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ORIGINAL ARTICLE

Phenotyping mitochondrial DNA-related diseases in childhood: A cohort study of 150 patients

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Abstract

Background and purpose: Mitochondrial diseases (MDs) are heterogeneous disorders caused by mutations in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) associated with specific syndromes. However, especially in childhood, patients often display heterogeneity. Several reports on the biochemical and molecular profiles in children have been published, but studies tend not to differentiate between mtDNA- and nDNA-associated diseases, and focus is often on a specific phenotype. Thus, large cohort studies specifically focusing on mtDNA defects in the pediatric population are lacking.

Methods: We reviewed the clinical, metabolic, biochemical, and neuroimaging data of 150 patients with MDs due to mtDNA alterations collected at our neurological institute over the past 20 years.

Results: mtDNA impairment is less frequent than nDNA impairment in pediatric MDs. Ocular involvement is extremely frequent in our cohort, as is classical Leber hereditary optic neuropathy, especially with onset before 12 years of age. Extraneurological manifestations and isolated myopathy appear to be rare, unlike adult phenotypes. Deep gray matter involvement, early disease onset, and specific phenotypes, such as Pearson syndrome and Leigh syndrome, represent unfavorable prognostic factors. Phenotypes related to single large scale mtDNA deletions appear to be very frequent in the pediatric population. Furthermore, we report for the first time an mtDNA pathogenic variant associated with cavitating leukodystrophy.

Conclusions: We report on a large cohort of pediatric patients with mtDNA defects, adding new data on the phenotypical characterization of mtDNA defects and suggestions for diagnostic workup and therapeutic approach.

KEYWORDS
mitochondrial disorder, mitochondrial DNA, pediatric, phenotypes

INTRODUCTION

Mitochondrial diseases (MDs) are heterogeneous disorders presenting with extreme phenotypic and genotypic variability. Both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) control mitochondrial functions; mtDNA encodes for 13 structural oxidative phosphorylation components, 22 transfer RNAs (tRNAs), and two ribosomal RNAs for mtDNA translation.

More than 250 potentially pathogenic mtDNA variants have been reported (https://www.mitomap.org/), associated with different phenotypes. Leigh syndrome (LS; Online Mendelian Inheritance in Man [OMIM] #256000) is the most common, defined by focal and/or bilateral symmetric deep gray matter lesions and associated with both nDNA and mtDNA mutations, especially in *MT-ND* genes encoding for complex I (CI) subunits and *MT-ATP6* gene. LS related to *MT-ATP6* point mutations (usually m.8993T>G) is conventionally known as maternally inherited LS. This variant is also associated with neuropathy, ataxia, and retinitis pigmentosa (NARP; OMIM #551500) syndrome, with a variable heteroplasmic rate. Mitochondrial encephalopathy with lactic acidosis and strokelike episodes (MELAS; OMIM #540000) syndrome, strongly associated with the m.3243A>G mutation in the *MT-TL1* gene, is characterized by recurrent strokelike episodes (SLEs), seizures, headaches, and hyperlactacidemia. Myoclonic epilepsy with ragged-red fibers (MERRF; OMIM #545000), related to the m.8344A>G change in the *MT-TK* gene, presents with seizures, myoclonus, and ataxia. Subacute, painless, bilateral optic nerve degeneration leading to vision loss is the hallmark of Leber hereditary optic neuropathy (LHON; OMIM #535000), associated with m.3460G>A, m.11778G>A, and m.14484T>C variants, in *MT-ND1*, *MT-ND4*, and *MT-ND6*, respectively. Ocular myopathy characterizes progressive external ophthalmoplegia (PEO; OMIM #530000), due to a single large scale mtDNA deletion (SLSMD). When additional neurological symptoms are present, the term PEO plus is used. The same SLSMD causes Pearson syndrome (PS; OMIM #557000) or Kearns–Sayre syndrome (KSS; OMIM #530000); PS is a multisystem MD primarily characterized by early onset refractory sideroblastic anemia; KSS is defined by early onset, PEO, pigmentary retinopathy, heart blocks, ataxia, sensory hearing loss, and endocrinopathy.

Several reports regarding MDs in children [1–8] are available; however, they tend to not differentiate between MDs due to mtDNA or to nDNA alterations [9–12]. The largest cohort of mtDNA-related MDs, recently published, comprises 27 patients [13]. Thus, data about the actual frequency of mtDNA pathogenic variants, phenotype distribution, and natural history in childhood remain unavailable.

The lack of knowledge on the impact of these diseases is a serious limitation for their clinical and genetic management and for experimental treatment approaches (e.g., oocyte donation, where the nDNA from an oocyte containing mutated mtDNA is transferred to an oocyte with functional mitochondria [14]).

The purpose of this study is to analyze a cohort of patients from a tertiary neuropediatric center, to identify genotype–phenotype correlations, define possible emerging phenotypes, investigate prognostic factors, and improve the diagnostic and therapeutic approach.

METHODS

We reviewed the findings of children with MDs diagnosed at Fondazione IRCCS Istituto Neurologico Carlo Besta from 2000 to 2020 and selected cases with confirmed mtDNA point mutations/SLSMDs. Analyzed data included family history, age at onset, presenting symptoms, clinical evolution, brain magnetic resonance imaging (MRI), metabolic data, respiratory chain enzyme activity in muscle and/or fibroblasts, and genetic diagnoses.

Molecular analysis was performed on genomic DNA extracted from muscle, blood, fibroblasts, or other tissues. mtDNA macrodeletions were detected by Southern blotting analysis [15]; the percentage of heteroplasmy was estimated by the densitometric analysis of the wild-type and mutant bands. Until 2016, mtDNA was polymerase chain reaction-amplified and then sequenced according to a standardized protocol [16], replaced since 2016 by next generation sequencing approaches [17].

Our study is retrospective, which could be a limitation. However, only few data were not available for all patients; we believe this does not affect the main results. Because our institution is a reference center for all MDs and not only those with neurological manifestations, we do not believe our cohort has any selection bias.

All the subjects' parents provided informed consent for MRI acquisition, muscle or skin biopsy, and genetic analysis. Parents consented to the use of anonymized personal data for scientific purposes according to the ethical standards of the 1964 Declaration of Helsinki.

RESULTS

One hundred fifty children (16.7%) with pathogenic mtDNA variants were identified among 902 children with a confirmed MD molecular diagnosis. Family history was available for 138 (92%), onset age and symptoms for 144 (96%) and 148 (99%), respectively, clinical findings for all, follow-up data for 70 (47%), brain MRIs for 110 (73%), and plasma and cerebrospinal fluid (CSF) lactate levels for 88 (59%) and 25 (17%), respectively. Biochemical analyses were performed in 87 (58%); in the other cases, molecular diagnosis was achieved without need for biochemical studies.

Patients were classified in phenotypes according to clinical, neuroimaging, and genetic findings (Figure 1, Table 1).

