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BIOCHEMICAL AND MOLECULAR DIAGNOSIS OF GLUTARIC ACIDURIA TYPE 1 IN A BLACK SOUTH AFRICAN MALE CHILD: CASE REPORT

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SUMMARY

Glutaric aciduria type 1 (GA-1) is an inborn error of metabolism caused by a deficiency of the mitochondrial enzyme glutaryl-Co enzyme A dehydrogenase. GA-1 is not uncommon amongst Caucasians but to the best of our knowledge, it has previously not been reported in black African children. We present a case of GA-1 in a black South African boy who was referred to hospital at the age of five years and ten 10 months with dyskinesia and dystonia accompanied by chorea and athetosis. Radiological examination revealed enlarged basal cisterns with bilateral fluid collection around the sylvian fissures suggestive of GA-1. Analysis of urine showed raised levels of glutaric acid at 520 µmol/mmol creatinine (normal < 2.0), 3-hydroxyglutaric acid at 113 µmol/mmol creatinine (normal <3.0) and a low blood carnitine level of 31.5 µmol/l (normal 35-84). A definitive diagnosis was reached through DNA analysis which revealed homozygosity for an A293T mutation in the glutaryl-Co-enzyme A dehydrogenase (GCDH) gene.

INTRODUCTION

The first case of glutaric aciduria type 1 (GA-1) was described by Goodman and colleagues in 1975(1). While several cases have subsequently been reported in Caucasian subjects, none has been described in black African children to the best of our knowledge.

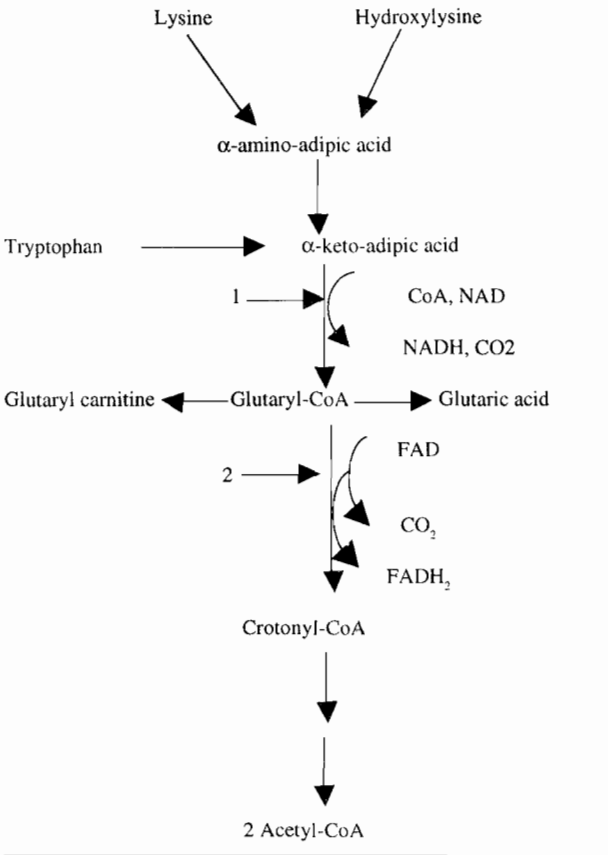
GA-1 is an autosomal recessive disorder caused by a deficiency of the mitochondrial enzyme glutaryl-Co enzyme A dehydrogenase (GCDH) which catalyses the conversion of glutaryl-CoA to crotonyl-CoA in the oxidative pathway of lysine, hydroxylysine and tryptophan (Figure 1). Unpublished reports(1) indicate that some other acyl-CoA dehydrogenase catalyses the conversion of glutaryl-CoA to glutaconyl-CoA which is then hydrated to 3-hydroxyglutaryl-CoA. Subsequent de-esterification converts the latter to 3-hydroxyglutaric acid.

