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Original Article

Evaluation of the Genetically Diagnosed Mitochondrial Disease Cases with Neuromuscular Involvement

Nöromusküler Tutulum Gösteren Genetik Tanılı Mitokondriyal Hastalık Tanılı Olgularımızın Değerlendirilmesi

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ABSTRACT

Objective: Due to the fact that mitochondrial diseases can involve different organ systems, neuromuscular involvement is frequently observed and has a substantial place in clinical practice. In this study, the clinical, radiological, electrophysiological and imaging features of the patients with mitochondrial disease with neuromuscular involvement were investigated.

Method: The clinical, radiological and genetic features of 16 patients with genetically diagnosed mitochondrial disease followed in the Departments of Pediatric Neurology and Pediatric Metabolism and Nutrition in Dokuz Eylül University Faculty of Medicine were retrospectively evaluated.

Results: The cases were between 3-17 years of age (mean: 8.8±4.2 years). 44% (n=7) of the cases were male. Clinical findings started at a mean age of 30 months (2-132 months). There was consanguineous marriage in 81% (n=13) of the cases. Leigh syndrome (LS), Charcot-Marie-Tooth disease (CMT) 2A, and CMT disease-axonal-type 2K were diagnosed in 5, 4, 2 cases, respectively. Alpers syndrome, combined oxidative phosphorylation deficiency-13, megalencephaly without cystic leukoencephalopathy, mt.9804G>A and m.11696G>A mutations which could not be phenotyped syndromicly were detected in one case each. SURF1 (n=2), MTATP6 (n=2) and PDSS2 (n=1) mutations were found in the patients with LS. NARS2, PNPT1, and RNASET2 mutations were found in the patients with Alpers syndrome, combined oxidative phosphorylation deficiency-13, cystic leukoencephalopathy without megalencephaly, respectively. Muscle weakness, developmental delay and skeletal deformity were the most common findings. The most common finding in brain magnetic resonance imaging was increased T2 signal in bilateral basal ganglia.

Conclusion: The most common genetically diagnosed mitochondrial disease was LS, the most common mutation was MFN2, and the most common clinical finding was muscle weakness.

Keywords: Mitochondrial diseases, Leigh disease, Charcot-Marie-Tooth disease, muscle weakness, genetics

ÖZ

Amaç: Mitokondriyal hastalıklar farklı organ sistemlerini tutabilmesine bağlı olarak nöromusküler tutulumlar sık gözlenmekte olup klinik pratikte aşamasında önemli yere sahiptir. Bu çalışmada nöromusküler tutulum gösteren mitokondriyal hastalık tanılı olguların klinik, radyolojik, elektrofizyolojik ve görüntüleme özelliklerinin incelenmesi amaçlandı.

Yöntem: Dokuz Eylül Üniversitesi Tıp Fakültesi, Çocuk Nörolojisi ve Çocuk Metabolizma ve Beslenme Bölümleri'nde takipli genetik tanılı mitokondriyal hastalığı olup genetik tanı almış 16 hastanın klinik, radyolojik ve genetik özellikleri retrospektif değerlendirildi.

Bulgular: Olgular 3-17 yaş aralığındaydı (ortalama: 8,8±4,2 yıl). Olguların %44'ü (n=7) erkekti. Klinik bulgular ortalama 30 aylıkken başlamıştı (2-132 ay). Olguların %81'inde (n=13) akraba evliliği vardı. Olguların 5 tanesi Leigh sendromu (LS), 4 tanesi Charcot-Marie-Tooth hastalığı (CMT) 2A, 2 tanesi CMT hastalığı-aksonal-tip 2K, 1 tanesi Alpers sendromu, 1 tanesi kombine oksidatif fosforilasyon eksikliği-13, 1 tanesi megalensefali eşlik etmeyen kistik lökoensefalopati tanısı almıştı ve birer olguda sendromik olarak fenotiplendirelemeyen mt.9804G>A ve m.11696G>A mutasyonu tespit edildi. LS alan iki olguda SURF1, iki olguda MTATP6, bir olguda PDSS2 mutasyonu saptandı. Alpers sendromu tanısı alan olguda NARS2, kombine oksidatif fosforilasyon eksikliği-13 tanısı alan

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olguda PNPT1, megalensefali eşlik etmeyen kistik lökoensefalopati tanısı alan olguda RNASET2 mutasyonu saptandı. Kas güçsüzlüğü, gelişimsel gerilik ve iskelet deformitesi en sık saptanan bulgulardı. Beyin manyetik rezonans görüntüleme incelemesinde en sık saptanan bulgu bilateral bazal gangliyonlarda T2 sinyal artışıydı.