Leigh syndrome

Fifty-four probands (36%) presented with LS, with an average onset age of 18 months (range = 0–13 years).

Family history was positive in 34.6%. In 40%, onset appeared to be associated with trigger factors; the main symptoms were psychomotor delay (PD; 31.4%) and hypotonia (18.5%).

Subsequently, hypotonia, ocular involvement, and seizures became frequent (66.6%, 51.8%, and 50%, respectively), whereas extraneurological involvement was rare: heart disease in 11.1%

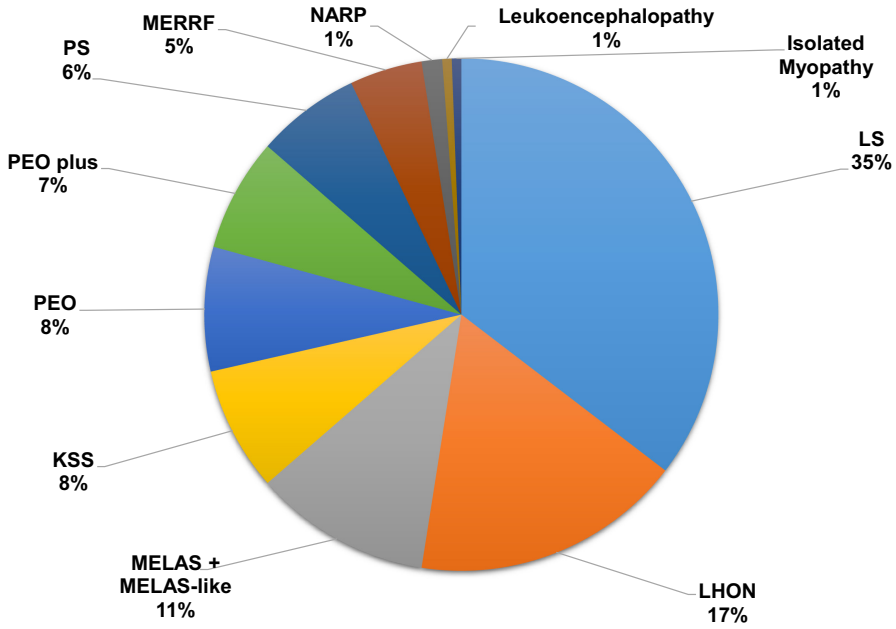

FIGURE 1 Phenotype distribution by percentage. KSS, Kearns–Sayre syndrome; LHON, Leber hereditary optic neuropathy; LS, Leigh syndrome; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF, myoclonic epilepsy with ragged-red fibers; NARP, neuropathy, ataxia, and retinitis pigmentosa; PEO, progressive external ophthalmoplegia; PS, Pearson syndrome.

(Wolf–Parkinson–White syndrome in one, hypertrophic cardiomyopathy in three, no details for two), diabetes in 9.2%.

On MRI, basal ganglia (BG) involvement was seen in 94.4%, followed by lesions in pontine/mesencephalic tegmentum (40.7%) or the thalamic/subthalamic region (24%). Other findings included cortical (18.5%) and subcortical (14.1%) atrophy and white matter (WM) lesions (9.2%).

Hyperlactacidemia was seen in 82.6% and elevated CSF lactate values in 94.7%.

The main biochemical defect was CI (49%), followed by complex V (13.7%) and multiple complex deficiency (MCD; 7%). Biochemical studies were normal in 23.5% (eight m.8993T>G and four *MT-ND* gene mutations). In three cases harboring m.8993T>G mutations, biochemical studies were not available.

Most patients harbored mutations in *MT-ND* genes (55.5%) and in *MT-ATP6* (42.6%).

Over time (data available for 36 patients [66.7%]; average = 66.1 months), 18 patients remained stable, one improved, five worsened, and 12 died. Patients were treated with a similar mitochondrial cofactor "cocktail" [18].

Data are summarized in Figure 2.

Leber hereditary optic neuropathy

Twenty-six children (17.3%), 23 males and three females, were affected by LHON. Average onset age, available for 25 patients, was 12.5 years (range = 3–17 years); one presented before 6 years of age (4%), six between 6 and 12 years (24%), and 18 between 12 and 18 years of age (72%).

Family history was positive in 43.4%. Brain MRIs, performed in eight patients, were normal. Molecular diagnoses included m.3460G>A in *MT-ND1* in five children (19%), m.11778G>A in *MT-ND4* in 17 (66%), and m.14484T>C in *MT-ND6* in four (15%).

Treatment data were available for four patients, treated with several vitamins including idebenone. Three patients (11.5%) had follow-up data available (average = 72 months); one was stable, one reported improvement, and one worsened.

MELAS and MELAS-like

Data from 17 children (11.3%) were collected; 15 of them (10%) were classified as MELAS and harbored the classic m.3243A>G transition, and two (1.3%) were classified as MELAS-like, with typical clinical and MRI MELAS findings, carrying the m.13514A>G variant in *MT-ND5* and the m.5521G>A transition in *MT-TW*. Mean onset age was 7.4 years (range = 0–15 years); family history was positive in nine children.

The most frequent onset manifestations were seizures (25%), and cognitive impairment and myopathy (12.4% each).

Subsequently, seizures became dominant (75%), followed by SLEs, (64.2%), pyramidal signs (31.2%), and headaches and myopathy (25% each). Hypertrophic cardiomyopathy was reported in one patient. Hyperlactacidemia was seen in 80%.

Follow-up data (average = 35.2 months) were available for eight patients (47%) treated with oral Bioarginina; three were stable, three worsened, and two died.

Data are summarized in Figure 3.

PEO and PEO plus

Progressive external ophthalmoplegia

Twelve probands (8%) were diagnosed with PEO. Average onset age was 11.1 years (range = 4–17 years). In 83%, brain MRI was normal; in one case, cortical atrophy was reported. Biochemical studies

TABLE 1 Genetic diagnoses and associated phenotypes in our cohort.

