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# Clinical, neuroimaging, genetic, and outcome characteristics of Leigh syndrome: Experience from a single tertiary center

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***Aim:*** *Leigh syndrome (LS) is a genetically heterogeneous mitochondrial disorder characterized by subacute necrotizing encephalopathy and early-onset neurological deterioration. We aimed to characterize the clinical presentation, biochemical profile, neuroimaging features, genetic background, and outcomes of children with LS followed at a national tertiary referral center.*

***Methods:*** *We conducted a retrospective cohort study including 17 patients diagnosed with LS between 2012 and 2022. Clinical data, triggering factors, neuroimaging findings, metabolic investigations, genetic results, treatment, and long-term outcomes were systematically extracted from medical records.*

***Results:*** *Median age at onset was 13 months, with 88% presenting before two years of age. Viral infections preceded encephalopathic crises in 41% of patients. All patients demonstrated bilateral symmetrical deep grey matter lesions on brain MRI. Extra-CNS involvement was common, particularly affecting the heart, vision, and hearing. Elevated blood lactate was observed in 88%, and elevated cerebrospinal fluid (CSF) lactate in 85%, including two patients with normal blood lactate but increased CSF concentration, highlighting a potential diagnostic pitfall. Pathogenic variants were identified in 15 patients (8 harbored variants in nuclear DNA and 7 in mitochondrial DNA), while 2 patients remained genetically unresolved. All patients developed permanent*

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*neurological sequelae, and 82% became non-ambulatory. Six patients (35%) died, predominantly those with early disease onset and nuclear DNA mutations.*

***Conclusion:*** *LS is associated with substantial morbidity and mortality. Neuroimaging is critical for diagnosis, even when biochemical markers are inconclusive, while CSF lactate assessment may increase diagnostic sensitivity. Early disease onset and nuclear gene mutations were associated with poorer outcomes.*

**Keywords:** LEIGH DISEASE; MITOCHONDRIAL DISEASES; LACTIC ACID; NEUROIMAGING

## INTRODUCTION

Leigh syndrome (LS) or subacute necrotizing encephalopathy is a common phenotype of mitochondrial disorders in children. The prevalence is approximately 1 in 30,000–40,000 individuals. LS occurs in all populations and races, affects both sexes equally, and typically manifests in infancy or early childhood (1, 2). LS is genetically heterogeneous and is caused by pathogenic variants in more than 100 genes located in both the mitochondrial and nuclear genomes (2, 3). Most cases are caused by nuclear DNA (nDNA) variants involving genes essential for mitochondrial structure or function, oxidative phosphorylation (OXPHOS), mitochondrial DNA (mtDNA) maintenance, mitochondrial protein synthesis, and respiratory chain complex assembly (4-6). Mutations in mtDNA are responsible for 21.5%–47.5% cases, with variants in MT-ATP6 representing a common cause of mtDNA-associated disease (3, 7, 8).

Pathogenic mutations impair OXPHOS capacities and adenosine triphosphate (ATP) production, particularly affecting high-energy-demand tissues such as the brain, heart, and skeletal muscle (9, 10). The hallmark pathological feature is bilateral, symmetrical necrosis of the basal ganglia, brainstem, and cerebellum, likely resulting from energy deficiency, oxidative stress, and excitotoxicity secondary to mitochondrial dysfunction (11).

LS exhibits variable clinical expression. Early-onset disease manifests during the first two years of life and is characterized by psychomotor delay, hypotonia, feeding difficulties, movement disorders, and respiratory failure, frequently leading to death or severe neurological sequelae (3, 12, 13). Late-onset disease occurs later in childhood or even early adulthood, typically after normal development, with a milder course and symptoms including ataxia, dystonia, psychiatric symptoms, and cognitive decline (3, 12, 14, 15). Beyond the central nervous system (CNS), other organs such as the heart, liver, kidneys, and muscles may also be affected (15, 16).

Brain magnetic resonance imaging (MRI) showing bilateral, symmetrical lesions in the deep grey matter is a key diagnostic feature, with the striatum most frequently affected, followed by the substantia nigra in the brainstem (17, 18). Impaired oxidative phosphorylation leads to pyruvate accumulation, which is converted to lactate and transaminated to alanine, resulting in elevated lactate and alanine concentrations in blood and cerebrospinal fluid (CSF), while urinary organic acid abnormalities may include increased excretion of lactate, pyruvate, 3-methylglutaconic acid, dicarboxylic acids, and Krebs cycle intermediates (3).

Therapeutic options remain limited and largely supportive. Although clear evidence of efficacy is lacking, so-called “mitochondrial cocktail” therapy is frequently prescribed, typically comprising a combination of coenzyme $Q_{10}$, L-carnitine, α-lipoic acid, creatine monohydrate, biotin, thiamine, riboflavin, and vitamins E and C (19-21). Several reports have documented the efficacy of high doses of coenzyme $Q_{10}$ and its more potent analogs, idebenone and EPI-743 (22, 23). Current research is exploring novel treatments, including toxic compound scavenging, deoxynucleoside and deoxynucleotide therapy, cell replacement, gene therapy, mitochondrial transfer, and modulation of mitochondrial DNA heteroplasmy (20, 24).

