An Inherited Metabolic Disease Being Candidate for The Turkish Newborn Screening Programme: Outcome of 41 Late-Diagnosed GA-1 Patients

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Keyword:	Glutaric aciduria type 1, acute encephalopathic crises, newborn screening program, dystonia
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Abstract:	**Background:**Glutaric aciduria type 1 (GA-1) is an inherited cerebral organic aciduria caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH). Untreated patients with GA-1 have a risk of acute encephalopathic crises during the first six years of life precipitated by catabolic processes. The outcome is better in countries where patients were diagnosed with newborn screening program (NBS) and put on a diet before the occurrence of an encephalopathic crisis. In so far as GA-1 desperately doesn't exist in Turkish NBS, most patients in our study were late-diagnosed. **Method:** This study included 41 patients diagnosed with acylcarnitine profile, urinary organic acids and mutation analyses in the symptomatic period. We presented clinical, neuroradiological and molecular data of our 41 patients. **Results:** The mean age at diagnosis was 14,8±13,9 (15 days-72 months), and the current age was 57,2± 43,3 (10-151 months). High blood C5DC and urinary glutaric acid levels in 41 patients were revealed. Seventeen different mutations in the GCDH gene were identified, 6 of which were novel. The patients, most of whom were late-diagnosed, had a poor neurological outcome. Treatment strategies made a little improvement in dystonia and frequency of encephalopathic attacks. **Conclusion:** All GA-1 patients in our study were severely affected since they were late-diagnosed, while others show that GA-1 is a treatable

	metabolic disorder if it is diagnosed with NBS. This study provides an essential perspective of the severe impact on GA-1 patients unless it is diagnosed with NBS. We immediately advocate GA-1 to be included in the Turkish NBS.

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ABSTRACT

Background: Glutaric aciduria type 1 (GA-1) is an inherited cerebral organic aciduria caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH). Untreated patients with GA-1 have a risk of acute encephalopathic crises during the first six years of life precipitated by catabolic processes. The outcome is better in countries where patients were diagnosed with newborn screening program (NBS) and put on a diet before the occurrence of an encephalopathic crisis. In so far as GA-1 desperately does not exist in Turkish NBS, most patients in our study were late-diagnosed.

Method: This study included 41 patients diagnosed with acylcarnitine profile, urinary organic acids, and mutation analyses in the symptomatic period. We presented clinical, neuroradiological, and molecular data of our 41 patients.

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Conclusion: All GA-1 patients in our study were severely affected since they were late-diagnosed, while others show that GA-1 is a treatable metabolic disorder if it is diagnosed with NBS. This study provides an essential perspective of the severe impact on GA-1 patients unless it is diagnosed with NBS. We immediately advocate GA-1 to be included in the Turkish NBS.

INTRODUCTİON:

Glutaric aciduria type 1 (GA-1, OMIM no. 231670) is an autosomal recessive disorder caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH) which is a riboflavin-dependent enzyme and converts glutaryl-CoA to crotonyl-CoA. GCDH is also a critical enzyme in the degradative pathways of L-lysine, L-hydroxylysine, and L-tryptophan. The estimated worldwide incidence is 1/110000, whereas in some high-risk populations, this can be increased to a ratio of 1 in 300 newborns. Patients usually experience encephalopathic crises in the first six years of life. As of yet, 244 disease-causing mutations in the GCDH gene have been reported, and are listed in the Human Gene Mutation Database (HGMD). GA-1 resulted from an accumulation of glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA), glutaconic acid, and glutarylcarnitine (C5DC) in body fluids such as plasma, urine, and cerebrospinal fluid. GA-1 is randomly classified into two biochemical subtypes because of urinary metabolite excretion. The former is a low excretory type that might have residual enzyme activity, and the latter is a high excretory type (1).

Dystonic movement disorder due to striatal injury will manifest after an acute encephalopathic crisis, usually in the first two years of life (between 3 and 24 months of age). Generally, these crises are characterized by acute bilateral striatal necrosis and movement disorders signifying a life-threatening disorder. Especially, dystonia is the primary symptom, together with spasticity (2). The neurologic disease has been shown in 90% of patients following an acute encephalopathic crisis, with the remaining 10 % without any crises (3). GA-1 is also considered as cerebral organic aciduria in which kidneys and the peripheral nervous system might also be involved (4, 5). Macrocephaly is a frequent symptom of presentation during infancy. However, macrocephaly is a nonspecific finding, and it is not realized as a constant finding of GA-1 by many clinicians (6). Muscular hypotonia and developmental motor delay occur in more than 50% of patients before eight months of age (7).

Life expectancy is relatively decreased in symptomatic and, likewise, late-diagnosed patients (3). If a special diet and L-carnitine supplementation start before the onset of neurological findings, encephalopathic crises may be prevented in most patients (8). Emergency treatment procedures during catabolic processes, considerably reduce the frequency and severity of acute encephalopathic crises from 80–90% to 10–20%, thereby morbidity and mortality (9, 10). So GA-I is now defined to be a treatable condition. The long-term management of GA-1 includes diet therapy using lysine free, and tryptophan reduced medical formulas, levocarnitine supplementation at 100 mg/kg/d that is increased up to 200 mg/kg/d when patients suffer from an acute crisis and riboflavin during maintenance treatment. Replenishing secondary carnitine deficiency reduces mortality by preventing the progression of neurological distortion (3, 7, 8). Education of parents about treatment protocols for catabolic processes is an essential topic to prevent acute crises, which are followed by neurological disabilities. Dietary treatment is relaxed at six-years of age, while lifelong carnitine supplementation is recommended. However, the GA-1 diet strategy has not been precisely determined yet for after six years of age (11). It is also reported that some neurodevelopmental deficits are detected despite the early treatment due to the restriction of tryptophan intake, which may lead to neurological dysfunction, sleep disturbances, and irritability by the subsequent depletion of serotonin (12).

