Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Genetics and Metabolism 88 (2006) 29–37
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

# Riboflavin-responsive glutaryl CoA dehydrogenase deficiency

Ronald A. Chalmers [a,b,*], Murray D. Bain [b], Johannes Zschocke [c]

[a] *CIMOA, London NW1 0NH, UK*
[b] *Paediatric Metabolism Unit, St. George's University of London, SW17 0RE, UK*
[c] *Institute of Human Genetics, University of Heidelberg, D-69120 Heidelberg, Germany*

Received 17 October 2005; received in revised form 10 November 2005; accepted 10 November 2005

**Abstract**

We report here riboflavin responsiveness in a patient with glutaryl CoA dehydrogenase (GCDH) deficiency, compound heterozygous for the S139L and P248L mutations and with 20% residual GCDH enzyme activity in vitro. Our results suggest the mitochondrial GCDH homotetramer remains intact with one of these mutations associated with the binding site of the single FAD cofactor and that pharmacological doses of the cofactor precursor may be sufficient to induce an increase in activity in the mutant GCDH enzyme, although not sufficient to normalise urinary organic acid excretion. Serine[139] is one of nine conserved amino acid residues that line the binding site of the protein and is in close proximity to both substrate and FAD cofactor. It is possible that steric alterations caused by substitution of serine with leucine at this position may be overcome with high cofactor concentrations. P248L is also associated with some residual GCDH activity in other patients and the unique combination of S139L with P248L may also explain the results in our patient. Responsiveness to riboflavin in our patient has been compared with two other patients with glutaric aciduria type 1 and minimal residual GCDH activity, one with homozygosity for the R257Q mutation and one with heterozygosity for the G354S mutation and a novel G156V mutation. A low lysine diet reduced glutaric acid excretion in our riboflavin-responsive GCDH-deficient patient almost to control values. She is now 21 years of age and clinically and neurologically normal.


*Keywords:* Glutaryl CoA dehydrogenase deficiency; GCDH; Glutaric aciduria; Riboflavin; Lysine metabolism; Dietary therapy; Carnitine; Enzymology; Fibroblasts; Mutation

## Introduction

Glutaryl CoA dehydrogenase (GCDH; EC 1.3.99.7) deficiency (glutaric aciduria type 1; McKusick 231670) is an autosomal recessive disorder, first described by Goodman et al. [1], caused by deficient activity of mitochondrial FAD-dependent GCDH in the catabolic pathway of L-lysine, L-hydroxylysine, and L-tryptophan. Most patients described have negligible measurable residual activity of GCDH in leucocytes or cultured skin fibroblasts [2,3]. The disorder is generally associated with increased urinary excretion of glutaric acid and 3-hydroxyglutaric acid, intermittent excretion of glutaconic acid, and increased urinary glutarylcarnitine, with severe L-carnitine deficiency [4]. Urinary 2-oxoglutaric acid is also always increased [4] and recent research has shown that glutaryl CoA specifically inhibits 2-oxoglutarate dehydrogenase activity [5]. Some patients have been described with low or almost absent urinary glutaric acid [6] but urinary 3-hydroxyglutaric acid and glutarylcarnitine [7] are always present and increased and together may be taken as diagnostic. Prevalence has been estimated variously between 1:40,000 and 1:100,000, being greatly increased in some inbred communities. The human GCDH gene has been mapped to chromosome 19p13.2 [8] and more than 100 disease-causing mutations have been identified [9–12]. Untreated, the disease may present with an acute encephalopathic crisis, especially during the vulnerable period of brain development between 3 and 18 months of age, subsequent to which severe striatal degeneration associated with almost complete destruction of the putamen is observed [13]. Subsequent course of the

* Corresponding author. Fax: +44 0 20 7681 9129.
*E-mail address:* rachalmers@cimoa.org.uk (R.A. Chalmers).

1096-7192/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2005.11.007

disorder is associated with a dystonic–dyskinetic movement disorder, choreoathetosis, dysarthria, and almost total helplessness. The morbidity of the condition is compounded by probable normal or near normal intelligence. Neurotoxicity has been ascribed variously to 3-hydroxyglutaric acid [13], glutaric acid, glutaryl CoA, and glutaconyl CoA [14] but with really little compelling evidence [15]. Quinolinic acid has also been implicated in the pathogenesis of the disorder [16,17] although other studies have found no evidence for quinolinate accumulation in GCDH deficiency [18]. Effective preventative treatment comprises dietary restriction with both low protein and low lysine/low tryptophan diets with L-carnitine to remove excess potentially toxic acyl moieties ([19] and Kölker et al. unpublished), although the precise mechanism of action of L-carnitine remains unknown.