mtDNA defect (%)	Gene	Patients, *n* (%)	Mutation (*n* patients)	Associated phenotypes (*n* patients)	Tissue [heteroplasmy][a] (range)
mtDNA mutations	*MT-ATP6*	25 (16.6%)	m.8993T>G (24)	LS (23)	M [97.5%] (95–100); L [91.4%] (80%–95%)
				NARP (1)	L [80%] (n.a.)
109 (72.6%)			m.8993T>C (1)	NARP (1)	L [90%] (n.a.); UEC [90%] (n.a.)
	MT-ND1	8 (5.3%)	m.3460G>A (5)	LHON (5)	L [100%] (n.a.)
			m.3481A>G (1)	LS (1)	M [80%] (n.a.); L [55%] (n.a.); F [60%] (n.a.); UEC [80%] (n.a.)
			m.3688G>A (1)	LS (1)	M [100%] (n.a.)
			m.3697G>A (1)	LS (1)	M [100%] (n.a.); L [100%] (n.a.); UEC [100%] (n.a.)
	MT-ND2	1 (0.6%)	m.4831G>>A (1)	Isolated myopathy (1)	M [95%] (n.a.); L [5%] (n.a.); F [0%] (n.a.); UEC [40%] (n.a.)
	MT-ND3	12 (8%)	m.10197G>A (5)	LS (5)	M [98%] (95%–100%); L [99.6%] (99%–100%)
			m.10191T>C (3)	LS (3)	M [74.3%] (63%–80%); L [49.3%] (40%–60%); UEC [68%] (60%–74%)
			m.10158T>C (4)	LS (4)	M [70.5%] (61%–80%); F [50%] (40%–60%)
	MT-ND4	18 (12%)	m.11778G>A (17)	LHON (17)	L [94.1%] (90%–100%)
			m.11777A>C (1)	LS (1)	M [60%] (n.a.)
	MT-ND5	11 (7.3%)	m.13084T>A (1)	LS (1)	M [85%] (n.a.); L [82%] (n.a.); F [72%] (n.a.); UEC [67%] (n.a.)
			m.13094T>C (1)	LS (1)	M [50%] (n.a.); L [40%] (n.a.); F [30%] (n.a.)
			m.13513G>A (6)	LS (6)	M [64.5%] (50%–80%); L [46.5%] (40%–53%); F [48.2%] (32%–70%)
			m.13514A>G (3)	LS (2)	M [60%] (50%–70%); L [34.4%] (4%–50%); F [27.3%] (12%–50%)
				MELAS-like (1)	
	MT-ND6	8 (5.3%)	m.14459G>A (1)	LS (1)	L [90%] (n.a.); F [90%] (n.a.)
			m.14484T>C (4)	LHON (4)	L [97.5%] (90%–100%)
			m.14487T>C (2)	LS (2)	M [98%] (95%–100%)
			m.14600G>A (1)	LS (1)	M [100%] (n.a.); F [100%] (n.a.)
	MT-CO3	1 (0.6%)	m.9907G>A (1)	Leukoencephalopathy (1)	M [100%] (n.a.); L [100%] (n.a.); F [100%] (n.a.); UEC [100%] (n.a.)
	MT-TL1	16 (10.6%)	m.3243A>G (15)	MELAS (15)	L [37.5%] (5%–70%)
			m.3242G>A (1)	LS (1)	M [100%] (n.a.); F [100%] (n.a.)
	MT-TI	1 (0.6%)	m.4309G>A (1)	PEO plus (1)	M [80%] (n.a.); L [0%] (n.a.); F [15%] (n.a.); UEC [60%] (n.a.)
	MT-TW	1 (0.6%)	m.5521G>A (1)	MELAS-like (1)	M [98%] (n.a.); L [38%] (n.a.); F [88%] (n.a.); UEC [91%] (n.a.)
	MT-TK	7 (4.6%)	m.8344A>G (7)	MERRF (7)	M [77.5%] (70%–90%); L [56%] (40%–70%)
Single large scale deletion		41 (27.3%)		KSS (9)	M [51.6%] (40%–60%)
				PS (7)	L [72%] (50%–85%)
41 (27.3%)				PS to KSS (3)	L [60%] (50%–70%)
				PEO (12)	M [63.5%] (40%–70%)
				PEO plus (10)	M [60%] (40%–70%)

Abbreviations: F, fibroblasts; KSS, Kearns–Sayre syndrome; L, lymphocytes; LHON, Leber hereditary optic neuropathy; LS, Leigh syndrome; M, muscle; MELAS, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes; MERRF, myoclonic epilepsy with ragged-red fibers; mtDNA, mitochondrial DNA; n.a., not available; NARP, neuropathy, ataxia, and retinitis pigmentosa; PEO, progressive external ophthalmoplegia; PS, Pearson syndrome; UEC, urinary epithelial cells.

[a]In square brackets, heteroplasmic rate for single patient or mean of heteroplasmy for group of patients.

