A Low-Excretor Biochemical Phenotype of Glutaric Aciduria Type I: Identification of Novel Mutations in the Glutaryl CoA Dehydrogenase Gene and Review of Literature from India

Sir,

Glutaric aciduria type-I (GA-I) (OMIM#231670) is a rare autosomal-recessively inherited dysfunction of glutaryl-CoA dehydrogenase enzyme (GCDH, E.C. 1.3.8.6), involved in the degradation of lysine and tryptophan.[1] This results in the accumulation of toxic by-products, glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA) and glutaconic acid in body fluids. The common clinical and neurological signs associated with GA-I include developmental delays, seizures, hypotonia,[1] hydrocephalus, brain atrophy, CSF space dilation and characteristic dilated Sylvian fissures etc.[2,3] There are limited genetic studies on Indian GA-I patients and till date, there is no case report on low excretor biochemical phenotype.

Here, we present a case of an 8-month-old male who was referred due to motor regression. He weighed normal (10.5 kg) for his age at the time of presentation. He was the second child of healthy, consanguineous (third degree) parents of Indian origin, with no history of GA-I or any related illness in the family. Developmental delay was not observed until 8 months of age. He further developed motor delay and chorea. The head circumference was 45 cm with < 98 percentile, normal for his age. The electroencephalography was normal. The Magnetic resonance imaging (MRI) suggested FLAIR hyper intensities in bilateral striatum, bilateral frontotemporal atrophy with CSF space dilation. Both MRI and computed tomography (CT) suggested widening of Sylvian fissure giving characteristic "bat-wing" appearance [Figure 1]. Biochemical investigations, including serum electrolytes, blood glucose, liver functions and urinary Orotic acid were within normal limits. Upon selective screening by tandem mass spectroscopy (TMS), C5DC was found elevated [1.28 μmol/L (Ref Range = 0.00-0.56 μmol/L)]. Subsequent, confirmation by urine organic acid analysis by gas chromatography mass spectrometry (GC-MS) detected a "Low-excretor" phenotype (GA 25.190 mmol/mol creatinine; 3-OH-GA 32.842 mmol/mol creatinine) (Ref GA: 0.02–3.8 and Ref: 3-OH-GA: 0–4.6 mmol/mol creatinine). Dried blood spots (DBS) were collected from the patient and his parents after obtaining informed consent. DNA was isolated from DBS using single-lysis salting-out method[3] and genetic testing was performed using polymerase chain reaction with exon specific primers of GCDH gene and

direct DNA sequencing using the ABI-3500 × 1 Genetic Analyzer (Applied Biosystems). This confirmed that the patient had homozygous and the parents had heterozygous R383S [g.11608(C > T] mutation [Figure 2]. Two intronic mutations, i.e. homozygous IVS10 + 125 [g.11829(C > G)] and heterozygous IVS10 + 180 [g.11884 (T > G)] [Figure 2] were also found.

The treatment with lysine and tryptophan-restricted protein diet with carnitine supplementation and riboflavin was initiated. Adequate emergency management was implemented according to current guidelines.[2] Baclofen, Gabapentin, Trihexyphenidyl and Risperidone was administered. Last seen at 3 years of age, the child's weight had improved. There were no observed metabolic crises. The child was able to sit independently and had no sign of seizure or dystonia. However, the child had motor delay cognitively well preserved.