Riboflavin has also been suggested at various times as a possible treatment for GCDH deficiency, based upon the requirement of the enzyme for FAD. In an early paper, Brandt et al. [20] described three patients with glutaric aciduria type 1 and suggested riboflavin might be of value in reducing urinary glutaric acid excretion in the two siblings studied. Both patients had low residual glutaryl CoA dehydrogenase activity in disrupted cultured skin fibroblasts [3]. Dungar and Snodgrass [21] also described a child with glutaric aciduria type 1 in whom further neurological deterioration was 'prevented' after treatment with riboflavin and a low protein diet although there was no appreciable reduction in glutarate excretion and fibroblast glutaryl CoA dehydrogenase activity was zero. Lipkin et al. [22] described a patient with GCDH deficiency but with low or absent glutaric excretion, in whom riboflavin alleviated clinical symptoms with increase in cerebrospinal fluid GABA levels, suggesting some responsiveness to this cofactor precursor. However, most of the described mutations of the GCDH gene result in almost complete abolition of enzyme activity and riboflavin treatment has been generally ascribed as being ineffective with no further reports of apparent riboflavin-responsive GCDH deficiency.

We report here biochemical and molecular studies on a patient diagnosed with glutaric aciduria in early infancy who showed considerable residual fibroblast glutaryl CoA dehydrogenase activity, marked biochemical response to riboflavin, with reduction in the urinary excretion of glutaric acid and normal clinical and neurological development. Comparison is made with studies undertaken on the patient described by Dungar and Snodgrass [21] and on another previously unreported patient with a novel mutation in the GCDH gene, absent residual activity of GCDH and no response to riboflavin.

## Methods

### *Organic acids*

Urinary and plasma organic acids were determined using capillary gas chromatography–mass spectrometry (GC–MS) as their corresponding ethoxime trimethylsilyl derivatives after extraction using DEAE–Sephadex and with use of extracted ion monitoring where necessary for accurate quantification of glutaric acid and 3-hydroxyglutaric acid [23].

### *Urinary and plasma carnitine and acylcarnitines*

Urinary and plasma free carnitine and esterified carnitine (acylcarnitines) were measured using an established radioenzymatic procedure [24]. Urinary acylcarnitines were identified using fast atom bombardment mass spectrometry of their isobutyl esters [25] and as their corresponding acyloxybutyrolactone (acyl-OBL) derivatives using gas chromatography–chemical ionisation–mass spectrometry (GC–CI–MS) with deuterated acylcarnitine internal standards [26]. Plasma and blood acylcarnitines were identified using electrospray tandem mass spectrometry [13].

### *Enzymology on cultured skin fibroblasts*

Skin fibroblasts were grown to confluence in 75 $cm^2$ Falcon flasks in Eagle's MEM supplemented with 10% fetal calf serum, antibiotics, and glutamine. Fibroblasts were subcultured for 8 days on the sides of 25 $cm^2$ Falcon flasks in Eagle's MEM (riboflavin 2.7 μM) supplemented with 15% dialysed fetal calf serum, antibiotics, and glutamine and were confluent for assays. Glutaryl CoA dehydrogenase activity was measured in PBS-washed fibroblasts growing in monolayer, by determination of the rate of oxidation of [6-$^{14}$C]L-lysine to $CO_2$ in PBS + 1% glucose. [6-$^{14}$C]L-lysine is decarboxylated specifically at the C-6 position and this method provides a physiological measure of the metabolic flux through the L-lysine pathway and specifically of the decarboxylation of glutaryl CoA (glutaconyl CoA) (Fig. 1). The oxidation of [1-$^{14}$C]butyrate, at the terminal end of the pathway, was measured as a control of activity of the pathway at the point immediately subsequent to the metabolic defect. The lysine/butyrate oxidation ratio was calculated to provide a sensitive assessment of residual enzyme activity and of cofactor responsiveness. A further control was provided by measurements in cells from a patient with multiple acyl CoA dehydrogenase deficiency, where both activities are deficient. Results are expressed as pmoles $CO_2$ produced/3 h/mg protein and all results are means of at least three duplicate measurements, each flask providing a separate experiment. Protein was measured by a modified Lowry method. For studies on riboflavin response, cells were subcultured from the third day in 199 medium (riboflavin 0.03 μM) supplemented with 15% dialysed fetal calf serum, antibiotics, and glutamine and with additional riboflavin at varying concentrations from 0 to 46 μM with 5 days of subculture in the supplemented medium before enzyme assay.

### *Molecular studies*

DNA was extracted from cultured skin fibroblasts from 75 $cm^2$ Falcon flasks, previously stored in liquid nitrogen after freezing down, using a QIAamp DNA minikit (Qiagen). Extracts contained >3 μg DNA. Denaturing gradient gel electrophoresis (DGGE) was performed of all exons and adjacent intron regions of the GCDH gene as previously described [11]. In addition, for Patient 1, exons 1, 5, 7, and 8 were sequenced; for Patient 2, exons 1 and 7–11 were sequenced; and for Patient 3 exons 1, 5, 8, 9, and 11 were sequenced using an automated fluorescent sequencer (Applied Biosystem 3100) and standard methods.

## Results

### *Case reports and results of clinical investigations and treatment*

The studies presented here were approved by the Local Research Ethics Committee and complied with the Declaration of Helsinki recommendations. Informed consent was