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The principal aim of this single-center study is to recommend the inclusion of GA-1 into the Turkish NBS program in order to prevent the undesirable neurological outcome, which was clearly demonstrated with 41 late-diagnosed patients. This report is also one of the first data about Turkish GA-1 patients.

METHODS:

This study was approved by the Çukurova University Ethical Committee of Adana-Turkey. Demographic, clinical, laboratory and radiological data were collected from patient charts retrospectively. Informed consent was obtained from all patients. This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review board. We have read and understood the journal's policies, and we believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare.

Following the diagnosis, patients were treated according to guideline recommendations, which include lysine-free, tryptophan reduced, arginine-containing amino acid supplements together with 100 mg/day riboflavin and 100 mg/kg/day L-carnitine that was increased up to 200 mg/kg during encephalopathic crises. Patients older than six-years-old were given a less restrictive diet (12). Benzodiazepines, antiepileptic drugs, GABA agonists were used for neurological symptoms. Carbohydrate-enriched, protein-restricted protocol was used during catabolic episodes. The follow-up period was 1-2 months until twelve months of age, every two-three months from 12 months to 6 years of age and every six months afterward. Laboratory tests, i.e., plasma amino acids, free and total carnitine levels, were evaluated in each clinical visit. Complete blood count, albumin, ferritin, and vitamin levels were checked during the follow-up period.

Gene sequence analysis was performed by using MiSeq next-generation sequencing (NGS) platform and an FDA approved diagnostic system (Illumina, San Diego, CA, USA). All coding exons and their flanking splice site junctions were amplified using PCR primers, designed with PRIMER©. PCRs were validated by using agarose gel electrophoresis. After PCR amplification, the libraries were prepared with the NexteraXT kit (Illumina Inc.), according to the manufacturer's instructions. Next-gene sequencing was carried on MiSeq (Illumina Inc.). Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc.). Visualization of the data was performed with IGV 2.3 (Broad Institute) software.

Data were analyzed using the software, "SPSS for Windows 22". Descriptive statistics were expressed as mean ± standard deviation or median (minimum-maximum) for discontinuous numeric variables, and categorical variables were expressed as case number and percentage. Means of groups were compared using the Mann-Whitney test. Significance was recognized when $p<0.05$.

RESULTS

The study included 41 (24 males, 17 females) patients who were referred to Çukurova University, Department of Pediatric Metabolism and Nutrition with suspicion of inherited metabolic disease. The complaints of patients were seizures (17 patients; 41,46%), neuromotor retardation (9 patients; 21,9%, owing to head trauma in 3 of them), macrocephaly (7 patients; 17%), and hypotonia (4 patients; 9,7%) at the first clinical visit. Although the age of recognizing the first symptoms by the parents was 6,6 ± 3,1 (1,5-15) months, the mean age at diagnosis was 14,8 ± 13,9 months (15 days-72 months). As the Turkish newborn screening program does not include GA-1, only two patients were diagnosed in the 7th day of life owing to family history. The oldest patient was diagnosed at 9,5 years of age during the etiological investigation of ataxia. Four patients had severe respiratory distress syndrome history, which required hospitalization in the newborn intensive care unit. All patients were symptomatic and had at least one encephalopathic crisis on admission. Thirty-five (89,7%) patients had parental consanguinity, ten patients were siblings, and two were second-degree cousins.

At initial physical examination; Seven patients (17%) had growth retardation and malnutrition, and fourteen (34%) had macrocephaly. Interestingly one patient was microcephalic. The age of diagnosis was significantly different between the macrocephalic (9,1± 6,93) and normocephalic (24±27,6) groups ($p<0,05$). Additionally, one patient had central hypothyroidism, and three patients had nephrocalcinosis. We did not detect any peripheral neuropathy. The first crisis, which was triggered by febrile infections and ended with movement disorders, mostly occurred by three months of age, and disappeared after the age of six, although one patient had the last crisis at 11 years of age. The clinical features of the patients were summarized in Table 1.

Mean C5DC level was 1,2 ± 0,95 µmol/l (0,36-4,23), mean total carnitine level was 21,4± 17,7 µmol/L (2,3-60,7) and free carnitine level was 12,7 ± 12,3µmol/L (1,59-39,6). Most patients had carnitine deficiency. Nevertheless, there was not any significant relation between the age of first attack and plasma free carnitine and C5DC levels.

Cranial MRI performed in 34 patients at the first clinical visit and revealed frontotemporal atrophy in 34, ventricular dilatation in 26, white matter changes in 23, basal ganglia changes in 20, cerebral arachnoid cysts in 19, subdural hematoma in 18, and delayed myelination in 18 patients. The affected basal ganglia lesions were globus pallidus (n=15 patients, 44%), putamen (n=15 patients, 44%), caudate nucleus (n=12 patients, 35%), and lentiform nucleus (n=11 patients, 32,4%), respectively. The incidence of basal ganglia involvement was 49 %. The MRI findings of the patients were summarized in Table 1.

The diagnosis was confirmed by mutation analysis (n=39). Seventeen different mutations were identified in 39 patients, and 5 of them were novel. A total of six patients had novel mutations. These included fourteen missense mutations, two frameshift mutations, and a nonsense change. Homozygosity was found in 37 (78,9 %) patients. Two patients had compound heterozygous mutations. The mutations were summarized in Table 2.

