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258th ENMC international workshop
Leigh syndrome spectrum: genetic causes, natural history and preparing for clinical trials
25-27 March 2022, Hoofddorp, Amsterdam, The Netherlands

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1. Introduction

A hybrid ENMC workshop was held in Amsterdam on the weekend of 25–27th March 2022 involving 24 clinical and basic scientists, an industry participant and 4 patient representatives to discuss the genetic causes, natural history and preparation for clinical trial readiness of the Leigh syndrome spectrum (LSS). Six participants (three of the organisers, two scientists and an industry representative) attended the workshop in person, and the other attendees participated online. This hybrid meeting followed three virtual conferences on the Leigh syndrome spectrum held on 16th October and 4th December 2020 and 9 July 2021 (https://www.enmc.org/download/genetic-epidemiology-and-clinical-trial-readiness-in-encephalomyopathy-of-leigh-syndrome-spectrum/).

The term LSS comprises both Leigh syndrome and Leigh-like syndrome. Leigh syndrome, also referred to as subacute necrotizing encephalomyelopathy, is the most frequently observed primary mitochondrial disease phenotype in children. Magnetic resonance imaging (MRI) is typically characterized by bilateral and symmetric lesions in the basal ganglia, thalamus, brainstem, and variable association with white matter and spinal cord involvement. Leigh-like syndrome is often used when clinical and other features strongly suggest Leigh syndrome but do not fulfill the stringent diagnostic criteria because of atypical (e.g. asymmetric lesions) or normal neuroimaging, lack of evidence of abnormal energy metabolism, atypical neuropathology (variation in the distribution or character of lesions or with the additional presence of unusual features such as extensive cortical destruction), and/or incomplete evaluation [1]. LSS is therefore a preferred terminology to encompass both Leigh and Leigh-like syndromes and will be used throughout this report.

LSS is one of the more frequently occuring neurodegenerative disorders of childhood with a birth prevalence of 1 in 40,000 births [2], although some populations have a higher incidence of specific genetic forms. We know that the frequency for each genetically distinct form of LSS varies, but most are ultra-rare. LSS has a diverse genetic background, can have complex multisystem clinical features, and is associated with an increased risk of early mortality. More than 100 genes with variable pathogenic variants across the nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) are known to cause LSS. The number of genes known to be associated with

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LSS continues to increase, but a definitive diagnosis of LSS does not require identification of a confirmed pathogenic mtDNA or nuclear DNA (nDNA) pathogenic variant(s). Much of the recent literature however includes those with confirmed mutational findings.

The most frequent clinical symptoms include developmental delay/regression, hypotonia, weakness, and extrapyramidal symptoms. Onset is most frequently < 2 years of age. An early onset seems to correlate with a worse outcome [3]. Apnoeas are frequently observed and reflect brainstem lesions and hence worse outcomes [3].

The overarching aims of this series of virtual ENMC workshops were to discuss the genetic causes together with indicative prevalence, disease course and development of therapies for this severe and progressive group of monogenic disorders.

2. Meeting outcomes

2.1. Genetic causes and data from published registries

LSS can be caused by pathogenic variants of mtDNA or nDNA. MtDNA-associated LSS is established in a proband fulfilling the criteria for Leigh syndrome in whom a heteroplasmic or homoplasmic pathogenic variant is identified in one of the mtDNA genes. Clinical expression of a mtDNA pathogenic variant is influenced not only by the pathogenicity of the variant itself but also by the amount of mutated and wild type mtDNA (the heteroplasmic mutant load), the variation among different tissues, and the energy requirements of brain and other tissues, which may vary with age [4]. The m.8993T>G and m.8993T>C pathogenic variants probably show the strongest genotype-phenotype correlation among the mtDNA pathogenic variants. Estimated probability of a severe outcome (95% CI) for an individual with the mtDNA m.8993T>G or m.8993T>C variant is based on the proportion of abnormal mtDNA (mutant load) in the individual. If the proportion of abnormal mtDNA is below 60% individuals are usually asymptomatic, or may have mild pigmentary retinopathy or migraine headaches; usually individuals with moderate levels (~70%–90%) of the m.8993T>*G* pathogenic variant present with the neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP) phenotype, while those with more than 90% abnormal mtDNA have maternally inherited Leigh syndrome (MILS) [5]. Genotype-phenotype correlations are weaker for other mtDNA pathogenic variants causing LSS.

Pathogenic variants in more than 90 nuclear genes have been associated with autosomal recessive, autosomal dominant, and X-linked nuclear gene-encoded LSS [6]. Nuclear pathogenic variants causing LSS include ones in genes encoding proteins needed for: 1) oxidative phosphorylation (OXPHOS) enzyme activity and assembly; 2) mtDNA maintenance and gene expression; 3) cofactor biosynthesis (lipoic acid and coenzyme Q_{10}); 4) mitochondrial membrane lipid remodelling, quality control, and dynamics; 4) pyruvate dehydrogenase, biotinidase, and B vitamin transport and metabolism; and 5) disorders of valine degradation associated with accumulation of toxic metabolites leading to secondary OXPHOS dysfunction [6]

2.1.1. ClinGen curation of gene disease relationships for Leigh syndrome spectrum

Rahman presented the findings of the NIH-funded ClinGen (https://clinicalgenome.org) initiative to curate gene-disease associations for 113 nuclear and mtDNA-encoded genes and LSS, including the internationally agreed consensus definition of LSS. The LSS inclusion criteria are: 1) a neuropathological diagnosis of LSS and/or neuroimaging-symptoms and signs; 2) metabolic markers (increased lactate in blood and/or CSF) and/or biochemical features (OXPHOS defects). During the gene curation process a total of 111 genes were identified to be associated with LSS: 97 nuclear and 14 mtDNA. For 2 of the 113 genes there was no evidence for a gene disease relationship with LSS. The factors that affected classification were: 1) year of gene discovery; 2) quality of reported cases; 3) associations with other mitochondrial disorder phenotypes; and 4) absence of compelling experimental data. Process challenges encountered during the gene curations have been both clinical and experimental. Clinical challenges were the rarity of many genetic causes of LSS, death from comorbidities before developing LSS, and necessity for exclusion of some published cases owing to insufficient clinical details. For these reasons, for many genes a maximum score for genetic evidence was not reached. Experimental challenges were mainly a lack of supporting experimental evidence for more recently discovered disease genes, particularly if the gene function is novel, and the difficulty of modelling LSS with in vivo models (McCormick et al. [7]; https://clinicalgenome.org/affiliation/40027/).