Sonuç: Genetik tanılı mitokondriyal hastalıklarda en sık görüleni LS, en sık saptanan mutasyon MFN2, en sık görülen klinik bulgu ise kas güçsüzlüğüdür.

Anahtar kelimeler: Mitokondriyal hastalıklar, Leigh hastalığı, Charcot-Marie-Tooth hastalığı, kas güçsüzlüğü, genetik

INTRODUCTION

Mitochondrial diseases (MDs) are the most common neurometabolic disease group with an estimated incidence of 1/5,000 [1]. This incidence is conjectured to be higher by virtue of the challenges in diagnosis [2-4]. About 5% of the human genome is thought to be in the mitochondria [5]. Especially thanks to the frequent use of genetic techniques such as next-generation sequencing, more than 250 genes associated with MD have been described in the literature so far [6-13]. MDs are inherited in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) [14,15]. Inheritance of MD may be autosomal or X-linked for nDNA, whereas maternal inheritance is true for mtDNA. Sporadic cases due to *de novo* mutations have been also reported [16].

MDs have many diverse clinical manifestations as they can involve different organ systems and can occur at any age, and are classified in two groups as primary mitochondrial diseases (PMDs) and secondary mitochondrial dysfunctions (SMDs). PMDs are caused by mutations in the mtDNA and/or nDNA genes encoding electron transport chain proteins. Leigh syndrome (LS), Alpers-Huttenlocher syndrome, MEGDEL syndrome (3 methylglutaconic aciduria, deafness, encephalopathy, Leigh-like syndrome), Pearson syndrome, Sengers syndrome, congenital lactic acidosis, mitochondrial neurogastrointestinal encephalomyopathy syndrome, Kearns-Sayre syndrome, mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like attacks syndrome, myoclonic epilepsy with irregular red fibers, neurogenic muscle weakness, ataxia, retinitis pigmentosa are common PMDs. SMD basically encompasses all mitochondrial disorders that are not PMD. Although PMDs are genetically based diseases, SMD can be inherited or acquired. Many myopathies and muscular dystrophies also cause SMD over time as the disease progresses. Examples include spinal muscular atrophy, limb-girdle muscular dystrophy, Bethlem myopathy, Charcot-Marie-Tooth disease, (CMT) and inflammatory myopathies [17].

In this study, the demographic, clinical, radiological, electrophysiological and genetic features of the cases with mitochondrial disease that were followed up in the department of pediatric neurology in Dokuz Eylül University Faculty of Medicine were analyzed.

MATERIALS and METHODS

Archive records of all cases followed up in pediatric neurology and pediatric metabolism and nutrition departments in Dokuz Eylül University Faculty of Medicine between November 2012 and November 2020 were reviewed retrospectively, and 16 cases of genetically diagnosed MDs with neuromuscular involvement were included. The cases without neuromuscular involvement and/or the diagnosis of mitochondrial disease which was not proven by genetic tests were excluded. Demographic, clinical, radiological, electrophysiological and genetic findings of the cases were analyzed with the information obtained from patient files and system data.

Ethical Approval

The present study was conducted in accordance with the 1964 Declaration of Helsinki and approved by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (approval number: 2021/14-59, date: 06.05.2021). Informed consent was obtained from patients and/or parents or legal guardians of the patients before their enrollment in the study.

Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA). The normality of distribution of numerical variables was evaluated using the Kolmogorov-Smirnov test. The numerical variables were expressed as median [minimum (min)-maximum (max)] and categorical variables as numbers and percentages (%).