In the last visit; axial hypotonia in 38 (92%), peripheral spasticity in 33 (%80), recurrent seizures in 28 (68,3%), and dystonia were present in 25 (61%) patients. Seizures of 19 patients were controlled with one antiepileptic drug (AED); however, nine patients needed three or more AEDs. Two out of 41 patients (%4,8) died because of pneumonia at the age of two and three.

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CASE PRESENTATİONS:

The onset of clinical symptoms in P1, P2, and P3 were 4, 5, and 10 months, respectively. Although P1 had the earliest onset of symptoms, clinical findings were slightly milder than the others. Macrocephaly was severely evident in P2 and P3. Additionally, cysts, renal atrophy, and nephrolithiasis in the right and hydroureteronephrosis and nephrolithiasis were detected in the left kidney of P2.

Symptoms of P4, P5, and P6 started when they were 15, 8, and 6 months of age, respectively. P4 and P6 had milder symptoms than P5, who was presented with epilepsy, spasticity, axial hypotonia, and dystonia.

P7 and P8 were siblings. The diagnosis age was seven days and two months. P8 had macrocephaly, severe axial hypotonia, and peripheral spasticity, even the diet and carnitine supplementation were initiated at the age of 2 months. On follow-up, he suffered from head trauma at the age of 4,5 months, hospitalized in the intensive care unit with a subdural hematoma, cerebral shift, and a ventriculoperitoneal shunt was implanted. Afterward, left hemiparesis and dystonia became evident. Although P7 was diagnosed when he was seven days old, his neuromotor milestones were lost. Both siblings also had nephrocalcinosis. Unfortunately, P7 died because of pneumonia.

P9, P10, P11 were severely affected cases. Although P9 and P10 were cousins, P10 was diagnosed after clinical symptoms were started. P11 had uncontrolled seizures and dystonia.

P12 showed neuroregression after acute gastroenteritis at seven months of age, and encephalopathic crises occurred at least once in a year on follow-up. His macrocephaly was prominent, and he was currently bedridden. P13 had the same mutation and similar clinical findings.

Siblings P14 and P15 were diagnosed with GA-1 and had a novel mutation. The current age of P15 was 11 years and had a better outcome than her sister in terms of school performance and motor skills, although the age of diagnosis was older than P14. She had only mild ataxic gait. The first child of the family was investigated for macrocephaly, psychomotor regression, and seizures at nine months old and unfortunately died at three years of age. As he had dysmyelination and frontotemporal atrophy on cranial MRI, Canavan disease was suspected. The phenotypic heterogeneity, even in the same family, could easily be seen.

The [Pro248Leu];[Pro248Leu] homozygous mutation was detected in 6 patients (P16, P17, P18, P19, P20, and P21) who had moderate to severe neurological involvement.

P22 and P23 were siblings, and P22 was diagnosed with GA-1 at four months of age. Currently, he had no seizures, could walk and speak. Another sibling who was diagnosed at eight months of age had more severe symptoms than P22.

P24 and P25 were siblings. P25 had been diagnosed at 72 months of age, owing to seizures. She was put on a lysine- restricted diet and L-carnitine treatment. Although deep white matter, periventricular, globus pallidus, lentiform nucleus, and corpus callosum diffusion restriction with signal changes were detected on cranial MRI, her school performance was average. Despite the fact that P26 had the same mutation and earlier diagnosis, he had psychomotor retardation, relative macrocephaly, severe dystonia, and spasticity.

P27 and P28 were siblings. Bilateral frontotemporal atrophy and white matter involvement were detected on cranial MRI.

P29 and P30 were Syrian refugees and severely affected.

The first encephalopathic attack of P31 occurred at 15 months of age, and she was able to walk with support, write, and read.

P32 was first misdiagnosed as having thiamin-biotin-responsive basal ganglia disease in terms of seizures, dystonia, and basal ganglia involvement on cranial MRI. Also, his metabolic investigations were not compatible with GA-1. However, he had a subdural hematoma after a head trauma at six months of age, and the clinical findings had started immediately after this event. When the patient was admitted to our department at 20 months of age with a slight increase in C5DC (0,78 μmol/l) level, GA-1 was the suspected diagnosis. His C5DC levels were in normal ranges several times before.

P33 was investigated for neuromotor retardation following a head trauma at the age of 12 months. After the initiation of diet and L-carnitine supplementation, the patient could smile, sit, and crawl at 15 months of age.

P34 and P35 were 15 months and ten months of age at the time of diagnosis; the former was severely affected. P34 was investigated for seizures and neuromotor retardation in different hospitals. Even though they had the same mutation, P34 had more severe neurological disabilities such as swallowing difficulties and spasticity when compared with others. Despite only a short time difference between the diagnosis ages, the clinical outcome tends to reveal heterogeneity in this group.

P37 had a head trauma at two months of age. Although she had mental-motor retardation together with frontotemporal atrophy and subdural hematoma in cranial MRI, GA-1 could not be suspected until she was eight months old. She was suffered from seizures when she was 14 months old.

P38 was diagnosed as GA-1 when he was investigating for psychomotor retardation at nine months of age. He was currently ataxic and could walk with support. His sisters, who were monozygotic twins, died when they were 2,5 and 3 years old. They had psychomotor retardation, which was erroneously attributed to premature birth and dystonia following infection before passing away.

P39 was diagnosed at two years old. He had extremely severe dystonia and drug-resistant seizures. Moreover, biting of tongue and lips resulted in painful episodes during encephalopathic crises.