While large international cohorts have been described, real-world data from smaller national

centers remain limited. Such data are valuable for understanding diagnostic challenges and patient outcomes in routine clinical practice. Therefore, this study aimed to characterize the clinical presentation, neuroimaging findings, biochemical hallmarks, genetic background, and long-term outcomes of children with LS followed at a national tertiary referral center.

## PATIENTS AND METHODS

This retrospective study included 17 patients with LS diagnosed and/or followed at the Department of Pediatrics, Division for Genetics and Metabolism, University Hospital Centre Zagreb, between 2012 and 2022. Following approval by the institutional ethics committee, anonymized data were retrospectively collected from electronic and paper medical records between January and April 2023. The study was conducted as part of the first author's graduate thesis.

Clinical, biochemical, neuroradiological, and genetic data were systematically extracted from official medical records. Neuroimaging findings and genetic results were obtained from final diagnostic reports generated during routine clinical care and were not reinterpreted for the purposes of this study. Due to the retrospective design, data availability depended on the completeness and accuracy of the medical records.

## RESULTS

### Clinical characteristics

Seventeen patients diagnosed with LS were enrolled in the study, comprising 10 females (59%) and seven males (41%). The median age at LS diagnosis was 13 months, with a range from five weeks to 7.5 years. Fifteen patients (88%) manifested LS within the first two years of life. Common initial symptoms included impaired consciousness, seizures, fatigue, hypotonia, inconsolable crying, ataxia, and respiratory failure. Table 1 summarizes the key clinical features, genetic findings, and clinical outcomes. In seven patients (41%), encephalopathic crises were triggered by viral infections, while two patients (12%) had seizures preceding the onset of encephalopathy (Figure 1). It remains unclear whether the seizures contributed to the development of the encephalopathic crisis or merely reflected the underlying LS-related brain injury. Neurological degeneration caused by LS led to persistent deficits in all patients of our cohort. Sixteen patients (94%) exhibited movement disorders and/or muscle tone abnormalities, including dystonia, bulbar paresis, hemiplegia, or spastic tetraplegia. Epilepsy was observed in five patients (29%), visual impairment in eight (47%), and hearing impairment in five (29%). Extra-CNS manifestations included cardiac, renal, endocrine, skeletal, gastrointestinal, and skin involvement. Cardiac manifestations were documented in six patients (35%), five of whom exhibited cardiomyopathy. Among these, one patient experienced severe hypertrophic cardiomyopathy with recurrent pericardial effusions. Additionally, one patient diagnosed with Kearns-Sayre syndrome (KSS) manifested heart block, a hallmark feature of the syndrome. Renal complications were identified in three subjects: one patient developed reversible kidney injury during an acute crisis, and two exhibited signs of chronic kidney disease. Gastrointestinal involvement included intestinal dysmotility in two patients. Skeletal abnormalities were identified in six patients, including four with osteopenia, one with hip dislocation, and one with severe scoliosis. Skeletal involvement appeared as a late manifestation, potentially increasing in frequency with longer follow-up. Dermatological manifestations were observed in four patients, including typical skin erythema and alopecia associated with NAXE-encephalopathy, hypertrichosis in two patients, and poikiloderma in one. Two patients exhibited endocrine disorders. One had growth hormone deficiency, while the other, diagnosed with KSS, initially presented with hypoparathyroidism and later developed diabetes mellitus. Clinical features are depicted in Figure 2.

### Biochemical characteristics

Lactate concentrations in the blood were available for 16 patients. Two patients (12%) exhibited normal values, while 14 (88%) had hyperlactatemia. The median concentration of lactate in the blood was 4.03 mmol/L, with a range of 1.3-12.0 mmol/L (reference range 1.0-2.2 mmol/L). Data on CSF lactate concentrations were available for 13 patients and were elevated in 11 (85%). The median concentration of lactate in the CSF

