Clinica Chimica Acta 411 (2010) 2087–2091

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Two inborn errors of metabolism in a newborn: Glutaric aciduria type I combined with isobutyrylglycinuria

Manuela Popek [a], Melanie Walter [a], Malkanthi Fernando [a], Martin Lindner [b], Karl Otfried Schwab [a], Jörn Oliver Sass [a,*]

[a] Zentrum für Kinder- und Jugendmedizin, Universitätsklinikum Freiburg, Freiburg, Germany
[b] Zentrum für Kinder- und Jugendmedizin, Universitätsklinikum Heidelberg, Heidelberg, Germany

ARTICLE INFO

Article history:
Received 8 August 2010
Received in revised form 5 September 2010
Accepted 6 September 2010
Available online 17 September 2010

Keywords:
Glutaric acid
Glutaric aciduria
Glutaryl-coenzyme A dehydrogenase
GCDH gene
Isobutyrylglycine
Isobutyrylglycinuria
Isobutyryl-coenzyme dehydrogenase
ACAD8 gene
Newborn screening
Tandem mass spectrometry
Acylcarnitines
Organic acids

ABSTRACT

Background: Glutaric aciduria type 1 (GA1) is an inborn error in the metabolism of the amino acids tryptophan, lysine and hydroxylysine due to mutations in the *GCDH* gene coding for glutaryl-CoA dehydrogenase. Affected individuals often suffer from an encephalopathic crisis in infancy or childhood which results in acute striatal injury leading to a severe dystonic–dyskinetic movement disorder. Isobutyryl-coenzyme dehydrogenase (IBD) is an enzyme encoded by the *ACAD8* gene and involved in the catabolism of the branched-chain amino acid valine. Both GA1 and IBD deficiency can be detected by expanded newborn screening using tandem-mass spectrometry, if they are considered screening targets.
Methods: Tandem-mass spectrometry and gas-chromatography with mass-selective detection were used for the assessment of key metabolites in body fluids of a patient with abnormal findings in newborn screening. Mutations were investigated by direct sequencing and by restriction fragment lengths analysis. Valine metabolism was studied *in vitro* in immortalized lymphocytes.
Results: Following accumulation of acylcarnitines C5DC and C4, of 3-hydroxyglutaric acid and isobutyrylglycine in body fluids, sequence analysis in the *GCDH* gene revealed homozygosity for a missense mutation in exon 6, c.482G>A, p.Arg161Gln, which had been reported in GA1 before. In the *ACAD8* gene a novel mutation c.841+3G>C was identified, which results in loss of exon 7 and predicts a premature stop of translation. Impaired valine degradation was corroborated by the increased post-load level of acylcarnitine C4 in lymphocytes.
Conclusion: The molecular basis of two inborn errors of metabolism in a newborn was elucidated. The metabolite studies underline the use of urinary C4 acylcarnitine as a sensitive marker of IBD deficiency. A functional test of IBD activity in lymphocytes may replace more invasive fibroblast studies. In view of the combination of two organic acidurias, which may both affect the level of free carnitine, careful follow-up including regular assessment of the carnitine status of the patient appears prudent.

1. Introduction

Glutaric aciduria type 1 (GA1; OMIM #231670) is an inborn error in the metabolism of the amino acids tryptophan, lysine and hydroxylysine due to an inherited (autosomal recessive) deficiency of glutaryl-CoA dehydrogenase (EC 1.3.99.7) [1,2]. Affected individuals often suffer from an encephalopathic crisis early in life which results in acute striatal damage leading to a severe dystonia and dyskinesia and secondary complications [2,3]. Recent publications have suggested that the development of such crises can often be prevented by dietary modifications combined with oral supplementation of L-carnitine and by aggressive treatment of intercurrent illness [3–6]. GA1 has been included in extended newborn screening by tandem mass spectrometry in a number of countries, e.g., in Germany and in the USA [7–9]. Based on newborn screening data its overall prevalence has been estimated to be 1:100,000 [7].

Isobutyryl-CoA dehydrogenase (IBD, EC 1.3.99.3) catalyzes the conversion of isobutyryl-CoA to methylacrylyl-CoA in the metabolism of the branched-chain amino acid valine [10]. So far, only 22 individuals have been reported with a deficiency of IBD (OMIM#611283), which follows an autosomal recessive trait of inheritance. Usually they were detected via an increased level of C4 acylcarnitine in newborn screening and may present with isobutyrylglycinuria [10–17]. So far, most individuals with IBD deficiency have remained asymptomatic, although the first patient showed failure to thrive, dilated cardiomyopathy and profound carnitine deficiency at the age of one year and was still carnitine dependent almost 10 years

* Corresponding author. Labor für Klinische Biochemie und Stoffwechsel, Zentrum für Kinder- und Jugendmedizin, Universitätsklinikum Freiburg, Mathildenstr. 1, 79106 Freiburg, Germany. Tel.: +49 761 270 4371; fax: +49 761 270 4527.
E-mail address: joern.oliver.sass@uniklinik-freiburg.de (J.O. Sass).

0009-8981/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2010.09.006

later [10,16]. An elevated concentration of C4 acylcarnitine in dried blood spots may also point to a defect of short-chain acyl CoA dehydrogenase (SCAD; EC 1.3.99.2; OMIM#201470). Differentiation is possible via urinary organic acids. An elevated concentration of ethylmalonic acid is characteristic for SCAD deficiency, while an increase in isobutyrylglycine is suggestive for IBD deficiency, although not always detectable in urine of patients with this inborn error of metabolism [14,15].

For both IBD deficiency and SCAD deficiency it is not proven that they really cause clinical disease. At present they are no screening targets in most European countries and have been designated only secondary targets in the USA [8,9].

