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Biochimica et Biophysica Acta 1406 (1998) 81–84

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

Identification of new mutations in the adenylosuccinate lyase gene associated with impaired enzyme activity in lymphocytes and red blood cells

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Received 29 September 1997; revised 29 October 1997; accepted 31 October 1997

Abstract

We determined the DNA sequence of the adenylosuccinate lyase (ASL) gene from a 13 year-old female, who showed a reduced ASL enzymatic activity in lymphocytes and red blood cells and suffered from severe psychomotor retardation. The patient was the offspring of a non-consanguineous marriage. She was found to be compound heterozygous for two missense-mutations located on different alleles (C_{300}–G and G_{1266}–T): the first mutation replaces Pro75 by Ala, the second mutation replaces Asp397 by Tyr. © 1998 Elsevier Science B.V.

Keywords: Adenylosuccinate lyase; Psychomotor retardation; Purine metabolism

1. Introduction

Adenylosuccinate lyase (ASL; EC 4.3.2.2) catalyses the *trans*-elimination of fumarate from both adenylosuccinate and succinylaminoimidazole (SAICA) ribotide in the pathway of purine biosynthesis [1,2]. Diminished ASL levels have been reported in about 20 patients exhibiting a profile of clinical features that include autistic behaviour, severe psychomotor retardation and, in some cases, epilepsy and muscle wasting. The defect is transmitted as an autosomal recessive trait and leads in body fluids to an accumulation of two normally undetectable compounds, succinyladenosine (S-Ado) and SAICA riboside, which are the dephosphorylation products of the two substrates of the enzyme [3].

ASL isoenzymes have not yet been identified. Nevertheless, the presence of different ASL isoenzymes in human tissues was hypothesised by considering that the inherited defect causes a marked decrease of enzyme activity in liver and kidney and a partial deficiency in lymphocytes, fibroblasts and muscle, but usually it does not affect erythrocyte and granulocyte activities [3–5].

Here we report the results of full-length analysis of ASL cDNA from a patient displaying, in the contrast to other cases of ASL deficiency, a significant decrease of enzyme activity in both erythrocytes and mixed peripheral blood lymphocytes [6]. The patient was compound heterozygous for two mutations (C_{300}–G and G_{1266}–T). Both parents and healthy sister carried only one of the mutations.

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PII S0925-4439(97)00086-0

2. Materials and methods

2.1. Patient and control subjects

The patient, a 13 year-old female, was previously described [6,7]. At the time of study, she presented severe psychomotor retardation and epilepsy. The concentration of S-Ado and SAICA riboside in the urine was 0.075 mol/mol creatinine and 0.06 mol/mol creatinine, respectively. ASL activity in erythrocytes and mixed peripheral blood lymphocytes was reduced to approximately 30% of the normal value. The erythrocyte enzyme showed normal substrate affinity, but decreased thermal stability. Father (51 years), mother (48 years) and sister (20 years) were asymptomatic, did not excrete detectable amounts of succinylnucleosides, but elicited abnormal heat denaturation curves of erythrocyte ASL, showing values between the patient and healthy subjects [6]. Normal controls, free of any neurological or muscular disorders, volunteered for the study. Informed consent was obtained in all cases.

2.2. mRNA extraction and first strand cDNA synthesis

Lymphocytes were purified from heparinized peripheral blood [8]. The cells were stimulated at 37°C in 5% CO_2 and RPMI 1640 medium containing 0.2 mg/ml phytohemagglutinin, 10% fetal bovine serum, 1% penicillin, 3.4 mM L-glutamine, and 0.024 mM 2-mercaptoethanol [9]. Poly $(A)^+$ RNA was purified by using a Micro-fast Track kit (Invitrogen, San Diego, CA) and reverse transcribed according to published procedures [10] by using either AMV Reverse Transcriptase (USB, Cleveland, OH) and 20-mer antisense primer (MWG-Biotech, Ebersberg, D) located at 1461–1480 bp of the human liver ASL cDNA sequence reported by Stone et al. [5] or SuperScript RNase H^- Reverse Transcriptase (Gibco BRL, NL) and oligo$(dT)_{12-18}$ (Pharmacia, Uppsala, S).