RESULTS

Demographic Features and Results of Genetic Evaluations

The mean age of 16 patients (7 males, 44%) included in the study was 8.8±4.2 years (minimum-maximum: 3-17 years). Clinical symptoms started at a mean age of 30 months (2-132 months). Consanguineous marriage was present in 81% (n=13) of the cases. The diagnoses of the cases were LS (n=5), CMT disease 2A (CMT2A)

(n=4), CMT disease axonal-type 2K (CMT2K) (n=1), Alpers syndrome (n=1), combined oxidative phosphorylation deficiency-13 (n=1), cystic leukoencephalopathy without megalencephaly (n=1). Furthermore, in two cases *mt.9804G>A* and *m.11696G>A* mutations that could not be syndromically phenotyped were detected. While nDNA mutation was detected in 12, and mutation on mtDNA was observed in the remaining 4 cases. SURF1 (n=2), *MTATP6* (n=2), *PDSS2* (n=1) mutations were detected in cases with LS. *MFN2* mutation was found in all four patients with CMT disease-2A, and *GDAP1* mutation in two patients with CMT disease-axonal-type 2K. *NARS2, PNPT1,* and *RNASET2* mutations were detected in the patients with Alpers syndrome, combined oxidative phosphorylation deficiency-13, and cystic leukoencephalopathy without megalencephaly (Table 1).

Clinical Findings

The most common clinical findings were muscle weakness (n=11, 68.7%), developmental delay (n=8, 50%), skeletal deformity (n=8, 50%), mental regression (n=6, 37.5%). ataxia (n=5, 31.2%), and spasticity (n=5, 31.2%). All five cases with LS, all two cases with CMT disease-

Table 1. Demographic characteristics, diagnoses, and mutations of the cases

	Syndrome	Age of onset (month)	Gender	Consanguineous marriage	Gene	Mutation site	Variant
Case 1	Leigh syndrome	6	Male	+	*SURF1*	nDNA	*c.484G>A*
Case 2	Leigh syndrome	18	Female	-	*SURF1*	nDNA	*c.845_846delCT*
Case 3	Charcot-Marie-Tooth disease-2A (CMT2A)	30	Male	+	*MFN2*	nDNA	*c.280C>T*
Case 4	Charcot-Marie-Tooth disease-2A (CMT2A)	12	Female	+	*MFN2*	nDNA	*c.296T>G*
Case 5	Charcot-Marie-Tooth disease-2A (CMT2A)	12	Female	+	*MFN2*	nDNA	*c.296T>G*
Case 6	Charcot-Marie-Tooth disease-2A (CMT2A)	56	Female	+	*MFN2*	nDNA	*c.404G>A*
Case 7	Charcot-Marie-Tooth disease, axonal, type 2K	18	Female	+	*GDAP1*	nDNA	*c.786delG*
Case 8	Charcot-Marie-Tooth disease, axonal, type 2K	36	Female	+	*GDAP1*	nDNA	*c.(695_1077)del*
Case 9	Leigh syndrome	12	Female	-	*MTATP6*	mtDNA	*m.8993T>G*
Case 10	*m.11696G>A* mutation (without a specific certain phenotype)	96	Male	+	*MTND4*	mtDNA	*m.11696G>A*
Case 11	Alpers syndrome	2	Male	+	*NARS2*	nDNA	*c.1096G>A*
Case 12	Leigh syndrome	132	Female	+	*PDSS2*	nDNA	*c.868G>A*
Case 13	Combined oxidative phosphorylation deficiency 13	4	Male	+	*PNPT1*	nDNA	*c.1576_1578dupGAT*
Case 14	*mt.9804G>A* mutation (without a specific certain phenotype)	42	Male	+	*mt.9804G>A*	mtDNA	*mt.980AG>A*
Case 15	Cystic leukoencephalopathy without megalencephaly	6	Female	+	*RNA SET2*	nDNA	*c.194A>G*
Case 16	Leigh syndrome	9	Male	-	*MTATP6*	mtDNA	*m.8993T>C*