Here we report a child who was initially detected in newborn screening with a suspicion for GA1. Follow-up investigations did not only confirm the initial suspicion, but revealed in addition IBD deficiency. The molecular basis of the two inborn errors of metabolism of the patient is characterized.

2. Patient and methods

2.1. Case report

Newborn screening in dried blood spots performed on day 3 of life identified a neonate with an elevated concentration of glutarylcarnitine (acylcarnitine C5DC) of 1.03 μmol/l (normal 0–0.17 μmol/l). The girl was the first child of consanguineous parents originating from Jordan. She was born at term after an uneventful pregnancy, which, however, ended with a premature rupture of membranes and a subsequent failure to progress in labor, which led to a secondary cesarean section. Postnatally, a neonatal infection was suspected. Therefore, the neonate was still hospitalized, when the positive result of the newborn screening was communicated on day 6 and samples were obtained for the further diagnostic work-up. Following the confirmation of the diagnosis of GA1 on the metabolite level, dietary treatment was initiated based on previously published guidelines [18]. At 7.5 months of age she was hospitalized because of pyelonephritis, at 11 months because of an infection of the upper respiratory tract. Up to the last examination, at the age of 12 months, the patient showed normal development. However, brain MRI, which was performed when the patient was 3.5 months old, revealed mildly retarded myelination. Sylvian fissure, subarachnoid space and cerebral ventricles were relatively enlarged.

2.2. Metabolites

Free carnitine and acylcarnitines were determined in dried blood or urine spots or lymphocyte homogenate by tandem mass spectrometry following the formation of the corresponding butyl esters [19]. Urinary organic acids were analyzed using an approach with special sensitivity towards glycine conjugates [20].

2.3. Lymphocyte culture

Lymphocytes from peripheral blood of the patient and of controls were immortalized by transformation with Epstein-Barr virus (EBV) using standard techniques. Cell culture was performed at 37 °C, 5% (v/v) carbon dioxide and >90% humidity.

2.4. In vitro valine loading test

The valine loading test was performed with transformed lymphocytes cultivated in RPMI 1640 medium supplemented with L-glutamine and 20% (v/v) fetal bovine serum. Penicillin G (sodium salt) and streptomycin sulphate were added (all from GIBCO-Invitrogen). At the onset of the incubation period of 72 h, the culture medium was replaced by a medium with additional supplements of 1 mM L-valine (Sigma) and 0.4 mM L-carnitine (Biocarn, Medice). Cell density was 10^6 cells per ml, each sample had a volume of 10 ml.

After 72 h, lymphocytes were harvested by centrifugation (300 *g* for 5 min at 4 °C), washed four times with phosphate-buffered saline and frozen at −80 °C until they were processed further. After thawing, the cell pellets were homogenized by sonication in distilled water. The protein concentrations of the homogenates were determined by the Lowry method [21] and adjusted to 0.3 mg protein/ml before the samples were submitted to acylcarnitine analysis.

2.5. Mutation analyses

2.5.1. Genomic DNA

Genomic DNA was extracted from peripheral blood by standard methods. All exons and exon–intron boundaries of the genes *GCDH* and *ACAD8* of the patient were amplified by PCR and prepared for direct sequencing by a commercial provider (AGOWA, Berlin, Germany). Primer sequences and PCR conditions are available upon request.

In order to exclude that the novel sequence variation c.841 + 3G > C of the ACAD8 gene represents merely a polymorphism [22], 109 samples (218 chromosomes) were analyzed by amplification of exon 7 and adjacent regions of the gene using primers 5'-GTG CTG AAA CCC ATA CCT-3' (forward) and 5'-TGT CCC GGA TCG CTA CCT C-3' and subsequent restriction fragment lengths analysis using Hph1 enzyme (New England Biolabs). 43% of the reference samples originated from the Middle East, as did the family of the patient.

2.5.2. Transcript analysis

Total RNA was isolated from EBV-transformed lymphoblasts using TRIzol reagent (GIBCO-Invitrogen). Synthesis of *ACAD8* cDNA was performed with RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas) according to the manufacturer's instructions. Nested PCR was made with two pairs of primers (primer sequences and PCR conditions are available upon request). PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide and photographed under UV light. Amplicons of the second (nested) PCR were excised from gel and purified using Zymoclean Gel DNA Recovery Kit (Zymo Research), followed by bidirectional sequencing by a commercial provider (Agowa).

3. Results

Repeated analyses of acylcarnitines in dried blood spots confirmed the newborn screening laboratory's observation of an elevated concentration of C5DC acylcarnitine, suggesting the diagnosis of GA1. Notably, elevated concentrations of C4 acylcarnitine were also observed. In dried blood spots, its concentration was mostly around 2 μmol/l. C4 acylcarnitine is no target and data are therefore blinded in German newborn screening, but retrospective disclosure of the data of the initial test card revealed a concentration of 1.23 μmol/l (normal 0–1.11 μmol/l). The level of free carnitine in dried blood spots remained always normal.

Urinary levels of acylcarnitines presented as sensitive markers of both inborn errors of metabolism, reaching several mmol/mol creatinine both for C5-DC acylcarnitine and – especially – C4 acylcarnitine, exceeding the upper limit of the reference range of the latter more than ten-fold, thus corroborating our earlier findings in IBD deficiency [14].

Urinary organic acids were also assessed repeatedly. However, their evaluation was partly impaired by high dilution of the urine samples, with only low creatinine concentrations. The concentrations of glutaric acid were normal, only trace amounts of 3-hydroxyglutaric acid were detected. Some urine samples revealed slight increases in isobutyrylglycine, while others were unremarkable in this regard. Except for a single value of isobutyrylglycine, urinary concentrations