2.1.2. Leigh syndrome registries

Several papers have now reported data from Leigh syndrome national registries worldwide. These data variably describe frequency and distribution, among the different patient cohorts, of time of onset, clinical symptoms, neuroimaging pattern, laboratory findings, biochemical profiles and genetic variants. Several national data registries that are yet to be published were also presented during the workshop, namely from France (Rötig), Australia (Thorburn), Japan (Muruyama) and the Czech Republic (Zeman). It was interesting to note that different registries show some data similarities. The most frequent symptoms in LSS are developmental delay/regression, hypotonia, weakness, and extrapyramidal symptoms. The onset is most frequently < 2 years of age and an early onset correlates with a worse outcome (Table 1).

Ardissone et al. (2021) published data from the Italian Mitochondrial Leigh syndrome registry [8]. Data from a total of 122 patients with LSS were analysed. The authors confirmed the prevalent onset before 1 year of age, but the median age of 3 months was earlier than previously reported cohorts. The most common symptoms at onset were psychomotor delay, hypotonia, and failure to thrive (22.5%). Despite basal ganglia involvement, extrapyramidal signs were less common than pyramidal tetraparesis. Plasma lactate levels were elevated in 69% of 113 patients and CSF lactate was elevated in 80% of 46 patients tested. The most common biochemical diagnosis was isolated complex IV deficiency followed by isolated complex I deficiency. Nuclear gene pathogenic variants were found in 67/110 patients in whom a genetic diagnosis was established, whilst the remainder harboured mtDNA mutations. Of the nuclear genes, *SURF1* pathogenic variants were the most frequent, whereas amongst the mtDNA pathogenic variants, variants in *MT- ATP6* were prevalent.

Barca et al. (2020) published data from patients with LSS enrolled in the North American Mitochondrial Disease Consortium (NAMDC) Registry [9]. NAMDC is an NIH-funded collaborative effort of 17 North American medical centres. LS was the clinical diagnosis in 97 patients. Disease onset was more frequent in early infancy (<2 years in 55 participants). The most frequent symptoms were developmental delay followed by developmental regression. Other neurological manifestations were ataxia, dystonia and seizures. Skeletal muscle was also commonly affected. Deficiencies of Complex I and Complex V were the most frequent biochemical defects. Variants in both nuclear and mitochondrial genomes were identified: *SURF1* was again the most frequently involved gene and the most common mtDNA pathogenic variants were in *MT-ATP6, MT-ND3*, and *MT-ND5*.

Table 1
Reported data from Leigh syndrome national registries worldwide.

	Nr of patients with LS/LSS	% of pts with genetic diagnosis	% of mtDNA mutations	% of nuclear DNA mutations	Most common mtDNA genes *(MT-)*	Most common nuclear genes	Most frequent clinical symptoms	% of pts with high blood lactate	% of pts with high CSF lactate	Most common OXPHOS defect
France (Rotig)	131	68%	22%	46%	*ND5, ND3, ATP6*	Complex I related	Hypotonia and developmental delay	84%	72%	Complex I
Australia (Thorburn)	67	80%	33%	47%	*ND5, ND3, ND6, ATP6*	*SURF1, PET100, NDUFV1, NDUFAF2, PDHA1*	NA	NA	NA	NA
Japan (Muruyama)	202	NA	NA	38/202	*ND3, ATP6*	17 pts *PDHA1*, 11 with *NDUFAF6*, 10 with *ECHS1*	NA	NA	NA	Complex I Combined defect
Czech Republic (Zeman)	35	100%	31%	69%	*ND5, ND1*	*SURF1*	Developmental delay/regression	80%	95%	Complex IV Complex I
GENOMIT	715	100%	NA	NA	*ATP6, ND3, ND5*	*SURF1, ECHS1, PDHA1*	Neurodevelopmental delay, Developmental regression, muscular hypotonia	NA	NA	NA
Italy (Ardissone, published)	122	90%	54%	46%	*ATP6*	*SURF1*	Psychomotor delay, hypotonia, failure to thrive	69% out of 113 pts tested	80% out of 46 pts tested	Complex IV Complex I
UK (Lim, published)	72	82%	22%	57%	*ATP6*	*SURF1, NDUFV1, ECHS1*	Extrapyramidal, failure to thrive, myopathy	68%	64%	Complex V Complex IV Complex I
CHOP (Alves, published)	53	100%	47%	53%	*ATP6, ND5*	*PDHA1, SURF1*	Hypotonia, Psychomotor regression/delay	58%	11%	NA
Northwestern Europe (Sofou, published)	130	96	32%	68%	*ATP6*	*SURF1* *NDUF-* related genes	NA	Not outlined	Not outlined	Complex I Complex IV
NAMDC (Barca, published)	97	100%	54%	46%	*ATP6, ND3, ND5*	*SURF1, PDHA1*	Developmental delay/regression	Not outlined	Not outlined	Complex I Complex IV Combined defects
China (Stenton published)	209	100%	42%	58%	*ATP6* *ND3, ND6*	*SURF1, PDHA1, ECHS1*	Global developmental delay, developmental regression, failure to thrive	74%	66%	NA

Sofou et al. (2018) reported a large cohort study recruited from eight European centres (Gothenburg, Rotterdam, Helsinki, Copenhagen, Stockholm, Brussels, Bergen and Oulu) within the Mitochondrial Clinical and Research Network [10]. Ninety-six patients with LSS who were diagnosed and followed at the participating centres fulfilled the inclusion criteria. Sixty-five patients had LSS caused by pathogenic variants in nuclear genes, while 31 patients had mtDNA-associated disease. The most common biochemical defects were of Complex I and Complex IV. Amongst the mtDNA genes, *MT-ATP6* and *MT-ND* genes were prevalent, whereas *SURF1* and genes encoding Complex I subunits and assembly factors were the most frequently involved nuclear genes. These defects also correlated with the most severe disease progression.

In 2020 Alves et al. published a review of the abnormal neuroimaging findings (MRI brain) in the Children's Hospital of Philadelphia (CHOP) cohort of patients with genetically confirmed LSS [11]. One hundred and nineteen children with a confirmed primary mitochondrial disorder and available brain MRI were reviewed; LSS was diagnosed in 53 children. The LSS diagnosis was based on clinical, genetic, and neuroradiological features, as previously described by Lake et al. [12]. Nuclear DNA pathogenic variants were predominant (mainly *PDHA1*) and for mtDNA, again, *MT-ATP6* and *MT-ND5* were the most frequently affected genes. Some of these findings were replicated in a subsequent Chinese study, but some associations reported in the CHOP study were not confirmed in the Chinese cohort [13]. Baseline MRI and follow-up MRI were reviewed in the CHOP study and the authors concluded that the definition of imaging findings needs to be expanded beyond "bilateral symmetric basal ganglia and/or brainstem lesions", and that imaging could be used for diagnosis, prognosis, longitudinal natural history, and response to treatment for clinical trials.