The clinical onset of GA-1 is variable with the majority of patients presenting between 6 and 12 months. In an acute form, a large number of patients present with recurrent episodes of vomiting and lethargy associated with metabolic acidaemia often precipitated by an acute infectious illness(2,3). More commonly, affected children manifest with a progressive neurological deterioration dominated by extrapyramidal features of dystonia, dyskinesia and choreoathetosis(4). Macrocephaly is a common feature and mental retardation, when present is widely variable(5,6). Computed tomography and magnetic resonance imaging of the brain in these patients have shown diffuse attenuation of cerebral white matter, enlarged cerebrospinal fluid containing spaces within the sylvian fissures and anterior to the temporal lobe and involvement of the basal ganglia(2,3,7,8). These neurological features seem to be consistent and often point to a diagnosis of GA-1 in children with unexplained neurological dysfunction.

Urine organic acid analysis forms the basis for an initial biochemical screen of the suspected cases. The profile shows a predominant presence of glutaric acid with the elevation of 3-hydroxyglutaric acid and occasionally glutaconic acid, especially during intercurrent illnesses(2). Patients with normal levels of urinary organic acids during periods of clinical stability have been described(9). The diagnosis of GA-1 can be made by measuring glutaryl-COA dehydrogenase activity in cultured skin fibroblasts or leucocytes(1,2), but recently genetic analysis of the GCDH gene has provided an alternative approach to the diagnosis of GA-1(10,11). In the present study, we describe a black South African boy in whom the definitive diagnosis was reached through DNA analysis.

Figure 1

Scheme showing metabolic pathways in lysine, hydroxylysine and tryptophan metabolism



Enzymes:
1 = α-ketoadipic dehydrogenase
2 = Glutaryl-CoA dehydrogenase

CASE REPORT

A five year nine months year-old boy, was referred to hospital by his pre-school teacher who expressed concern about poor gross and fine motor co-ordination, slurred speech and general developmental delay. He was an only child living with his maternal grandparents. Details of his birth and early infancy were therefore, incomplete. He was born at term, in hospital by normal vertex delivery with an average birth weight but unknown Apgar scores. Early milestones were all delayed. He started crawling after his first birthday, walking after his second and speaking after his third. He commenced attendance at a pre-school just after his fifth birthday where he was noted to have been functioning at the level of a 3-year-old with associated motor inco-ordination and slurred speech. According to his family his speech had always been thick and slurred and from infancy erratic uncordinated movements of both limbs and trunk had been noted.

He was fully immunised and had no history of any significant illnesses or injuries, particularly no seizures or unexplained episodes of vomiting. There was no family history of epilepsy or neurologic disorders.

Examination revealed a co-operative, emotionally stable child with normal growth parameters and no neuro-cutaneous stigmata. On neurologic assessment he was alert and responsive. Apart from bilateral hibbus the cranial nerves were all intact. Sensation was intact and the motor system revealed normal tone but reduced deep tendon reflexes and flexor plantar responses bilaterally. Bilateral choreiform movements, a 'Jack in the Box' tongue and 'milk-maids' grip were all present. A provisional diagnosis of chorea was made.

Full blood count was normal. ESR was 1 mm/hr; anti streptolysin O titre - 320 IU; ECG was normal with a PR interval of 0,12; CT scan of the brain showed enlarged basal cisterns, bilateral extracranial fluid collections around the sylvian fissures and an abnormal gyral pattern in both frontal lobes - features suggestive of glutaric aciduria type 1 or schizencephaly. In light of the CT scan findings urinary amino acids and organic acid were assessed for possible glutaric aciduria type 1, followed by DNA analysis.

MATERIALS AND METHODS

Approximately 10 ml of random urine was collected for organic acid analysis. Blood samples were collected in EDTA tubes for amino acid, carnitine and DNA analysis. Blood and urine samples from the parents were not available.

Organic acid analysis: Quantitative determination of urinary glutaric acid and 3-hydroxyglutaric acid was performed as described by Sweetman *et al*(12) using gas chromatography-mass spectrometry. Total plasma carnitine was determined by a radioenzymatic method(13). The amino acids were analysed on plasma and urine by gas chromatography as previously described(14).

