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Glutaric Acidemia Type I: A Case Report in an Egyptian patient

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Abstract:

To characterize an Egyptian patient with glutaric acidemia type I (GA I) and to identify the causative mutation(s) that may be responsible for the disease phenotype. biochemical analysis was carried out using gas chromatography/mass spectrometry on the patient's dried blood spot, and the patient's organic acids were measured in dried blood and a urine sample using MS/MS and GC/MS, respectively. Total RNA was isolated from the patient's peripheral blood, and the synthesized cDNA was bi-directionally sequenced The patient exhibited clinical features compatible with a diagnosis of GA I. The abnormal elevation of organic acids in the urine supported the presence of glutaryl-CoA dehydrogenase deficiency. The mutation analysis on the cDNA of the GCDH gene revealed Nucleotide Mutation: c.1204C>T, P.Arg402Trp in exon 11. This mutation has been described as one of the most severe and common mutations that account for about 20% of the mutations in Caucasians. It affects the binding site of the enzyme leading to the drastic pathological effect of the mutation.

Keywords: Glutaric acidemia type I, GAI , Glutaryl-CoA dehydrogenate.

1. Introduction

Glutaric aciduria type I (GAI, MIM 231670) is an autosomal recessive disease caused by defects of the mitochondrial matrix protein glutaryl-CoA dehydrogenase (GCDH, EC 1.3.99.7), an enzyme involved in the degradation of the amino acids lysine, hydroxylysine and tryptophan (Kölker et al.,2006). It has an estimated prevalence of 1 in 100,000 newborns (Lindner et al., 2004).

Infants with glutaric acidemia type I often are born with unusually macrocephaly that is amongst the earliest signs of GAI (Chow et al., 2003). Newborn screening techniques enabled the early identification of affected patients, and allow the implementation of therapy prior to the development of encephalopathic crises. The clinical course of GAI shows a high degree of variability, and some patients remain asymptomatic (Kölker et al., 2006). Therefore GAI has been included in the disease panel of

newborn screening in some countries. DNA-based mutation analysis is used for the high-risk screening in one cohort of low excretors (Greenberg et al., 2002). GCDH gene is localized on chromosome 19p13.2 and encodes a flavin adenine dinucleotide-dependent mitochondrial matrix protein that is involved in the degradative metabolism of L-lysine, L- hydroxylysine and L-tryptophan (Greenberg et al., 1995). Mutations in the GCDH gene prevent production of the enzyme or result in the production of a defective enzyme with very low residual activity, or an enzyme with relatively high residual activity but still phenotypic consequences (Christensen et al., 2004). The gene spans about 7 kb on chromosome 19p13.2 and is composed of 11 exons and 10 introns (Goodman et al., 1998). The encoded protein comprises 438 amino acids. After import into mitochondria 44 N-terminal amino acid residues are cleaved off (Biery et al., 1996). More than 200 disease-causing mutations are known (Zschocke et al., 2000). Here, we report an Egyptian patient diagnosed with GAI after taking the consent of parents.

2.Patient and methods

2.1.Patient

A 4 years old male, 6th order of birth of 1st cousin consanguineous Egyptian parents. The patient was delivered at full term by cesarean section. His birth weight was 2.500 kg. No problems were noted by the mother during pregnancy. The patient was referred to the Genetics Clinic, Pediatric Hospital, Ain Shams University due to family history of previous sibling diagnosed with glutaric aciduria type 1. At birth, GA 1 diagnosis was suspected and confirmed by the quantification of organic acids in the urine and dried blood using MS/MS and GC/MS, and genetic testing of the GCDH gene. The patient started treatment immediately in the form of milk formula free of lysine low in tryptophan, duocal formula and protein restriction with carnitine supplementation 100mg/kg/day and riboflavin 100mg/day. The patient had normal developmental milestones till the age of 5 month then he had attack of fever and gastroenteritis followed by attack of convulsions and disturbed conscious level which necessitated admission to hospital for 1 week. After discharge, the patient had lost all acquired milestones and he developed dystonia which was treated by baclofen. At the age of 1 year, he had another attack of fever and gastroenteritis which necessitated admission to hospital for 5 days. He was admitted again to the hospital at the age of 3 years for gastroenteritis for 1 week. The patient started physiotherapy. At the age of 3.6 year, he was admitted to the hospital for gastroenteritis for 2 days. The patient had global developmental delay as he cannot support his neck or vocalize till now .His response to treatment was noticeably poor. Dystonia evaluated using the Fahn-Marsden rating dystonia scale, with a score of (98, 30). Family history showed a previous sib was diagnosed with the same condition. On examination, his weight was 10 kg (below 5th percentile), his height was 87 cm (below 5th percentile) and his skull circumference was 49 cm (below 5th percentile). Cardiac examination and abdominal examination were normal. Neurologic examination revealed hypertonia and hyperreflexia with positive babinski sign. Intact superficial sensation, no cerebellar manifestations. Genital examination was apparently normal.

2.2.Biochemical analysis

MS/MS

A blood sample was taken from the patient via a finger prick and placed on a Guthrie card (Whatman 903 filter paper, GE Healthcare, New Jersey, USA). The blood spots were analyzed for acylcarnitines and amino acids by triple-quadruple tandem mass spectrometer (ACQUITY UPLCH-Class. Waters® Corporation, Massachusetts, USA), with a positive electrospray

ionization probe utilizing MassChrom® amino acids and acylcarnitine from the Dried Blood kit (Chromsystems Instruments and Chemicals GmbH, München, Germany) according to the manufacturer's instructions. The data from the Multiple Reaction Monitoring (MRM) scan were analyzed using the Neolynx® application (Waters ® Corporation, Massachusetts, and USA).