**Table 1.** Basic clinical characteristics, genotype, and disease outcome of patients included in the study

| Patient | Gender | Psychomotor development / symptoms before the onset of encephalopathy | Age at onset of encephalopathy | Presenting symptoms of subacute encephalopathy | Genotype | Hearing and vision impairment | Systemic manifestations | Outcome |
|---|---|---|---|---|---|---|---|---|
| P1 | M | Weak head control, restlessness and inconsolable crying | 7.5 months | Hypotonia, dystonia, reduced spontaneous movements, high-pitched cry | *SCO2* gene, c.418G>A (p.Glu140Lys) homozygous | / | Obstructive cardiomyopathy and recurrent pericardial effusions | Died at 14 months |
| P2 | F | IUGR, oligohydramnios delayed psychomotor development from birth | NA* | Hypotonia, psychomotor retardation | *FBXL4* gene, c.513-3C>T (p.?) and c.1058C>T (p.Ser353Phe) | Vision impairment | Osteopenia, intestinal pseudo-obstruction, hypertrichosis | Died at 3 years and 9 months |
| P3 | M | Hypoglycemia, hepatopathy, developmental delay since early infancy | 16 months | Hypotonia, involuntary movements | *SERAC1* gene c.202C>T (p.Arg68*) homozygous | Hearing and vision impairment | Osteopenia, hepatopathy | Died at 16 years |
| P4 | F | Mild psychomotor delay | 14 months | Hypotonia, disturbed consciousness, apnea | *MT-ATP6* gene, m.8993 T>C (p.Leu156Pro), 98% heteroplasmy in peripheral blood | / | Recurrent acute respiratory infections | Severe motor disability, wheelchair-bounded at 6.5 years |
| P5 | M | Normal development | 15 months | Weakness, unsteady gait, ataxia | *MT-ND6* gene, m.14487T>C (p.Met63Val), homoplasmy in peripheral blood | / | / | Mild delay in motor skills and expressive speech at 2 years and 3 months |
| P6 | F | Normal development | 14 months | Breathing difficulties, tonic seizures, vomiting | *ECHS1* gene, c.676G>A (p.Ala226Thr) and c.518C>T (p.Ala173Val) | / | / | 7 years, spastic tetraplegia |
| P7 | F | Mildly delayed psychomotor development | 17 months | Impaired consciousness, acute respiratory failure | *NAXE* gene, c.196C>T (p.Gln66*) and c.516+1G>A (p?) | Hearing and vision impairment | Skin changes (intertriginous and perigenital rash, erythema on both hands and soles and around the mouth and eyes), alopecia | Died at 2 years |
| P8 | F | Delayed psychomotor development | 1 year | Fatigue, feeding and sleeping problems | *MT-ND5* gene, c.370T>C (p.Phe124Leu), 97% heteroplasmy in peripheral blood | Hearing impairment | Right ventricular hypertrophy, preexcitation | Died at 3 years and 8 months |
| P9 | F | Delayed psychomotor development | 6 months | Hypotonia | Unknown (complex I deficiency) | / | / | 15 years, severe motor disability, relatively spared cognition, no sphincter control |

**Table 1.** Continued

| Patient | Gender | Psychomotor development / symptoms before the onset of encephalopathy | Age at onset of encephalopathy | Presenting symptoms of subacute encephalopathy | Genotype | Hearing and vision impairment | Systemic manifestations | Outcome |
|---|---|---|---|---|---|---|---|---|
| P10 | F | Normal development | 2 years | Psychomotor regression | *SURF1* gene c.845_846delCT (p.Ser282Cysfs*9) homozygous | / | Hypertrophic cardiomyopathy, tubulopathy, hypercalciuria | Severe psychomotor retardation at 4 years and 3 months |
| P11 | F | Delayed psychomotor development | 9 months | Fatigue, disturbed consciousness, vomiting | *SURF1* gene, c.836-IG>A and c.845_846delCT (p.Ser282Cysfs*9) | Vision impairment | Hypertrophic cardiomyopathy, growth hormone deficiency, intestinal dismotility, hypertrichosis | Died at 3 years and 6 months |
| P12 | M | Delayed psychomotor development | 10 months | Seizures, dehydration | *MT-ND6* gene, m.14459G>A (p.Ala72Val), 96% heteroplasmy in peripheral blood | / | Hip dislocation | Immobile, severe psychomotor retardation at 6.5 years |
| P13 | M | Normal development | 5 weeks | Seizures, inconsolable high-pitched cry, hyperexcitability | *SLC19A3* gene, c.541T>C (p.Ser181Pro) homozygous | / | Osteopenia | 7 years, severe psychomotor retardation |
| P14 | M | Delayed development of motor skills and expressive speech | 5 years and 5 months | Fatigue, ataxia, leg pain, sleep problems | *MT-ATP6* gene, m.9185T>C (p.Leu220Pro), homoplasmy in muscle tissue | Vision impairment | Hypertrophic cardiomyopathy, progressive acute renal failure, osteopenia | Non-ambulatory at 8.5 years |
| P15 | F | Normal development | 7 months | Seizures, psychomotor delay, poor reaction to external stimuli | *MT-ATP6* gene, m.8993T>G (p.Leu156Arg), 96% heteroplasmy in peripheral blood | Vision impairment | / | Bradykinesia, ataxia, assisted walking at 4 years and 10 months |
| P16 | F | Delayed psychomotor development | 3 months | Seizures, impaired consciousness, spasticity, inconsolable screaming crying | Unknown | Vision and hearing impairment | Scoliosis and deformation of the chest | 13 years, spastic tetraparesis and severe psychomotor retardation |
| P17 | M | Delayed development of motor skills and expressive speech, hypotonia, progressive external ophthalmoplegia, diagnosed with Kearns Sayre syndrome at 5.5 years | 7 years and 6 months | Disturbed consciousness | 5.5 kb mtDNA deletion | Vision and hearing impairment | Heart block, hypoparathyroidism, diabetes mellitus, chronic kidney disease, poikiloderma | 10 years, cognitive problems, ataxia, assisted walking |

*In patient P2, disease manifestations were evident from birth, and a diagnosis of mitochondrial disease was made during the neonatal period. Subacute encephalopathy progressed gradually without an overt encephalopathic crisis.