P40 and P41 were diagnosed with clinical and laboratory results without a genetic confirmation. Their urinary organic acid analysis showed elevated GA and 3-OH-GA, together with elevated plasma C5DC levels. P40 had severe spasticity of which required baclofen. Seizures and motor retardation of p40 were detected at six months of age, although her brother had the same disease. The patient died at four years of age. Interestingly, the first symptoms of her brother had started after three years of age following an upper respiratory tract infection. He was currently 23 years old and using a wheelchair.

DİSCUSSİON:

Glutaric aciduria type 1 (GA-1) is an inherited cerebral organic aciduria in which late-diagnosed patients have a huge risk of recurrent encephalopathic crises that result in mortality or undesirable neurological sequels. The outcome is better in countries where patients are diagnosed with newborn screening program and put on a diet before the occurrence of an encephalopathic attack. NBS for GA-I has begun in 1999 in Germany and then hold

into nationwide in 2005 (13) American College of Medical Genetics recommends GA-I as a candidate disease for NBS (14). Kölker et al. demonstrate that outcome of GA-1 is certainly connected to the age of symptom onset, the severity of encephalopathic crises that affects brain development, the genotype, the compliance to restricted lysine diet, and L-carnitine supplementation in a natural history study. Nevertheless, all of these should be neuroprotective when the patients can be diagnosed pre-symptomatically (3). The diagnosis age of our patients and the initiation of a restricted lysine diet and L-carnitine supplementation was approximately six months. A big majority of them had at least one encephalopathic attack before diagnosis. This reflects presumably the time of the injury, i.e., associated with perinatal onset in a previous study (15). Another prospective follow-up study put forth that compliance to diet and especially to an early or preventive start of emergency treatment is crucial for the prevention of irreversible neurological sequelae (13). The severity of clinical symptoms generally seems to be related to the development of encephalopathic crises rather than the GA-I genotype or biochemical phenotype. We also partially observed the unfavorable effect of treatment cessation in two early diagnosed siblings who had not completely normal neurological development. Probably, multifactorial impacts on GA-1 patients tend to cause different clinical symptoms. Encephalopathic crises triggered by catabolic episodes (e.g., febrile illness) result in irreversible bilateral striatal injury following that a complex movement disorder. Life expectancy significantly reduces in these patients. Generalized dystonia, dysarthria, axial hypotonia, swallowing difficulties are the main findings of severely affected patients. Hypertonicity becomes more evident with age, and dystonia combined with akinetic-rigid parkinsonism could be seen. Choreatic movements have also been demonstrated in a small group of individuals. Subtle findings such as deficits in speech development and fine motor skills must alert clinicians about GA-1 (16). Should clinicians realize mild axial hypotonia and macrocephaly in the newborn period, GA-1 patients will most likely not miss out (3, 13).

Although it seems to be crucial to detect elevated C5DC and 3-OH-glutaric acid levels in the diagnosis of GA-1 patients, Canda et al. reported a case that shows completely normal C5DC and 3-hydroxy glutaric acid levels with a compound heterozygous p.Tyr123Cys (c.368A> G) /p. Leu340Phe (c.368A> G) mutation in GCDH gene (17). Our study also showed that normal C5DC and 3-hydroxyglutaric acid levels, as in P31, do not exclude the diagnosis of GA-1. So, diagnosis is difficult in low excretor patients, as demonstrated in previous reports (3, 18). Postmortem observations realized that GA and 3-OH-GA concentrations are quite higher in brain tissue than plasma, which indicates a nearly similar risk of neurological impairment in both low and high excretor groups (3, 18). The homozygous p.Glu365Lys mutation is defined as a low excretor, and patients with this mutation manifest fewer neurological disabilities compared with high excretors. Our three patients with the same mutation exhibited a neurological improvement with treatment even though this mutation is also reported with a poor prognosis in another case (19).

Lysine-restricted dietary treatment and L-carnitine supplementation have a significant neuroprotective impact on pre-symptomatically diagnosed patients (3). Except for a few siblings, all of our patients were late-diagnosed and severely affected. Even though neurological disabilities cannot be completely reversed after the onset of symptoms, dietary treatment is beneficial in slowing down neurological deterioration and partially improving the clinical findings. Patient 33 literally demonstrated satisfactory progress in speech and motor functions with treatment, although he had presented with moderate extrapyramidal and neurological symptoms.

Riboflavin supplementation was reported as having a limited effect on GA-1 patients. A compound heterozygous p.Pro248Leu/p.Ser139Leu mutation was reported as being riboflavin responsive in one study (20). We initiated

B2 treatment with a dosage of 100 mg to every patient, and one from three patients with homozygous p.Pro248Leu mutation showed neurological improvement. Additionally, two patients who have compound heterozygous p.Pro248Leu/p.Arg402Trp mutation also demonstrated a little improvement in clinical findings. Future studies are conducted to clarify these results.

In previous studies, it has been demonstrated that approximately 80-90% of individuals diagnosed in the newborn period by NBS and treated properly are completely asymptomatic. (3, 9, 13, 21-23). The neurological outcome of NBS (n=38) group had been compared with the historical cohort (n=62) group in a study and demonstrated that encephalopathic crises were prevented (89%) in the NBS group (10). Heringer et al. pointed out that complete adherence to treatment guidelines lead to mild movement disorder in 2 of 37 patients (5%) and nonadherence to basic treatment (carnitine and low-lysine) resulted in 44% of mild chronic movement disorder whereas nonadherence to emergency treatment resulted in 100% of severe chronic movement disorder with secondary complications. (13). We highlight that emergency treatment and intensive care management, together with timely diagnosis is likely to prevent the devastating outcome of neurologic crises. So, diagnosing patients in the newborn period seems extremely important to prevent early damage in the neurological system. After the onset of neurologic crises, treatment efficacy significantly decreases in GA-1 patients (24).

