Molecular Genetics and Metabolism Reports 36 (2023) 100979

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

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

# Glutaric aciduria and L-2-hydroxyglutaric aciduria: Clinical and molecular findings of 35 patients from Turkey

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ARTICLE INFO

*Keywords:*
Glutaric aciduria type 1
L-2-hydroxy glutaric aciduria
Flavin adenine dinucleotide
Vitamin B2
Cerebral organic aciduria

ABSTRACT

*Background:* Cerebral organic acid disorders are progressive neurometabolic diseases characterized by neurologic dysfunction. Glutaric aciduria type I (GA-I) and L-2-hydroxyglutaric aciduria (L2HGA) are the main cerebral organic acid disorders. They are both classified as, and it is suggested that these two disorders may share a common metabolic pathway. Current treatment strategies are based on levocarnitine, vitamin B2, and diet. Recent guidelines recommend a lysine-restricted diet up to six years of age, but there is no consensus for patients over the age of six. Vitamin B2 is exists in the blood as riboflavin and its cofactors, flavin mononucleotide and flavin adenine dinucleotide (FAD). FAD, the cofactor of L2HGD, accelerates the conversion of L-2-hydoxy glutarate to alpha-ketoglutarate. Levocarnitine stimulates the formation and excretion of derivatives of glutaric acid. Also, lysine-associated organic acidurias some results provide principal proof for the beneficial effects of riboflavin in GA-I. It has been previously reported that combination therapy with riboflavin and levocarnitine is effective for L2HGA as well as GA-I. Riboflavin and levocarnitine have been reported to improve not only clinical symptoms but also urinary 2-HGA levels. In our study, we aimed to evaluate the effect of the current treatment strategies and genotype on urinary metabolites and IQ scores in GA-I and L2HGA patients.

*Methods:* The presented retrospective multicenter study included patients followed up in Diyarbakir Children's Hospital and Izmir Katip Celebi University Faculty of Medicine, Division of Pediatric Metabolism. Between 2016 and 2021, we retrospectively evaluated 35 patients with confirmed diagnosis of GA-I and L-2HGA. We analyzed the clinical, biochemical, neuroradiological, molecular data and treatment of the patients. The follow-up period was every 2 months until 12 months old, every 3 months until 6 years of age, and every 6 months thereafter. Therapy monitoring was undertaken during follow-up visits that included evaluation of clinical parameters, laboratory parameters, and dietary consumption records. Denver II was applied in order to evaluate children aged 0–6 years in terms of development. Patients between 6 and 16 years of age were evaluated using the Wechsler Intelligence Scale for Children-Revised.

*Results:* We identified 25 with GA-I and 10 with L2HGA. The most common clinical symptoms were developmental delay, intellectual disability, and movement disorders. Behavioural problems were more common in L2HGA than in GA-I patients. In the same family, there were patients with severe developmental delay despite early diagnosis and treatment and individuals with normal IQ scores. In our study group, we used diet (lysine restricted or protein controlled), levocarnitine and vitamin B2 for GA-I patients. The mean urinary glutaric acid levels were decreased with treatment in GA-I patients. Group I consisted of 14/25 patients receiving lysine restricted diet and levocarnitine, Group II (8/25) received protein-controlled diet and levocarnitine. Group III (3/

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https://doi.org/10.1016/j.ymgmr.2023.100979
Received 16 March 2023; Received in revised form 15 May 2023; Accepted 15 May 2023

25) patients whom had p.Pro248Leu (P248L) variant, received riboflavin in combination with protein-controlled diet and levocarnitine. When we evaluated according to the treatment groups, a significant decrease was observed in urinary glutaric acid levels in group I. But there were no significant difference in Group II and III. The patients with c.1018C > T variant in GCDH gene had higher pre-treatment urinary metabolites and significant reduction in urinary metabolites with treatment was detected. In L2HGA patients, we used levocarnitine and vitamin B2. In all L2HGA patients, there was a significant decrease in the mean urinary 2- hydoxy glutarate with treatment. However, there was no significant difference between the c.164G > A and c.1115delT variants. The mean pre- and post-treatment IQ scores of GA-I patients, no significant difference was observed. Relative neurologic improvement was seen in three L2HGA patients. We found two novel variants, including the c.221A > G (p.Tyr74Cys) in the GCDH gene and the c.738 + 5A > G splice variant in the L2HGDH gene.

*Conclusions:* Glutaric aciduria type I and L2HGA are the most common cerebral organic acidurias. Early and correct diagnosis is crucial. Poor prognosis based on metabolic crises and progressive deterioration still appears. In countries where newborn screening is not performed, a clinical suspicion index is required for cerebral organic aciduria. GA-I and L-2HGA are difficult to examine by medical evidence standards because of the small sample size, regional differences in newborn screening, and medical care limits. More clinical studies are needed to identify effective treatments. However, the significant decrease in urinary glutaric acid levels after treatment in patients on lysine-restricted diet raises the question of whether lysine-restricted diet should be continued after six years of age. We also reported our experience in order to contribute to the literature.

## 1. Introduction

Cerebral organic acidurias (COA) are rare neurometabolic diseases characterized predominantly by central nervous dysfunction. Glutaric aciduria type I (GA-I), D-2-hydroxyglutaric aciduria (D2HGA), L-2-hydroxyglutaric aciduria (L2HGA), succinate semialdehyde dehydrogenase deficiency, *N*-acetylaspartic aciduria and ethylmalonic encephalopathy are the main COA [1]. Accumulated organic acids are structurally similar to the excitatory neurotransmitter or show neurotransmitter/neuromodulatory properties [2]. Cerebral energy metabolism plays an important role in pathophysiology. General metabolic abnormalities are absent. Elevated characteristic organic acid excretions, mostly dicarboxylic acids, are frequently observed [2]. GA-I and L2HGA are both classified as lysine-associated organic acidurias. To our knowledge, it is thought that these two disorders may share a common metabolic pathway.

Primarily a neurological disorder, GA-I is cerebral organic aciduria caused by mitochondrial glutaryl-CoA dehydrogenase (GCDH, EC 1.3.8.6) enzyme deficiency. GCDH catalyzes the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA. Its deficiency results in the accumulation of glutaryl-CoA, glutaconic acid, glutaric acid (GA) and 3-hydroxyglutaric acid (3-HGA). Cerebral GA and 3-HGA accumulation in the brain occurs through the blood-brain barrier, which has a low permeability for dicarboxylic acids [2]. The accumulation of 3HGA is thought to play a role in cell damage and striatal injury. Depending on the urinary GA concentration, two biochemical subtypes have been identified, low excretors (LE) with 3–30% residual GCDH activity and high excretors (HE) with 0–2% residual activity. The genotype correlates with the biochemical phenotype However, patients with LE may present with a clinical phenotype that can be as severe as those with HE. Extra-striatal abnormalities and macrocephaly are more common in HE patients. These findings are associated with higher intracerebral GA and 3-HGA levels and result in poorer cognitive outcome. In early diagnosed individuals, the frequency of acute encephalopathic crises and movement disorders was significantly reduced with current treatment strategies [3]. NBS for GA-I has an overall positive effect on neurological outcomes. The quality of treatment is critical [4].

