

## Mutation Analysis in a Patient with Succinic Semialdehyde Dehydrogenase Deficiency: A Compound Heterozygote with 103–121del and 1460T > A of the *ALDH5A1* Gene

Tsutomu Aoshima<sup>a</sup> Mitsuharu Kajita<sup>a</sup> Yoshitaka Sekido<sup>b</sup>  
Yoshiko Ishiguro<sup>a</sup> Ikuya Tsuge<sup>a</sup> Masahiko Kimura<sup>c</sup> Seiji Yamaguchi<sup>c</sup>  
Kazuyoshi Watanabe<sup>a</sup> Kaoru Shimokata<sup>b</sup> Toshimitsu Niwa<sup>b</sup>

<sup>a</sup>Department of Pediatrics, Nagoya University School of Medicine, Nagoya, <sup>b</sup>Department of Clinical Preventive Medicine, Nagoya University Hospital, Nagoya, and <sup>c</sup>Department of Pediatrics, Shimane Medical College, Shimane, Japan

### Key Words

Mutation · Succinic semialdehyde dehydrogenase deficiency · 4-Hydroxybutyric aciduria · 4-Aminobutyric acid

### Abstract

We saw a 17-month-old boy with moderate psychomotor retardation, and enzymatically diagnosed succinic semialdehyde dehydrogenase (SSADH) deficiency. After extracting mRNA and genomic DNA from his cultured lymphoblasts, we analyzed the entire coding region of the *ALDH5A1* gene using reverse transcription-polymerase chain reaction (RT-PCR) and genomic PCR followed by sequencing. He was demonstrated to be a compound heterozygote with two novel mutations (103–121 del and 1460T>A). The former leads to a frameshift and premature termination, and the latter is a missense mutation, V487E. Both mutations were also detected in the genomic DNA. Taken together with previous mutation reports, genetic heterogeneity was suspected for SSADH deficiency, and may account for the wide range of its phenotype.

### Description of the Mutation

A patient with succinic semialdehyde dehydrogenase (SSADH) deficiency was found to be a compound heterozygote. One mutation is a 19-bp deletion at nucleotides 103–121 of the *ALDH5A1* gene (GenBank accession No. NM\_001080) that leads to a frameshift and premature termination. Another mutation in another allele is a T to A substitution at nucleotide 1460, which leads to an amino acid change from valine to glutamic acid at codon 487. Both mutations were also seen in the genomic DNA, 38752–38770del and 77217T > A (GenBank accession No. AL031230).

### Source of the Material

We previously reported a 17-month-old boy with SSADH (EC 1.2.1.24) deficiency (MIM 271980) [1]. He was the second child of nonconsanguineous parents. His 4-year-old brother showed normal development. A developmental delay was diagnosed in early infancy. He controlled his head at the age of 6 months and crawled at 14 months. When he was brought to our hospital, he could not sit without support nor speak meaningful words. Although he showed marked nonparalytic hypotonia, he was hyperactive, crawling around and hitting his head against the wall of the consultation room.

Copyright © 2002 S. Karger AG, Basel

### KARGER

Fax +41 61 306 12 34  
E-Mail karger@karger.ch  
www.karger.com

© 2002 S. Karger AG, Basel  
0001-5652/02/0531-0042\$18.50/0

Accessible online at:  
www.karger.com/journals/hhe

Toshimitsu Niwa  
Department of Clinical Preventive Medicine  
Nagoya University Hospital  
65 Tsuruma-cho, Showa-ku, Nagoya 466-8560 (Japan)  
Tel. +81 52 744 1980, Fax +81 52 744 1954, E-Mail tniwa@med.nagoya-u.ac.jp

