheral blood leukocytes mitochondrial respiratory chain complex of Patients 1 and 2 were analyzed as described previously (Birch-Machin and Turnbull, 2001).

### 2.7. Amniocentesis for prenatal diagnosis

At the gestational age of 16–20 weeks, 15–20 mL amniotic fluid were collected from each mother in the Department of Obstetrics and Gynecology, Peking University First Hospital. The amniotic fluid samples were centrifuged at 3000 rpm for 5 min at 4 °C. The cell-free amniotic fluid was then separated for the metabolites assays. The amniocytes were cultured using standard methods for gene analysis (Hamerton, 1982).

**Table 1**
Clinical and laboratory data of four patients with SSADH deficiency.

| Patients | 1 | 2 | 3 | 4 | Normal range | Units |
|---|---|---|---|---|---|---|
| Gender | F | M | F | F | | |
| Age of onset | 1d | 12d | 6mo | 3d | | |
| Age of diagnosis | 50d | 3 m | 1y | 7 m | | |
| Present age | 5y6m | 5 mo (dead) | 4y | 3y7m | | |
| Symptoms and signs | | | | | | |
| Seizure | | + | + | + | + | |
| Nystagmus | | + | − | − | − | |
| Hypotonia | | + | + | − | + | |
| Ataxia | | + | − | + | − | |
| Hyperactivity | | − | − | + | − | |
| Feeding difficulty | | + | + | − | + | |
| Vomiting | | − | + | − | − | |
| Sleep disturbance | | + | + | + | + | |
| Psychomotor retardation | | + | + | + | + | |
| Positive family history | − | + | + | − | | |
| Abnormal cranial EEG | + | + | + | + | | |
| Abnormal cranial MRI/CT | + | + | + | + | | |
| Anemia | − | + | − | + | | |
| Metabolic acidosis | − | + | − | − | | |
| Blood ammonia | 91 | 161 ↑ | 67 ↑ | 64 ↑ | 0–60 | μmol/L |
| Serum lactic acid | 2.9 ↑ | 4.3 ↑ | 2.8 ↑ | 2.6 ↑ | 0.5–2 | mmol/L |
| Serum pyruvic acid | 170 ↑ | 120 ↑ | 113 ↑ | 109 ↑ | 30–100 | μmol/L |
| Urine 4-hydroxybutyric acid | | | | | | |
| Before treatment | 35.05 | 204.75 | 35.05 | 0.19 | 0 | mmol/mol creatinine |
| After treatment | 8.46 | N/A | 10.35 | N/A | 0 | mmol/mol creatinine |
| Outcome | Regression | Died | Improved | Regression | | |

Notes: N/A (not applicable, not tested), M = male, F = female, y = years, m = months, d = days.