L2HGA is an autosomal recessive COA caused by L-2-hydroxyglutarate dehydrogenase (L2HGDH, EC 1.1.99.2) enzyme deficiency which is involved in the oxidation of L-2-hydoxyglutarate (L-2-HG) to alpha 2-ketoglutarate. 2-Hydroxyglutarate (2-HGA) has structural similarity to alpha 2-ketoglutarate, an intermediate of the tricarboxylic acid (TCA) cycle. To prevent the loss of carbon fragments in the TCA cycle and to protect the potentially toxic effects, L2HGA is reconverted to 2-ketoglutarate by L2HGDH with cofactor flavin adenine dinucleotide sodium (FAD). To our knowledge, L-2-HG has no physiological function in a human, so it can be considered a metabolite repair disorder. Increased L-2-HG in tissues induces the free radical formation and increases glutamate uptake in synaptosomes and synaptic vesicles. It is toxic to the brain, causes leukoencephalopathy, and is also an oncometabolite. Therefore, L2HGA is a progressive and potentially neurodegenerative disorder. Especially in L2HGA patients present with cerebellar ataxia, extrapyramidal and pyramidal symptoms and slowly progressive neurodegeneration with seizures and macrocephaly. These symptoms occur as a result of acute or chronic pathological changes in the brain. Delayed myelination, dysmyelination, cerebellar/cortical atrophy, and symmetrical changes in basal ganglia are suspicious for COA [1]. The development of malignant brain tumors has also been reported in the clinical course of L2HGA [5]. Subtle findings such as speech delay and deficits in fine motor skills must alert clinicians about COA. Even elevations in diagnostic metabolites may be very slight, and therefore may be overlooked in routine organic acid analysis [6]. In countries where newborn screening is not available, a clinical suspicion index for cerebral organic aciduria is required.

Current treatment strategies are based on levocarnitine, vitamin B2, and diet. Vitamin B2 exists in the blood as riboflavin and its cofactors, flavin mononucleotide (FMN) and FAD. FAD, the cofactor of L2HGD, accelerates the conversion of L2HG to alpha-ketoglutarate [3,6]. Also, levocarnitine stimulates the formation and excretion of derivatives of glutaric acid. It has been previously reported that combination therapy with riboflavin and levocarnitine is effective for L2HGA as well as GA-I. Riboflavin and levocarnitine have been reported to improve not only clinical symptoms but also urinary 2-HGA levels. In our study, we aimed to evaluate the effect of the current treatment strategies and genotype on urinary metabolites in GA-I and L-2-HGA.

## 2. Methods

### 2.1. Study design and data acquisition

Individuals were included based on a retrospective collection of the data of the L2HGDH and GA-1 patients that were diagnosed in three metabolism centers in Turkey. Inclusion criteria were rare biallelic variants in GCDH and L2HGDH genes classified as likely pathogenic or pathogenic according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines. All data were retrieved via standardized proformas agreed by participating centers. For phenotyping, the following variables were analyzed within this study: individual's genetic ancestry, sex, age at last assessment, and clinical status. In addition, detailed data on neurological disease, laboratory values, and clinical features involved were scrutinized and recorded according to Human Phenotype Ontology terminology.

The patients were diagnosed by abnormal biochemical profile (3-HGA and GA in urinary organic acid and C5DC in acylcarnitine profile for GA-I and 2HGA in urinary organic acid for L2HGA). Diagnosis confirmed by molecular analysis. After the diagnosis patients were treated according to guideline recommendations. The follow-up period was every 2 months until 12 months, every 3 months until 6 years of age, and every 6 months thereafter. Therapy monitoring was undertaken during follow-up visits that included evaluation of clinical parameters, laboratory parameters, and dietary consumption records. At each visit, physical examination including detailed neurological examination, plasma amino acids, carnitine-acylcarnitine analysis with tandem MS and urine organic acid analysis were evaluated. Last three-days dietary consumption records were requested from the patients/parents. Protein, lysine, energy, carnitine and vitamin B2 intake were calculated. Denver II was applied in order to evaluate children aged 0–6 in terms of development. Patients between 6 and 16 years of age were evaluated using the Wechsler Intelligence Scale for Children-Revised (WISC-R), the test includes verbal and performance subscales. 2HGA excretion can be determined by urinary organic acid screening with gas chromatography–mass spectrometry (GC–MS). However, order to determine the chiral configuration, either D-2HGA or L-2HGA, measurement with liquid chromatography-tandem mass spectrometry is required. We could not determine the chiral configuration due to technical impotence; differential diagnosis was made by molecular analysis.

### 2.2. Molecular analyses

All exons and exon-intron junctions of the genes were evaluated by the next-generation sequencing method. Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Standardized PCR pools were prepared using NexteraXT sample preparation kit for next-generation sequencing analysis with the Miseq device (Illumina, Inc.).

#### 2.2.1. Whole exome sequencing

Whole exome sequencing was performed using an Ion S5 TM Sequencer. Ion AmpliSeq Exome RDY Kit was used according to the manufacturer's protocol. Ion reporter software was used to analyze pathogenic variants. All variants were assessed individually according to the clinical phenotype, MAF (minor allele frequency) score and pathogenicity scores calculated by prediction programs. Variants were filtered to retain nonsynonymous changes with a minor allele frequency (MAF) of $<0.01$ using combined datasets from the 1000 Genomes Project, the Exome Variant Server pro- ject, and Genome Aggregation Database (gnomAD). The potential func- tional impacts of the disease candidate variants were assessed using SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (www.mutationtaster.org/) and VarSome. All genetic variants were screened by pathogenicity, mode of inheritance and clinical phenotypes. Finally, candidate pathogenic variants identified by WES were verified with Sanger sequencing.

#### 2.2.2. Validation by sanger sequencing

Sanger sequencing of the genomic variants identified by exome sequencing or targeted gene sequencing was performed for all patients and their families. Sanger sequencing was used to validate the pathogenic variants within families on 3500 genetic analyzer (Applied Biosystems, Foster City, USA). The sequencing results were analyzed using CLC genomic workbench software. For the clinical interpretation of variants, allele frequency data were obtained from various databases, including gnomAD (http://gnomad.broadinstitute.org/) and ExAc (http://exac.broadinstitute.org/). The pathogenicity of variants was assessed using in silico prediction tools, such as PolyPhen-2 (http://genetics.bwh. harvard.edu/pph2), SIFT (http://sift.jcvi.org), and MutationTaster (http://www.mutationtaster.org) and Human Splicing Foundation (http://www.umd.be/hsf/). Variants were classified according to The American College of Medical Genetics and Genomics (ACMG).

#### 2.2.3. Nomenclature

All pathogenic variants are described according to accepted HGVS nomenclature. Nucleotide numbers are derived from complementary DNA (cDNA) sequences (GenBank accession no. GCDH, NM_000159.2 and L2HGDH, NM_024884.2).