In the paper by Stenton et al. [13] a total of 209 genetically diagnosed patients were enrolled; 207 patients met the disease criteria for LS and 2 patients for "LS-mimicking". The median age of onset was 1.1 years, and the most frequent clinical features were developmental delay/regeression. Serum lactate was increased in 47% (144/195) of patients, compared to 66% in CSF (43/65). Across both genomes, most frequently, disease-causing variants were identified in *MT-ATP6* and *SURF1*, similarly to other large LS cohorts reported in the literature

In summary, the most common nuclear genes among the different cohorts are *SURF1* and Complex I-related genes, whereas the most common mtDNA genes involved are *MT-ND3, MT-ND5,* and *MT-ATP6.* Earlier onset is reported to be related with a worse outcome [8,10], as is brainstem involvement [3,8]. A worse disease progression is reported for *SURF1* and *MT-ATP6* related LS [8,13], and prognosis of LS due to nuclear-encoded complex I deficiency was poor in the cohort described by Sofou et al. [10].

GENOMIT (https://genomit.eu/project/index.html) is an EJP RD funded network of eight partners (Germany, Austria, Italy, France, UK, Japan and China) which act in close collaboration with Mitochondrial Patient organizations to improve the diagnosis and care of patients with mitochondrial disease. The project aims are: 1) to define mitochondrial disease natural history in order to identify outcome measures for future clinical trials; 2) to integrate patient-reported outcome measures into the registry; 3) to increase the diagnostic yield by integrating exome and whole genome sequencing (WGS) data from 3000 cases and implementing RNA-sequencing and proteomics in diagnostics; and 4) to validate and characterize novel disease variants, genes and pathomechanisms. The registry and GENOMIT WES database covers 2958 pediatric patients including 623 with LSS, 415 of whom have a genetic diagnosis. An additional 297 patients with LSS were included in the study from published national studies summing up to 715 genetically confirmed patzients with LSS. The majority harbour pathogenic variants in LSS genes whereas 9% have pathogenic variants in other mitochondrial disease genes and 6% have non mitochondrial genetic causes. The most frequent genes identified in the registry were *MT-ATP6, MT-ND3, MT-ND5, SURF1, ECHS1* and *PDHA1.* Some clinical features appeared to be more associated with particular genes: for example, cerebellar atrophy with *MT-ATP6*, hearing loss with *ECHS1*, cerebral atrophy and high lactate on MRS with no basal ganglia lesions for *EARS2*, and sensorineural hearing loss plus feeding difficulties for *SUCLA2/SUCLG1*. Blood lactate was increased in 83% of patients. A higher diagnostic yield was obtained when CSF lactate was elevated. Untargeted metabolomics was performed on 2000 plasma samples. There are initial promising data, which require further analysis and replication. Some limitations of the project must be considered: the HPO terms lack severity grading, the collected data were derived from both paper and electronic records, and there was a mixture of in-house and referred patients.

2.1.3. Clinical novelties in Leigh syndrome spectrum

Pennisi presented several cases of LSS due to non-mitochondrial gene variants in the following genes: *SCN1A, VPS13D, SUOX, GDCH, ALDH5A1, MORC2,* and *RANBP2*. The dataset included 8 patients and focused on neuroimaging data. Apart from two genes, *RANBP2* and *VPS13D*, the other genes did not have a specific neuroradiological presentation that allowed them to be differentiated from primary mitochondrial disease genes.

Rötig presented emerging evidence regarding incomplete penetrance of *SDHB* variants. Complex II defects represent a rare cause of mitochondrial diseases and few pathogenic variants in genes encoding either complex II subunits or assembly factors have been reported. *SDHB* encodes one of four structural subunits of complex II. The same homozygous *SDHB* pathogenic variant (NM_003000, c.143A), p.(Asp48Val) has been reported in two unrelated families [14] and in one unpublished family in which the proband exhibited LSS including basal ganglia and white matter lesions [15]. Functional validation in yeast demonstrated the deleterious nature of this variant [14]. Surprisingly, in two of these families, the homozygous variant was also present in asymptomatic individuals suggesting reduced penetrance. With the aim of identifying the mechanisms underlying this incomplete penetrance, Rötig's group performed WGS on blood DNA and transcriptomic analyses (RNAseq) on RNA extracted from cultured skin fibroblasts. In WGS data, they searched for: 1) another *SDHB* variant in homozygous *SDHB* individuals in coding and non-coding regions, 2) uniparental disomy of *SDHB*, 3) variants in other complex II subunits or assembly factors and pseudogenes, 4) recessive mutations in another gene with the hypothesis that *SDHB* is not the disease causing gene, 5) de novo variants in affected individuals, 6) one variant present in homozygous asymptomatic individuals and absent in homozygous affected individuals, 7) one variant absent in asymptomatic healthy individuals and present in homozygous affected individuals, and 8) mtDNA variants, but failed to detect anything. Transcriptomic analysis revealed normal expression of all complex II subunits (including *SDHB*) and assembly factors and no significant deregulation of genes encoding OXPHOS proteins, TCA cycle or involved in mitochondrial functions. Comparison of transcript expression profiles of symptomatic (2 individuals from unrelated families) and one asymptomatic individual with SDHB variation revealed opposite gene regulation patterns with a profound enrichment of transcripts involved in cell cycle and a significant downregulation of transcripts involved in lysosomal and endosomal functions in affected individuals suggesting defective degradation of cellular material in affected SDHB deficient fibroblasts. Further studies in additional families with symptomatic and asymptomatic individuals should

be performed to confirm these preliminary results. Transcriptomic analysis in fibroblasts of patients with other SDH gene variants should help to determine the specificity of the profile observed in individuals affected with *SDHB* variants.