Physical examination revealed no other abnormalities. His development quotient according to Tsumori and Inage was decreased to 45. The ECG and head MRI showed no abnormalities. Analysis of organic acids using gas chromatography-mass spectrometry revealed an increase in urinary excretion of 4-hydroxybutyric acid (GHB), glutaric acid and adipic acid. GHB levels were increased in serum (7,450  $\mu\text{mol/l}$ ), cerebrospinal fluid (CSF) (200  $\mu\text{mol/l}$ ) as well as urine (107.4  $\text{mmol/mol Cr}$ ). 4-Aminobutyric acid (GABA) levels were also increased in both serum (1,626  $\text{pmol/ml}$ ) and CSF (393  $\text{pmol/ml}$ ). Based on these data, he was suspected to suffer from SSADH deficiency. Then we measured the activity of SSADH in his cultured lymphoblasts according to the method of Gibson et al. [2], and found that it was decreased to 5% of the normal control level (0.06  $\text{nmol/mg protein/min}$ ). Thus, we enzymatically diagnosed SSADH deficiency. The activity of SSADH in his mother's cultured lymphoblasts was 60% of normal control (0.83  $\text{nmol/mg protein/min}$ ), indicating that she is a heterozygote for the disease. We could not get a sample from the patient's father.

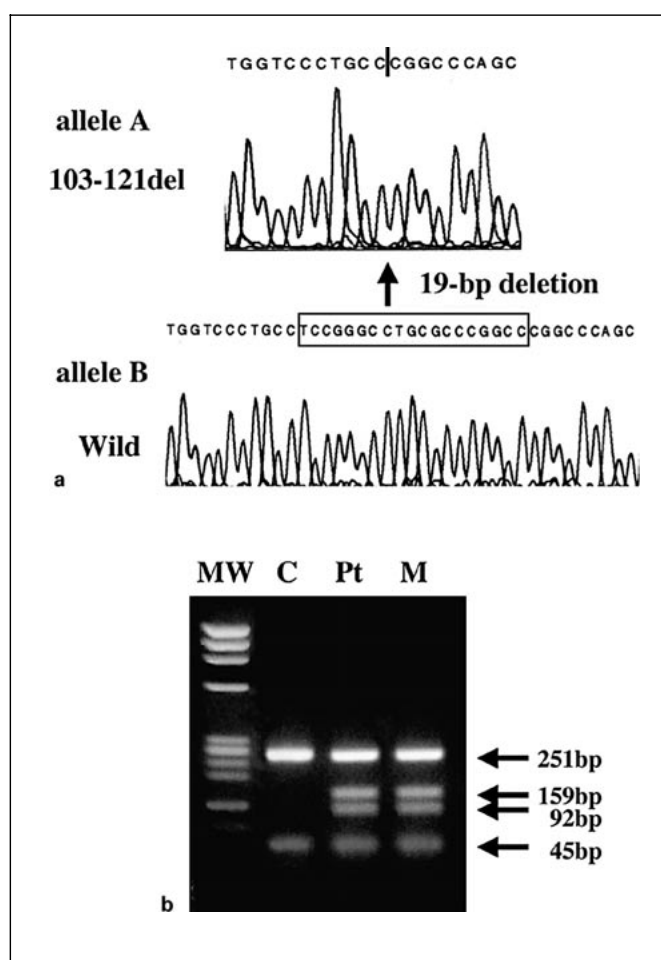
## Methods

After extraction of mRNA from his cultured lymphoblasts using a QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech), the *ALDH5A1* gene was analyzed by standard reverse transcription-polymerase chain reaction (RT-PCR). Then, we synthesized a pair of PCR primers (5'-TTGCCTGTTTCCTGTCGCCGT-CGTT-3' and 5'-AGAATCCTACAAGCCCCCG-3') to cover the entire coding region (1,608 bp), and performed PCR using TaKaRa LA *Taq* with the GC buffer kit (Takara). The 1,648-bp amplified fragments were subcloned into pGEM-T easy plasmid (Promega), and sequenced. Further, genomic DNA was extracted from his cultured lymphoblasts using the High Pure PCR Template Preparation Kit (Roche Molecular Biochemicals). The mutations detected in the cDNA were confirmed by both genomic sequencing and PCR-restriction fragment length polymorphism analysis.

