Review

The Path to Precision Medicine in Leigh Syndrome Spectrum: A Four-Decade Chronicle of Genetic Discovery and Targeted Treatment

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Abstract

Leigh syndrome (LS), first reported in 1951, is the most common primary mitochondrial disease. The overarching term, Leigh Syndrome Spectrum (LSS) was proposed by a ClinGen Expert Panel to encompass the wide continuum of neurodegenerative and non-neurologic manifestations which were associated with classic LS and Leigh-Like Syndrome (LLS). Notably, LSS typically presents developmental regression or delay by two years of age, with about 20% of cases presenting as late-/adult-onset forms after 2 years. Historically defined by clinical, biochemical, and neuropathological findings, the genetic basis of LSS has been elucidated through the use of Sanger and next-generation sequencing (NGS), resulting in the discovery of over 120 causative genes. Moreover, LSS can be caused by mutations in both nuclear-encoded genes and mitochondrial DNA (mtDNA), with overlapping clinical characteristics that occur at similar frequencies. This review aims to summarize the clinical and onset characteristics of LSS, genetic testing-aided diagnosis criteria, and the development of treatments. Furthermore, this review organizes the years since the first reports of gene and mutation discoveries into four consecutive eras: Clinical-Biochemical Era (1990–1999), Early Genomics Era (2000–2009), NGS Revolution Era (2010–2019), and Modern Era (2020–Present). Thus, using this framework, this review chronicles the evolution of LSS molecular genetics and treatment development, highlighting the shift from supportive care to targeted therapies driven by modern technologies. Cornerstone experimental models, such as the $Ndufs4^{-/-}$ knockout mouse and patient-derived induced pluripotent stem cells (iPSCs), have facilitated mechanistic studies and drug repurposing screens, including the identification of sildenafil as a potential therapeutic agent, which has led to medical improvements in patients. Current advances in gene editing, including mitochondrial single-base editors such as eTd-mtABE and mitoBEs, are enabling gene therapy with precise introduction and correction of LS-causing variants in rat and mouse models. On the preventative front, Mitochondrial Replacement Therapy (MRT), guided by precise maternal mtDNA genotyping, has been successfully applied in clinical practice, allowing mothers carrying LSS-causing mtDNA variants to have healthy babies free of the LS manifestation. Collectively, these advances in gene discovery, genetic diagnosis, sophisticated disease modeling, rapid screening of small molecule drugs, precise gene editing for gene therapy, and innovative treatment strategies, such as MRT, are ushering in an era of precision medicine for LSS.

Keywords: Leigh Syndrome (LS); Leigh Syndrome Spectrum (LSS); targeted therapy; precision medicine; mitochondrial replacement therapy (MRT); gene therapy; mitochondrial DNA (mtDNA); $Ndufs4^{-/-}$ knockout mouse

1. Introduction—The History and Nomenclature—From Leigh Syndrome (LS) to Leigh-Like Syndrome (LLS) and Leigh Syndrome Spectrum (LSS)

Leigh syndrome (LS) (OMIM: 25600, Orphanet ID 506) was originally described by Archibald Denis Leigh in 1951 as Subacute Necrotizing Encephalomyelopathy [1], which was later renamed Leigh syndrome. LS is the most frequent manifestation of primary mitochondrial diseases (PMDs), with a prevalence of approximately 1 in 40,000 live births [2]. LS is a devastating disease with a highly heterogeneous symptom spectrum and disease course. This first reported case carried hallmark symptoms for LS, including early-onset development of necrotizing lesions in the grey nuclei of the brain stem, subthalamic region, and basal ganglia; bilateral optic atrophy; deafness; and bilateral spasticity [3].