### 2.3. Statistics

Statistical analyses of the data were performed using the SPSS software package for Windows software package (ver.18.0; SPSS Inc., Chicago, IL, USA). As descriptive statistics, numbers, and percentages for categorical variables, mean ± standard deviation or median (minimum-maximum) were used for numerical variables. The distribution of data was evaluated using the Shapiro-Wilk test. For numerical comparisons, the Student's *t*-test or Mann-Whitney *U*- test were used to assess differences between two groups according to the normal distribution of the measured parameters. The study was performed in accordance with the declaration of Helsinki and was approved by the Local Ethics Committee of the Diyarbakir Gazi Yaşargil Research and Training Hospital (Date.1.5.2021/No944).

## 3. Results

### 3.1. Study population

A total of 35 individuals (18/17, F/M) from 27 different families residing in two cities of Turkey, were included in the study. 25 patients were diagnosed with GA-I and 10 with L2HGA. None of them previously published. Data pertaining to brain image, serum amino acid levels, organic acid profiles, and further laboratory findings are documented. The cohort comprised 35 individuals, of whom, 33 were alive at the time of data collection (median age 36 months, range: 11–190 months). All individuals in our cohort were born to consanguineous marriages. First symptoms were recognized at a median age of 7 months, and the genetic diagnosis was made at a median age of 12 months (range:1–180). The total duration of follow up of the cohort was five years, individually ranging from 6 to 69 (median = 24.6) months. Initial neurological findings, demographic, clinical, and molecular characteristics of all patients are presented in Table 1. The mean age at the time of diagnosis and the mean diagnostic delay time were presented at Table 2.

### 3.2. Glutaric aciduria type 1

The neurological findings at the time of diagnosis were seizure (15/25), developmental delay (9/25), intellectual disability (7/25), movement disorders (2/25), and tone abnormality (1/25), behavioural problems (1/25) in GA-I patients. Non-neurological findings at the time of diagnosis were failure to thrive (1/25). P1 had a diagnosis of both GA-I and Griscelli syndrome. P7, P12, P15 and P20 (4/25) with GA-I were diagnosed with family screening in the presymptomatic period, at a median age of 2 months (range: 1–12 months). Two patients were diagnosed during the etiological investigation of ataxia. Additional neurological findings that developed during follow-up in GA-I patients were movement disorders (7/25), intellectual disability (9/25) and behavioural problems (2/15), non-neurological findings were feeding difficulties (5/25) and failure to thrive (4/25). Neurological findings at last visit in GA-I patients were intellectual disability and developmental delay (16/25), movement disorders (9/25), epilepsy (13/25), tone abnormality (5/25), behavioural problems (3/25), nonneurological findings were failure to thrive (5/25) and feeding difficulties (6/25). Acute encephalopathic crisis was seen in five patients during follow-up. The encephalopathic crisis developed during an intercurrent infectious

**Table 1**
Clinical and molecular findings of GA-I and L-2-HGA patients.

| Family | Patient | Gender | Consanguinity | Diagnosis | Initial neurological symptoms | Neurological symptoms onset of age (Month) | Age at diagnosis (month) | Gene | DNA change | Protein Change | Type of mutation | Zygosity | ACMG classification | Previous Reports |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F1 | P1 | F | + | GA-I | DD | 5 | 7 | GCDH | c.221A > G | p.Tyr74Cys | Missense | Homozygous | Likely Pathogenic | **Novel** |
| F2 | P2 | M | + | GA-I | ID | 12 | 180 | GCDH | c.383G > A | p. Arg128Gln | Missense | Homozygous | Pathogenic | rs755586631 |
| F3 | P3 | M | + | GA-I | Seizure, DD, nystagmus | 7 | 7 | GCDH | c.541G > C | p. Glu181Gln | Missense | Homozygous | Pathogenic | rs745852738 |
| F4 | P4 | F | + | GA-I | Seizure, ID, movement disorders | 6 | 73 | GCDH | c.743C > T | p. Pro248Leu | Missense | Homozygous | Pathogenic | rs1057516344 |
| F5 | P5 | F | + | GA-I | ID | 6 | 72 | GCDH | c.743C > T | p. Pro248Leu | Missense | Homozygous | Pathogenic | rs1057516344 |
| F6 | P6 | F | + | GA-I | Seizure | 6 | 76 | GCDH | c.743C > T | p. Pro248Leu | Missense | Homozygous | Pathogenic | rs1057516344 |
| F7 | P7 | M | + | GA-I | Family Screening, DD | 24 | 2 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
|  | P8 | F | + | GA-I | Seizure, ID, behavioural problems | 72 | 84 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
| F8 | P9 | F | + | GA-I | Seizure, ID | 9 | 12 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
| F9 | P10 | M | + | GA-I | Movement disorder | 84 | 86 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
|  | P11 | M | + | GA-I | Seizure, DD | 12 | 12 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
|  | P12 | M | + | GA-I | Seizure, DD | 7 | 7 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
|  | P13 | F | + | GA-I | Family Screening | 12 | 1 | GCDH | c.1018C > T | p. Leu340Phe | Missense | Homozygous | VUS | rs1599617735 |
| F10 | P14 | F | + | GA-I | Seizure, DD | 1 | 5 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
| F11 | P15 | F | + | GA-I | Family Screening | 12 | 2 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
|  | P16 | M | + | GA-I | Seizure, DD | 3 | 4 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
| F12 | P17 | M | + | GA-I | Sibling death history |  | 1 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
|  | P18 | F | + | GA-I | Seizure, ID | 5 | 78 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
| F13 | P19 | M | + | GA-I | Seizure, DD | 6 | 16 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
| F14 | P20 | M | + | GA-I | Family Screening | 15 | 12 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
|  | P21 | M | + | GA-I | Seizure, ID | 6 | 88 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
| F15 | P22 | F | + | GA-I | Seizure | 4 | 87 | GCDH | c.1204C > T | p. Arg402Trp | Missense | Homozygous | Pathogenic | rs121434369 |
| F16 | P23 | F | + | GA-I | Tone abnormality, DD | 8 | 12 | GCDH | c.1205G > A | p. Arg402Gln | Missense | Homozygous | Pathogenic | rs786204626 |
| F17 | P24 | F | + | GA-I | Seizure | 12 | 92 |  | NA |  |  |  |  |  |
| F18 | P25 | F | + | GA-I | Seizure | 2 | 78 |  | NA |  |  |  |  |  |
| F19 | P26 | M | + | L2HGA | Hypotonia | 1 | 6 | L2HGDH | c.164G > A | p.Gly55Asp | Missense | Homozygous | Pathogenic | rs118204021 |

(*continued on next page*)