2.3. PCR amplification

cDNA generated by AMV Reverse Transcriptase was amplified with different 20-mer primer pairs (Gibco BRL, NL; MWG-Biotech, Ebersberg, D) derived from the published sequence of human liver ASL. These sets of primers allowed the synthesis of three fragments with the following nucleotide coordinates: 25–942, 273–1201, 1073–1480 [5]. These PCR products were subjected to direct sequencing after asymmetric PCR.

PCR reactions were performed in a 100 μl volume containing 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 320 μM dNTP, 2.5 U Taq polymerase (Pharmacia, Uppsala, S), 38 pmol of each primer (or 38 pmol of the primer in excess and 7.7 pmol of the limiting primer, in the case of asymmetric amplification), and 0.5–1 μl cDNA (or PCR product). The reaction mixture was heated to 94°C for 5 min, followed by 35 cycles of DNA denaturation (30 s at 94°C), annealing (60 s at 48–55°C, depending on primers) and extension (60 s at 72°C). These cycles were followed by a single extension period at 72°C for 15 min [10].

PCR products were analysed by electrophoresis on a 1–2% agarose gel (depending on band sizes) and stained with ethidium bromide [10].

2.4. Sequencing of PCR products

Asymmetric PCR products were purified with a Geneclean II kit (BIO 101, La Jolla, CA) and sequenced in both directions using appropriate sense and antisense PCR primers and a Sequenase kit (Version 2.0; US Biochemicals, Cleveland, OH) according to the manufacturer's instructions [11].

2.5. Mutational analysis by restriction enzyme digestion

cDNA generated by SuperScript RNase H^- Reverse Transcriptase was subjected to a full length amplification using a 20-mer primer pair (MWG-Biotech, Ebersberg, D) based on the published sequence of human liver ASL that allowed the synthesis of a single fragment with nucleotide coordinates 75–1515. The PCR product was then symmetrically amplified in the presence of specific 20-mer primer pairs (Gibco BRL, NL), generating two different fragments with nucleotide coordinates 273–462 and 1073–1345. PCR conditions were as described above with minor modifications.

After precipitation with 0.3 M sodium acetate and 2.5 volume ethanol, the 273–462 and 1073–1345

PCR products (20 μl) were digested with 15 U MwoI (New England Biolabs; 2 h at 60°C) and with 15 U Tsp509I (New England Biolabs; 2 h at 65°C), respectively. The digestion products were separated on a 2% agarose gel and stained with ethidium bromide [10].

3. Results and discussion

To characterise the molecular defect associated with ASL deficiency, we isolated the cDNA encoding human liver ASL from cultured lymphoblasts of patient, both parents and sister, and control subjects. PCR amplification of control samples resulted in a product with the coding sequence identical to the published sequence [5]. As shown in Fig. 1, the patient was compound heterozygous for two mutations not yet described: a C_{300}–G transversion corresponding to a Pro75Ala substitution and a G_{1266}–T transversion corresponding to an Asp397Tyr substitution. The mother was heterozygous only for the second mutation, the father and the healthy sister only for the first mutation. We did not find a mutation in the codon for Ser413 responsible for the decreased ASL activity found in the only other patient reported in detail [5].