We assume that low diet compliance and additionally delay in the management of acute crises due to unawareness of families about the alerting signs generally deteriorate neurological symptoms in late-diagnosed patients. All of our patients suffered from neurological problems (ranging from mild to a bedridden status) because the diagnosis was mostly made owing to an acute encephalopathic crisis. We continued a strict diet to P39 whose symptoms had started at 2,5 years of age and had severe encephalopathic crises. However, only a little improvement was observed. Additionally, a few other late-diagnosed patients with mild phenotype manifested a partial recovery in terms of the motor function and speech in our study. Even though the diet and carnitine supplementation were started to seven days old P7, neurological involvement and cerebral MRI findings could not be prevented. Although P7, P10, and P28 had diagnosed much earlier than their siblings, their clinical symptoms worsened rapidly and severely. Our study clearly exhibited that siblings present with phenotypic heterogeneity in terms of clinical severity, symptom onset, and neurological symptoms. So, diet compliance, genotype, enzyme level, and some other unknown genetic and epigenetic factors might have an influence on the prognosis.

On the whole, we revealed severe neurological disabilities and diminished life expectancy in our cohort when compared with other case series. The most common clinical complications were epilepsy 28 (68%) and movement disorders 25 (61%). Dystonia frequency in our study group was 61% (25 patients). Our patients did not optimally benefit from baclofen and diazepam, which are used as centrally acting muscle relaxants. Although macrocephaly was reported as a nonspecific finding, 34 % of our patients had macrocephaly. Subdural hemorrhage is caused by the disruption of elongated bridging veins and minor head traumas. It peaked when the maximal extent of macrocephaly became evident (7, 24, 25). The diagnosis age of our patients with subdural hemorrhage (n=18 patients) is (mean ± SDS): 13 ± 11,3 (7 days-44) months and six patients (36,8%) had macrocephaly in this group. There was no significant difference between subdural hemorrhage (+) and subdural hemorrhage (-) groups related with macrocephaly. Head trauma was a triggering factor of subdural hemorrhage in our P5.

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Bitemporal arachnoid cysts were described to be an indicator of GCDH deficiency. Indeed, its prevalence is 56 % in our study. Nineteen out of 34 patients (56 %) who were scanned for MRI, had arachnoid cysts. There have been several poor outcomes because those cysts were treated surgically without knowledge about the diagnosis and thus missing care for the anabolic state of the patient all the time (26). MRI findings comprise chronic white matter abnormalities, deep grey matter injury, delay in myelination, and basal ganglia involvement. Putaminal atrophy and dilated ventricles have been demonstrated as the most reliable predictors for movement disorders. Severe movement disorders have been mostly seen with cortical atrophy; however, it is not a distinctive finding (23). Cranial MRI findings in our patients (n=34) at first clinical visit were frontotemporal atrophy 100 % (n=34), ventricular dilatation 76 % (n=26), white matter changes 68% (n=23), cerebral arachnoid cysts 56 % (n=19), basal ganglia changes 56 % (n=19), subdural hematoma 53% (n=18), and delayed myelination 50% (n=17). Cortical atrophy incidence in our patients was 18%. Neither in our study, nor Garbade et al's, was it found that globus pallidus signal changes and cortical atrophy are specific findings for predicting movement disorders.

The mutational analysis results of our study showed substantial differences from the literature. The most common mutations in our cohort were p.Pro248Leu in 8 patients, p.Arg402Trp in 5 patients, p.Leu340Phe in 5 patients, and p.Glu365Lys in 4 patients, respectively. Even though p.Arg402Trp is reported as being with a relatively high incidence in the European and Russian population and Caucasians (9, 27), we found it as the second most common mutation in our study. This mutation shows a clinical variability in the literature in terms of mild and severe phenotypes such as our patients (18, 28). p.Pro248Leu, and p.Leu340Phe were the most common mutations in our patient group. The former was defined as a severe, mild, and riboflavin responsive type; the latter was defined in only one Turkish patient before (28, 29). The severity of the clinical phenotype depends on the development of encephalopathic crises (30). To date, only a small correlation has been determined between the genotype and clinical or biochemical phenotype in GA-1 patients, as it is clearly demonstrated in our study group (18, 31). p.Glu365Lys mutation was shown with a severe phenotype, dystonic cerebral palsy, and late-onset GA-1 with headache and vertigo in different studies. Similarly, we saw from mild to severe clinical variability in our patients with the same mutation (19, 32, 33).

If patients are not diagnosed in earlier stages, most of them will come to the emergency department between 6 and 18 months of age with a brain injury. Several reasons for the misdiagnosis and delay of GA-1 are low awareness, investigations of child abuse due to the subdural hemorrhage, and similarity with more common pediatric conditions. The outcome will improve with the aggressive management of common childhood infections that trigger catabolism. (34). Clinicians should be aware of acute encephalopathic crises during any febrile illness and febrile reactions due to vaccinations and surgery, especially between 0-6 years of age. Repeated vomiting, diarrhea, reduced nutrient intake, new or severe neurological symptoms, i.e., dystonia, muscular hypotonia, irritability, spasticity, rigor, chorea, tremor, and reduced consciousness are the alarming symptoms for the family and pediatrician (7) If these alarming symptoms occur, patients should urgently be treated at the closest hospital or metabolic center. The pediatricians immediately should start emergency treatment, which includes high energy

intake, low or no natural protein intake for 24-48h, and levocarnitine treatment. Emergency treatment should always be supervised by the metabolic center and start immediately. In condition, the individual is clinically well with body temperature <38.5 °C (101 °F) and no alarming symptoms, an oral emergency treatment in an outpatient setting might be recommended to the family with tight monitoring and prompt presentation to the hospital in case of deterioration (9, 13). Close contact with a metabolic center is essential.