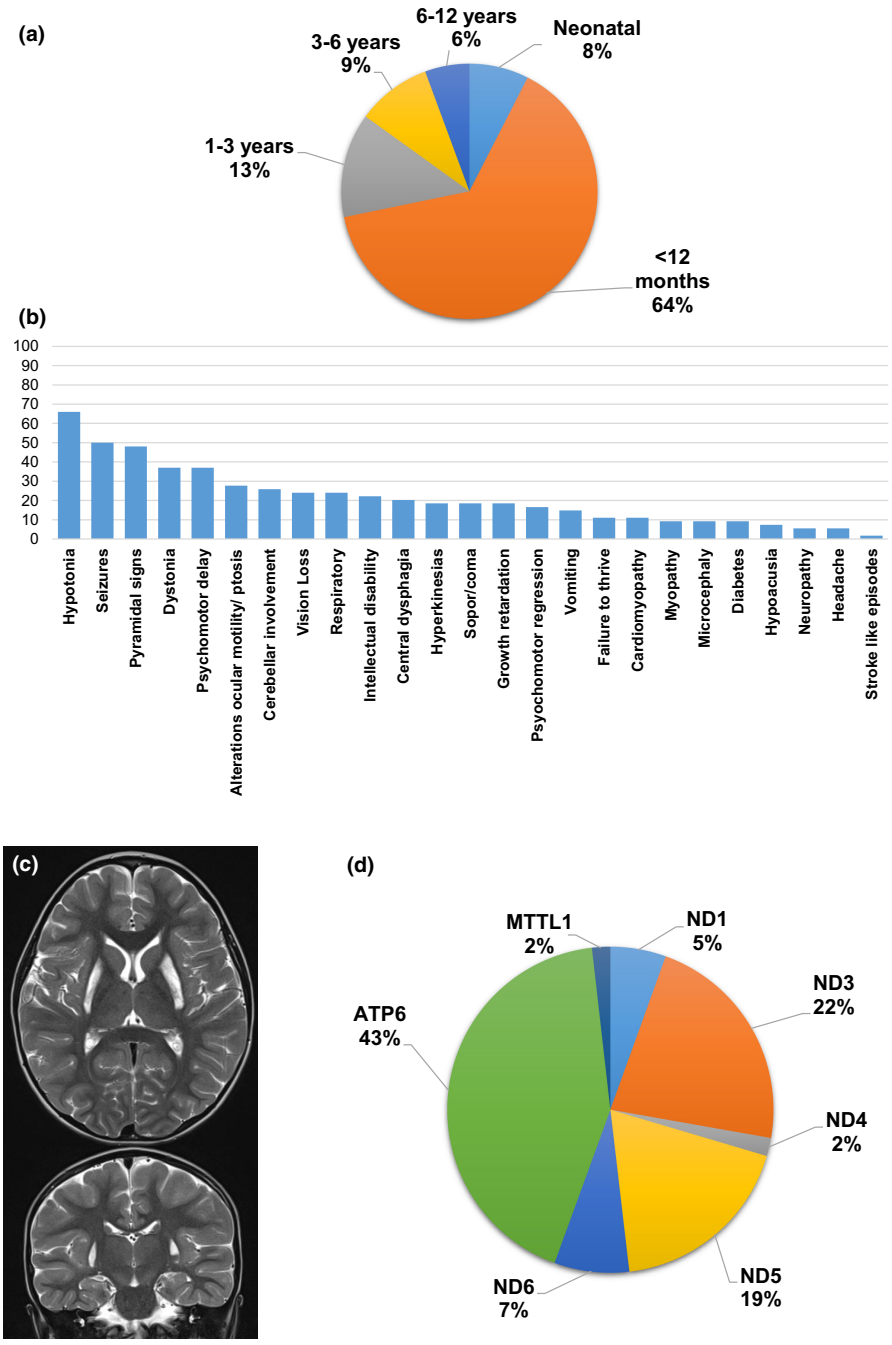

FIGURE 2 Leigh syndrome. (a) Age at onset distribution. (b) Clinical findings. (c) Magnetic resonance imaging axial (above) and coronal (below) T2 sequences show hyperintensities of putamen in a patient with complex I deficiency associated with *MT-ND3* mutation. (d) Genetic diagnosis distribution.

showed CIV deficiency in two and normal results in four. All patients harbored an SLSMD, (mean heteroplasmy in muscle = 63.5%, range = 40%–70%). The two patients with follow-up data (16.7%, average = 72 months) worsened but are alive.

PEO plus

In 11 children (7.3%), a "PEO plus" phenotype was identified. Mean onset age was 7.6 years (range = 1–14 years). Onset symptoms included ocular motility alterations and/or ptosis (81.4%) and cerebellar involvement (18.1%), which in time was found in 63.6%. Other symptoms included myopathy (45.4%), hypoacusia (45.4%), hypotonia (36.3%), failure to thrive (18.1%), cardiac manifestations (27.7%, one conduction block and one dilatative cardiomyopathy), renal involvement (18.1%), and diabetes (9%).

MRI findings included BG lesions (62.5%), pontine/mesencephalic tegmentum involvement (50%), and WM abnormalities (37.5%). Hyperlactacidemia was seen in 55.5%. Biochemical studies found MCDs (42.8%), isolated CI and CIII deficiency (one case each), and normal values (one patient). An SLSMD was harbored by 90.9% of children; one child was positive for the m.4309G>A mutation in *MT-TI* (9.1%).

At follow-up, available for five patients (45.5%), three children worsened, one died, and one remained stable.

Data are summarized in Figure 4.

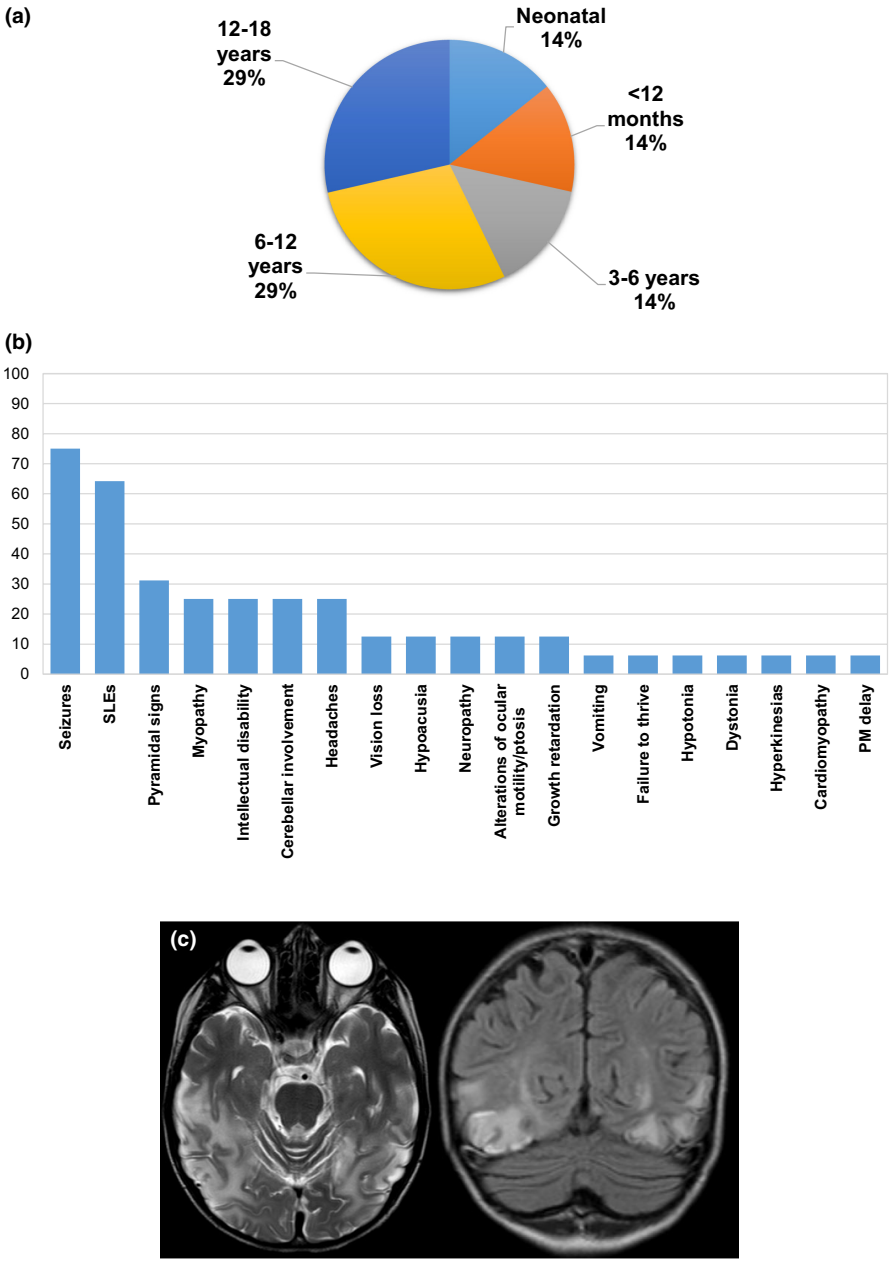

FIGURE 3 Mitochondrial encephalopathy with lactic acidosis and strokelike episodes (MELAS) and MELAS-like. (a) Age at onset distribution. (b) Clinical findings. (c) Axial (left) and coronal (right) fluid-attenuated inversion recovery sequences show strokelike lesions in occipital, temporal, and parietal lobes. PM, psychomotor; SLE, strokelike episode.