nDNA: Nuclear deoxyribonucleic acid, mtDNA: Mitochondrial deoxyribonucleic acid

axonal-type 2K, one of the cases with CMTdisease-2A, the cases with Alpers syndrome, and those with *mt.9804G>A* and *m.11696G>A* mutations had muscle weakness. Four cases with LS and cases with Alpers syndrome, combined oxidative phosphorylation deficiency-13, *mt.9804G>A* mutation, cystic leukoencephalopathy without megalencephaly had developmental delay. Skeletal deformity was observed in all four patients diagnosed with CMT disease-2A, and in patients with *m.11696G>A* mutation and combined oxidative phosphorylation deficiency-13. Three of the cases with mental regression were among the patients with LS. Other mental regression cases had combined oxidative phosphorylation deficiency-13, *mt.9804G>A* mutation, and cystic leukoencephalopathy without megalencephaly. Ataxia was seen in two patients with CMT disease-axonal-type 2C, in two patients with LS and the patient with Alpers syndrome. Three patients diagnosed with LS had spasticity which was also present in patients with combined oxidative phosphorylation deficiency-13 and cystic leukoencephalopathy without megalencephaly. Hearing loss, cardiac involvements such as restrictive cardiomyopathy, left ventricular hypertrophy and/or dysfunction, renal involvement, epilepsy, recurrent respiratory failure, hypertrichosis and ocular involvements such as ptosis, nystagmus, strabismus, and optic atrophy were other clinical findings (Table 2).

Radiological Findings

While brain magnetic resonance imaging (MRI) results could not be obtained in four (25%) cases, 12 (75%) patients were examined by brain MRI which yielded normal signs in four (25%) of them. The most common abnormality was increased T2 signal intensity in bilateral basal ganglia. To assess other accompanying clinical findings, seven cases (43.7%) were evaluated with spinal MRI which was normal in five (31.2%) of them. Spinal MRI revealed abnormality in one of the cases with LS (increased T2 signal intensity in cervical spinal cord) and CMT disease-2A (S1-2 transitional vertebra anomaly). Magnetic resonance spectroscopy (MRS) was performed

Table 2. Symptoms and signs of the cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9
Syndrome	**Leigh syndrome**	**Leigh syndrome**	**CMT2A**	**CMT2A**	**CMT2A**	**CMT2A**	**Charcot-Marie-Tooth disease, axonal, tip 2K**	**Charcot-Marie-Tooth disease, axonal, tip 2K**	**Leigh syndrome**
Muscle weakness	+	+	+	-	-	-	+	+	+
Seizure	-	-	-	-	-	-	-	-	+
Swallowing dysfunction	-	-	-	-	-	-	-	-	+
Mental regression	+	+	-	-	-	-	-	-	+
Developmental delay	+	+	-	-	-	-	-	-	+
Dystonia	-	-	-	-	-	-	-	-	-
Spasticity	+	+	-	-	-	-	-	-	+
Recurrent	-	+	-	-	-	-	-	-	-
Skeletal deformity	+	+	+	+	+	+	-	-	-
Ptosis	-	+	-	-	-	-	-	-	-
Nystagmus	NA	NA	NA	NA	NA	NA	NA	NA	NA
Strabismus	-	-	-	-	-	-	-	-	-
Hypertrichosis	-	-	-	-	-	-	-	-	-
Ataxia	+	-	-	-	-	-	+	+	-
Hearing loss	-	-	NA	NA	NA	NA	-	NA	-
Renal involvement	-	-	-	-	-	-	-	-	-