Conclusion: To the best of our knowledge, this is the first report of Turkish patients diagnosed with GA1. Unfortunately, the last clinical status of our patients: 25; severely affected, 10; bedridden, and 2; died due to severe pneumonia. Dietary treatment and L-carnitine supplementation after being symptomatic did not reveal adequate improvement in the neurological functions of our patients. So with the highlights of this study, we strongly recommend diagnosing GA-1 patients by newborn screening program for a better outcome. Knowing that GA-1 is a treatable disorder, it is necessary to include GA-1 in expanded Turkish NBS. Time is that wherein there is an opportunity, and opportunity is that wherein there is no great time. Healing is a matter of time, but it is also a matter of opportunity (Hippocrates, Epidemics).

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Footnotes

Ethics

Ethics Committee Approval: The study was approved by the Ethics in Research Committee of Çukurova University Faculty of Medicine, Adana, Turkey (approval number: 2019/85-58).

Informed Consent: Consent form was filled out by all participants.

Authorship Contributions

Surgical and Medical Practices: N.Ö.M., S.K, B.Ş.Y., Concept: S.K., N.Ö.M.,F.D.B, Design: S.K.,D.K, Data Collection and Processing: N.Ö.M., S.K.,F.İ, N.Ö, B.O Analysis and Interpretation: N.Ö.M., S.K, G.C., Literature Search: F.D.B., S.K., D.K, Writing: S.K,

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Table I. Clinical and MRI Findings of Glutaric Aciduria Type 1 Patients

THE LAST CLINICAL VISIT												MRI FINDINGS												
Patient	Gender (Nationality)	Cause of Selective Screening	Crisis Onset of Age (Months)	Age at diagnosis (Months)	Macrocephaly	Hypotonia	Dystonia	Spasticity	PMR	Seizure	Date of MRI (Months)	Subdural Hematoma	FTA	Caudate Nucleus	Putamen	Globus Pallidus	Lentiform Nucleus	Supratentorial White Matter	Delayed Myelination	Ventricular Dilatation	Cerebral Cyst	Centrum Semiovale	Current Age at Last Visit (Months)	Clinical Status at Last Visit
p1	F(T)	Seizure	4	12	+	+	+	+	+	+	13	-	+	+	+	-	-	+	+	-	-	-	24	Smiling, sitting with support
p2	M(S)	Hypotonia	5	7	-	+	+	+	+	+	18	+	+	-	-	-	-	-	-	+	+		33	Severely affected
p3	M(T)	Macrocephaly	10	9	+	+	+	+	+	+	84	-	+	+	+	+	+	+	-	+	+	-	98	Severely affected, bedridden
p4	F(T)	Seizure	15	20	-	+	-	+	+	+	22	-	+				+	+	+	+	+	-	66	Walking with support
p5	M(T)	Macrocephaly	9	10	+	+	+	+	-	-	24	-	+		+	+	-	+	+	+	+	+	132	Severely affected
p6	F(T)	Seizure	7	9	-	+	-	-	+	+	9	-	+	+	+	+	-	+	-	+		+	76	Walking with support, ataxia
p7*	M(T)	Family screening	0,5	7 days	-	+	+	+	+	+	8	+	+	-	-	-	-	+	-	-	-	-	20	Severely affected, passed away at 2 years old because of pneumonia
p8*	M(T)	Macrocephaly	8	2	+	+	+	+	+	+	37	-	+	-	-	-	-	-	+	+	-		19	Severely affected (only smiling),bedridden

p9**	M(T)	Seizure, fever	8	17	-	+	+	+	+	+	23	-	+	-	+	-	-	+	+	+	+	-	54	Severely affected, bedridden
p10**	F(T)	PMR	5	6	+	+	+	+	+	+	11	+	+	-	-	-	-	+	-	+	-	-	16	Severely affected
p11	M(T)	PMR	8	10	+	+	+	+	+	+	13	-	+	-	-	-	-	-	-	+	-	_	16	Severely affected
p12	M(T)	Macrocephaly	7	8	-	+	-	+	+	+													27	Severely affected
p13	F(T)	PMR	8	11	-	+	+	+	+	-	9	+	+	-	-	-	-	-	-	+	+	-	40	Severely affected
p14#	F(T)	Seizure	6	30	-	+	+	+	+	+	27	+	+	+	+	+	-	-	+	+	-	-	53	Severely affected
p15#	F(T)	PMR	6	114	-	-	-	-	+	-	120	-	+	-	-	-	-	-	-	+	+	-	132	Mildly affected with ataxia, successful at school
p16	M(T)	Hypotonia	3	4	+	+	+	+	+	-	66	-	+	-	+	+	+	+	-	+	+	-	144	Sitting with support ,smiling
p17	F(T)	PMR	4	17	-	+	+	+	+	-	16	-	+	-	-	+	-	+	+	-	-	-	48	Severely affected
p18	M(T)	Hypotonia	10	12	+	+	-	+	+	-													105	Severely affected,bedridden
P19	M(S)	Seizure	7	10	-	+	-	+	+	+												-	13	Only head control
P20	F(T)	Hypotonia after gastroenteritis	6	36	-	+	-	+	+	-													38	Walking with support, speaking
P21	M(T)	PMR	6	96	-	+	+	+	+	+													116	Bedridden with tracheostomy
P22##	M(T)	Macrocephaly/ family screening	4	4	+	+	+	-	+	+	16	+	+	-	-	-	-	-	-	+	+	-	20	Walking, speaking