Kearns–Sayre syndrome

Twelve children (8%) were diagnosed with KSS; three progressed from PS to KSS during childhood. Symptoms began at an average of 7.2 years (range = 2–18 years) and included growth retardation (GR; 37.5%) and ocular motility alterations, renal involvement, hypoacusia, and vision loss (12.5% each). In time, all patients showed vision loss, 58.3% GR, 75% ataxia, 50% myopathy, and 41.6% hypoacusia. One child developed a cardiac conduction defect, one diabetes.

MRI data (available in 11 patients) showed typical KSS WM lesions (72.7%), pontine or mesencephalic tegmentum lesions (65.4%), and BG involvement (36.6%). Spinal dorsal column lesions were presented by 33.3%.

Hyperlactacidemia was seen in 66.6%. Biochemical studies on muscle were performed in five of 12; four of five were normal, and one patient showed an MCD. All patients harbored SLSMDs.

Folinic and coenzyme Q_{10} supplementation was reported for five patients. Follow-up data of eight patients (66.7%, average time = 50.6 months) showed improvement in one, stable conditions in four, worsening in two, and exitus in one.

Data are summarized in Figure 5a–c.

Pearson syndrome

Ten children (6.6%) were diagnosed with PS, with an average onset age of 3.7 months (range = 0–12 months); three of them

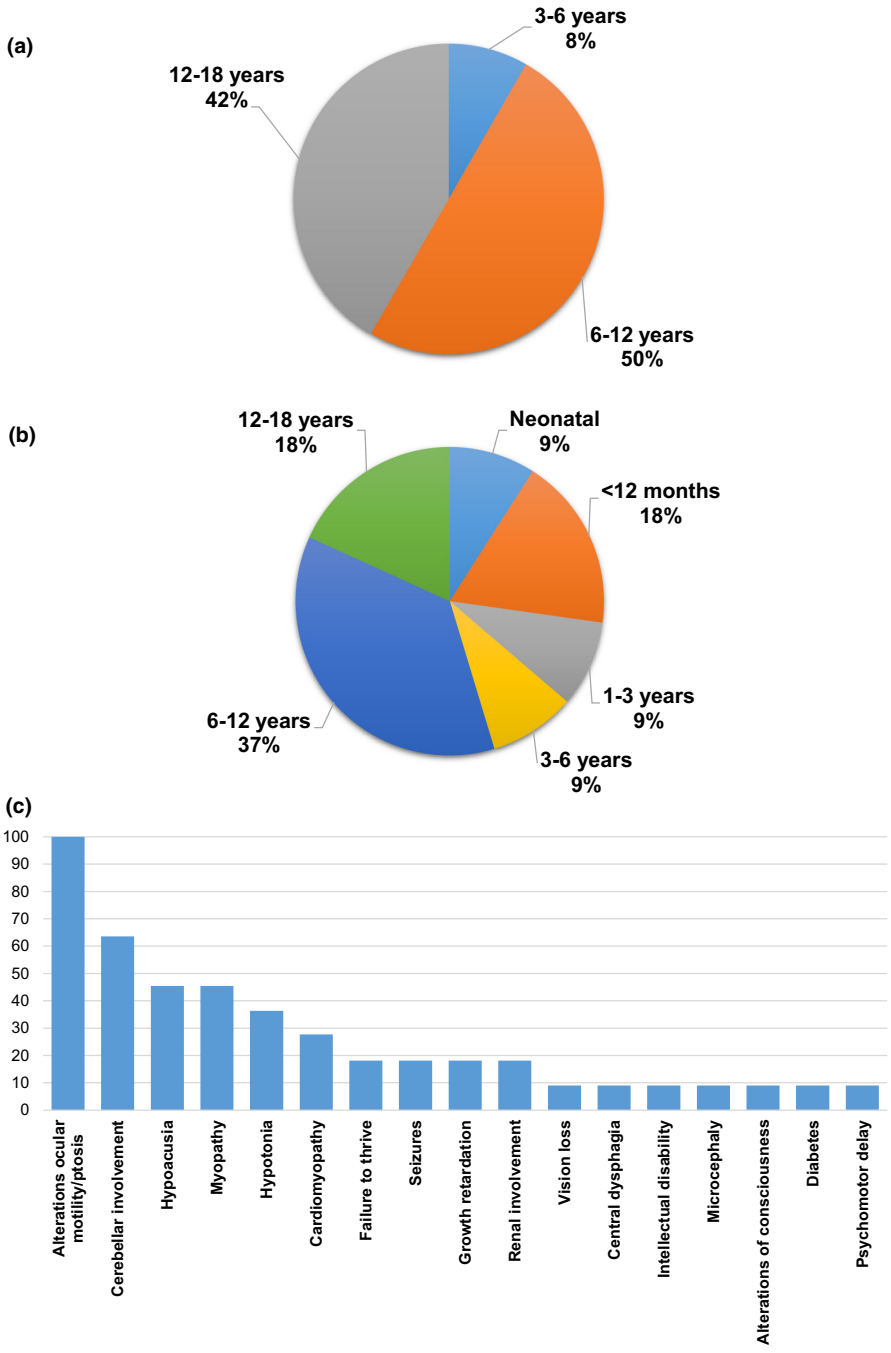

FIGURE 4 Progressive external ophthalmoplegia (PEO) and PEO plus. (a, b) Age at onset distribution of (a) PEO and (b) PEO plus patients. (c) Clinical findings of PEO plus patients.

developed KSS and are previously described. Along with hematological features, GR was reported in two (20%). At follow-up, pancreatic dysfunction, seizures, hypotonia, PD, and renal involvement were seen in 14.2% each. Hyperlactacidemia was seen in two of three children with available data. MRI findings (two patients) showed WM alterations and cortical atrophy in one patient each.

All children harbored an SLSMD in blood (mean heteroplasmy = 72%, range = 50%–85%).

Follow-up of five children (50%) showed exitus in two patients and progression to KSS in three (two died, one remained stable).

Clinical features are summarized in Figure 5d.

Mitochondrial encephalopathy with ragged-red fibers

Seven patients (4.6%), with an average onset age of 5.4 years (range = 0–13 years), harbored the m.8344A>G mutation in *MT-TK*, associated with MERRF. Seizures were present at onset in 42.8%, hyperkinetic movements in 28.5%, and vomiting, failure to thrive, hypoacusia, neuropathy, and cerebellar involvement in 14.2% each.

Subsequently, 71.4% showed epilepsy, 57.1% cerebellar involvement, 42.8% hypoacusia and hyperkinetic movements, and 28.6% myopathy and cognitive impairment. MRI findings were normal in 80%; one patient showed BG alterations. Hyperlactacidemia was seen in 25%.