**Table 1** (*continued*)

| Family | Patient | Gender | Consanguinity | Diagnosis | Initial neurological symptoms | Neurological symptoms onset of age (Month) | Age at diagnosis (month) | Gene | DNA change | Protein Change | Type of mutation | Zygosity | ACMG classification | Previous Reports |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F20 | P27 | F | + | L2HGA | DD, Movement disorder | 12 | 38 | L2HGDH | c.164G > A | p.Gly55Asp | Missense | Homozygous | Pathogenic | rs118204021 |
| F21 | P28 | M | + + | L2HGA | Speech Delay | 12 | 180 | L2HGDH | c.164G > A | p.Gly55Asp | Missense | Homozygous | Pathogenic | rs118204021 |
| F22 | P29 | F | + | L2HGA | Seizure, behavioural problems | 30 | 60 | L2HGDH | c.528G > T | p. Glu176Asp | Missense | Homozygous | Pathogenic | rs2029895356 |
| F23 | P30 | M | + | L2HGA | DD | 18 | 19 | L2HGDH | c.738 + 5A > G | | Splice site | Homozygous | VUS | **Novel** |
| F24 | P31 | F | + | L2HGA | Seizure | 12 | 18 | L2HGDH | c.845G > A | p. Arg282Gln | Missense | Homozygous | VUS | rs765312282 |
| F25 | P32 | M | + | L2HGA | Movement disorder, behavioural problems | 84 | 85 | L2HGDH | c.1115delT | p.Met372fs | Frameshift | Homozygous | Pathogenic | rs786200869 |
| F26 | P33 | M | + + | L2HGA | Seizure | 18 | 18 | L2HGDH | c.1115delT | p.Met372fs | Frameshift | Homozygous | Pathogenic | rs786200869 |
| F27 | P34 | M | + + | L2HGA | Family Screening | 56 | 30 | L2HGDH | c.1115delT | p.Met372fs | Frameshift | Homozygous | Pathogenic | rs786200869 |
| | P35 | F | + | L2HGA | Seizure | 48 | 49 | L2HGDH | c.1115delT | p.Met372fs | Frameshift | Homozygous | Pathogenic | rs786200869 |

DD: Developmental Delay, ID: Intellectual disability, GA-I: Glutaric aciduria type 1, L2HGA: L-2 hydroxy glutaric aciduria, NA: Not available.

**Table 2**
The mean age of patients at diagnosis, first neurological findings, diagnostic delay, and clinical findings at last visit.

| | Total | GA I | L2OHGA |
|---|---|---|---|
| The mean age at diagnosis of index patients (month) | 37.88 ± 49.35 (min:4-max:180) | 28.35 ± 41.58 (min:4-max:180) | 55.7 ± 49.13 (min:6-max:180) |
| The mean age at initial neurological findings (month) | 16.39 ± 21.02 (min:1 max:84) | 14 ± 19.95 (min:1-max:84) | 27 ± 24.29 (min:1 max:84)r |
| The mean diagnostic delay (month) | 23.64 ± 47.17 (min:0 max:168) | 15.94 ± 37.66 (min:0 max:168) | 31.6 ± 50.95 (min:0 max:91.5) |
| The mean age at first acute encephalopathic crisis (month) | – | 8.2 ± 11.43 (min:3-max:52) | – |

illness and after delayed start of emergency treatment and subsequently developed movement disorder. WISC-R was performed on 11 GA-I patients (11/25). The mean Intelligence Quotient (IQ) scores of these patients before treatment were 67.3 ± 34.6 for verbal, 65.2 ± 41.3 for performance, and 66.8 ± 37.1 for total. Four patients had no intellectual disability. The mean IQ scores of GA-I patients (10/25) at 6th month of treatment were 68.4 ± 36.1 for verbal, 62.8 ± 32.6 for performance, and 67.8 ± 34.2 for total. During this period, one patient was excluded because of poor condition due to status epilepticus. When the pre- and post-treatment IQ scores of 10 patients were compared, no significant difference was observed. Denver II was performed on 14 GA-I patients. While five (5/14) patients showed normal development, nine patients (9/14) had a variable degree of developmental delay. In total, nine GA-I patients (9/25) had no intellectual disability or developmental delay at last visit. Movement disorders were characterized by dystonia in GA-I patients. Three patients were diagnosed and treated after family screening and six patients were diagnosed and treated before 12 months of age. P10, who presented with dystonia and ataxia at the age of 84 months, was the first individual to be diagnosed with GA-I among the F9 members. Siblings P11 and P12, who were not allowed family screening by parents, were diagnosed after seizure at 12 months and 7 months, respectively. P13 was diagnosed in the presymptomatic period. While P13 and P10 have no intellectual disability or developmental delay, P11 and P12 have poor neurological condition, despite early diagnosis and treatment. Three patients (3/4) who were diagnosed at presymptomatic had normal development.

At the time of diagnosis, 22 patients (22/25) had normal C0 carnitine and three (3/25) had low C0 carnitine. Laboratory findings were presented in Table 3. P21 had normal C0 carnitine (C0:24.14 μmol/L), and C5DC carnitine (C5DC:0.056 μmol/L, $N < 0.40$) at first admission. P21

**Table 3**
Glutaryl carnitine levels of GA-I patients before and after carnitine supplementation.

| | At the time of Diagnosis | | After Carnitine Supplementation | |
|---|---|---|---|---|
| | **C0** (μmol/L) | **C5DC** (μmol/L) | **C0** (μmol/L) | **C5DC** (μmol/L) |
| Normal C0 ($n$ = 21) | 35.2 ± 21.52 [a] (min:14.2-max:72.2) | 2.84 ± 1.37 [a] (min:0.056-max:5.6) | 47.3 ± 19.2 [a] (min:18-max:72.3) | 2.91 ± 1.61[a] (min:0.97-max:5.7) |
| Low C0 ($n$ = 3) | 4.44 ± 0.84* (min:3.63 max:5.6) | 0.42 ± 0.18 [a] (min:0.27-max:0.69) | 28.7 ± 7.78 [a] (min:20.5 max:36) | 2.63 ± 1.56 [a] (min:0.84 -max:4.1) |

C0 N: 8.6–90 μmol/L, C5DC N < 0.40 μmol/L, Normal C0: GA-I patients with normal C0 carnitine levels, Low C0: GA-I patients with low C0 carnitine levels. [a]: Mean.

diagnosed with molecular analysis by family screening. C5DC levels increased to 1.2 μmol/L after carnitine supplementation at the follow-up. However, there was no significant association between C5DC levels and disease severity or encephalopathic crises. Carnitine supplementation was administered to all GA-I patients at an initial dose of 100 mg/kg/day. During the follow-up, the dose varied according to the plasma carnitine levels. Elevated GA and 3-HGA excretion were detected in all GA-I patients. The mean urinary metabolite excretion before and 6th month under treatment is presented in Table 4. Group I, II, III included GA-I patients and Group IV included L2HGA patients.GA-I patients ($n = 25$) had normal plasma lysine, also the mean pre- and post-treatment plasma lysine levels were 149.5 ± 45.6 and 104.34 ± 56.7 μmol/L, respectively. Controlled protein intake was achieved by using natural protein with low lysine content and lysine-free, tryptophan-reduced amino acid supplements for GA-I patients up to 6 years of age. In our cohort, 14 patients (group I) received low-lysine diet and levocarnitine. Treatment was initiated at a mean age of 7.14 ± 4.77 months (min:1 max:16) in group I. Treatment was started in four patients when they were asymptomatic. Mean daily lysine intake was 103% (SD: ±3.1%) of recommendations in group I. After 6 years of age, patients were switched from a low-lysine diet to a protein-controlled diet. 11 patients received protein-controlled diet. 8/11 patients (Group II) received protein-controlled diet and levocarnitine. The mean age at initiated of the treatment was 96.62 ± 31.83 months (min:78 max:180) in group II. 3/11 patients (Group III) with the c.743C > T (p.Pro248Leu) mutation received protein-controlled diet, levocarnitine and riboflavin. The mean age at the initiated of the treatment was 73.66 ± 1.69 months (min:72 max:76) in group III.