P23[#][#]	M(T)	Seizure	6	8	+	+	+	+	+	+	20	-	+	+	+	+	+	+	+	+	+	83	Severely affected, wheelchair bound
p24[j]	M(T)	Seizure	14	27	-	+	-	+	+	+	27	-	+	-	+	+	-	+	+	-	+	-	80	Severely affected wheelchair bound
P25[j]	F(T)	Seizure	7	72	-	-	-	-	+	+	105	-	+	-	+	+	+	+	+	-	-	-	151	Walking, writing, reading, going to school
P26	M(T)	PMR	6	18	-	+	+	+	+	-													18 Lost to follow up	Severely affected,
P27[q]	F(T)	Seizure	1,5	44	-	-	-	+	+	+	112	+	+	-	-	-	-	+	-	+	+	-	125	Going to school,walking,writing and reading
p28[q]	M(T)	Macrocephaly, family screening	2	2,5	+	+	-	-	-	-	12	+	+	-	-	-	-	+	-	+	+	-	24	Severely affected
p29	M(S)	Seizure	5	10	-	+	+	+	+	-	10	-	+	+	-	-	+	+	+	+	-	+	17	Severely affected
P30	M(S)	Macrocephaly	6	7	+	+	-	+	+	+	11	+	+	-	-	-	-	-	+	+	-	-	11	Severely affected
P31	F(T)	PMR	15	16	-	-	+	+	+	-	28	-	+	+	+	+	+	+	-	+	+	-	88	Walking with support, writing, reading, going to school
Patient no	Gender (Nation of origin)	Cause of Selective Screening	Crisis Onset Age	Age at diagnosis	Macrocephaly	Hypotonia	Dystonia	Spasticity	PMR	Seizure		Subdural Hematoma	FTA	Nucleus Caudatus	Putamen	Globus Pallidus	Nucleus Lentiformis	Supratentorial White matter	Delayed myelination	Ventricular dilatation	Cerebral cyst	Centrum semiovale	Current Age(months)	Clinical status at last follow-up visit
P32	M(T)	Seizure	6	22	-	+	+	+	+	+	12	+	+	+	+	+	+	-	-	-	-	-	22	Severely affected, sitting with

																						support
p33 Lost to follow-up	F(T)	Seizure	9	12	+	+	-	-	+	+	13	+	+	+	-	-	-	-	+	+	-	-	29	Sitting, walking with support
p34	M(T)	Seizure	5	15	-	+	+	+	+	+	24	+	+	+	-	+	+	+	+	-	+	-	78	Severely affected, bedridden
p35	F(T)	Head trauma	9	10	-	+	+	-	+	-	12	+	+	+	+	+	+	+	+	-	+	+	30	Sitting
p36	F(T)	Seizure	6	7	-	+	+	+	+	+													19	Head control, sitting with support
p37	M(T)	PMR, head trauma	2	8	-	+	-	+	+	+	32	+	+	-	+	+	-	+	-	+	-	-	69	Severely affected, bedridden
p38	M(T)	PMR, family screening, twin sister ex with GA-1	8	9	-	+	-	-	+	-	21	+	+	-	-	-	-	+	+	+	+	-	53	Walking with support, ataxia, speaking, stammering
p39	M(T)	Head trauma	6	30	-	+	+	+	+	+	66	+	+					+	+	+	+		128	Severely affected, bedridden with gastrostomy
P40	F(T)	Seizure	4	11	-	+	+	+	+	+	7	+	+							+	-	+	78	Severely affected, bedridden
P41	F(T)	Seizure/ family screening	6	6	-	+	+	+	+	+	23	+	+	+	+	+	+	+	-	+	-	+	27	Severely affected, bedridden and passed away at 4 years old because of

																									pneumonia.

S: Syrian, T: Turkish, FTA; Frontotemporal Atrophy, PMR;Psychomotor Retardation
P9 and p10 are the 2nd degree cousins

Table II: Details of Genetic Results of Patients

Patient no	Intron or exon affected	Known/ Novel	Base Change	Effect	Type of Mutation	Mutation Class	Zygosity	dbSNP NM_000159.4:
p1	E11	Known	c.1228G>A	p.Val410Met	Missense	Pathogenic	homozygous	rs760155287
p2	E11	Known	c.1228G>A	p.Val410Met	Missense	Pathogenic	homozygous	rs760155287
p3	E11	Known	c.1228G>A	p.Val410Met	Missense	Pathogenic	homozygous	rs760155287
p4	E11	Known	c.1204C>T	p.Arg402Trp	Missense	Pathogenic	homozygous	rs121434369
p5	E11	Known	c.1204C>T	p.Arg402Trp	Missense	Pathogenic	homozygous	rs121434369
p6	E11	Known	c.1204C>T	p.Arg402Trp	Missense	Pathogenic	homozygous	rs121434369
p7*	E11	Known	c.1156C>T	p.Arg386Ter	Nonsense	Pathogenic	homozygous	rs752127949
p8*	E11	Known	c.1156C>T	p.Arg386Ter	Nonsense	Pathogenic	homozygous	rs752127949
p9**	E11	Known	c.1148G>A	p.Arg383His	Missense	Likely pathogenic	homozygous	rs764608975
p10**	E11	Known	c.1148G>A	p.Arg383His	Missense	Likely pathogenic	homozygous	rs764608975
p11	E11	Known	c.1147C>T	p.Arg383Cys	Missense	Pathogenic	homozygous	rs150938052
p12	E6	Known	c.481C>T	p.Arg161Trp	Missense	Pathogenic	homozygous	rs1173575355
p13	E6	Known	c.481C>T	p.Arg161Trp	Missense	Pathogenic	homozygous	rs1173575355
p14#	E6	Novel	c.496_497delCC	p.Gln167AlafsTe	Frameshift	Pathogenic	homozygous	