CMT2A: Charcot-Marie-Tooth disease-2A

Table 2. continued

	Case 10	Case 11	Case 12	Case 13	Case 14	Case 15	Case 16
Syndrome	**m.11696G>A mutation**	**Alpers syndrome**	**Leigh syndrome**	**Combined oxidative phosphorylation deficiency 13**	**mt.9804G>A mutation**	**Cystic leukoencephalopathy without megalencephaly**	**Leigh syndrome**
Muscle weakness	+	+	+	-	+	-	+
Seizure	-	-	-	-	-	+	-
Swallowing dysfunction	-	-	-	-	NA	-	-
Mental regression	-	-	-	+	+	+	-
Developmental delay	-	+	-	+	+	+	+
Dystonia	-	-	-	-	-	-	-
Spasticity	-	-	-	+	-	+	-
Recurrent	-	-	-	-	-	-	+
Skeletal deformity	+	-	-	+	NA	-	-
Ptosis	-	-	-	-	-	-	-
Nystagmus	NA	+	-	NA	-	-	-
Strabismus	-	NA	-	-	+	-	-
Hypertrichosis	-	NA	-	-	+	-	-
Ataxia	-	+	-	-	-	-	-
Hearing loss	NA	+	+	-	+	NA	-
Renal involvement	-	-	+ (CRF)	-	+ (CRF)	NA	-

CMT2A: Charcot-Marie-Tooth disease-2A

in eight cases (50%), and normal findings were detected in 3 (18.7%), lactate peaks in 4 (25%), and both lactate and lipid peaks in 1 (6.3%) patient. Three (18.7%) cases with lactate peaks had LS and one (6.3%) patient had Alpers syndrome. The case with LS had both lactate and lipid peaks (Table 3).

Electrophysiology

Electroencephalography (EEG) was performed in six (37.5%) cases in that the most common abnormality was generalized epileptic discharges. While EEG examination was normal in one case, focal epileptic discharge and encephalopathy were observed in one case each. However, most of these cases did not experience clinical seizures and only two of them were diagnosed with epilepsy. Ten patients (62.5%) were evaluated with electromyography which revealed sensorimotor polyneuropathy in eight cases and myopathic changes in one. One patient had normal electromyographic findings.

Other Diagnostic Approaches

Muscle biopsy was performed in five cases (31.2%) which yielded normal results in three patients, while complex I, II, III, IV deficiency was detected in one LS case. Two cases with normal biopsy findings had CMT disease-2A and one patient had LS. Mitochondrial staining could not be performed in one muscle biopsy specimen due to inappropriate sample collection. Elevated serum lactate (>2 mmol/L) levels were detected in eight patients including cases with LS (n=4), and elevated serum lactate levels were observed in cases with CMT disease-axonal-type 2C (n=1), combined oxidative phosphorylation deficiency (n=1), *m.11696G>A* (n=1) and *mt.9804G>A* mutations (n=1). A slight increase in creatine kinase (CK) values was observed in a case with LS (CK: 225 U/L) (n=1), and another one with *mt.9804G>A* mutation (CK: 219 U/L) (n=1) (Table 4).

Table 3. Magnetic resonance imaging results of the cases

	Syndrome	Brain MRI	Spinal MRI	MRS
Case 1	Leigh syndrome	Signal increase in bilateral basal ganglia	NA	Lactate and lipid peak
Case 2	Leigh syndrome	Signal increase in bilateral mesencephalon and bulbus	T2 hyperintensity in the cervical spinal cord	Lactate peak
Case 3	CMT2A	Normal	S1-2 transitional vertebral anomaly	NA
Case 4	CMT2A	NA	NA	NA
Case 5	CMT2A	NA	NA	NA
Case 6	CMT2A	Normal	Normal	NA
Case 7	Charcot-Marie-Tooth disease, axonal, type 2K	Normal	Normal	NA
Case 8	Charcot-Marie-Tooth disease, axonal, type 2K	NA	NA	NA
Case 9	Leigh syndrome	Bilateral signal increase in basal ganglia	Normal	Lactate peak
Case 10	*m.11696G>A* mutation	Normal	NA	NA
Case 11	Alpers syndrome	Leukodystrophy pattern in cerebral white matter, involvement in brainstem and bilateral middle cerebellar peduncles	NA	Lactate peak
Case 12	Leigh syndrome	Bilateral signal increase in cerebral and cerebellar white matter	Normal	Lactate peak
Case 13	Combined oxidative phosphorylation deficiency 13	Bilateral signal increase in cerebral white matter, thin corpus callosum, hypomyelination of posterior limb of capsula interna	Normal	Normal
Case 14	*mt.9804G>A* mutation	Thin corpus callosum, posterior cerebral periventricular gliotic changes	NA	Normal
Case 15	Cystic leukoencephalopathy without megalencephaly	Cerebellar atrophy, bilateral signal increase in the cerebral hemispheres	NA	Normal
Case 16	Leigh syndrome	NA	NA	NA