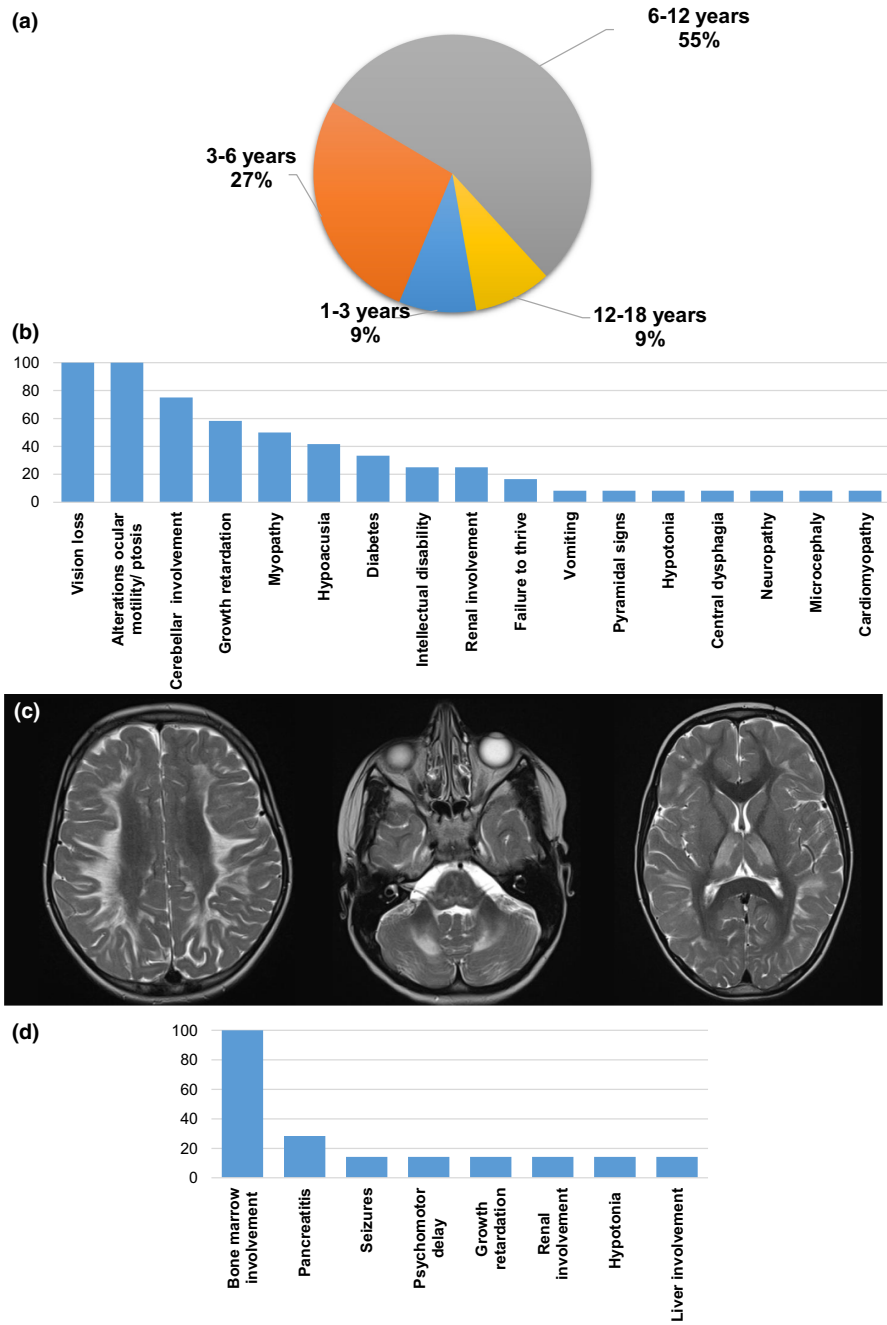

FIGURE 5 Kearns–Sayre syndrome (KSS) and Pearson syndrome (PS). (a) Age at onset distribution and (b) clinical findings of KSS patients. (c) Axial T2 sequences in a KSS patient shows white matter (WM) hyperintensities involved subcortical U-fibers with sparing of periventricular WM (left), cerebellar WM (middle), and thalami (right). (d) Clinical findings of patients affected by PS.

Follow-up of two patients (28.6%, average = 10.5 years), showed worsening in one case, stable condition in the other.

Neuropathy, ataxia, and retinitis pigmentosa

Two children (1.3%) exhibited the classic NARP syndrome associated with an *MT-ATP6* variant: m.8993T>G in one patient and m.8993T>C in the other. Average onset age was 4.5 months (range = 2–7 months). Family history was positive in the first patient.

At onset, the first patient showed cerebellar signs and the second PD. Subsequently, both showed neuropathy, ataxia, and retinopathy; pyramidal signs, dystonia, and hearing impairment were observed only in the second child. On MRI, both showed cerebellar atrophy. They remained stable at follow-up (average = 3 years).

Isolated myopathy

One proband presented at age 7 years with a pure myopathic phenotype due to the m.4831G>A mutation in *MT-ND2* and has been previously described [19].

Leukoencephalopathy

One patient (aged 6 years) presented with PD and hypotonia. At 30 months, retinopathy, hypoacusia, and hyperlactacidemia were found; brain MRI showed a supratentorial cavitating leukoencephalopathy (Figure 6a). Clinical and MRI follow-up were stable.

Isolated CIV defect was detected in muscle and fibroblasts. The novel m.9907G>A variant in *MT-CO3* was identified,