				r20				
p15[#]	E6	Novel	c.496_497delCC	p.Gln167AlafsTer20	Frameshift	Pathogenic	homozygous	
p16	E8	Known	c.743C>T	p.Pro248Leu	Missense	Likely pathogenic	homozygous	rs1057516344
p17	E8	Known	c.743C>T	p.Pro248Leu	Missense	Likely pathogenic	homozygous	rs1057516344
p18	E8	Known	c.743C>T	p.Pro248Leu	Missense	Likely pathogenic	homozygous	rs1057516344
P19	E8	Known	c.743C>T	p.Pro248Leu	Missense	Likely pathogenic	homozygous	
P20	E8	Known	c.743C>T	p.Pro248Leu	Missense	Likely pathogenic	homozygous	
P21	E8	Known	c.743C>T	p.Pro248Leu	Missense	Likely pathogenic	homozygous	
P22[##]	E8/ E11	Known	c.743C>T /c.1204C>T	p.Pro248Leu/ p.Arg402Trp	Missense/Missense	Likely pathogenic	Compound heterozygous	rs1057516344/ rs121434369
p23[##]	E8/ E11	Known	c.743C>T /c.1204C>T	p.Pro248Leu/ p.Arg402Trp	Missense/Missense	Likely pathogenic	Compound heterozygous	rs1057516344/ rs121434369
p24[φ]	E10	Known	c.1018C>T	p.Leu340Phe	Missense	Likely pathogenic	homozygous	
p25[φ]	E10	Known	c.1018C>T	p.Leu340Phe	Missense	Likely pathogenic	homozygous	
p26	E10	Known	c.1018C>T	p.Leu340Phe	Missense	Likely pathogenic	homozygous	
p27[θ]	E10	Known	c.1018C>T	p.Leu340Phe	Missense	Likely pathogenic	homozygous	
p28[θ]	E10	Known	c.1018C>T	p.Leu340Phe	Missense	Likely pathogenic	homozygous	
p29	E7	Known	c.553G>A	p.Gly185Arg	Missense	Likely pathogenic	homozygous	rs576948027

P30	**E6**	**Known**	**c.349G>A**	**p.Gly117Arg**	**Missense**	**pathogenic**	**homozygous**	
P31	**E5**	**Novel**	**c.296A>T**	**p.Glu99Val**	**Missense**	**Likely pathogenic**	**homozygous**	
P32	**E5**	**Novel**	**c.295G>C**	**p.Glu99Gln**	**Missense**	**Likely pathogenic**	**homozygous**	
p33	**E11**	**Known**	**c.1093G>A**	**p.Glu365Lys**	**Missense**	**Pathogenic**	**homozygous**	**rs121434370**
p34	**E11**	**Known**	**c.1093G>A**	**p.Glu365Lys**	**Missense**	**Pathogenic**	**homozygous**	**rs121434370**
p35	**E11**	**Known**	**c.1093G>A**	**p.Glu365Lys**	**Missense**	**Pathogenic**	**homozygous**	**rs121434370**
p36	**E11**	**Known**	**c.1093G>A**	**p.Glu365Lys**	**Missense**	**Pathogenic**	**homozygous**	**rs121434370**
p37	**E12**	**Novel**	**c.1253A>T**	**p.Asp418Val**	**Missense**	**Likely pathogenic**	**homozygous**	
p38	**E7**	**Known**	**c.561C>A**	**p.Asp187Glu**	**Missense**	**Likely pathogenic**	**homozygous**	**rs377580992**
p39	**E2**	**Novel**	**c.52_53insC**	**p.His18ProfsTer 25**	**Frameshift**	**pathogenic**	**homozygous**	
P40								
P41								

AUTHOR DECLARATION FORM

We wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other people who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are o impediments to publication, including the timing of publication, with respect to intellectual property. In so doing, we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that this manuscript that has involved human patients has been conducted with the ethical approval of the institutional Ethics Committee for Human Research and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from (dr.skilavuz@gmail.com)

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Toplantı Sayısı	Tarih
85	1 Şubat 2019

KARAR NO 58- Çocuk Metabolizma ve Beslenme Bilim Dalı'nda, Uzman Dr. Sebile Kılavuz, Prof. Dr. Halise Neslihan Önenli Mungan, Doktor Öğretim Üyesi Deniz Kor, Uzm. Dr. Fatma Derya Bulut, Prof. Dr. Faruk İncecik, Uzm. Dr. Neslihan Özcan tarafından yürütülmesi öngörülen, "Türkiye Yenidoğan Tarama Programı İçin Aday Bir Metabolik Hastalık – 41 Geç Tanı Almış Glutarik Asidüri Tip 1 Hastası (A Candidate Inherited Metabolic Disease for Turkish Newborn Screening Programme: 41 Late - Diagnosed Glutaric Aciduria Type 1 Patients)" başlıklı proje araştırma etiği yönünden değerlendirildi. Toplantıya katılan üyelerin oybirliğiyle uygun olduğuna karar verildi.

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