2.2. Natural history of Leigh syndrome spectrum

Prospective natural history studies in Leigh syndrome are lacking; they are important in order to define, as far as possible, the disease course of the most frequent forms and thus be prepared for future interventional trials. McFarland outlined a retrospective natural history study undertaken in children with a diagnosis of LSS [16]. In summary, 72 (38F, 34 M) children fulfilled inclusion criteria and 59 (82%) had a confirmed genetic diagnosis. Median length of follow-up was 2.6 years. Median height was on the 9th centile while median head circumference was on the 2nd centile. Blood lactate was elevated in 34/50 (68%) and CSF lactate elevated in 23/36 (64%). 37/72 children had undergone muscle biopsy with complex I deficiency identified in 7/37, complex IV deficiency in 6/37 and multiple OXPHOS deficiencies in 5/37. Median baseline total Newcastle Paediatric Mitochondrial Disease Scale (NPMDS) score was 18 rising to 24 at follow-up ($p<0.001$). Rate of change of NPMDS was most notably high for patients with *SURF1*-related LSS and this was reflected in poor survival rates for children with LSS due to pathogenic variants in this gene. LSS due to PDH deficiency and mtDNA variants showed moderate disease progression while other nDNA-related LSS progressed more slowly. Mobility, self-care, communication, educational outcomes and extrapyramidal movement disorder were the areas that showed most disease progression between baseline and follow-up. McFarland also discussed a prospective natural history study, "**L**eigh syndrome: **I**nvestigating **O**utcome measures and **N**atural history (LION)" and explained that this will involve baseline, 3–6 month and 6–9 month assessments using a range of established and exploratory outcome measures in addition to the NPMDS. The two principal aims of the LION study are to quantify disease burden of LSS and understand disease progression (natural history) with secondary aims of exploring outcome measures that might serve as clinical trial endpoints.

2.3. Biomarkers in Leigh syndrome spectrum

Body tissues have local responses to OXPHOS deficiency, which result in the release of metabolites or signalling molecules into the bloodstream, urine, or CSF; these molecules provide a disease-associated metabolic signature. Many mitochondrial oxidative defects present with a raised blood or CSF lactate, and this is often (but not always) associated with a raised lactate/pyruvate ratio, which means a change in the cellular redox state [17]. Increased serum and/or CSF lactate, or elevated lactate peaks found with magnetic resonance spectroscopy, can be considered a mitochondrial disease biomarker but are neither specific nor sufficiently sensitive for mitochondrial disease. A fasting-related increase in plasma lactate >3 mmol/l is suggestive of mitochondrial disease, but a raised value can also occur during or after epileptic seizures, suboptimal sampling (stasis, struggling child), or improper sample management. Moreover, CSF lactate can be increased in central nervous system (CNS) infection, stroke, malignancy, inflammation, and seizures [18]. LSS can often present with infantile lactic acidosis. A review of all published cases of nuclear-encoded complex I deficiency revealed that plasma and CSF lactate are frequently elevated in the reported cases and do not seem to discriminate between different molecular genetic defects, nor was there any consistent difference between patients with nuclear subunit pathogenic variants and those with assembly factor defects. Furthermore, plasma lactate levels did not correlate with residual complex I activity [19]. In PDH deficiency, laboratory investigations show increased lactate concentration in blood, urine, and CSF. Pyruvate level in blood and urine is also elevated, often resulting in a normal or low lactate to pyruvate ratio in blood and CSF [20]. Depending on the presentation, amino acids, acylcarnitines, and urinary organic acid concentrations may help to define the global picture and raise the suspicion of mitochondrial disease, but their value remains limited [18].

Suomalainen discussed two serum proteins, fibroblast growth factor 21 (FGF21), and growth differentiation factor 15 (GDF15) that have been reported as biomarkers with greater sensitivity and specificity compared to the traditional biomarkers listed above in diagnosing muscle-manifesting mitochondrial disorders. FGF21 is a hormone-like cytokine that is involved in intermediary metabolism of carbohydrates and lipids, and is upregulated in the liver during fasting to induce lipolysis in adipose tissue of healthy individuals. FGF21 was first reported as a potentially sensitive and specific biomarker in mitochondrial disorders since its serum concentration was found to be elevated in mitochondrial myopathies, correlating with disease severity and respiratory chain-deficient muscle fibres [21]. On the other hand, FGF21 is reported to be elevated in a range of non-mitochondrial diseases, including cancer, obesity, renal disease, diabetes, and liver disease, with the latter two frequently associated with mitochondrial disorders [18,22]. Lehtonen et al. (2016) [23] reported that the highest induction of FGF21 response occurs in disorders that primarily or secondarily affect mitochondrial translation such as direct mutations of translation machinery or mtDNA deletions, but not in mitochondrial diseases caused by pathogenic variants in structural OXPHOS complexes or their assembly factors. GDF15 is a cytokine of the transforming growth factor β (TGF-β) superfamily, which is expressed in many tissues including placenta, kidney, liver, lung, pancreas, and prostate. It is involved in regulating the cellular response to stress signals and inflammation, including suppression of inflammation in early pregnancy, cancer, and cardiovascular diseases.

Recently, Lehtonen et al. (2021) [24] published data from 194 patients with a suspected mitochondrial disorder in which they compared the diagnostic yield of FGF21 and GDF15 to the routine diagnostic histological and biochemical analyses of muscle. They confirmed that the induction of FGF21 and GDF15 is highly restricted to muscle-manifesting disorders caused by defects in mtDNA expression, showing that mitochondrial encephalopathies without muscle involvement usually exhibited normal FGF21 and GDF15 values.

2.4. Diagnostic algorithm

The diagnosis of LSS relies on clinical findings, neuroimaging pattern and molecular genetic testing. Laboratory findings such as elevated lactate levels in blood and/or CSF or, in some cases, other metabolic analyses (urinary organic acid profile, plasma acylcarnitines) can support or reinforce the diagnosis. OXPHOS enzyme activities can be tested in muscle biopsies and can help to confirm the genetic diagnosis. In recent years, widespread use of next generation sequencing technology in first-line genetic diagnostic tests has led to a decrease in the number of muscle biopsy procedures, which are now rarely performed if the genetic result is diagnostic.

2.5. Treatment of Leigh syndrome spectrum disorders

To date, there are no effective treatments for most LSS disorders.

2.5.1. Targeted therapies

Specific treatment is possible for three nuclear gene-encoded LSS disorders: 1) Biotin-thiamine-responsive basal ganglia disease (also known as thiamine transporter-2 deficiency, caused by pathogenic variants in the *SLC19A3* gene) in which biotin (5–10 mg/kg/day) and thiamine (in doses ranging from 300 to 900 mg) are given orally as early as possible and continued lifelong; 2) Biotinidase deficiency (BTD) where all symptomatic children improve if treated with 5–10 mg of oral biotin per day; 3) Coenzyme Q_{10} biosynthesis deficiency (caused by pathogenic variants in CoQ_{10} biosynthesis pathway genes) [25]. In genetically confirmed coenzyme Q_{10} biosynthesis deficiency, supplementation with oral coenzyme Q_{10} (CoQ_{10}, 30 mg/kg/day in children and 1200–3000 mg/day in adults) should be commenced as early in the disease course as possible and continued lifelong, although clinical responses are variable [26]. PDH deficiency caused by mutations in *PDHA1*, encoding the E1alpha subunit, can respond to a ketogenic diet [27]. Ketogenic diet may also be effective in seizure control more generally in mitochondrial disorders [28], although efficacy is yet to be formally established.