In GA-I patients ($n = 25$), the mean pre- and post-treatment urinary GA were detected 669.56 ± 602.78 and 551.07 ± 28.81 mmol/mol creatine, respectively. The mean pre- and post-treatment urinary 3-HGA were detected 75.52 ± 43.93 and 73.83 ± 43.88 mmol/mol creatine, respectively. A decrease was observed in urinary GA levels, but there was no significant difference in urinary 3HGA levels with treatment. When we evaluated according to the treatment groups, a statistically significant decrease was found in the mean pre- and post-treatment urinary GA levels in group I ($p < 0.026$). However no significant difference was found for pre- and post-treatment urinary 3-HGA ($p > 0.05$). When the mean urinary GA and 3-HGA levels for Group II and Group III (one by one) were evaluated, no significant difference was observed with the treatment. The mean pre- and post-treatment urinary GA levels of the patients under protein-controlled diet (11/25, group II-III) were 436.42 ± 318.42 and 396.32 ± 232.62 mmol/mol creatine, respectively. The mean pre- and post-treatment urinary 3-HGA levels of the patients under protein-controlled diet were 56.09 ± 25.76 and 57.36 ± 30.04 mmol/mol creatine, respectively. There were no significant differences between pre and post treatment urinary GA and 3-HGA levels.

**Table 4**
Comparison of urinary metabolites pre- and post-treatment.

| | Before the Treatment | | After the Treatment | |
|---|---|---|---|---|
| | The mean urinary GA (mmol/mol creatinine) | The mean urinary 3-HGA (mmol/mol creatinine) | The mean urinary GA (mmol/mol creatinine) | The mean urinary 3-HGA (mmol/mol creatinine) |
| Group I ($n$ = 17) | 752.15 ± 643.1 [a] (min:154.2 - max:3131) | 73.16 ± 46.10 [a] (min9.9:- max:198) | 597.31 ± 289.41 [a] (min:167- max:976) | 71.76 ± 46.66[a] (min:21- max:224) |
| Group II ($n$ = 5) | 239.4 ± 140.28* (min:125.2- max:437) | 41.9 ± 16.56 (min:28.1- max:65.2) | 214.96 ± 155.4 (min:92.1- max:435) | 55.4 ± 27.14 (min:30.2 -max:93.1) |
| Group III (n = 3) | 443.56 ± 182.18 (min:186- max:578.1) | 68.6 ± 39.4 (min:12.9- max:98) | 428.36 ± 175.79 (min:180- max:562.1) | 72.8 ± 10.38 (min:78.3- max:102.1) |

| | The mean urinary 2-HGA (mmol/mol creatinine) | The mean urinary 2-HGA (mmol/mol creatinine) |
|---|---|---|
| Group IV ($n$ = 10) | 260.86 ± 129.7 (min:98-max:456) | 143.81 ± 61.54 (min:67-max:237) |

3-hydroxyglutaric acid (3-HGA) N: 4.6–29.55 mmol/mol creatinine, Glutaric acid (GA) N:3.8–92.43 mmol/mol creatinine, 2-hydroxyglutaric acid (2-HGA): 5–26.8 mmol/mol creatinine. Group I: The GA-I patients who used lysine restricted diet and levocarnitine. Group II: The GA-I patients older than 6 years of age, who used protein-controlled diet and levocarnitine. Group III: The GA-I patients with the c.743C > T (p.Pro248Leu) mutation who used lysine-restricted diet, levocarnitine, and vitamin B2 combination. Group IV: The L2HGA patients who used levocarnitine and vitamin B2.

In 17 families with GA-I, we detected seven different homozygous variants of the GCDH gene including the novel c.221A > G (p.Tyr74Cys) variant and previously reported c.383G > A (p.Arg128Gln), c.541G > C (p.Glu181Gln), c.743C > T (p.Pro248Leu), c.1018C > T (p.Leu340Phe), c.1204C > T (p.Arg402Trp) and c.1205G > A (p.Arg402Gln) variants. The most common variant in GCDH gene was c.1204C > T (p.Arg402Trp) in our study group. The second common variants were c.1018C > T (p.Leu340Phe) and c.743C > T (p.Pro248Leu). The mean pre-treatment urinary GA levels of patients with c.1204C > T, c.1018C > T and c.743C > T variants were 526.32 ± 359.15, 999.38 ± 912.97 and 443.56 ± 182.18 mmol/mol creatine, respectively. The mean pre-treatment urinary 3HGA levels of the same patients were 71.36 ± 42, 140.22 ± 139.54 and 68.6 ± 39.4 mmol/mol creatine, respectively. The patients with c.1018C > T variant had higher pre-treatment urinary GA and 3HGA levels. However, the patients were in different neurocognitive status. The mean post-treatment urinary GA levels of patients with c.1204C > T, c.1018C > T and c.743C > T variants were 534.51 ± 329.03, 557.97 ± 331.84 and 428.36 ± 175.79 mmol/mol creatine, respectively. The mean post-treatment urinary 3HGA levels of the same patients were 68.66 ± 34.23, 89.18 ± 65.83 and 92.8 ± 10.38 mmol/mol creatine, respectively. When we compared the mean urinary GA and 3HGA levels before and after treatment, there was no significant difference in patients with c.1204C > T and c.743C > T variants, but significant decrease was found in patients with variant c.1018C > T.

The most common neuroradiological findings were cerebral atrophy for GA-I and subcortical white matter changes for L-2HGA. Cranial MRI findings in our cohort were wide Sylvian fissures in 72%, WM involvement in 56%, ventricular dilatation in 48%, cerebral cysts in 8% and subdural hematoma in 4%. Basal ganglia injury was observed in 60% ($n$ = 15) of GA-I patients. 14 patients already had basal ganglia changes at the time of diagnosis. The outcome was poor in patients who were diagnosed after acute encephalopathic crises.

### 3.3. L-2-Hydroxy glutaric aciduria

The neurological findings in L2HGA patients at the time of diagnosis were seizure (3/10), developmental delay (2/10), movement disorders (2/10), behavioural problems (2/10), speech delay (1/10), and tone abnormalities (1/10). No non-neurological findings were detected in L2HGA patients. Additional neurological findings during follow-up were movement disorders (3/10) and behavioural problems (2/10), and epilepsy (2/10). At last visit, L2HGA patients had movement disorders (5/10), epilepsy (2/10), delay of the developmental milestones (4/10) and behavioural problems (4/10). Behavioural problems were more common in L2HGA (5/10) than in GA-I (4/25) patients. Movement disorders were characterized by ataxia in L2HGA. Seizures of nine patients were controlled with one antiepileptic drug, while five patients needed three or more. WISC-R was performed on two L2HGA patients (2/10). Pre-treatment IQ scores of two patients were 67 and 71 for verbal, 62 and 69 for performance, 65 and 70 for total. The post-treatment IQ scores were 69 and 73 for verbal, 67 and 71 for performance, and 68 and 72 for total.