DNA and sequence analysis: Exons 3 and 5-11 of the GCDH gene were screened for a range of 20 mutations by allele specific oligonucleotide hybridisation using 32P-ATP end labelled probes against wild type and mutant sequences as previously described by Nyhan *et al*(15). Exon 8 of the GCDH gene was subsequently amplified by polymerase chain reaction (PCR) using flanking intronic oligonucleotide primers, and sequenced in both directions using the ABI Prism 310 Automated Genetic Analyser. The primers used were: 5' CTGCATAGGCCCTCTTGGTG 3' (forward); 5' CCCCACACCCCCAGAGM 3' (reverse).

RESULTS

The levels of urinary glutaric acid, 2-hydroxyglutaric acid and 3-hydroxyglutaric acid are shown in Table 1. The urinary concentrations of glutaric acid and 3-hydroxyglutaric acid are markedly increased and lie within the classical GA-1 range.

Table 1

Organic and amino acid levels in urine and plasma from patient LZ

Urine (μmol/mmol creatinine)	Patient	Reference Range
Glutaric acid	520	<2
2-OH glutaric acid	18.9	<16
3-OH glutaric acid	113	<3
Threonine	103	14-56
Serine	230	50-137
Glutamic acid	27	0-18
Ornithine	13	0-8
Lysine	140	13-79
Histidine	445	92-278
Plasma (μmol/L)		
Carnitine	31.5	35-84
Glycine	290	97-390

The concentrations of plasma amino acids were all within the normal reference range but mild increases of threonine, serine, glutamate, ornithine, lysine and histidine were found in the urine (Table 1). Plasma total carnitine was found to be low at 31.5 µmol/l (normal, 35—84).

Figure 2

Auto radiograph showing (1) homozygous hybridisation with a mutant A293T probe (patient LZ), (2&4) wild-type A293 probe and (3) heterozygous A293T control

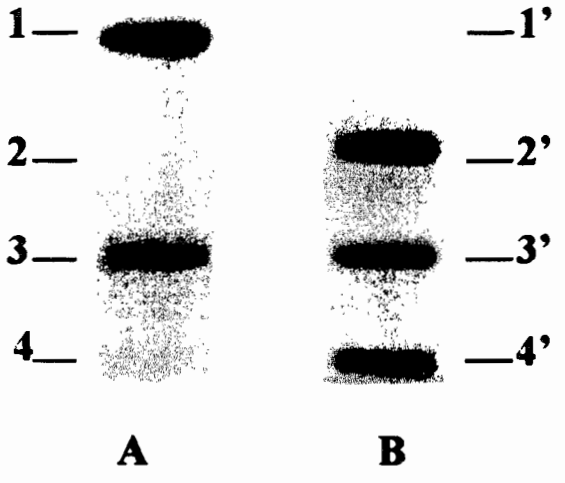
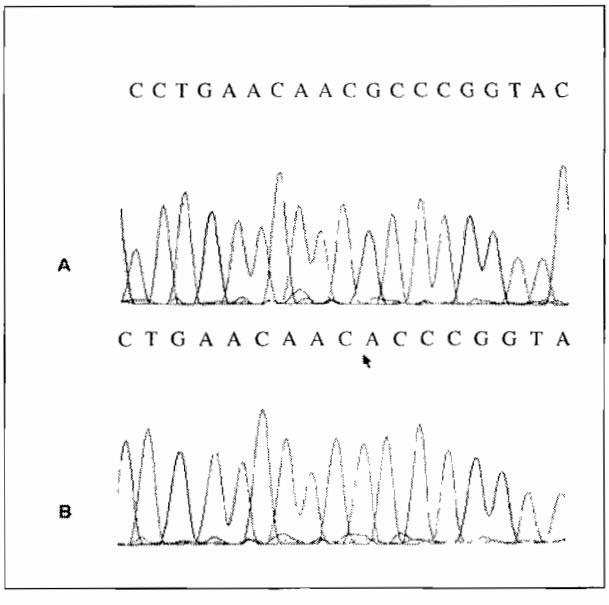


Figure 3

Electropherogram showing nucleic acid sequence of the wild type GCDH gene (upper panel A) and the G→A transition in patient LZ indicated by an arrow (lower panel B)



DNA from the subject revealed homozygous positive hybridisation (Figure 2) with the A293T probe indicating an alanine to threonine substitution at codon 293. This was confirmed to be a G⟶A base transition by direct nucleic acid sequencing (Figure 3).