P – patient, M – male gender, F – female gender, NA – not applicable, IUGR – intrauterine growth retardation.

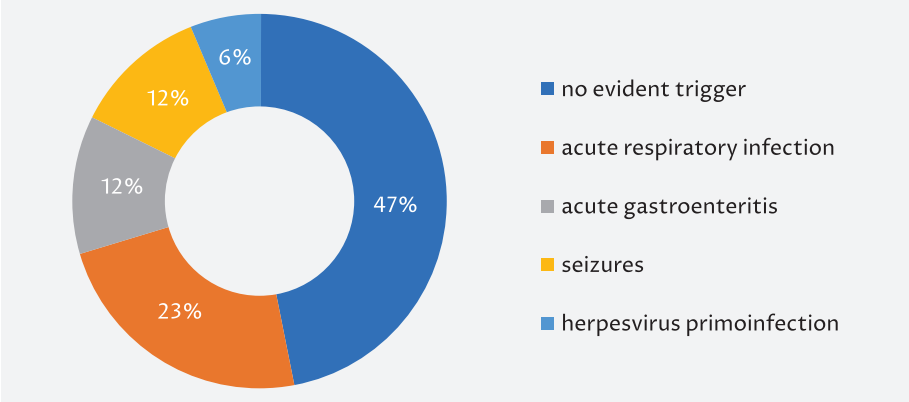


**Figure 1.** Graphical representation of possible triggering factors for the development of Leigh syndrome in our cohort.

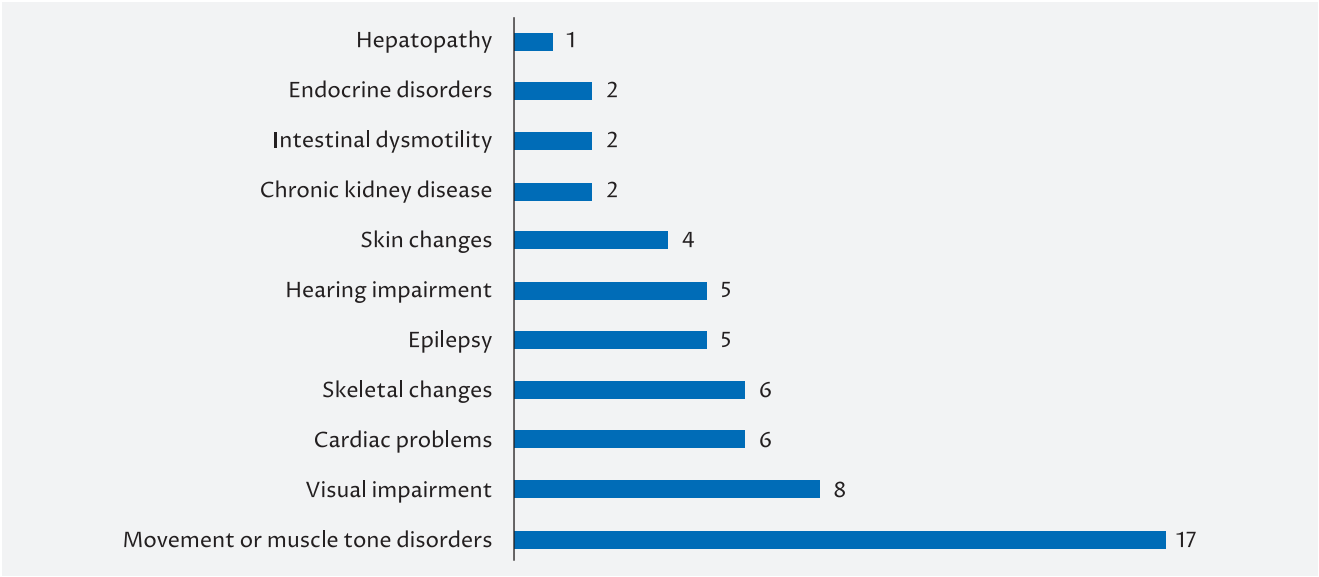


**Figure 2.** Clinical features, including extra-central nervous system involvement, of 17 patients included in this study.

was 4.01 mmol/L, with a range of 1.32 to 7.2 mmol/L (reference range 1.0–2.1 mmol/L). Plasma amino acid concentrations were available for 14 patients, revealing that six (43%) had elevated plasma alanine, while alanine was within the reference range in the remaining eight (57%). Among the 13 patients with available alanine concentrations in CSF, elevated values were observed in five (38%), while eight (62%) had alanine within the reference range. Organic acid profiles in urine were available for 15 patients, with pathological excretion detected in nine (60%). The abnormalities included increased excretion of pyruvate, fumaric acid, 3-methylglutaconic acid, 2-oxoglutarate, 2-oxoisovaleric acid, 3-hydroxyisovaleric acid, suberic acid, sebacic acid, and 3-hydroxydodecanoic acid. Detailed individual biochemical findings are presented in Table 2.