MRI: Magnetic resonance imaging, MRS: Magnetic Resonance Spectroscopy, NA: Not available, CMT2A: Charcot-Marie-Tooth disease-2A

DISCUSSION

Except for erythrocytes, every cell in the human body is dependent on mitochondria to function properly. Thence, mitochondrial dysfunctions give rise to multisystem involvement, especially in brain, heart and muscle tissues which all are in a bind for high energy levels [18]. A wide range of findings such as muscle weakness, developmental delay, mental regression, ataxia, spasticity, hearing loss, cardiological and renal involvements, seizures, recurrent respiratory failure, swallowing dysfunction, ptosis, nystagmus, strabismus were observed in this study.

Table 4. Results of the laboratory evaluation, electrophysiological study and muscle biopsy of the cases

	Syndrome	Lactate	EEG	EMG	ECHO	ECG	Muscle biopsy
Case 1	Leigh syndrome	Elevated	NA	Sensorimotor polyneuropathy	AF	Normal	NA
Case 2	Leigh syndrome	Elevated	NA	Sensorimotor polyneuropathy	MF, PFO	Normal	Inappropriate sampling
Case 3	CMT2A	NA	NA	Sensory loss in the upper extremities and axonal loss in the lower extremities	Normal	NA	NA
Case 4	CMT2A	NA	NA	Motor-predominant sensorimotor polyneuropathy in the lower extremities	NA	NA	Normal
Case 5	CMT2A	NA	NA	Motor-predominant sensorimotor polyneuropathy in the lower extremities	NA	NA	Normal
Case 6	CMT2A	NA	NA	Sensorimotor polyneuropathy	Normal	NA	NA
Case 7	Charcot-Marie-Tooth disease, axonal, type 2K	Elevated	NA	Axonal-predominant sensorimotor polyneuropathy	Normal	Normal	NA
Case 8	Charcot-Marie-Tooth disease, axonal, type 2K	NA	NA	Axonal-predominant sensorimotor polyneuropathy	NA	NA	NA
Case 9	Leigh syndrome	Elevated	Generalized	NA	Normal	Normal	Normal
Case 10	m.11696G>A mutation (without a specific certain phenotype)	Elevated	Generalized	Myopathic changes	NA	NA	NA
Case 11	Alpers syndrome	Normal	NA	NA	Normal	Normal	NA
Case 12	Leigh syndrome	Elevated	Encephalopathy	NA	HCMP, LVD (mild), MF, AS, AF	NA	NA
Case 13	Combined oxidative phosphorylation deficiency 13	Elevated	Normal	NA	Normal	Normal	NA
Case 14	*mt.9804G>A* mutation (without a specific certain phenotype)	Elevated	NA	Normal	LVH (mild), MF	Normal	NA
Case 15	Cystic leukoencephalopathy without megalencephaly	Normal	Focal	NA	MF	Normal	NA
Case 16	Leigh syndrome	Normal	Generalized	NA	RCMP, LVD (mild), MF, TF	NA	Complex 1, 2, 3, 4 deficiency

EEG: Electroencephalography, EMG: Electromyography, ECHO: Echocardiography, ECG: Electrocardiography, NA: Not available, AF: Aortic valve failure, MF: Mitral valve failure, PFO: Patent foramen ovale, HCMP: Hypertrophic cardiomyopathy, LVD: Left ventricular dysfunction, AS: Aortic valve stenosis, LVH: Left ventricular hypertrophy, RCMP: Restrictive cardiomyopathy, TY: Tricuspid valve failure, CMT2A: Charcot-Marie-Tooth disease-2A

