CASE REPORT

Adenylosuccinate lyase deficiency in a Malaysian patient, with novel adenylosuccinate lyase gene mutations

Bee Chin Chen · Ivan N. McGown · Meow Keong Thong · James Pitt · Zabedah M. Yunus · Teck Beng Khoo · Lock Hock Ngu · John A. Duley

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Abstract Most cases of adenylosuccinate lyase (ADSL OMIM 103050) deficiency reported to date are confined to the various European ethnic groups. We report on the first Malaysian case of ADSL deficiency, which appears also to be the first reported Asian case. The case was diagnosed among a cohort of 450 patients with clinical features of psychomotor retardation, global developmental delay, seizures, microcephaly and/or autistic behaviour. The patient presented with frequent convulsions and severe myoclonic jerk within the first few days of life and severe psychomotor retardation. The high performance liquid chromatography (HPLC) profile of the urine revealed the characteristic biochemical markers of succinyladenosine (S-Ado) and succinyl-aminoimidazole carboximide riboside (SAICAr). The urinary S-Ado/SAICAr ratio was found to be 1.02 (type I ADSL deficiency). The patient was compound heterozygous for two novel mutations, c.445C > G (p.R149G) and c.774_778insG (p.A260GfsX24).

Abbreviations

ADSL adenylosuccinate lyase
S-Ado succinyl-adenosine
SAICAr succinyl-aminoimidazole carboximide riboside

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References to electronic databases: Adenylosuccinate lyase deficiency; OMIM 103050. Nomenclature of mutations or genetic variants was based on HUGO/HGVS recommendations: http://www.hgvs.org/mutnomen/

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B. C. Chen (✉)
Biochemical Genetic Unit, Department of Genetics, Kuala Lumpur Hospital, Jalan Pahang 50586, Malaysia
e-mail: beechinmy@yahoo.com

I. N. McGown · J. A. Duley
Pathology Department, Mater Health Services, Brisbane, Australia

M. K. Thong
Genetic & Metabolism Unit, Dept of Pediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

J. Pitt
The Victorian Clinical Genetics Services (VCGS), Pathology, Murdoch Children’s Research Institute, Melbourne, Australia

Z. M. Yunus
Division of Biochemistry, Institute for Medical Research, Kuala Lumpur, Malaysia

T. B. Khoo
Division of Pediatric Neurology, Kuala Lumpur, Malaysia

L. H. Ngu
Division of Clinical Genetics, Department of Genetics, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia

J. A. Duley
School of Pharmacy, The University of Queensland, Brisbane, Australia

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HPLC  high performance liquid chromatography  
MS/MS  tandem mass spectrometry

Introduction

Adenylosuccinate lyase deficiency (ADSL OMIM 103050) is a rare autosomal recessive disorder associated with severe neurological abnormalities. The main clinical presentations are psychomotor retardation, epilepsy, hypotonia and autistic features. Clinical symptoms usually evolve within the first few days or months of life. Since the first report of adenylosuccinate lyase (ADSL) deficiency in a child with mental retardation and seizures (Jaeken and Van den Bergh 1984), approximately 56 patients, all Caucasians, have been reported (Ariyananda et al. 2009).

Adenylosuccinate lyase catalyses two steps of purine nucleotide metabolism: the conversion of succinyl-aminoimidazole carboxamide ribotide (SAICAR) into aminoimidazole carboxamide ribotide (AICAR) in the eighth step of the de novo purine biosynthetic pathway, and the conversion of adenylosuccinate (S-AMP) into adenosine monophosphate (AMP). In ADSL deficiency, the two accumulating substrates (SAICAR and S-AMP) are dephosphorylated into SAICA riboside (SAICAr) and succinyladenosine (S-Ado). These substances accumulate in the biological fluids and are thought to be pathogenic, particularly the former one (Stone et al. 1998). Screening for ADSL deficiency relies on the detection of these two diagnostic markers, SAICAr and S-Ado, in cerebrospinal fluid and/or urine, using thin-layer chromatography (de Bree et al. 1986; Sebesta et al. 1995) or high performance liquid chromatography (HPLC) with ultraviolet (UV) detection or mass spectrometry (Krijt et al. 1999).