## Neuroimaging characteristics

All patients underwent brain MRI at the time of diagnosis, and characteristic changes indicative of LS were observed in each subject. Signal abnormalities were identified in the putamen (14/17, 82%), globus pallidus (11/17, 65%), caudate nucleus (8/17, 47%), substantia nigra (4/17, 24%), thalamus (4/17, 24%), and subthalamic nucleus (2/17, 12%). Additionally, signal alterations were observed in the supratentorial white matter of 11 (65%) in the frontal lobe and 9 (53%) in the parietal and/or occipital lobes. Regarding infratentorial structures, pathological signal changes were noted in the mesencephalon (7/17, 41%), pons (6/17, 35%), medulla oblongata (4/17, 24%), and cerebellum (10/17, 59%). Detailed distribution of brain MRI changes in our cohort is provided in

**Table 2**. Biochemical characteristics of patients included in the study. Patient identifiers correspond to those listed in Table 1.

| Patient | Blood lactate (mmol/L) | CSF lactate (mmol/L) | Plasma alanine | CSF alanine | Urine organic acids |
|---|---|---|---|---|---|
| P1 | 4,06 | 6,5 | Increased | Increased | Normal finding |
| P2 | 12 | 5,74 | Increased | Increased | Significantly increased excretion of pyruvate, mildly increased excretion of fumaric acid, ketosis |
| P3 | 1,85 | 2,91 | / | Normal | Increased excretion of 3-methylglutaconic acid |
| P4 | 4 | / | Normal | Increased | Normal finding |
| P5 | 2,9 | 2,6 | Normal | Increased | Normal finding |
| P6 | 3,8 | 1,32 | Normal | Normal | Mildly increased excretion of 3-methylglutaconic acid and pyruvate |
| P7 | 1,3 | 4,09 | Increased | Normal | Normal finding |
| P8 | / | / | Normal | Normal | Mildly increased excretion of fumaric acid, 3-hydroxyisovaleric acid, and lactate |
| P9 | 4,5 | 4,01 | Normal | Increased | Mildly increased excretion of glutaric acid |
| P10 | 7,9 | 6,83 | Increased | Normal | Mildly increased excretion of fumaric and malic acids, pyruvate, 3-hydroxyisobutyrate, and 2-ethyl-3-hydroxypropionic acid |
| P11 | 3,88 | 6,6 | Normal | Normal | Increased excretion of pyruvate, lactate, and Krebs cycle metabolites, moderately elevated sebacic, suberic, and 3-hydroxydecanedioic acids |
| P12 | 4,25 | 3,57 | Increased | Normal | Normal finding |
| P13 | 3,1 | 1,85 | Normal | Normal | Mildly increased excretion of 3-hydroxyadipic lactone, octenedioic, suberic, decadienedioic, 3-hydroxysebacic, 3-hydroxysuberic, and 2-hydroxysebacic acids |
| P14 | 5,16 | 3 | / | / | / |
| P15 | 4,6 | / | / | / | / |
| P16 | 2,4 | / | Normal | / | Normal finding |
| P17 | 4,02 | 7,2 | Increased | / | Increased excretion of 3-methylglutaconic, fumaric, malic, methylmalonic, and 2-oxoglutaric acids |
| **Total** | Increased in 14/16 (median 4,03 mmol/L) | Increased 11/13 (median 4,01 mmol/L) | Increased in 6/14 | Increased in 5/13 | Abnormal finding in 9/15 |

Table 3 (Supplementary Data). Figure 3 shows the neuroimaging findings in selected patients.

## Genetic characteristics

Causative gene variants were confirmed in 15 patients, seven (41%) harbored mtDNA mutations, eight (47%) nDNA mutations, and two (12%) remained genetically unsolved (Figure 4). Whole-exome sequencing was used in most patients to establish the genetic diagnosis, whereas in one patient with clinical suspicion of KSS, long-range polymerase chain reaction (PCR) of mtDNA confirmed the diagnosis. Pathogenic variants in *MT-ATP6* were detected in three patients, pathogenic variants in *MT-ND6* and *SURF1* in two patients each, and pathogenic variants in *MT-ND5*, *SCO2*, *FBXL4*, *SERAC1*, *ECHS1*, *NAXE*, *SLC19A3*, and a single large-scale mtDNA deletion of 5.5 kb in one patient each. In one of the two patients without genetic confirmation, complex I deficiency was diagnosed based on decreased complex I activity measured in snap-frozen muscle tissue. The absence of genetic confirmation in these two patients likely reflects the limitations of earlier diagnostic approaches, as next-generation sequencing was not yet available in our setting at the time of their diagnosis.