2.5.2. Supportive management

In LSS treatment the commonly used compounds are antioxidants such as CoQ_{10} and its derivatives idebenone and vatiquinone (PTC-743, previously known as EPI-743). Idebenone is taken up more readily by cells and has been suggested as a replacement for CoQ_{10}, although it does not function in electron transport in the respiratory chain. Idebenone has been used mainly as a treatment for Leber Hereditary Optic Neuropathy (LHON), but it is emerging as a possible treatment option for LSS. Vatiquinone has also shown some promise in patients with LSS but its efficiency is still being evaluated. In most cases treatment for LSS is supportive and focused on management of complications such as ventilatory assistance/support, and treatment of acidosis, seizures, dystonia and/or spasticity, major organ involvement such as cardiomyopathy, liver and renal failure, and nutritional needs.

2.5.3. Treatabolome

Although historically the treatment for LSS is largely supportive, new treatments are on the horizon. Due to the rarity of LSS, large-scale interventional studies are scarce, limiting dissemination of information of therapeutic options to the wider scientific and clinical community. Horvath presented the Leigh Syndrome Treatabolome, a systematic review of pharmacological therapies of LS following the guidelines for FAIR-compliant datasets as part of the Treatabolome project within Solve-RD (www.solve-rd) [29]. They searched for interventional studies within Clincialtrials.gov and European Clinical trials databases. Randomised controlled trials, observational studies, case reports and case series formed part of a wider MEDLINE search. Of the 1193 studies initially identified, 157 met their inclusion criteria, of which 104 were carried over into a final analysis. Treatments for LS included a few interventional trials using EPI-743 and cysteamine bitartrate. Wider literature searches identified case series and reports of treatments depleting glutathione stores, reduction of oxidative stress and restoration of OXPHOS. They concluded that although interventional randomised controlled trials have begun for LSS, most of the evidence remains in case reports and case series for a number of treatable genes, encoding cofactors or transporter proteins of the mitochondria. Their findings form part of the international expert-led Solve-RD efforts to assist clinicians initiating treatments in patients with treatable variants of LSS.

2.6. Clinical trial readiness

2.6.1. Selection of outcome measures

Mitochondrial diseases are heterogeneous multisystem disorders mostly affecting the brain, muscle and heart. As a requirement for preparation of clinical trials in children with mitochondrial diseases, the phenotype and natural disease course should be well documented [30]. The complexity of these disorders often makes it difficult to determine which symptoms to measure during a clinical trial and a major barrier to establishing approved therapies is the lack of mitochondrial disease specific outcome measures [31]. In defining outcome measures, it is extremely important to consider differences between children and adults with mitochondrial diseases.

The Newcastle Mitochondrial Disease Adult Scale (NMDAS) and its pediatric version the NPMDS are validated semi-quantitative clinical rating scales designed to reflect the multi-system burden of mitochondrial disease and have been utilized in clinical trials to measure change over short treatment intervals (although they have not been validated for this timescale) with negative results. The International Paediatric Mitochondrial Disease Scale (IPMDS) was adapted from the NPMDS through a Delphi method [32] to create a more clinically relevant and detailed scoring system for future clinical trials in pediatric patients with mitochondrial disease, covering more symptoms indicated by patients and parents as “burdensome”. On the other hand, this scale requires a longer time to be administered, which perhaps might explain its poor uptake in clinical trials and studies

In 2016 an International Workshop was held in Rome which aimed to define outcome measures and clinical trial readiness in primary mitochondrial myopathies (PMM) in children and adults. PMM, as defined by this consortium of international experts in mitochondrial disease, are genetically defined disorders leading to defects of OXPHOS affecting predominantly, but not exclusively, skeletal muscle [33]. While muscle weakness is the predominant symptom in other genetic neuromuscular disorders, such as Duchenne Muscular Dystrophy (DMD) or Spinal Muscular Atrophy (SMA), exercise intolerance and muscle fatigue often exist in the absence of overt muscle weakness in PMM. Therefore, an assessment that focuses solely on muscle strength would not fully reflect PMM disease severity. The group then identified, through a Delphi method, a set of recommended outcome measures to be implemented in PMM clinical studies, and proposed a set of clinical scales, functional tests, performance, and patient reported outcome measures, and biomarkers to be used in both adults and children with PMM. Another Delphi-based international workshop was held in 2018 in the Netherlands to design a natural history protocol for children with mitochondrial myopathy, with a strong focus on which outcome measures to use for children with mitochondrial myopathy and mitochondrial encephalopathy. The 6-minute walk test (6MWT), 30 Second Sit to Stand, and CHOP Infant Test of Neuromuscular Disorders (CHOP-INTEND) reached the highest consensus for children with PMM. PEDICAT, GMFM and SARA scales were suggested for children with mitochondrial encephalopathy. NPMDS and IPMDS were chosen for both categories.

During the workshop Zolkipli-Cunningham discussed the Mitochondrial Myopathy-Composite Assessment Tool (MM-COAST), a validated quantitative tool [34] that aims to capture the mutually interactive key domains of muscle strength, muscle fatigue, balance, dexterity, and exercise intolerance which are frequently observed in primary mitochondrial disease including in LSS. The MM-COAST provides a quantitative assessment that can be utilized for longitudinal studies and future intervention trials. Zolkipli-Cunningham presented MM-COAST assessment data in a cohort of adult and pediatric patients with LSS followed at

the Children's Hospital of Philadelphia, highlighting the ability of the MM-COAST to capture abnormalities in these key domains for longitudinal studies. Longitudinal patient/caregiver reported outcome measure data (PedsQL, Karnofsky-Lansky, Modified Fatigue Impact Scale) was also presented. Lastly, Zolkipli-Cunningham showed preliminary results of the Leigh Syndrome Roadmap Project assessments conducted at the Children's Hospital of Philadelphia.