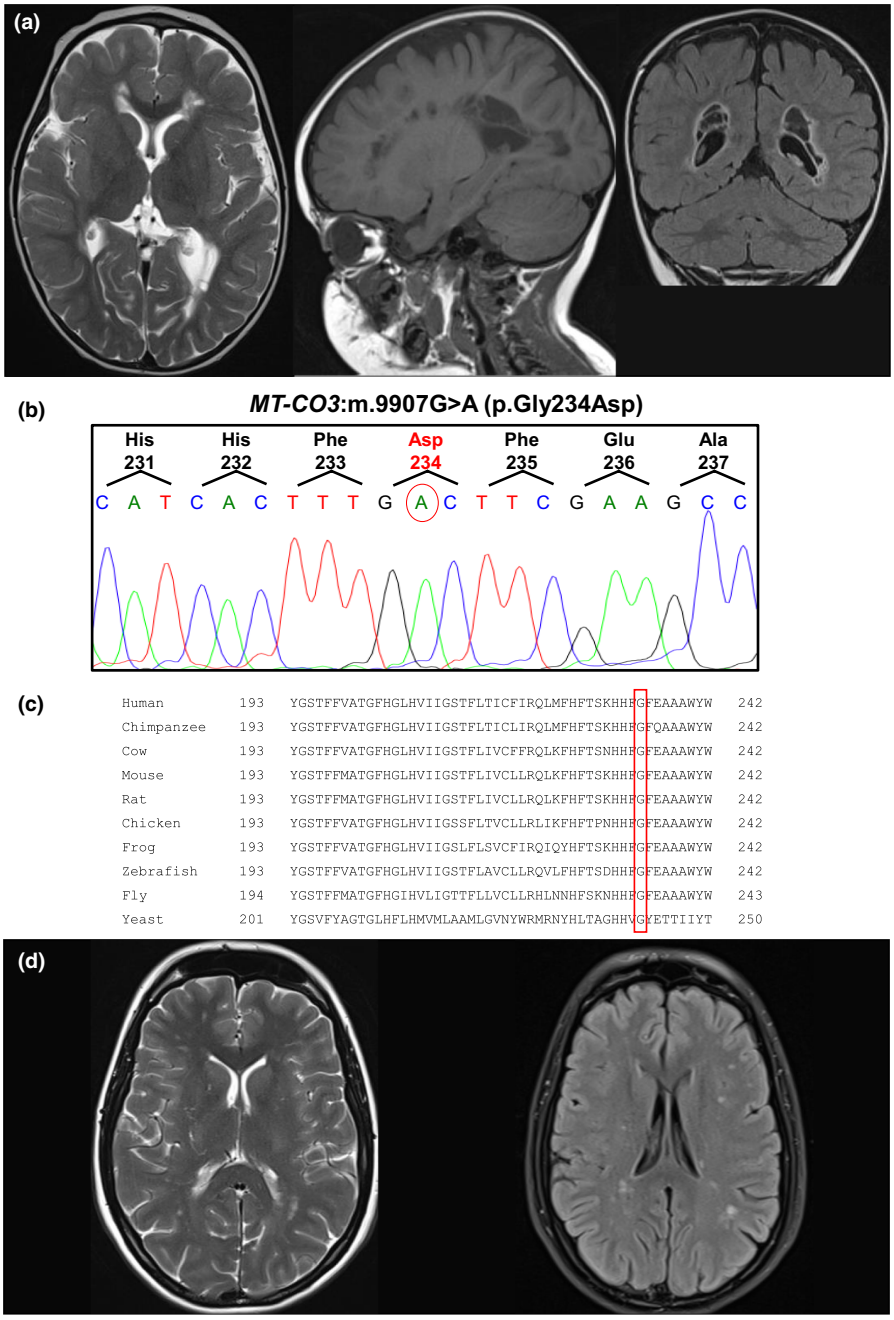

FIGURE 6 Patient with leukoencephalopathy and novel *MT-CO3* mutation. (a) Magnetic resonance imaging (MRI) findings in patient; axial T2-weighted (left), coronal fluid-attenuated inversion recovery (FLAIR; middle), and sagittal T1-weighted (right) sequences show cavitating leukoencephalopathy involving deep white matter (WM) and corpus callosum. (b) Mitochondrial DNA sequence analysis of *MT-CO3* gene with the m.9907G>A mutation and (c) alignment of CoIII protein in different species show the high conservation of Gly234 residue. (d) MRI findings of patient's mother; axial T2-weighted (left) and axial FLAIR (right) sequences reveal focal gliosis area in supratentorial WM.

predicting the substitution of glycine in position 234 with aspartic acid (p.Gly121Asp) in the CoIII protein (Figure 6b). This change scored very high for likelihood to be deleterious according to ad hoc software for pathogenicity prediction (PolyPhen2, MutPred, SIFT, and SNP&Go), and the involved amino acid is highly conserved (100%) in the phylogenies (Figure 6c).

Almost 100% of mtDNA was mutated in the patient's muscle, blood, fibroblasts, and urinary epithelial cells; the patient's mother, apparently healthy, showed 69% mutation in urinary epithelial cells and 2.3% in blood [17].

Her clinical evaluation, performed after her son's diagnosis, revealed hypomimia, rhinolalia, hypotonia, and neuropathy. MRI detected areas of focal gliosis (Figure 6d).

DISCUSSION

In 20 years, among 902 children with a confirmed MD molecular diagnosis, we found 150 patients (16.7%) with an mtDNA pathogenic defect, similar to other pediatric cohort studies (14.4%–20.5%) [1–3] but unlike Loos et al. (30%) [13]. Our data confirm mtDNA impairment is less frequent than nDNA defects in pediatric MDs.

Neonatal onset of symptoms was reported in 7.5%, similar to Honzik and colleagues (10%) [6]. Other studies rarely report mtDNA mutations causing symptoms in newborns (<2%) [4, 5]. Similar to previous studies, early onset of MDs was mostly found in LS and PS [6].

Most symptoms observed at disease onset—PD, hypotonia, seizures, and visceral involvement—were consistent with previous reports [2, 8, 20, 21]. However, central respiratory involvement was quite rare (2.5% vs. 25.6% in Hu et al. [22]). Ocular motility alterations and/or ptosis were more frequent both at onset (17.3%) and during disease progression (33.3%) than previously reported [3, 13, 22, 23]. Vision loss was similarly more frequent than expected in mtDNA-related MDs (22.6% at onset and 36.6% at follow-up, vs. 12%–18.5% in literature) [7, 13, 22]. Although this symptom is part of the diagnostic criteria for some phenotypes (LHON, KSS, NARP), we also found it in other phenotypes (e.g., LS and MELAS, 24% and 14.2%, respectively).

Our data underline the need to suspect mtDNA-related MDs in children presenting with optic atrophy and/or progressive bilateral ocular motility defects. Furthermore, a full ophthalmological examination must be performed in all pediatric patients suspected of an MD.

Extraneurological manifestations and myopathy, frequent manifestations in adult MDs, were rare in our cohort [24].

The heart was the most frequent organ involved, similar to previous series [4, 5]; Honzik et al. [6] reported 40% of subjects having neonatal heart failure; however, they analyzed newborns with both mtDNA and nDNA mutations, including children with *TMEM70* mutations, frequently associated with hypertrophic cardiomyopathy.

LS (35.8%), LHON (17.7%), and MELAS/MELAS-like (11.2%) constituted the three main phenotypes in our cohort.

LS was mainly caused by mutations in *MT-ND* genes (55.5%) or in the *MT-ATP6* gene (42.5%), similar to previous LS series [25–28], which included nDNA-related cases.

Among *MT-ATP6* mutations, LS was the most frequent phenotype (88.4%), whereas NARP syndrome was encountered in only two patients. Our data are consistent with recent literature reports [29], whereas previously NARP was regarded as a frequent *MT-ATP6*-related disorder [30] in children and adults. Notably, these reports often included children who presented with NARP symptoms but showed typical LS MRI lesions, which would account for the difference in frequency. Furthermore, all patients with NARP syndrome presented additional features and a quarter of the LS children showed ataxia, contributing to the emerging concept that *MT-ATP6* variants cause a continuous disease spectrum [29].