In our cohort, 10 patients with L2HGA (Group IV) received B2 and levocarnitine after the diagnosis. The mean age at initiated of the treatment was 53.36 ± 47.43 months (min:6 max:180). We compared the pre- and post-treatment urinary 2-HGA levels of L-2-HGA patients. There was a significant decrease in urinary 2-HGA with the treatment (Table 4). Relative neurologic improvement was seen in three patients (3/10).

In nine families with L-2-HGA, we detected five different homozygous variants of the L2HGDH gene including the novel c.738 + 5A > G splice variant and previously reported c.164G > A (p.Gly55Asp), c.528G > T (p.Glu176Asp), c.845G > A (p.Arg282Gln), and c.1115delT (p.Met372fs) variants. The most common variants were c.164G > A (p.Gly55Asp) and c.1115delT (p.Met372fs). The mean pre-treatment urinary 2-HGA levels in patients with c.164G > A and c.1115delT were 173.15 + 152.6 and 217.23 + 116.90 mmol/mol creatine, respectively. The mean post-treatment urinary 2-HGA levels of the same patients, were 284.55 + 302.73 and 267.84 + 249.65 mmol/mol creatine, respectively. There was no significant difference between the mean urinary 2HGA levels of c.164G > A and c.1115delT variants. Also, no significant difference was found between pre- and post-treatment mean urinary 2HGA levels. The mutations were summarized in Table 1.

In the same family, there were patients with severe developmental delay despite early diagnosis and treatment and individuals with normal IQ scores. No correlation was found between mutation analyzes of patients without intellectual disabilities. Magnetic resonance imaging (MRI) findings of GA-I and L2HGA patients are presented in Table 5. Diffusion restriction in basal ganglia during AEC (P22) and the L2HGA patient's (P26) MRI are shown in supplementary material. Functional analysis could not be performed on novel variants due to technical incompetence. However, maternal and paternal segregation analyzes were performed and both parents were found to be carriers. According to in silico analysis tools, the pathogenicity score of novel variants was found to be high. Since the biochemical findings of the patients were compatible with the disease, novel variants were considered disease-causing. The limitations of our study were the small sample size, the diet which was calculated according to patients consumptions from diet diaries.

## 4. Discussion

In COA, unlike classical organic acidemias, acute metabolic decompensation with metabolic acidosis, hyperammonemia, high lactate and ketosis is rare. The incidence of COA varies by subtype. We described 25 patients with GA-I and 10 patients with L2HGA. The estimated incidence of the GA-I is 1/30.000–110.000 worldwide [3,4].

The phenotypic spectrum of untreated GA-I is established with infantile-onset (three months-six years) and late-onset (after six years of age) [1,6]. In 80–90% of untreated patients, neurological damage accelerates after an AEC, usually in the first six years of life [12]. Crises are usually triggered by febrile illness and catabolic stress [1,3]. In the literature, the mean age at first symptom varies between 1 and 6 months, and the mean age at first diagnosis varies between 4 and 14 months [1,3,7–11]. In our cohort, while the age of first AEC was consistent with the literature, the mean age at first symptoms was older. This may be due to physicians' lack of awareness about clinical findings. While the early diagnosed patients had better outcome, late diagnosed patients had severe neurologic deterioration. All patients were high excreters (HE) and three patients were late-onset phenotype in our cohort. Macrocephaly (75%) and epilepsy are common but nonspecific [11,14,15]. Consistent with the literature, DD and intellectual disability were the most common clinical findings in our cohort. The frequency of macrocephaly was higher in our cohort than in previous studies in Turkey [7,8].

L2HGA develops insidiously without metabolic decompensation episodes. Consistent with the literature DD, seizure, and behavioural problems were the most common clinical findings in our L2HGA cohort [16]. The development of malignant brain tumors is also known. Macrocephaly was reported at a frequency of 48% in large study groups however it was 18.18% in our group [16]. The most common clinical findings are intellectual disability, movement disorders, epilepsy, and macrocephaly. Movement disorders and behavioural problems (ADHD, oppositional defiant disorder, conduct disorder) may be the first clinical findings in COA. It was most frequently seen in L2HGA. In our cohort, movement disorder was observed in three patients at first admission and increased during follow-up ($n = 12$). It was characterized by ataxia in L2HGA patients, dystonia, and spasticity in GA-I. Behavioural problems were more common in L2HGA.

Two biochemical subtypes, LE and HE, have been identified in GA-I and show a similar clinical course [1,17]. However, a recent study reported that HE patients had worse neurocognitive outcomes than LE [17]. All patients in our cohort were HE. One patient had normal C0 and C5DC levels as well as elevated urinary GA excretion. Patients with normal C5DC levels and normal urinary GA metabolites have been reported in the literature [7,18]. We know that normal C5DC and 3HGA levels don't exclude the diagnosis. Increased urinary 3HGA levels are considered the most reliable diagnostic marker.

The recent guideline for GA-I recommends a low lysine diet up to six years of age, followed by continued dietary therapy using a less strict protocol. Since the late 1990s, lysine-restricted diet with carnitine supplementation and intensified emergency treatment during illness episodes have been associated with optimal neurological outcomes in several studies [6,14,19–21]. The main goal of the metabolic therapy in GA-I is reduction of glutaryl-CoA, GA and 3HGA. Increasing awareness of the disease in countries where newborn screening is not available for GA-I will enable early diagnosis and treatment [4]. In our study group, the mean urinary GA levels were decreased with treatment. When we evaluated according to the treatment groups, a significant decrease was observed in urinary GA levels in the lysine restricted diet and levocarnitine receiving group. However, when we compared the mean pre- and post-treatment IQ scores of GA-I patients, no significant difference was observed. This may be since most patients were diagnosed in the symptomatic period. Pre- and post-treatment IQ scores were not compared according to the treatment groups, due to small sample size. The effectiveness of diet after six years of age has not been systematically evaluated, but continued diet using a less strict protocol is recommended. In our study group, we compared the mean urinary GA and 3HGA levels of the patients on a protein-controlled diet ($n = 8$, Group II and III) pre-treatment and at 6th months of treatment. There was no significant difference ($p > 0.05$). It is difficult to know the reality since the dietary consumption records of the patients were arranged according to the patient's statements. However, the significant decrease in urinary GA levels after treatment in patients on lysine-restricted diet raises the

**Table 5**
MRI Findings of GA-I and L-2-HGA patients.

| MRI Findings | GA-1 (n = 25) | L2HGA ($n = 10$) |
|---|---|---|
| Atrophy | | |
| Cerebral | 60% | 20% |
| Cerebellar | – | 50% |
| White matter signal changes | | |
| Subcortical | 4% | 100% |
| Periventricular | 56% | 10% |
| Deep | 8% | – |
| Internal capsule | 4% | 80% |
| External capsule | – | 80% |
| Wide sylvian fissures | 72% | 30% |
| Wide ventricle | 48% | – |
| Caudate nuclei Inv. | 52% | 60% |
| Putamen Inv. | 52% | 60% |
| Globus Pallidus Inv. | 60% | 80% |
| Dentate nuclei Inv. | 8% | 90% |
| Cerebral Cyst | 8% | 50% |
| Subdural Hematoma | 4% | – |

Inv: Involvement.

question of whether lysine-restricted diet should be continued after six years of age.