2.6.2. Patient advocacy group perspectives

Katie Waller and Alison Maguire from The Lily Foundation presented the role of patient advocacy groups (PAGs). They began by giving an update on the Leigh Syndrome Roadmap Project, an international collaboration led by a consortium of the world's leading patient advocacy groups to improve diagnosis, develop treatments, and improve clinical care. They acknowledged the challenges associated with collecting and analysing data for a disease as variable as LSS, whilst emphasizing that this work is hugely valuable to patients and crucial to provide a baseline for future clinical trials. They concluded that collaboration is key to the success of this project and highlighted its potential to become an important international resource to advance research. They then presented the example of the development of international care standards for DMD and called for similar guidelines for LSS. The presentation detailed how an innovative approach, driven by PAGs and other key stakeholders, led to the development of NICE accredited care guidelines for DMD. These have not only been associated with improved standards of care and survival rates but have also provided a key framework to allow for the development of global, multi-centre clinical trials into the disease. The Lily Foundation called upon the experts gathered for the workshop to consider how to develop similar care guidelines for LSS, and proposed that consideration of this, including how to engage key stakeholders, gather resources and funding and overcome potential barriers, could be the topic for a future ENMC workshop.

Faye Wylie (Leigh Network, UK) gave a personal account of the lived experience of Leigh syndrome, and also shared a poem with the group (Box 1).

Battery of Life

Imagine you are a mitochondria
A pocket of energy powering,
The body
Run, run, run,
Power, power, power
Mi-to, mi-to, mi-to
Must reach brain, must reach heart, must reach muscles
Pedal, pedal,
Faster faster
Climb to the eyes
This mountain is so high
Flash, flash
Low battery, low battery
Reserve, reserve
Important organs only
A power cut has occurred
A power cut has occurred
A power cut has occurred
Reserve what we can
Body in failure

By Faye Wylie

Finally, Phil Yeske (United Mitochondrial Diease Foundation (UMDF), USA) discussed patient registries from the PAG perspective and introduced MitoSHARE, a global robust research database with multi stakeholder governance, and discussed the importance of collaboration.

2.7. Research perspectives

2.7.1. The Leigh syndrome roadmap project

Cohen presented further details about the Leigh Syndrome Roadmap Project (LSRP), an international initiative funded through the International Leigh Syndrome Consortium (a collaboration between UMDF, the Lily Foundation, MitoCon, Mito Foundation (Australia) and People Against Leigh Syndrome), based on a rich history of scientific collaboration. LSS was chosen as the first disease to investigate for the following reasons: 1) it is a progressive illness with poor prognosis; 2) worst disease outcomes of the major pediatric mitochondrial phenotype disorders; 3) there are no effective therapies; 4) it involves pathogenic variants of mtDNA and nDNA. LSRP's main goals are 1) to define the natural history, 2) to improve diagnosis, and 3) to investigate preclinical therapeutics and biomarkers that will improve disease management. The intent will allow for Clinical Trial Readiness as new therapies enter the human evaluation arena. In the almost three years of the project, eight preclinical trials have been funded internationally, with some of the therapies involving medications already approved by the FDA and EMA for other indications. A natural history and outcomes study is also funded, as described above.

2.7.2. Vatiquinone

Klein presented a trial of vatiquinone for mitochondrial disease associated seizures. Vatiquinone is an oral small molecule that has been in development for the treatment of mitochondrial disorders and related diseases characterized by high levels of oxidative stress and neuroinflammation. Vatiquinone targets the oxidoreductase enzyme 15-lipoxygenase, which is a key regulator of an oxidative stress and cell death pathway that has been shown to be an important upstream modulator of CNS disease, including seizure disorders. The MIT-E trial is a double-blind, placebo-controlled trial being conducted in children with mitochondrial disease associated seizures, including LSS. The study is being conducted globally and was actively enrolling at the time of the workshop. The rationale for focus on seizure was based on previously data collected as well as the need to identify symptoms that are common and meaningful in mitochondrial disease given that the extreme disease heterogeneity is a significant challenge in therapeutic development.

2.7.3. Apomorphine

Osaka presented the work of his group at Jichi Medical University, Tochigi, Japan. They examined the cell-protective effect of an existing commercially available chemical library on fibroblasts from patients with LSS and MELAS and identified apomorphine as a potential therapeutic drug for mitochondrial disease. They conducted a cell viability assay under oxidative stress induced by L-butionine-sulfoximine (BSO), a glutathione synthesis inhibitor. Among the chemicals in the library, apomorphine rescued cells from death induced by oxidative stress much more effectively than idebenone. The EC50 value was ~100 nM. In an extracellular flux analysis, apomorphine significantly improved all assessed oxygen consumption rate values. Furthermore, elevation of GDF15, a biomarker of mitochondrial disease as discussed above, was significantly reduced by apomorphine. The dopamine agonistic action of apomorphine appears to be independent of the cell-protective effect, as other dopamine agonists showed no cell-protective effects in their analysis. Among 441 apomorphine-responsive genes identified by microarray analysis, apomorphine induced the expression of genes that inhibit mTOR activity

and inflammatory responses [35]. Moreover, daily injection of apomorphine prolonged survival and improved rota-rod test results in the *Ndufs4-/-* mouse model of LSS ($p<0.01$). Hitoshi and colleagues have applied for Japanese and international patents for apomorphine (PCT/JP2018/041,354). They are now in discussion with the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) about appropriate toxicity studies in juvenile animals to enable progression to clinical trials of apomorphine in children with mitochondrial disease.

2.7.4. Drug repurposing screen in iPSC models

Prigione and Schuelke presented their collaborative work based on the generation of induced pluripotent stem cells (iPSCs) derived from patients affected by LSS. They focused on two major hotspot genes in LSS patients: the nuclear gene *SURF1* and the mtDNA gene *MT-ATP6*. They showed that iPSCs can be differentiated into dopaminergic neurons, which are predominantly affected in LSS, and in three-dimensional brain organoids. They used this approach to demonstrate that dopaminergic neurons carrying *SURF1* pathogenic variants exhibit defective neuronal outgrowth capacity. By analysing brain organoids, they found that *SURF1* pathogenic variants impair not only neurons but also the organization of neural progenitor cells (NPCs) [36]. They had previously demonstrated that NPCs carrying *MT-ATP6* mutations show aberrant calcium homeostasis. They linked this defect to an abnormal increase in mitochondrial polarization [37]. They then showed how they developed an assay to monitor mitochondrial polarization in live NPCs [38]. Using this assay, they carried out a compound screening in NPCs carrying *MT-ATP6* defects, and identified phosphodiesterase-5 (PDE5) inhibitors as a potential therapeutic strategy [37]. Finally, they presented unpublished observations. They showed how they applied the mitochondrial polarization assay to NPCs carrying *MT-ATP6* defects and identified an approved drug as a potential treatment strategy that could be repositioned for treating patients carrying *MT-ATP6* mutations. They presented how five patients with *MT-ATP6* mutations appeared to have amelioration of their clinical features following treatment with a PDE5 inhibitor under compassionate use. However Prigione and Schuelke acknowledged that the unpredictable natural history of LSS means that it is impossible to make robust conclusions regarding drug efficacy from compassionate use data. They concluded their presentation by showing their ongoing efforts to receive an orphan drug designation (ODD) status for this treatment and for starting a discussion with EMA for a repositioning approval, since their request .was selected as a “Champion” project within EMA’s Drug Repurposing Pilot program.