## Vitamin and cofactor treatment

The majority of patients received vitamins and cofactors, with the most commonly used being coenzyme Q10 (14/17), thiamine (13/17), ribofla-

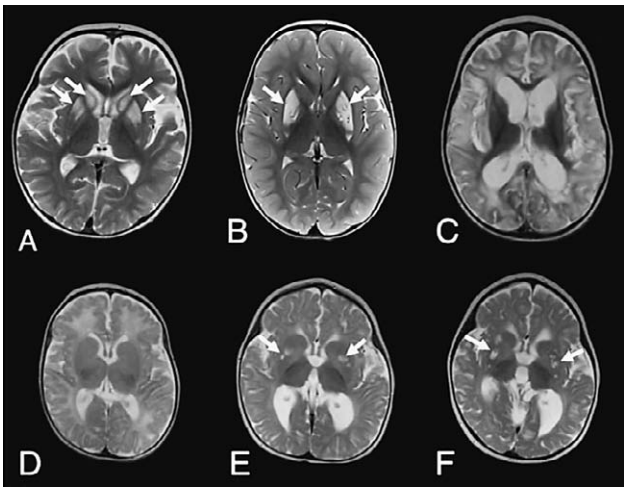


**Figure 3.** Axial T2-weighted brain MRI images. **(A)** Symmetrical bilateral hyperintensities of the caudate nucleus, globus pallidus, and putamen (arrows) in patient P4. **(B)** Bilateral hyperintensities in the putamen and globus pallidus (arrows) in patient P5. **(C)** Diffuse hyperintensities throughout the parenchyma, predominantly in the white matter, with relatively preserved basal ganglia morphology and advanced deep white matter atrophy in patient P7. **(D–F)** Gradual progression of basal ganglia hyperintense lesions (arrows) and diffuse parenchymal volume loss, including basal ganglia and thalamus, at 2 months **(D)**, 8 months **(E)**, and 2 years 8 months **(F)** in patient P2.

vin (11/17), vitamin C (10/17), and biotin (9/17). The doses used were mostly consistent with those reported in the medical literature. In two cases, parents opted to discontinue therapy after observing no apparent benefit. It was not possible to determine whether the remaining patients who continued treatment experienced clinical benefit, particularly in the absence of standardized outcome measures.

## Outcome

Seven patients (41%) required mechanical ventilation due to respiratory failure. Of these, six had respiratory failure during acute metabolic decompensation, while one developed respiratory insufficiency during the chronic phase of the disease, two and a half years after LS diagnosis. Among the six patients with acute respiratory failure, three required permanent respiratory support, whereas the remaining three were successfully weaned from the ventilator after tracheostomy. Median age of onset of respiratory failure requiring mechanical ventilation was 2.2 years, with a range of 10 months to 7.5 years. Dysphagia and feeding difficulties were present in 13 patients (76%). Of these, seven patients (41%) were fed via nasogastric tube, three (18%) had percutaneous endoscopic gastrostomy, and three (18%) were fed orally with pureed foods. Following the onset of encephalopathy, all patients developed persistent motor impairments. Fourteen patients (82%) had severe mobility limitations or were immobile, while three (18%) exhibited an unsteady gait. Sixteen patients (94%) had some intellectual difficulties or delayed speech and language development, ranging from mild delay in expressive speech development to severe cognitive problems. Six patients (35%) died during the chronic phase of their disease, all of whom had severe psychomotor disabilities as a consequence of encephalopathy. The median age at death was 3.5 years, ranging from 14 months to 16 years. Five of the six deceased patients had nDNA mutations, while one had mtDNA-associated disease. In five patients with a lethal outcome, disease manifestation occurred within the first year of life.

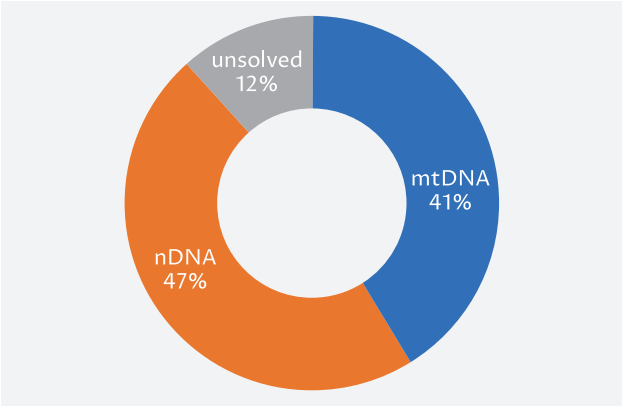


**Figure 4.** Distribution of genetic etiology indicating the proportions of patients with mtDNA- and nDNA-associated Leigh syndrome.