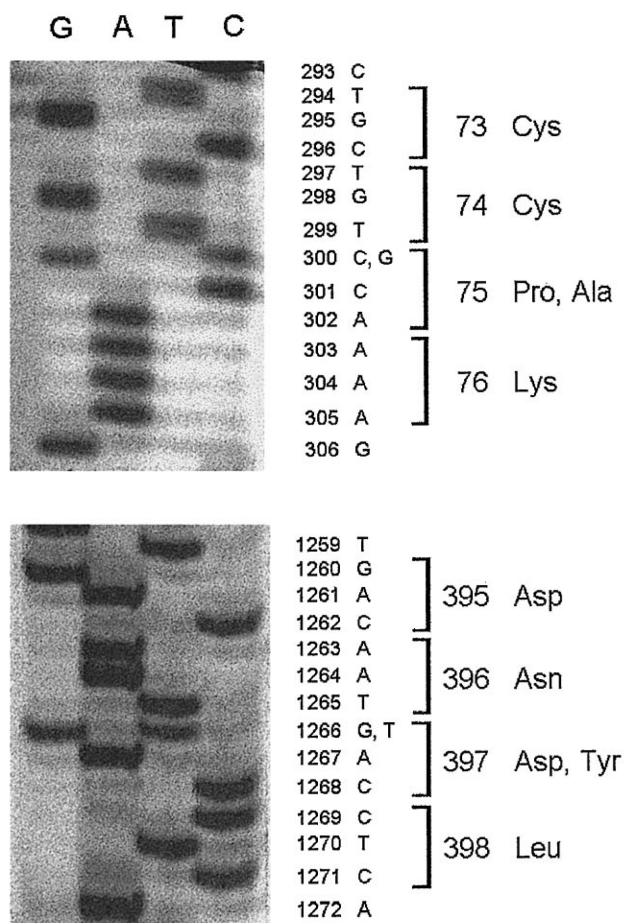

Fig. 1. Partial nucleotide sequence of PCR-amplified ASL cDNA from the patient that has been found compound heterozygous for a C_{300}–G transversion in codon 75 and for a G_{1266}–T transversion in codon 397.

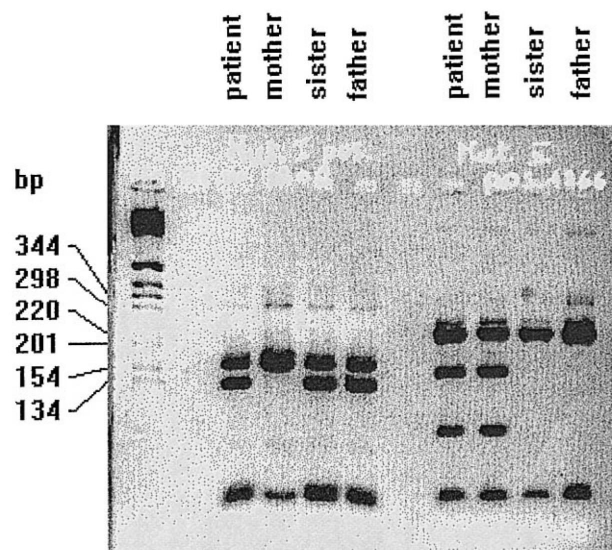

Fig. 2. Restriction analysis of ASL cDNA fragments encompassing the C_{300}–G and G_{1266}–T transversions. The fragments with nucleotide coordinates 273–462 (*left*) and 1073–1345 (*right*) were amplified, purified, and digested with MwoI and Tsp509I restriction endonucleases, respectively.

The patient and her relatives were confirmed to be heterozygous for the mutant alleles by demonstrating the presence of new restriction endonuclease sites generated by the point mutations (Fig. 2). Digestion of the 273–462 segment by MwoI generated two fragments of 163 and 27 bp in the wild-type situation and four fragments of 163, 129, 34, and 27 bp in the heterozygotes for the C_{300}–G transversion, owing to the cleavage of the 163 bp fragment carrying the new restriction site into two smaller products (the 34 bp and 27 bp fragments cannot be separated in the agarose gel shown in Fig. 2). The 1073–1345 segment was cut by Tsp509I into two fragments of 240 and 33 bp in the wild-type situation, while hetero-

zygotes for the G_{1266}–T transversion showed, beside the 240 and 33 bp fragments, two additional products of 157 and 83 bp resulting from the cleavage of the 240 bp fragment at the new restriction site created by the point mutation.

The genetic defects identified by using the human liver ASL sequence provide circumstantial evidence that the decrease in ASL activity in the patient's erythrocytes and lymphocytes could be caused by a structural lability of the protein. This suggests that the main ASL activities in erythrocytes and lymphocytes are derived from the same gene. It is striking that the point mutations occur in ASL regions that are not conserved in prokaryotes, but conserved between humans and birds [5,12]. At least the Pro397Ala substitution would significantly alter ASL flexibility, as predicted by secondary structure algorithms [13].

The case reported here demonstrates the genetic heterogeneity of ASL defect, which could, at least in part, account for the marked variability in the clinical expression of the disease [3,4,6,14].

Acknowledgements

This research was done in the framework of the European Community Concerted Action contract no. BMH1-CT-1384.

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