These unpublished findings prompted active discussions among members of the workshop. Major points focused on the strategy to follow in order to effectively engage with the EMA to establish a clinical trial for probing effectiveness of PDE5 inhibitors in patients carrying *MT-ATP6* defects. There appear to be unique critical challenges. First, there is a well-known heterogeneity of clinical features exhibited by patients with *MT-ATP6* pathogenic variants. Hence, it is not trivial to decide which patients to select and most importantly which outcome measures to assess. Second, given the rarity of this condition (with these patients being a subgroup of a rare disease), it remains to be decided which kind of trial to perform (e.g. pilot trial or multi-centric, placebo-control or crossover). Third, given the paucity of prospective clinical studies, it is challenging to define what is the expected disease course to which the treatment should be compared against. Members of the workshop expressed their willingness to support these efforts to conduct a formal clinical trial going forwards. This collaborative spirit will be instrumental in tackling the challenges and enable the establishment of a well-designed clinical trial.

3. Conclusion

In summary, LSS is an expanding group of genetic primary mitochondrial disorders for which specific therapies are still lacking. Numerous compounds are being tested in vitro or in vivo in animal models in an effort to identify potential therapies for LSS. Existing patient registries and prospective natural history studies will be of utmost importance in order to gather strong and reproducible natural history data and devise future clinical trials for this group of rare and heterogeneous mitochondrial diseases.

4. Chairpersons

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Declaration of Competing Interest

Matthew Klein is Chief Executive Officer and President at PTC Therapeutics.

The other authors have nothing to declare.

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References

[1] Rahman S. GeneReviews®. seattle (WA), Seattle: University of Washington; 2015. 1993–2022Oct 1 [updated 2020 Jul 16].
[2] Rahman S, Blok RB, Dahl HH, Danks DM, Kirby DM, Chow CW, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. Ann Neurol 1996;39(3):343–51. doi:10.1002/ana.410390311.
[3] Sofou K, De Coo IF, Isohanni P, Ostergaard E, Naess K, De Meirleir L, et al. A multicenter study on Leigh syndrome: disease course and predictors of survival. Orphanet J Rare Dis 2014;9:52. doi:10.1186/1750-1172-9-52.
[4] Thorburn D.R., Rahman J., Rahman S. Mitochondrial DNA-Associated Leigh Syndrome and NARP. 2003 Oct 30 [updated 2017 Sep 28]. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2023.
[5] Fryer A, Appleton R, Sweeney MG, Rosenbloom L, Harding AE. Mitochondrial DNA 8993 (NARP) mutation presenting with a heterogeneous phenotype including 'cerebral palsy'. Arch Dis Child 1994;71(5):419–22. doi:10.1136/adc.71.5.419.
[6] Rahman J, Noronha A, Thiele I, Rahman S. Leigh map: A novel computational diagnostic resource for mitochondrial disease. Ann Neurol 2017;81(1):9–16. doi:10.1002/ana.24835.
[7] McCormick EM, Keller K, Taylor JP, Coffey AJ, Shen L, Krotoski D, Harding B; NICHD ClinGen U24 Mitochondrial Disease Gene Curation Expert Panel; Alves CAPF, Ardissone A, Bai R, de Barcelos IP, Bertini E, Bluske K, Christodoulou J, Clause AR, Copeland WC, Diaz GA, Diodato D, Dulik MC, Enns G, Feigenbaum A, Fratter C, Ghezzi D, Goldstein A, Gropman A, Haas R, Karaa A, Koenig MK, Monteleone B, Parikh S, Duenas BP, Rajkumar R, Saada A, Saneto RP, Sergeant K, Shoffner J, Smith C, Stanley C, Thiffault I, Thorburn D, Walker M, Wallace D, Wong LJ, Gai X, Falk MJ, Zolkipli-Cunningham Z, Rahman S. Expert panel curation of 113 primary mitochondrial disease genes for the Leigh syndrome spectrum. Ann Neurol. 2023 May 31. doi:10.1002/ana.2671.
[8] Ardissone A, Bruno C, Diodato D, Donati A, Ghezzi D, Lamantea E, et al. Clinical, imaging, biochemical and molecular features in Leigh syndrome: a study from the Italian network of mitochondrial diseases. Orphanet J Rare Dis 2021;16(1):413. doi:10.1186/s13023-021-02029-3.
[9] Barca E, Long Y, Cooley V, Schoenaker R, Emmanuele V, DiMauro S, et al. Mitochondrial diseases in North America: An analysis of the NAMDC Registry. Neurol Genet 2020;6(2):e402. doi:10.1212/NXG.0000000000000402.
[10] Sofou K, de Coo IFM, Ostergaard E, Isohanni P, Naess K, De Meirleir L, et al. Phenotype-genotype correlations in Leigh syndrome: new insights from a multicentre study of 96 patients. J Med Genet 2018;55(1):21–7. doi:10.1136/jmedgenet-2017-104891.
[11] Alves CAPF, Teixeira SR, Martin-Saavedra JS, Guimarães Gonçalves F, Lo Russo F, Murarescu C, et al. Pediatric Leigh Syndrome: Neuroimaging Features and Genetic Correlations. Ann Neurol 2020;88(2):218–32 Epub 2020 Jun 13PMID32445240.ù. doi:10.1002/ana.25789.
[12] Lake NJ, Compton AG, Rahman S, Thorburn DR. Leigh syndrome: One disorder, more than 75 monogenic causes. Ann Neurol 2016;79(2):190–203. doi:10.1002/ana.24551.
[13] Stenton SL, Zou Y, Cheng H, Liu Z, Wang J, Shen D, et al. Leigh Syndrome: A Study of 209 Patients at the Beijing Children's Hospital. Ann Neurol 2022;91(4):466–82. doi:10.1002/ana.26313.
[14] Alston CL, Davison JE, Meloni F, van der Westhuizen FH, He L, Hornig-Do HT, et al. Recessive germline SDHA and SDHB mutations causing leukodystrophy and isolated mitochondrial complex II deficiency. J Med Genet 2012;49(9):569–77. doi:10.1136/jmedgenet-2012-101146.
[15] Ardissone A, Invernizzi F, Nasca A, Moroni I, Farina L, Ghezzi D. Mitochondrial leukoencephalopathy and complex II deficiency associated with a recessive SDHB mutation with reduced penetrance. Mol Genet Metab Rep 2015;5:51–4. doi:10.1016/j.ymgmr.2015.10.006.
[16] Lim AZ, Ng YS, Blain A, Jiminez-Moreno C, Alston CL, Nesbitt V, et al. Natural History of Leigh Syndrome: A Study of Disease Burden and Progression. Ann Neurol 2022;91(1):117–30. doi:10.1002/ana.26260.
[17] Robinson, B.H. Lactic acidemia and mitochondrial disease. Mol Genet Metab 89, 3–13, 10.1016/j.ymgme.2006.05.015.
[18] Boenzi S, Diodato D. Biomarkers for mitochondrial energy metabolism diseases. Essays Biochem 2018;62(3):443–54. doi:10.1042/EBC20170111.
[19] Fassone E, Rahman S. Complex I deficiency: clinical features, biochemistry and molecular genetics. J Med Genet 2012;49(9):578–90. doi:10.1136/jmedgenet-2012-101159.
[20] Patel KP, O'Brien TW, Subramony SH, Shuster J, Stacpoole PW. The spectrum of pyruvate dehydrogenase complex deficiency: clinical, biochemical and genetic features in 371 patients. Mol Genet Metab 2012;106(3):385–94. doi:10.1016/j.ymgme.2012.03.017.
[21] Suomalainen A, Elo JM, Pietiläinen KH, Hakonen AH, Sevastianova K, Korpela M, et al. FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. Lancet Neurol 2011;10(9):806–18. doi:10.1016/S1474-4422(11)70155-7.
[22] Suomalainen A. Blood biomarkers of mitochondrial disease-One for all or all for one? Handb Clin Neurol 2023;194:251–7. doi:10.1016/B978-0-12-821751-1.00006-3.
[23] Lehtonen JM, Forsström S, Bottani E, Viscomi C, Baris OR, Isoniemi H, et al. FGF21 is a biomarker for mitochondrial translation and mtDNA maintenance disorders. Neurology 2016;87(22):2290–9. doi:10.1212/WNL.0000000000003374.
[24] Lehtonen JM, Auranen M, Darin N, Sofou K, Bindoff L, Hikmat O, et al. Diagnostic value of serum biomarkers FGF21 and GDF15 compared to muscle sample in mitochondrial disease. J Inherit Metab Dis 2021;44(2):469–80. doi:10.1002/jimd.12307.
[25] Alcázar-Fabra M, Rodríguez-Sánchez F, Trevisson E, Brea-Calvo G. Primary Coenzyme Q deficiencies: A literature review and online platform of clinical features to uncover genotype-phenotype correlations. Free Radic Biol Med 2021;167:141–80. doi:10.1016/j.freeradbiomed.2021.02.046.
[26] Drovandi S, Lipska-Ziętkiewicz BS, Ozaltin F, Emma F, Gulhan B, Boyer O, et al. Oral Coenzyme Q10 supplementation leads to better preservation of kidney function in steroid-resistant nephrotic syndrome due to primary Coenzyme Q10 deficiency. Kidney Int 2022;102(3):604–12. doi:10.1016/j.kint.2022.04.029.
[27] Wexler ID, Hemalatha SG, McConnell J, Buist NR, Dahl HH, Berry SA, et al. Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. Studies in patients with identical mutations. Neurology 1997;49(6):1655–61. doi:10.1212/wnl.49.6.1655.
[28] Zweers H, van Wegberg AMJ, Janssen MCH, Wortmann SB. Ketogenic diet for mitochondrial disease: a systematic review on efficacy and safety. Orphanet J Rare Dis 2021;16(1):295. doi:10.1186/s13023-021-01927-w.