Genetic analysis

The ethics committee at Ain-Shams University, Cairo, Egypt approved this study, and written informed consent was obtained from the parents of the patient.

GC/MS

The urine sample of the patient was collected and frozen until derivatization by silylation of organic compounds. The GC/MS analysis was performed using the Agilent 7890 system gas chromatography instrument interfaced with a model 5975 mass spectrometer and a gas chromatography capillary column HP 5 MS (25 × 0.2 mm i.d., 0.33 µm film, Agilent, USA). The column temperature was initially maintained at 80 °C for 2 min and then increased from 80 °C to 280 °C by 4 °C/min where it was held for 3 min. Run time was 55 min. The results were calculated in µmol/mmol creatinine using a calibration curve of the organic acid of interest, which was processed under the same conditions.

Genetic analysis was done according to method used by (Moseilhy et al.,2016). Total RNA was extracted from the peripheral blood of the patient using the Gene JET Whole Blood RNA purification Mini Kit (Thermo Scientific, Germany), and cDNA was synthesized using the Quanti Tect Reverse Transcription Kit (Qiagen, Belgium) according to the manufacturer's instructions. The cDNA was further amplified by PCR using GoTaq Green polymerase Mix 2× (Promega, Fitchburg, WI) according to the manufacturer's instructions. Forward and reverse primers designed to cover the entire sequence of the GCDH cDNA were used to amplify four amplicons for further sequencing as shown in table (1).

	Primers Forward / Reverse	start	stop	Annealing temperature	size (bp)
Set 1	F 5'-TTGCTCCGCTCGCTCTGAGAG -3'	85	481	54 °C	397 bp
	R 5'-GCCCATAGGCCACAGACGAA- 3'				
Set 2	F5'-GATGGGGGAGTTGGGTGT-3'	405	904	55 °C	500 bp
	R 5'-TGATGATCATGCCTGTGG-3'				
Set 3	F 5'-GTGTGAAGATGGCTGCATTC-3'	789	1372	59 °C	584 bp
	R 5'-GGGCGTGAATGTCATGTGTA-3'				
Set 4	F 5'-GGAATGGGATTTCTGACGAG-3'	1280	1857	63 °C	578 bp
	R 5'-GGGGTCAGATGTGCAGGTCTTT-3'				

Table (1): The primers were designed using the NCBI primer designing tool ***(http://www.ncbi.nlm.nih.gov/tools/primer-blast/),*** with the NCBI nucleotide reference sequence of NM_000159.3. The selective specificity of the primers was confirmed using the in silico PCR tool of the UCSC genome browser ***(http://genome.ucsc.edu/cgi-bin/hgPcr).*** The four different amplicons of the GCDH gene were bi-directionally sequenced using the

sequencing service of GATC Biotech, Germany ***(https://www.gatc-biotech.com/en/index.html).***

3.Results

LC-MS/MS was performed on his dried blood spot. The MS/MS measurement revealed a significant elevation in the C5DC value of 2.06 µmol/L (normal <0.34 µmol/L). Urine examination for organic acids using GC/MS showed an increase in glutaric acid, elevated 3-OH-GA and an exaggerated excretion of glutaconic acid.

3.1.Molecular genetics findings

The mutation analysis on the cDNA of the GCDH gene revealed Nucleotide Mutation: c.1204C>T, P.Arg402Trp in exon 11.

4.Discussion

GAI patients are prone to the development of encephalopathic crises triggered by catabolic stress induced by fever, infections, vomiting and/or diarrhea as detected in our patient. About 75% of the patients suffer an acute encephalopathic crisis, usually associated with an upper respiratory and/or gastrointestinal infection, immunization, or surgical intervention between the ages of 2 and 37 months (Goodman and Frerman 2001). During these catabolic crises, concentrations of the metabolites show a further increase. The development of an encephalopathic crisis is accompanied by destruction of striatal neurons with a subsequent irreversible disabling movement disorder, especially dystonia. After recovery, the patients lost motor skills and function and a severe dystonic-dyskinetic syndrome in children with relatively well-preserved intellectual functions may be recognized as detected in our patient (Goodman et al., 1995).

Our patient was diagnosed shortly after birth. Despite treatment, our patient developed dystonia. This is due to the nature of the disease which the outcome might not be predictable despite treatment (Hedlund et al., 2006). Other factors for poor final outcome could be lack of awareness amongst physicians, distance to the appropriate medical facility, lack of home management and poor patient compliance due to illiteracy and socioeconomic status.

Our patient had a c.1204C>T, P.Arg402Trp in exon 11. This mutation has been described as one of the most severe and common mutations that account for about 20% of the mutations in Caucasians (Goodman et al., 1998). It affects the binding site of the enzyme leading to the drastic pathological effect of the mutation.

In conclusion, we have characterized both the clinical and molecular aspects of Egyptian patient with GAI. The setting up of biochemical and molecular testing for GAI should allow for detection of pre-symptomatic patients that can be treated early. However, this should be associated with the implementation of adequate health facilities and health education of physicians to minimize the risk of adverse neurological outcome in case of acute decompensation.

Compliance with ethical standards

All authors declare that they have no conflict of interest.

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