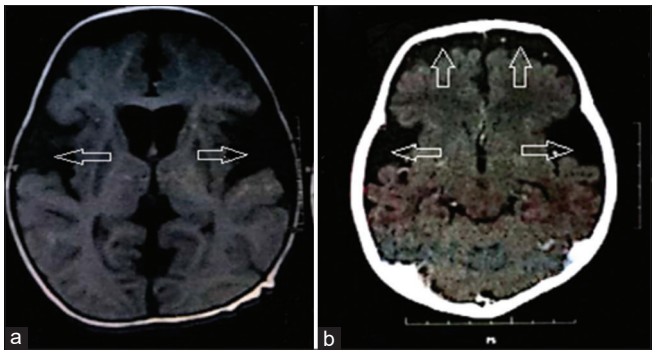

Figure 1: Brain (a) MRI (b) CT scan of the patient at 8 months of age. T1-weighted axial image showing fronto-temporal atrophy and bat-wing dilatation of the Sylvian fissures with open opercula (arrow)

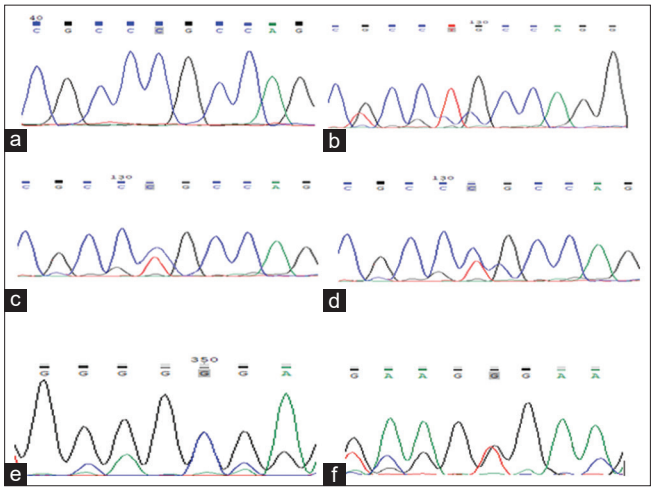

Figure 2: Sequencing Chromatograms showing site highlighted in grey of (a) Control peak for mutation (g.11608C) (B) R383S (g.11608C > T) mutation in patient; (c) Two peaks (C and T) representing heterozygous alleles in Father (d) Two peaks (C and T) representing heterozygous alleles in Mother (e) Intron 10 region of the mutant site IVS10 + 125 (g.11829(C > G) highlighted in grey of patient depicting two peaks (C and G) and (f) Intron 10 region of the mutant site IVS10 + 180 (g.11884 (T > G) highlighted in grey of the patient depicting two peaks (T and G)

Among the few Indian studies on GA-I, only 11% (5/42) patients were of low-excretor phenotype. Till now, only 5 novel missense mutations associated with low-excretor GA-I phenotype are reported from India. Most common symptoms found are motor delay and cerebral atrophy with low GA and 3-OH-GA concentrations. Detectable GA concentration was not present in all; however, 3-OH-GA was present in all the patients. Other details, and associated mutations are presented in Table 1. No genotypic, biochemical or clinical correlation was found.

In GA-I, clinical symptoms are varied and macrocephaly is frequent.[2] However, in the present case, macrocephaly was absent, which could probably be due to slow intracerebral accumulation of GA and 3-OH-GA and their low concentrations. Phenotypic differential diagnoses of GA-I include disorders such as urea cycle defects, mitochondrial disorders etc,[2] hence confirmatory biochemical diagnosis was performed using TMS and GCMS. Biochemical differential diagnosis of GA-I with elevated C5DC and GA (multiple acyl-CoA dehydrogenase deficiency, renal insufficiency, maternal GA-I etc) and 3-OH-GA (Short-chain 3-hydroxyacyl-CoA dehydrogenase deficiency) are also present.[2] Hence, initially, the child was tested for urinary Orortic acid using GCMS, to rule out the possibility of metabolic disorders such as Orotic aciduria and any other urea cycle disorders.[4] Upon TMS, elevated C5DC levels were indicative of GA-I. Biochemically, GA-I patients are classified into high (GA > 100 mmol/mol Cr in urine) and low (GA < 100 mmol/mol Cr levels in urine) excretors.[1] Subsequently, upon GC-MS analysis, the patient under study was classified as low-excretor as GA concentration in urine was <100 mmol/mol Cr. GA-I patients with a low-excretor phenotype, either typically show mild to nil GA elevations, or only 3-OH-GA and few cases have mild to nil GA elevations with comparatively, higher 3-OH-GA levels.[5] Detecting Low-excretors has various diagnostic pitfalls[2] as these patients with no intermittent increase in C5DC level or with mild biochemical phenotype can be missed. Hence, confirmatory diagnosis using DNA based mutational screening, is an important diagnostic method. Exome sequencing followed by sanger validation is a useful alternative.[2] According to the new guidelines, GA-I should be confirmed by mutation analysis, which has a sensitivity of 98–99%[2] and has been recommended to be considered for new-born screening in a cohort with GA-I incidence due to a common GCDH mutation and a low excretor phenotype. In this case, irrespective of no macrocephaly, clinical and neuroimaging findings along with biochemical investigations suggested GA-I and genetic analysis confirmed the diagnosis.