Mitochondria need about 1500 proteins to function properly [19,20]. Of which, 13 are encoded by mtDNA and the rest by nDNA. Oxidative phosphorylation occurs by virtue of electron transport through the mitochondrial respiratory chain. There are four complexes [complex I (NADH: ubiquinone oxidoreductase), complex II (succinate dehydrogenase), complex III (Coenzyme Q-cytochrome c reductase), complex IV (cytochrome c oxidase)] and two mobile electron carriers [ubiquinone (coenzyme Q10) and cytochrome c] in the mitochondrial respiratory chain. The proton gradient formed by the mitochondrial respiratory chain induces ATP production via complex V (ATP synthase) [21]. While the coding of Complex II is entirely under the control of nDNA, both nDNA and mtDNA play a role in the coding of other structures involved in the respiratory chain [22]. A Poland cohort study have shown that pediatric MDs are caused by nDNA and mtDNA mutations at the rates of 91% and 9%, respectively [23]. In a study conducted in China, patients diagnosed with pediatric MDs were evaluated, and mtDNA and nDNA mutations were found to be responsible for 67.2%, and 32.8% of the cases, respectively [24]. Another study in China revealed these rates as 65% for mtDNA and 35% for nDNA mutations [25]. In our study, nDNA and mtDNA mutation rates were found to be 75% and 25%, respectively.

LS is the most common PMD in childhood [26]. In our study, similar to the literature, LS was the most common diagnosis with a rate of 31.2% in the PMD cases. Hu et al. [24] reported that LS was found to be the third most common diagnosis with a rate of 25.6% in 58 patients with pediatric MDs and the most common diagnoses were mitochondrial myopathy (33.3%) and MELAS (28.2%). Although the first signs and/or symptoms of LS become manifest usually between 3-12 months, in the literature onset times varying from birth to adult life have been reported [27,28]. The age of the onset of LS findings was between 6-132 months (mean 35.4 months) in our study. In LS, which presents a wide spectrum of clinical features such as global developmental delay or regression, hypotonia, dystonia, ataxia, ophthalmological abnormalities like nystagmus or optic atrophy [29], the most common symptoms in our study were muscle weakness (n=5, 100%), developmental delay (n=4, 80%), mental regression (n=3,60%). Ataxia (n=2,40%), cardiac involvement (n=2,40%), ptosis (n=1,20%), optic atrophy (n=1, 20%), swallowing dysfunction (n: 1, %) 20), hearing loss (n=1, 20%), renal involvement (n=1, 20%), respiratory failure (n=1, 20%) were other clinical features in LS. Ma et al. [30] reported that the most common findings in 75 patients with LS in China were motor retardation (55%), muscle weakness (29%), and epilepsy (25%). In the same study, ataxia was found in 11%, swallowing dysfunction in 7%, and ptosis in 4% of their patients. In a multicenter study of Sofou et al. [27], the most common clinical findings in LS cases were abnormal motor findings (99.2%), abnormal ocular findings (60.8%), and feeding difficulties (45.4%). In terms of abnormal motor findings, hypotonia with a rate of 74.6% was the most common feature, while ataxia, and muscle weakness were found at a incidence rates of 34.6%, and 26.2%, respectively. Among abnormal ocular findings, nystagmus was the most common finding (23.8%), as optic atrophy was observed in 14.6% and ptosis in 13.1% of the cases. Rates of the respiratory failure, hearing loss, cardiac, and renal involvement were 37.7%, 19.2%, 17.7, and 5.4%, respectively [27]. The low number of cases in our study and the diversity of underlying genetic factors in the cohorts can be shown as the reason for our diverse incidence rates compared to the literature. T2-weighted hyperintensities in the basal ganglia and/or brainstem are classic findings on brain imaging in LS [29]. This radiological finding was also obtained in 60% of our cases, and increased signal intensities in cerebral-cerebellar white matter were observed in one case. Similar to the clinical features, LS is a heterogeneous disease from a genetic perspective, as well. While more than 75 genes associated with LS have been identified 17 new genes have been reported in a recent study [29,31]. In our study, SURF1 (n=2, 12.5%), MTATP6 (n=2, 12.5%), *PDSS2* (n=1, 6.2%) mutations were found in cases diagnosed with LS. Li et al. [32] found SURF1 mutation in 12 of 178 cases with suspected mitochondrial disease in that one of them was later diagnosed with Leigh-like syndrome and the others with LS. In the genetic analysis of 64 cases clinically diagnosed with LS in South Korea, 3.1% of the cases had SURF1 and 7.8% of them MTATP6 mutations [31]. In the literature, MTATP6 mutation has been found in approximately 10% of LS cases [29]. López et al. [33] reported LS due to *PDSS2* mutation in a patient who presented with hypotonia and neonatal pneumonia, followed up with epilepsy, swallowing dysfunction and nephrotic syndrome, and died at the age of 8 months due to status epilepticus. The findings of our case with *PDSS* mutation started at a later age (132 months) compared to the literature, and muscle weakness, ataxia, hearing loss, nephrotic syndrome and chronic kidney failure were observed during the clinical course of the disease. Although in the case reported by López et al. [33], typically increased signal intensity in basal ganglia consistent with LS was