## Comments

SSADH deficiency is a rare autosomal recessive disorder. The deficient activity of SSADH in the GABA degradative pathway results in accumulation of GHB. GHB is a neuropharmacologically active compound used intravenously as an anesthetic drug, which acts as a direct or indirect GABA receptor agonist. Patients with SSADH deficiency show nonspecific neurological abnormalities, such as psychomotor retardation, speech delay and seizures. High levels of GHB in CSF probably reflect its abnormally high concentration in the brain; it may show neurotoxicity and impair the neurophysiological role of GABA in the development and function of the brain. GHB has also been found in nonneural tissues of animals, although its role is unclear.

The boy was demonstrated to be a compound heterozygote with a 19-bp deletion at nucleotides 103–121



**Fig. 1.** **a** Electropherograms of sequence analyses with the cDNAs of the *ALDH5A1*. A 19-bp deletion at nucleotide 103–121 exhibiting a heterozygous pattern was seen. **b** 3% agarose gel electrophoresis of the *Mbo*II-digested PCR fragments amplified with the genomic DNAs using a pair of primers, 5'-GTCATCAATGGTGCCCT-CATC-3' and 5'-AGAATCCTACAAGCCCCCG-3'. The amplified 296-bp fragments were digested to 251- and 45-bp in the wild type, whereas 159- and 92-bp bands were detected in the patient. The patient showed a heterozygous mutation pattern. MW:  $\Phi$ X174/*Hae*III digest; C: normal control; Pt: patient; M: patient's mother.

(fig. 1a) and 1460T > A in the cDNA of the *ALDH5A1* gene, which were also detected in the genomic DNA, 38752–38770del and 77217T > A. The deletion mutation leads to a frameshift and premature termination, which may cause a loss of SSADH activity. His mother did not carry this deletion mutation. Since another mutation 1460T > A creates an *Mbo*II restriction site, PCR digestion was performed. His mother was heterozygous for the 1460T > A mutation (fig. 1b). This substitution was not seen in 104 alleles of normal Japanese controls. It leads to

an amino acid change from valine to glutamic acid at codon 487. Since we did not perform an expression analysis for this change, we cannot deny that the 1460T > A is a rare polymorphism. However, because no other substitution was seen in the same allele, we suspect that the V487E caused a loss of SSADH activity in the patient and his mother. Taken together with previous mutation reports, genetic heterogeneity was suspected for SSADH deficiency, and may account for the wide range of its phenotype.

The cDNA sequence of the *ALDH5A1* gene and mutational analyses were reported by Chambliss et al. [3] in two families with SSADH deficiency in 1998. However, full-length cDNA clones of the *ALDH5A1* gene had not been isolated, because of the high G + C content in the 5' end. The sequence data of the 5' end of the gene at nucleotides 1–257 was that inferred from amino acid sequence and the genomic clone sequence. Their recombinant human SSADH was considerably less active (22-fold less) than recombinant rat SSADH. They could not exclude a possibility that there may be another intron somewhere within this region. However, in the present study, we ana-

lyzed the full-length cDNA which contains the GC-rich region, and confirmed that the sequence data were correct. Cloning of the full-length cDNA of the *ALDH5A1* gene permitted a further mutational study and understanding of the relationship between the phenotype and the genotype of SSADH deficiency.

## References

- 1 Ishiguro Y, Kajita M, Aoshima T, Watanabe K, Kimura M, Yamaguchi S: The first case of 4-hydroxybutyric aciduria in Japan. *Brain Dev* 2001;23:128–130.
- 2 Gibson KM, Lee CF, Chambliss KL, Kamali V, Francois B, Jaeken J, Lakobs: 4-Hydroxybutyric aciduria: Application of a fluorometric assay to the determination of succinic semialdehyde dehydrogenase activity in extracts of cultured human lymphoblasts. *Clin Chim Acta* 1991;196:219–221.
- 3 Chambliss KL, Hinson DD, Trettel F, Malaspina P, Novelletto A, Jakobs C, Gibson KM: Two exon-skipping mutation as the molecular basis of succinic semialdehyde dehydrogenase deficiency (4-hydroxybutyric aciduria). *Am J Hum Genet* 1998;63:399–408.