The gene for ADSL occurs on chromosome 22 (22q13.1–13.2). Most patients with ADSL deficiency have missense mutations (Stone et al. 1992), with the most commonly identified mutation causing the amino acid change R426H (Marie et al. 1999; Nyhan 2005).

In this paper we describe the clinical, biochemical and molecular findings in the first Malaysian patient, also the first Asian patient, with adenylosuccinate lyase deficiency. The case illustrates that ADSL deficiency is not restricted to European or, indeed, Caucasian patients but may be more widely distributed.

Case history

The male patient was the first child of non-consanguineous Malay parents and was born at full term via a spontaneous vaginal delivery with a birth weight of 2.6 kg following an uncomplicated pregnancy. The Apgar scores were 5 after 1 min, 6 after 5 min and 8 after 10 min, as the mother had been sedated prior to delivery. After 24 h, he developed apnoea and required assisted ventilation, and, on the third day of life, he had convulsions. Cranial ultrasound revealed bilateral grade 2 intraventricular haemorrhages and work-up for sepsis was negative. On discharge, he was mildly hypotonic and had a weak sucking reflex, but he tolerated oral feeding.

He had recurrent apnoea and myoclonic jerks after 2 weeks and 1 month, which required brief ventilator support. He developed generalised epilepsy. At 1.5 months the diagnosis was made. Currently, at 2 years old, he has microcephaly (head circumference 43.5 cm, <3rd centile) and profound psychomotor retardation. He cannot hold his head up or roll over and has no speech or visual fixation.

Materials and methods

Investigations were performed to exclude inborn errors of metabolism and included urine testing by dipstick (glucose, ketones, pH and reducing sugars), acylcarnitine profiling, plasma phytanic acid, and determination of plasma amino acids, urine organic acids and sulphocysteine. Urinary purines and pyrimidines, including the succinylpurines, were analysed by reversed-phase HPLC with diode array detector. Peaks were eluted with a mobile phase of 25 mmol/l potassium dihydrogen phosphate (KH2PO4) (solvent A), pH 4.6, and a gradient up to 40% of solvent B (a mixture of solvent A and methanol, 75:25 by volume), as previously described by Vidotto et al. (2003), and the method was further modified for this study (Chen 2008).

The urine was further analysed by negative ion electro-spray HPLC–tandem mass spectrometry (MS/MS) using a Waters Quattro LC instrument. Initial screening was performed using multiple reaction monitoring (Pitt et al. 2002) and included the transition 382>206 m/z for S-Ado, which indicated a gross increase in this metabolite. This finding was confirmed by our acquiring product ion spectra 382 m/z (S-Ado) and 373 m/z (SAICAr), which matched with authentic compounds.

Subsequently, mutation screening of the ADSL gene was performed. Genomic DNA was isolated, and the 5′UTR and coding region of the ADSL gene was amplified using a previously described methods (Marie et al. 1999, 2000). The polymerase chain reaction (PCR) products were purified with a Roche High Pure kit and bi-directionally sequenced with an Applied Biosystems (ABI) Big Dye Terminator v3.1 cycle sequencing kit, according to the manufacturer’s instructions. Sequences were read on an ABI Genetic Analyser 3130xl.
Results

Initial biochemical investigations of inborn errors of metabolism in our patient did not show any positive findings. The plasma amino acids profile showed moderately raised glycine (712 µmol/l), glutamic acid (442 µmol/l), alanine (764 µmol/l) and aspartic acid (66 µmol/l) on day 9 of the patient’s life. A mildly low carnitine level was observed from the analysis of acylcarnitines. In view of the raised glycine level, the initial suggestion was that the patient had non-ketotic hyperglycinæmia.

Plasma amino acid analysis was repeated when he was 1.5 months of age and revealed consistently raised levels of glycine and glutamic acid. The parents declined a lumbar puncture. Purine and pyrimidine analysis of the patient’s urine showed excessive excretion of S-Ado (380 mmol/mol creatinine) and SAICAr (370 mmol/mol creatinine), pointing to ADSL deficiency. The identity of these compounds was confirmed by HPLC-MS/MS.