DISCUSSION

Most patients with GA-1 present by the age of 14 months with the clinical picture dominated by neurological dysfunction. The clinical findings include acute, intermittent or chronic acidaemia and motor retardation characterised by dystonia, dyskinesia, chorea and athetosis. The patient in this study lived in a remote rural community with his grandparents and whilst he is reported to have had delayed early milestones, he was not referred to hospital until the age of nearly six years when he started school. He appeared to have poor concentration but normal intellectual development. He exhibited abnormal movements with a tendency to fall easily and was unable to participate actively in sports. On clinical examination, the abnormal movements were found to be consistent with choreoathetosis, and radiological examination revealed evidence of enlarged basal cisterns with bilateral fluid collection around the sylvian fissures commonly seen in GA-1(15-17).

The biochemical picture was compatible with the clinical diagnosis of GA-1 as supported by the presence of large amounts of glutaric and 3-OH glutaric acid in the urine and reduced plasma carnitine (Table 1). The excretion of glutaric acid is, however, not always constant in children with GA-1 and may be normal during periods of clinical stability with levels increasing only during intercurrent infection(4,18). The excretion of both glutaric acid and 3-hydroxyglutaric in our patient was consistent with levels in the classic GA-1 range on more than two occasions.

The mechanism of toxicity in glutaric aciduria type 1 is related to the accumulation of glutaryl CoA and glutaric acid or possibly to toxic metabolites produced by alternative pathways of glutaric acid metabolism. Due to the widely variable biochemical findings and clinical manifestations, the possibility of more than one neurotoxic metabolite or mechanism exists. The neurodegeneration may be related to nerve cell lesions caused by 3-hydroxyglutaric acid(18,19). Alternatively, a substrate derived from dietary tryptophan which is normally metabolised by glutaryl-CoA dehydrogenase, may in states of enzyme deficiency, be converted to quinolinic acid in the brain. Quinolinic acid has been shown to be a potent neurotoxin and a convulsant when injected into the central nervous system of experimental animals(20). The dystonic syndrome has been associated with decreased cerebral gamma-aminobutyric acid (GABA) biosynthesis, caused by inhibition of neuronal glutamate decarboxylase by glutaric acid(21).

The clinical significance of reduced plasma carnitine is not clearly understood. L-carnitine may, however, play an important pathobiochemical role in the pathogenesis of GA-1(22). Reduced levels occur secondarily during the first months of life due to the excretion of carnitine as glutarylcarnitine. This leads to marked depletion of neonatal carnitine reserves in muscles and other organs(23). Blood lactate, ammonia and glycine are reported to be elevated in some patients(6). Our patient had significantly reduced

blood carnitine concentration, but blood glycine concentration was normal (Table 1).

The diagnosis of GA-1 is commonly made by the analysis of glutaryl-CoA dehydrogenase activity in fibroblasts or lymphocytes. In most symptomatic cases, the enzyme activity is undetectable but residual activity has been observed in others. Undetectable enzyme activities in keeping with GA-1 have also been confirmed in asymptomatic individuals(3).

DNA analysis is increasingly being used in the definitive diagnosis of GA-1. The gene encoding glutaryl-CoA dehydrogenase has been localised to the short arm of chromosome 19(10). It spans 7kb and contains 11 exons. More than 60 pathogenic mutations have been identified but the R402W mutation is reported to be the most common(24). The relationship between genotype and clinical phenotypic expression is not fully established and some mutations, even when present in the homozygous state, seem to be associated with normal or only minimal excretion of glutaric acid(24). Our patient was homozygous for the A293T mutation, and this appears to be associated with significant excretion of both glutaric acid and 3-hydroxyglutaric acid.

Whilst GA-1 appears to be a rare genetic disorder in Africa, this case serves to confirm its distribution among all racial groups. The development of facilities for genetic analysis will play an increasingly important role in the diagnosis of this disorder, particularly in cases where the biochemical results are not in concordance with the phenotype, and may lead to increased detection.

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