LHON represents the second most frequent phenotype (17.7%). Among pediatric MD series, only some authors found a high incidence of LHON-associated mutations (12%–20%) [2, 6, 7], whereas in most cases this phenotype does not appear as frequently [3, 12, 20, 22]. To date, the only available treatment for LHON is idebenone, limited to subjects older than 12 years [31]. In our cohort, 27% of LHON patients began showing symptoms of disease before age 12 years, stressing the need for treatment in this age group.

Similar to previous data [32, 33], in our report the phenotype associated with the MELAS mutation was very variable, ranging from mildly symptomatic to a lethal multisystem disease. Suggestive symptoms were extremely rare in the neonatal period, and the first manifestations occurred in childhood, confirming literature data [33]. SLEs occurred in approximately 60% of our cohort; interestingly, no patients presented with hearing impairment and/or diabetes, reported in up to 50% of cohorts comprising adult populations [32, 33]. Overall, our data underline that the absence of the pathognomonic symptoms of MELAS syndrome at disease onset should not exclude a possible MELAS diagnosis.

SLSMD-associated phenotypes were seen in 26.9%, the most frequent being KSS and PEO (29.2% each), followed by PS and PEO plus (24.3% each). A similar distribution has been recently reported [12]. In our cohort, renal involvement was seen in one patient only, contrary to the 50% of Broomfield et al. [12]. Progression from PS into KSS was slightly higher than previous studies (30% vs. 18%–27%) [12, 34].

All patients affected by MERRF syndrome showed central nervous system symptoms of disease; myopathy and/or exercise intolerance was reported in only 28%, but previously described in up to 70% of m.8344A>G mutated subjects in adult and pediatric cohorts [11]. Our data suggest a predominant neurological phenotype and rare muscle involvement in childhood.

Most patients had elevated lactate levels. Associated phenotypes were LS (82.6%), MELAS and MELAS-like (80%), and KSS and PS (66.6% each). This finding confirms that normal blood lactate does not exclude an MD diagnosis [8, 28] and more specific biomarkers are needed.

The most frequent neuroradiological feature was BG involvement (56.7%), consistent with recent reports (40% of mtDNA mutated patients in Fang et al. [7], 47% in Loos et al. [13]). These were seen not only in LS children, as expected, but also in other phenotypes, including PEO plus (62.5%) and KSS (33.3%).

Other findings included WM abnormalities, especially in KSS (72.7%) and PEO plus (37.5%), similar to previous studies [35]. No patients with predominant WM involvement showed a CI deficiency; therefore, CI-linked leukoencephalopathy appears restricted to nuclear-encoded genes [36]. One third of KSS patients also presented spinal cord involvement, as recently described [37].

Our report confirms that spinal cord impairment in MDs is more frequent than expected; this recommends including spinal cord MRI in the diagnostic pathway of suspect MDs or of unexplained neurodegenerative diseases.

In the patient with the novel m.9907G>A mutation in *MT-CO3*, cavitating WM lesions were reported. Infantile mitochondrial leukoencephalopathies are mostly related to isolated complex deficiencies and nDNA gene mutations, especially those associated with aminoacyl-tRNA synthetase dysfunctions [38, 39], mitochondrial proteins [40, 41], iron–sulfur cluster-related leukoencephalopathies [42–45], and more recently, *POLG* mutations [46]. However, mitochondrial leukoencephalopathies are rarely associated with mtDNA mutations [47].

To date, an *MT-CO3* mutation in pediatric MDs has been reported in two patients associated with LS and MELAS, respectively [48, 49]. Our case expands the phenotype related to *MT-CO3* and suggests looking for mtDNA variants in children with undefined leukodystrophies.

Similar to other reports [29, 35], in our cohort the phenotypes most frequently leading to exitus were PS, LS, KSS, and MELAS. Other unfavorable prognostic factors are onset within the first year (46% of the patients who died in our cohort were in this age group) and BG involvement (56% of the patients who presented exitus). Similar unfavorable prognostic factors have been reported in other series [4, 25, 28].

The most frequently detected mtDNA defects were mutations in *MT-ND* genes (38%), mostly associated with a LS (51.2%) or LHON (44%) phenotype, similar to previous reports [10].

The m.8993T>G variant in *MT-ATP6* was seen in 17% of our patients, in most cases (95%) associated with LS, similar to other series [6, 21].

We reported the MELAS m.3243A>G mutation in approximately 10% of patients, similar to Cruz et al. (7.4%) [23], whereas in other pediatric series it appears to be among the most frequently reported mtDNA defects (26%–40%) [2, 3, 7, 13, 20]. Prevalence is especially high (51.2%) in a recent childhood Chinese cohort study [22].

Although SLSMDs are a common cause of adult MDs, accounting for approximately 16% of all adult mtDNA defects [50], prevalence is usually absent [7, 21] or lower in pediatric cohorts (8%–18%) [2, 13, 20]. In contrast, SLSMDs were frequent in our cohort (26.9%).

CONCLUSIONS

We present the results of a large cohort of patients with mtDNA-associated MDs. Our data confirm that mtDNA impairment is less frequent than nDNA in pediatric MDs. Nevertheless, because early onset neurological manifestations occur in different point mutations, our evidence suggests mtDNA sequencing must be included in the diagnostic workup of neonates and children with a suspected MD.

Ocular involvement is extremely frequent both at onset and throughout disease course, underlining the necessity of a full ophthalmological examination in these patients. Classical LHON was quite frequent in our cohort, especially with onset before 12 years of age, highlighting the need for suitable therapy for this age group. Extra neurological manifestations and isolated myopathy appear rare, unlike adult phenotypes. Unfavorable prognostic factors are deep gray matter involvement, early disease onset, and specific phenotypes such as PS and LS. SLSMD-related phenotypes appear very frequent, whereas MELAS mutation is less frequent than expected; we add novel data on the distribution of mtDNA defects in childhood.

Furthermore, we report a new mtDNA pathogenic variant associated with cavitating leukodystrophy, expanding the phenotype related to mtDNA mutations.

AUTHOR CONTRIBUTIONS

Anna Ardissone: Supervision (lead); conceptualization (lead); investigation (equal); writing–original draft (equal); formal analysis (equal); writing–review and editing (equal). **Giulia Ferrera**: Writing–original draft (equal); formal analysis (equal); writing–review and editing (equal). **Costanza Lamperti:** Formal analysis (equal); writing–review and editing (equal). **Valeria Tiranti:** Formal analysis (equal); writing–review and editing (equal). **Daniele Ghezzi:** Investigation (equal); formal analysis (equal); writing–review and editing (equal). **Isabella Moroni**: Investigation (equal); writing–review and editing (equal). **Eleonora Lamantea**: Conceptualization (supporting); investigation (equal); writing–original draft (supporting); formal analysis (equal); writing–review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors report no competing interests.

DATA AVAILABILITY STATEMENT

Data supporting the study findings are available from the corresponding author upon reasonable request.

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