# DISCUSSION

In this study, we present the characteristics of 17 patients with LS who were diagnosed and/or followed at a tertiary care center over an 11-year period. The age at symptom onset ranged from 5 weeks to 7.5 years, with the majority of patients experiencing an encephalopathic crisis within the first two years of life. This is consistent with published data, which indicates that LS is most commonly diagnosed by the age of two years (12). Three patients were diagnosed with mitochondrial disease prior to the development of encephalopathy. The initial symptoms and signs of LS in our cohort included impaired consciousness, seizures, weakness, hypotonia, inconsolable high-

pitched crying, ataxia, and respiratory failure. The most common precipitating factor was a viral infection. Neurological sequelae were only partially reversible and included severe motor impairment and movement disorders. Systematic assessment revealed frequent extra-CNS involvement, particularly visual impairment and cardiomyopathy, emphasizing the importance of evaluating other highly energy-dependent organs even in the absence of overt symptoms. As expected, the most common pattern of neuroimaging signal changes comprised symmetrical, bilateral lesions of the putamen and globus pallidus, consistent with findings reported in larger cohorts (17). Nevertheless, the incidence of supratentorial white matter changes in our cohort was higher than expected. According to an Italian study from 2021, which included 122 patients with LS, white matter lesions were present in 28% of subjects (25). The higher occurrence of white matter changes in our cohort is likely explained by the comprehensive inclusion of all abnormalities, including non-specific findings, reported in the neuroradiological assessment.

Most patients showed elevated lactate concentrations in blood and CSF, consistent with observations from other studies (13, 25). Notably, two patients in this study with normal blood lactate exhibited elevated CSF lactate, underscoring the importance of CSF analysis in the diagnostic evaluation of mitochondrial disorders with CNS involvement. This represents an important diagnostic pitfall, as normal blood lactate may falsely reassure clinicians and delay further metabolic and genetic evaluation. Additional widely available metabolic tests that may aid in the diagnostic process include measurement of alanine concentrations in plasma and CSF and organic acid profile in urine. The spectrum of identified genes illustrates the genetic heterogeneity underlying LS. In this cohort, seven out of 15 patients with a known genotype carried a pathogenic mtDNA mutation, including three with *MT-ATP6* variants. The relatively higher proportion of mtDNA-associated LS in our cohort may be attributable to the small sample size. Nevertheless, similar findings have been reported in larger, recently published cohorts. For instance, 35% and 42% of genetically confirmed LS patients in Italian and Chinese cohorts, respectively, harbored mtDNA mutations (13, 25).

All patients included in this study experienced permanent neurological sequelae. The most common were motor impairments, ranging from mild hemiparesis to immobility and paralysis, with the majority of the patients unable to walk unassisted. Furthermore, the majority of patients demonstrated varying degrees of cognitive or speech impairment. Speech difficulties were likely influenced by delayed speech development and impaired expressive abilities secondary to oropharyngeal dysmotility.

In this cohort, 76% of the patients had psychomotor delay, 29% had epilepsy, and 24% experienced weakness and fatigue. Among the seven patients who developed respiratory failure and required mechanical ventilation, three were weaned from the ventilator but continued to require a tracheostomy. These findings are consistent with those reported by *Chang et al.*, who observed psychomotor retardation in 57% of patients, movement disorders in 42%, epilepsy in 33%, general weakness in 27%, and respiratory dysfunction in 34% (26). Additionally, 47% of the patients in this study had visual impairment, and 35% had cardiac problems. Our findings are in line with published data, indicating that the eyes and heart, in addition to the CNS and muscles, are the most commonly affected organs in LS (27). Notably, two patients exhibited intestinal dysmotility, a well-recognized manifestation of certain mitochondrial disorders (28).

While the majority of our patients received high-dose vitamins and cofactors, an evidence-based indication was present in only one patient diagnosed with biotin-thiamine-responsive basal ganglia disease. This patient received high-dose thiamine and biotin, a well-established, disease-specific therapy with proven clinical benefit (29). In the remaining patients, the use of high-dose vitamins and cofactors was largely empirical. The administered doses were based on regimens commonly reported in the literature and were adjusted according to body weight. Although some patients appeared clinically stable, any treatment effect remains speculative. This highlights a persistent challenge in the field: although “mitochondrial cocktails” are widely used in clinical

practice, convincing evidence supporting their efficacy remains scarce (30).

LS is associated with high mortality. Approximately half of patients die, usually by the age of three years, with the poorest survival observed in those who develop encephalopathy during early infancy (31). In this cohort, six patients (35%) had died by the time of data collection, all during the chronic phase and due to complications of their severe condition. The median age at death was 3.5 years, and five of the six patients with a fatal outcome presented with disease onset during the first year of life. Similarly, in a study by *Ardissone et al.*, 42 out of 44 patients who died had developed clinical symptoms within the first year of life (25). These findings further support that patients with an early presentation are likely to have a more severe clinical course and a poorer prognosis.