[29] Tiet MY, Lin Z, Gao F, Jennings MJ, Horvath R. Targeted Therapies for Leigh Syndrome: Systematic Review and Steps Towards a 'Treatabolome. J Neuromuscul Dis 2021;8(6):885–97. doi:10.3233/JND-210715.
[30] Keshavan N, Rahman S. Natural history of mitochondrial disorders: a systematic review. Essays Biochem 2018;62(3):423–42. doi:10.1042/EBC20170108.
[31] Goldstein A, Rahman S. Seeking impact: Global perspectives on outcome measure selection for translational and clinical research for primary mitochondrial disorders. J Inherit Metab Dis 2021;44(2):343–57. doi:10.1002/jimd.12320.
[32] Koene S, Hendriks JCM, Dirks I, de Boer L, de Vries MC, Janssen MCH, et al. International Paediatric Mitochondrial Disease Scale. J Inherit Metab Dis 2016;39(5):705–12 Epub 2016 Jun 9Erratum inJ Inherit Metab Dis2017 May403463. doi:10.1007/s10545-016-9948-7.
[33] Mancuso M, McFarland R, Klopstock T, Hirano M. consortium on Trial Readiness in Mitochondrial Myopathies. International Workshop:: Outcome measures and clinical trial readiness in primary mitochondrial myopathies in children and adults. Neuromuscul Disord 2017;27(12):1126–37. doi:10.1016/j.nmd.2017.08.006.
[34] Flickinger J, Fan J, Wellik A, Ganetzky R, Goldstein A, Muraresku CC, et al. Development of a Mitochondrial Myopathy-Composite Assessment Tool. JCSM Clin Rep 2021;6(4):109–27.
[35] Miyauchi A, Kouga T, Jimbo EF, Matsuhashi T, Abe T, Yamagata T, et al. Apomorphine rescues reactive oxygen species-induced apoptosis of fibroblasts with mitochondrial disease. Mitochondrion 2019;49:111–20. doi:10.1016/j.mito.2019.07.006.
[36] Inak G, Rybak-Wolf A, Lisowski P, Pentimalli TM, Jüttner R, Glažar P, et al. Defective metabolic programming impairs early neuronal morphogenesis in neural cultures and an organoid model of Leigh syndrome. Nat Commun 2021;12(1):1929. doi:10.1038/s41467-021-22117-z.
[37] Lorenz C, Lesimple P, Bukowiecki R, Zink A, Inak G, Mlody B, et al. Human iPSC-Derived Neural Progenitors Are an Effective Drug Discovery Model for Neurological mtDNA Disorders. Cell Stem Cell 2017;20(5):659–74 e9. doi:10.1016/j.stem.2016.12.013.
[38] Zink A, Haferkamp U, Wittich A, Beller M, Pless O, Prigione A. High-content screening of mitochondrial polarization in neural cells derived from human pluripotent stem cells. STAR Protoc 2022;3(3):101602. doi:10.1016/j.xpro.2022.101602.