found, in our case increased signal intensity in bilateral cerebral and cerebellar white matter in brain MRI and a lactate peak in MRS were observed.

Several types of CMT with SMDs have been described in the literature including CMT2A and CMT2K, in which mutations in *MFN2* and *GDAP1* genes were causative factors [17]. While all four of our six cases with CMT disease-2A had *MFN2* mutation, *GDAP1* mutation was found in two cases with CMT disease-axonal-type 2K. In a multicenter study on the genetic etiology of CMT disease, after *PMP22* deletion/duplication was excluded in typical demyelinating CMT cases, the most common mutations revealed by next -generation sequencing were *GJB1* (5.5%), *SH3TC2* (3.6%), *MFN2* (3%). In the same study, *GDAP1* mutation was found with a rate of 1.8% [34].

CMT type 2A, the most common axonal hereditary polyneuropathy with *MFN2* mutations, has been typically associated with distal extremity muscle weakness, atrophy, and unlike other CMTs; optic atrophy [35]. In our study, muscle weakness was found in 25% of the cases with CMT2A. Although visual impairment has been reported in approximately 20% of these cases in the literature, in our study visual signs/symptoms were not observed in this particular disease [35]. According to the age of disease onset, CMT2A is divided into two groups as early (<10 years) and late- onset (>10 years) disease. Early- onset disease is associated with more severe clinical findings and earlier loss of ambulation. However, in some reported cases any correlation could not be found between the age of disease onset and its severity [35]. In support of the literature, in our study early-onset CMT2A cases aged between 12 and 56 months. However, due to the lack of long follow-up periods in our study, the relationship between the onset of the disease and its severity could not be demonstrated.

CONCLUSION

LS was the most common genetically diagnosed PMDs, while CMT2A was the most common SMDs. MDs, which can progress with multisystem involvements and have many various clinical presentations, should be considered in the differential diagnosis of the cases with neuromuscular involvement. Diagnosis of MDs remains challenging in many cases, and detailed evaluation of clinical findings, biochemical screening, histopathological studies, neuroimaging and molecular genetic testing play a substantial role in the diagnostic process.

Ethics

Ethics Committee Approval: The present study was conducted in accordance with the 1964 Declaration of Helsinki and approved by the Ethics Committee of Dokuz Eylül University Faculty of Medicine (approval number: 2021/14-59, date: 06.05.2021).

Informed Consent: Informed consent was obtained from patients and/or parents or legal guardians of the patients before their enrollment in the study.

Peer-review: Externally peer-reviewed.

Author Contributions

Surgical and Medical Practices: Ç.G., C.P., P.E., G.S.U., A.S.H.K., Z.A.G., P.T.K., N.A., U.Y., Concept: Ç.G., C.P., P.E., G.S.U., A.S.H.K., Z.A.G., P.T.K., N.A., U.Y. Design: Ç.G., C.P., P.E., G.S.U., A.S.H.K., Z.A.G., P.T.K., N.A., U.Y., Data Collection and/or Processing: Ç.G., C.P., P.E., G.S.U., A.S.H.K., Z.A.G., P.T.K., N.A., U.Y., Analysis and/or Interpretation: Ç.G., C.P., P.E., G.S.U., A.S.H.K., Z.A.G., P.T.K., N.A., U.Y., Literature Search: Ç.G., U.Y., Writing: Ç.G.

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