Molecular analysis of the 5′UTR and coding region of the proband’s ADSL gene revealed two novel mutations: c.445C > G (p.R149G) and c.774_778insG (p.A260GfsX24). Testing of the parents showed that these mutations had been inherited from the mother and father, respectively. Nucleotide numbering was based on the complementary DNA (cDNA) sequence NM_000026.1, with nucleotide no. 1 denoting the first coding base. Nomenclature was based on the recommendations of the Human Gene Organisation/Human Genome Variation Society (HUGO/HGVS) (http://www.hgvs.org/).

Discussion

We first established a diagnostic service for purine and pyrimidine metabolic disorders in Malaysia in 2007. Since then, we have detected eight patients with various purine and pyrimidine metabolic defects from 450 urine samples of patients with appropriate clinical features. Our analyses led to the identification of the first Malaysian patient with ADSL deficiency, described here. Screening of purines and pyrimidines had not been introduced as a routine diagnostic service in south-east Asian countries, which might explain why there are no reported Asian cases of purine defects. The clinical, biochemical and genetic findings in our first Malaysian patient with ADSL deficiency are in keeping with what has been described in the literature (Van den Berghe and Jaeken 2001), more specifically as ‘type I’ ADSL deficiency (Jurecka et al. 2008). The patient had neonatal apnoea and developed early intractable seizures. At 2 years of age, he has severe psychomotor retardation, constant myoclonic jerk, microcephaly and poor visual ability.

Our discovery of a case of ADSL in a child of Malay ethnicity demonstrates that this condition is not restricted to European populations. As a result, it may be under-diagnosed, particularly outside Europe. However, recognition of the presenting features of ADSL deficiency may improve the diagnosis of this disorder by neonatologists and neurologists. This is important, because early diagnosis may facilitate genetic counselling for the family. Our case also illustrates the feasibility of S-Ado testing as part of routine high-throughput urine metabolic screening using tandem mass spectrometry (Pitt et al. 2002), and this may facilitate the diagnosis of unsuspected cases in patients in the future.

In our patient, the urine sample showed the characteristic biochemical markers, with an S-Ado/SAICAr ratio of 1.02, which suggested a poor prognosis, as indicated by the clinical conditions described by Van den Berghe (2009), with the neurological manifestation being similar to that of the ‘type I’ ADSL deficiency reported in the literature (Jurecka et al. 2008; Gitiaux et al. 2009). More severe presentations tend to be associated with a S-Ado/SAICAr ratio <2, whereas, in milder clinical pictures, these ratios range from 2 to 4. According to Gitiaux et al. (2009), more than 40 different disease-causing mutations have been described, with a high prevalence of missense mutations.

Analyses for correlations between the ADSL gene mutation, activity of the enzyme, and the severity of the phenotype have been performed (Kmoch et al. 2000; Marie et al. 2002; Spiegel et al. 2006). For our patient, the maternal c.445C > G (p.R149G) mutation within exon 4 of the ADSL gene had resulted in an amino acid change from arginine to glycine at codon 149. The arginine residue is highly conserved in mammals, and the change is non-conservative from a large basic amino acid to a small neutral amino acid. The paternal c.774_778insG (p.A260GfsX24) mutation occurs within exon 7 of the ADSL gene and results in the introduction of a stop codon in position 284 of the protein. Although we have not been able to perform expression studies to confirm the functionality of these unique mutations, the production of a stop codon and the non-conservative substitution on the two alleles, combined with the severe clinical presentation and low S-Ado/SAICAr ratio, all point to a rather severe enzymatic defect.

At present, there is no effective therapy for ADSL deficiency, but early diagnosis is important for genetic counselling and prenatal diagnosis.

In conclusion, ADSL deficiency may be suggested in any infant with typical clinical features, and screening for disorders of purine and pyrimidine metabolism should be included in the routine investigations. There is a need for further studies in wider population samples to ascertain the epidemiology of the condition in Asian populations. An early diagnosis will facilitate treatment, genetic counselling and pre-natal testing for affected families.
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References