This study provides real-world data from a single-center cohort, highlighting diagnostic challenges and prognostic indicators relevant to routine clinical practice. Limitations include the small sample size and retrospective design, with reliance on potentially incomplete medical records. Genetic confirmation of LS diagnosis for most patients was performed through international collaboration with Paracelsus Medical University Salzburg and the Technical University of Munich. Four patients from this cohort have been reported previously: one in a study on NAXE-encephalopathy, two in a study on MT-ATP6–associated disease, and one in two studies on MEGDEL syndrome (32-35).

## CONCLUSION

The results of this single-center retrospective study highlight that LS is a severe neurodegenerative disease of early childhood, characterized by significant neurological sequelae and high mortality. In almost half of our patients, acute viral infections preceded subacute necrotizing encephalopathy. Therefore, it is important to consider LS in patients presenting with encephalopathy or other neurological symptoms during acute infections. Although not pathognomonic, symmetrical lesions of the deep grey matter on the brain MRI, particularly affecting the striatum, were observed in all patients and served as a key criterion for establishing the diagnosis of LS. Conversely, characteristic biochemical features, such as elevated lactate and alanine in the blood and CSF, were not present in all patients. Thus, negative biochemical results should not exclude the diagnosis of LS, and further genetic testing should be pursued. Patients harboring nDNA mutations exhibit higher mortality compared to patients with mtDNA mutations, reflecting a more severe disease course.

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SAŽETAK

# Klinička, neuroradiološka i genetska obilježja te ishodi bolesnika s Leighovim sindromom u skupini hrvatskih bolesnika

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***Cilj:*** *Leighov sindrom (LS) je genetski heterogena skupina mitohondrijskih bolesti, karakteriziranih subakutnom nekrotizirajućom encefalopatijom i progresivnim neurodegenerativnim tijekom. Cilj ovog istraživanja bio je opisati kliničku sliku, biokemijska i neuroradiološka obilježja, genetsku podlogu te ishode djece s LS praćene u tercijarnom referentnom centru.*

***Metode:*** *Provedeno je retrospektivno istraživanje koje je obuhvatilo 17 bolesnika s LS, kod kojih je dijagnoza postavljena u razdoblju od 2012. do 2022. godine. Iz medicinske dokumentacije sustavno su prikupljeni podaci o kliničkoj slici, mogućim provocirajućim čimbenicima za razvoj encefalopatije, rezultati neuroradioloških, metaboličkih i genetskih pretraga te podatci o primijenjenom liječenju i dugoročnom ishodu pacijenata.*

***Rezultati:*** *Medijan dobi pri nastupu encefalopatije bio je 13 mjeseci, pri čemu se u 88 % ispitanika bolest manifestirala prije druge godine života. U 41 % slučajeva encefalopatskoj krizi prethodile su virusne infekcije. Magnetska rezonancija mozga otkrila je obostrane simetrične lezije duboke sive tvari kod svih ispitanika. Česta je bila i zahvaćenost drugih organskih sustava, osobito srca, vida i sluha. Povišene vrijednosti laktata u krvi zabilježene su u 88 % ispitanika, dok su u cerebrospinalnoj tekućini povišene vrijednosti zabilježene u 85 %, uključujući dvoje pacijenata s urednim vrijednostima laktata u krvi, ali povišenima u likvoru, što naglašava važnost analize cerebrospinalne tekućine u dijagnostici mitohondrijskih bolesti. Patogene varijante gena identificirane su kod 15 bolesnika (8 ispitanika imalo je mutacije u genima jezgrine DNA, a 7 u mitohondrijskoj DNA), dok kod dvoje genetski uzrok nije razjašnjen. Svi ispitanici razvili su trajne neurološke posljedice, a 82 % bilo je nepokretno. Smrtni ishod zabilježen je u šest bolesnika (35 %), uglavnom kod onih s ranim početkom bolesti i mutacijama u genima jezgrine DNA.*

***Zaključak:*** *LS dovodi do teških neuroloških oštećenja i obilježen je visokom smrtnošću. Ključnu ulogu u postavljanju sumnje na LS ima neuroradiološka obrada, dok biokemijski pokazatelji, iako korisni, nisu u potpunosti pouzdani. Određivanje laktata u cerebrospinalnoj tekućini kod pacijenata sa sumnjom na LS može povećati dijagnostičku osjetljivost. Rana encefalopatska kriza i mutacije u genima jezgrine DNA povezane su s nepovoljnijim ishodom.*

**Ključne riječi:** LEIGHOV SINDROM; MITOHONDRIJSKA BOLEST; LAKTAT; NEURORADIOLOŠKA DIJAGNOSTIKA