Riboflavin supplementation is not recommended currently as standard therapy for GA-I. In the literature, a decrease in urinary GA and 3HGA levels with riboflavin has been reported in affected individuals, but there is no evidence that it improves clinical outcome [19,21]. Chalmers et al. reported that a patient who had S139L and P248L compound heterozygous mutations and 20% residual GCDH enzyme activity, was riboflavin responsive [22]. They reported the patient's urinary GA levels decreased to control values and neurologically normal with riboflavin in combination with lysine restricted diet. Also, the P248L variant is associated with some residual GCDH activity in other patients. In addition, in the p.Val400Met homozygous mutation study, riboflavin was reported to be a driving force for the mutant enzyme [23]. These results provide principal proof for the beneficial effects of riboflavin in GA-I. In our study group, we administered riboflavin in combination with protein-controlled diet and levocarnitine to three patients with the p.Pro248Leu (P248L) variant. There was no statistically significant difference between mean urinary GA and 3HGA levels before and after treatment ($p > 0.05$). When Chalmers et al.'s study was compared with our study, the dietary treatments received were different from each other. Therefore, it has been difficult to understand whether the decrease in urinary GA levels in the literature with P248L mutation is responsive or unresponsive to riboflavin. When the pre- and post-treatment IQ scores were compared, no significant difference was found in our study. There is no standard protocol for how to assess riboflavin sensitivity or no predictor based on GCDH gene mutation analysis [19]. The fact that the patients were diagnosed in the symptomatic period may explain this. 64% of patients have a poor neurologic outcome in our study group. We know that it has been included in newborn screening programs in many countries due to effect of early diagnosis and treatment on mortality and neurocognitive outcome. No randomized controlled trials have demonstrated a significant positive effect of carnitine on clinical outcomes. However, Kölker et al. reported that L-carnitine supplementation contributed to a reduction of striatal injury in early diagnosed patients and reduced mortality rates in symptomatic patients [3].

In our study group, the most common variant in GCDH gene was the c.1204C > T (p.Arg402Trp) missense variant, also reported as the most common in Europe, Brazil, Egypt and Russia. The previously reported c.1018C > T (p.Leu340Phe) and c.743C > T (p.Pro248Leu) mutations ranked as the second frequent mutations in our patients. The c.221A > G (p.Tyr74Cys) variant, found in one patient, was the only novel variant of the GCDH gene. This novel variant is classified as likely pathogenic with the evidence that it is not found in the GnomAD population database (PM2), and computational predictions are pathogenic (PP3). We evaluated the patients' mean urinary metabolites according to the variants. Among the c.1204C > T, c.1018C > T and c.743C > T mutations, the patients with c.1018C > T variant had higher pre-treatment urinary GA and 3HGA levels. Also, significant reduction in urinary metabolites with treatment was detected in patients with c.1018C > T variant. However, the patients had different neurocognitive status.

The most common variants in L2HGDH gene were c.164G > A (p.Gly55Asp) and c.1115delT (p.Met372fs). The c.164G > A mutation in the L2HGDH gene was previously reported only in Turkish patients [9,10]. The novel variant of the L2HGDH gene was the c.738 + 5A > G splice variant. This variant is classified as VUS because of the evidence of extremely low frequency in the GnomAD population database (PM2). Although there are no specific therapeutic approaches in L2HGA, there are studies showing that the use of riboflavin is effective in some patients. Therefore, riboflavin would be expected to increase any residual activity. Samuraki et al. reported a neurologic improvement and a decrease in urinary 2HGA levels with riboflavin and carnitine [24]. Yılmaz et al. reported similar clinical and laboratory findings [25]. When we evaluated the urinary metabolites of the c.164G > A and c.1115delT variants, there was no significant difference between the variants and between the pre- and post-treatment levels. However, in all L2HGA patients, there was a significant decrease in the mean urinary 2HGA with treatment. Relative neurologic improvement was seen in three patients (3/10). Clinical improvement with riboflavin is primarily based on patient functionality and parental observations.

According to the literature, the most characteristic neuroimaging feature of GA-I is widening Sylvian fissure, found in approximately 90% of patients [13]. Restricted diffusion referred to as acute striatal necrosis is observed in GA-I at the AEC. Consistent with the literature, basal ganglia changes were detected with a frequency of 60% in our cohort. Brain MRI findings are very specific for L2HGA disease. Subcortical WM involvement was observed in all L2HGA patients of our cohort. Steenweg et al., reported that the periventricular WM was spared in 41 of the 56 patients with subcortical WM involvement [16]. Consistent with this study, deep WM was spared in our L2HGA patients and periventricular WM involvement was observed in P26.

## 5. Conclusions

Glutaric aciduria type I and L2HGA are the most common cerebral organic acidurias. Early and correct diagnosis is crucial. Poor prognosis based on metabolic crises and progressive deterioration still appears. In countries where newborn screening is not performed, a clinical suspicion index is required for cerebral organic aciduria. Current treatment strategies are based on levocarnitine, vitamin B2 and diet. We reported the evaluation of the effect on urinary metabolites and quality of life with current treatment strategies. GA-I and L-2HGA are difficult to examine by medical evidence standards because of the small sample size, regional differences in newborn screening, and medical care limits. In our study with a limited number of patients, we think that more reliable results will be achieved by large cohorts. More clinical studies are needed to identify effective treatments. We also reported our experience in order to contribute to the literature.

## Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the local Diyarbakir Gazi Yasargil Research and Training Hospital Ethics Committee (Date.1.5.2021/No944).

## Submission declaration and verification

All data not published within this article will be made available by request from any qualified investigator.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## CRediT authorship contribution statement

**Ayse Ergül Bozaci:** Conceptualization, Methodology, Software, Data curation, Writing – original draft. **Esra Er:** Visualization, Investigation. **Aysel Tekmenuray Ünal:** Software, Validation, Writing – review & editing. **İbrahim Taş:** Data curation, Visualization. **Ercan Ayaz:** Data curation, Writing – review & editing. **Mehmet Nuri Ozbek:** Data curation, Visualization, Investigation. **Asude Durmaz:** Data curation. **Ayçe Aykut:** Data curation. **Melis Kose:** Conceptualization, Writing – review & editing, Supervision.

## Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

## Data availability

Data will be made available on request.

## Acknowledgments

We thank all patients and their families who participated in the study for giving their consent.

## References

[1] G.F. Hoffman, S. Kölker, Cerebral organic acid disorders and other disorders of lysine catabolism, in: J.M. Saudubray, M.R. Baumgartner, J. Walter (Eds.), Inborn Metabolic Diseases Diagnosis and Treatment, 6th ed., Springer, Heidelberg, 2016, pp. 333–346.

[2] S. Kölker, E. Mayatepek, G.F. Hoffmann, White matter disease in cerebral organic acid disorders: clinical implications and suggested pathomechanisms, Neuropediatrics 33 (2002) 225–231.