Glutaric aciduria type-I is treatable when diagnosed on time before crisis occurs. In this patient, excessive restriction of tryptophan is avoided to prevent irritability and sleep disturbances. Riboflavin is a cofactor of GCDH, and hence was administrated. L-carnitine supplementation corrects

Table 1: Summary of Low excretors found in India

Case	Age at onset (months)/Sex	History/age at diagnosis	Clinical manifestation	Neurological signs	C5DC (μmol/L)	GA/3-OH-GA levels (mmol/mol of Creatinine)	Associated Mutations	Reference
1	8/M	Yes/24m]	Developmental delay	Frontotemporal atrophy and basal ganglia hyper intensity	0.08 (Ref: 0.00-0.56)	773.65 (GA 0-31.2)	NA	[7]
2	9/NA	NA	Motor delay	Minimal cerebral atrophy	9.2 (Ref: 0.01-0.18)	82/52 (Ref GA: 0.02-3.8) and (Ref 3-OH-GA: 0-4.6)	p.G391G	[5]
3	8/NA	NA	Motor delay	Minimal cerebral atrophy	2.9 (Ref: 0.01-0.18)	77.4/86 (Ref GA: 0.02-3.8) and (Ref 3-OH-GA: 0-4.6)	p.P286S; p.G391G	[5]
4	7/F	Yes/24m	Macrocephaly, extrapyramidal symptoms	Abnormal Neuro radiological changes	*+	*-	p.L221P	[6]
5.	6/M	Yes/12m	extrapyramidal symptoms	Abnormal neuro radiological changes	*-	*-	p.R94Q	[6]

*Values were not provided, NA: Not available

severe secondary carnitine deficiency. Baclofen, Gabapentin and Trihexyphenidyl was administered to control spasticity, seizures and poor muscle control respectively. Baclofen is a GABA analogue, used to alleviate neurological symptoms. Risperidone was given to control chorea.

In conclusion, this case report augments to the growing evidence of the existence of low-excretor GA-I patients in India. Further, the absence of macrocephaly and false negative biochemical parameters cannot rule out the possibility of GA-I. Hence, emphasis should be laid on the need for genetic confirmation. As the differential diagnosis and disease course in low-excretor GA-I patients can be misleading, it becomes necessary to correlate clinical, biochemical, radiologic characteristics and molecular genetic analysis.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

Muntaj Shaik, Mahesh Kamate[1], Kruthika-Vinod T.P.[2], Anakala Basappa Vedamurthy

Department of Biotechnology and Microbiology, Karnataka University, Dharwad, [1]Department of Pediatrics, Jawaharlal Nehru Medical College, KLE University, Belgaum, [2]Department of Neurochemistry, National Institute of Mental Health and Neurosciences, Bengaluru, Karnataka, India

Address for correspondence: Prof. Anakala Basappa Vedamurthy, Department of Microbiology and Biotechnology, Karnataka University, Dharwad, Karnataka, India.
E-mail: vedamurthybt@gmail.com

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