[3] S. Kölker, S.F. Garbade, N. Boy, E.M. Maier, T. Meissner, C. Mühlhausen, et al., Decline of acute encephalopathic crises in children with glutaryl- CoA dehydrogenase deficiency identified by neonatal screening in Germany, Pediatr. Res. 62 (3) (2007) 353–362.

[4] N. Boy, K. Mengler, E. Thimm, K.A. Schiergens, T. Marquardt, N. Weinhold, et al., Newborn screening: a disease-changing intervention for glutaric aciduria type 1, Ann. Neurol. 83 (2018) 970–979.

[5] Z. Patay, J.C. Mills, U. Löbel, A. Lambert, A. Sablauer, D.W. Ellison, Cerebral neoplasms in L-2 hydroxyglutaric aciduria: 3 new cases and meta-analysis of literature data, AJNR Am. J. Neuroradiol. 33 (2012) 940–943.

[6] J. Heringer, S.P. Boy, R. Ensenauer, B. Assmann, J. Zschocke, I. Harting, et al., Use of guidelines improves the outcome in glutaric aciduria type I, Ann. Neurol. 68 (2010) 743–752.

[7] S. Kılavuz, D. Bulut, D. Kor, B. Şeker-Yılmaz, N. Özcan, F. Incecik, et al., The outcome of 41 late-diagnosed Turkish GA-1 patients: a candidate for the Turkish NBS, Neuropediatrics. 52 (2021) 358–369, https://doi.org/10.1055/s-0040-1722691.

[8] B.B. Gürbüz, D.Y. Yılmaz, T. Coşkun, A. Tokatlı, A. Dursun, H.S. Sivri, Glutaric aciduria type 1: genetic and phenotypic spectrum in 53 patients, Eur. J. Med. Genet. 63 (2020), 104032, https://doi.org/10.1016/j.ejmg.2020.104032.

[9] T. Zübarioğlu, C. Yalçınkaya, Ç. Oruç, E. Kıykım, M.S. Cansever, A. Gezdirici, et al., L-2-hidroksiglutarik asidüri hastalarında klinik, nöroradyolojik ve genetik bulguların değerlendirilmesi, Turkish Arch. Pediatr. 55 (2020) 290–298.

[10] M. Topçu, Aydin OF, C. Yalçinkaya, G. Haliloğlu, S. Aysun, B. Anlar, et al., L-2-hydroxyglutaric aciduria: a report of 29 patients, Turk. J. Pediatr. 47 (2005) 1–7.

[11] J. Heringer, S.P. Boy, R. Ensenauer, B. Assmann, J. Zschocke, I. Harting, et al., Use of guidelines improves the neurological outcome in glutaric aciduria type I, Ann. Neurol. 68 (2010) 743–752, https://doi.org/10.1002/ana.22095.

[12] S. Kölker, A. Garcia-Cazorla, V. Valayannopoulos, A.M. Lund, A.B. Burlina, J. Sykut-Cegielska, et al., The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation, J. Inherit. Metab. Dis. 38 (2015) 1041–1057.

[13] E. Huishi, L. Liang, H. Zhang, W. Qiu, J. Ye, F. Xu, et al., Evaluation of the clinical, biochemical, neurological, and genetic presentations of Glutaric aciduria type 1 in patients from China, Front. Genet. 7 (12) (2021 Jul) 702374, https://doi.org/10.3389/fgene.2021.702374.

[14] V.M. McClelland, D.B. Bakalinova, C. Hendriksz, R.P. Singh, Glutaric aciduria type 1 presenting with epilepsy, Dev. Med. Child Neurol. 51 (3) (2009) 235–239, https://doi.org/10.1111/j.1469-8749.2008.03240.x.

[15] L. Healy, M. O'Shea, J. McNulty, G. King, E. Twomey, E. Treacy, et al., Glutaric aciduria type 1: diagnosis, clinical features and long-term outcome in a large cohort of 34 Irish patients, JIMD Rep. 63 (4) (2022 Jun 14) 379–387, https://doi.org/10.1002/jmd2.12302.

[16] M.E. Steenweg, G.S. Salomons, Z. Yapici, G. Uziel, E. Scalais, D.I. Zafeiriou, et al., L-2-Hydroxyglutaric aciduria:pattern of MR imaging abnormalities in 56 patients, Radiology. 251 (3) (2009) 856–865.

[17] E.M.C. Märtner, E. Thimm, P. Guder, K.A. Schiergens, F. Rutsch, S. Roloff, et al., Publisher correction: the biochemical subtype is a predictor for cognitive function in glutaric aciduria type 1: a national prospective follow-up study, Sci. Rep. 11 (1) (2021), https://doi.org/10.1038/s41598-021-00137-5, 20618. 12.

[18] E. Canda, H. Yazıcı, E. Er, C. Eraslan, Y. Atik Altınok, H.M. Serin, et al., Glutaric aciduria type I diagnosis case with normal glutaryl carnitine and urine organic acid analysis, J. Pediatr. Res. 5 (2018) 60–62.

[19] N. Boy, C. Mühlhausen, E.M. Maier, J. Heringer, B. Assmann, P. Burgard, et al., Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision, J. Inherit. Metab. Dis. 40 (2017) 75–101.

[20] K. Viau, S.L. Ernst, R.J. Vanzo, L.D. Botto, M. Pasquali, Longo N Glutaric acidemia type 1: outcomes before and after expanded newborn screening, Mol. Genet. Metab. 106 (4) (2012) 430–438.

[21] K.A. Strauss, K.B. Williams, V.J. Carson, L. Poskitt, L.E. Bowser, M. Young, et al., Glutaric acidemia type 1: treatment and outcome of 168 patients over three decades, Mol. Genet. Metab. 131 (3) (2020 Nov) 325–340, https://doi.org/10.1016/j.ymgme.2020.09.007.

[22] R.A. Chalmers, M.D. Bain, J. Zschocke, Riboflavin-responsive glutaryl CoA dehydrogenase deficiency, Mol. Genet. Metab. 88 (1) (2006 May) 29–37, https://doi.org/10.1016/j.ymgme.2005.11.007 (PMID: 16377226).

[23] J.V. Ribeiro, C.M. Gomes, B.J. Henriques, Functional recovery of a GCDH variant associated to severe Deflavinylation-molecular insights into potential beneficial effects of riboflavin supplementation in Glutaric aciduria-type I patients, Int. J. Mol. Sci. 21 (19) (2020 Sep 25) 7063, https://doi.org/10.3390/ijms21197063.

[24] M. Samuraki, K. Komai, Y. Hasegawa, M. Kimura, S. Yamaguchi, N. Terada, M. Yamada, A successfully treated adult patient with L-2-hydroxyglutaric aciduria, Neurology. 70 (13) (2008 Mar 25) 1051–1052, https://doi.org/10.1212/01.wnl.0000287141.90944.95.

[25] K. Yilmaz, Riboflavin treatment in a case with l-2-hydroxyglutaric aciduria, Eur. J. Paediatr. Neurol. 13 (1) (2009 Jan) 57–60, https://doi.org/10.1016/j.ejpn.2008.01.003. Epub 2008 Mar 17. PMID: 18343698.