In earlier years, clinical and research work into LS was largely observational, limited to physical and biochemical characterization with little dissection of the molecular genetic basis due to the lack of DNA sequencing techniques. In the 1990s, the advent of Sanger sequencing technique enabled the gene discovery through family segregation study and positional cloning. The causative genes and mutations were discovered in both mitochondrial genome DNA (mtDNA) [4] and nuclear genome [5], which gradually led to the discovery of dozens classic LS genes by the early 2000s. High-throughput next generation sequencing (NGS) technology, introduced in 2007 by Illumina, revolutionized the speed and width of LS gene discovery, enabling the broad availability of genetic testing products covering LS diagnosis for patients across the world. NGS-based patient tests led not only to more classic LS gene discovery, but also frequent Leigh-Like Syndrome (LLS) gene discovery
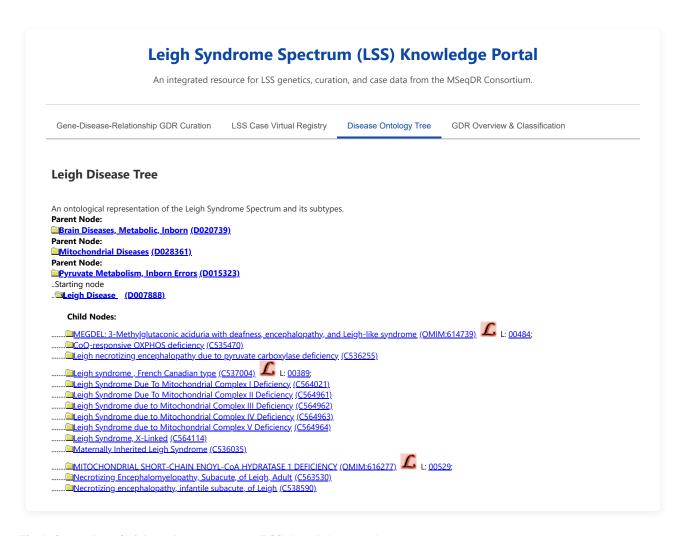
Leigh Syndrome Spectrum (LSS) Knowledge Portal

An integrated resource for LSS genetics, curation, and case data from the MSeqDR Consortium.

Gene-Disease-Relationship GDR Curation   LSS Case Virtual Registry   Disease Ontology Tree   GDR Overview & Classification

Leigh Disease Tree

An ontological representation of the Leigh Syndrome Spectrum and its subtypes.

Parent Node:
📁 **Brain Diseases, Metabolic, Inborn (D020739)**
Parent Node:
📁 **Mitochondrial Diseases (D028361)**
Parent Node:
📁 **Pyruvate Metabolism, Inborn Errors (D015323)**
..Starting node
..📁 **Leigh Disease (D007888)**

    Child Nodes:

.........📁 MEGDEL: 3-Methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome (OMIM:614739) *L* L: 00484;
.........📁 CoQ-responsive OXPHOS deficiency (C535470)
.........📁 Leigh necrotizing encephalopathy due to pyruvate carboxylase deficiency (C536255)

.........📁 Leigh syndrome , French Canadian type (C537004) *L* L: 00389;
.........📁 Leigh Syndrome Due To Mitochondrial Complex I Deficiency (C564021)
.........📁 Leigh Syndrome Due To Mitochondrial Complex II Deficiency (C564961)
.........📁 Leigh Syndrome due to Mitochondrial Complex III Deficiency (C564962)
.........📁 Leigh Syndrome due to Mitochondrial Complex IV Deficiency (C564963)
.........📁 Leigh Syndrome due to Mitochondrial Complex V Deficiency (C564964)
.........📁 Leigh Syndrome, X-Linked (C564114)
.........📁 Maternally Inherited Leigh Syndrome (C536035)

.........📁 MITOCHONDRIAL SHORT-CHAIN ENOYL-CoA HYDRATASE 1 DEFICIENCY (OMIM:616277) *L* L: 00529;
.........📁 Necrotizing Encephalomyelopathy, Subacute, of Leigh, Adult (C563530)
.........📁 Necrotizing encephalopathy, infantile subacute, of Leigh (C538590)

Fig. 1. Screenshot of leigh syndrome spectrum (LSS) knowledge portal.

where atypical symptoms may disqualify the patients being strictly diagnosed with classic LS and thus the genes may be overlooked and molecular diagnosis missed. The quick accumulation of LLS cases and genes with variable overlapping symptoms with classic LS cases led to the introduction of the overarching nomenclature, Leigh Syndrome Spectrum (LSS), by Mito-GCEP Expert Panel [6].

Like other PMDs, LS is caused by mitochondrial dysfunction due to mutations in mitochondrial DNA (mtDNA)-encoded genes or nuclear-encoded genes. There are 14 subtypes (child nodes) according to disease ontology tree at MSeqDR (Fig. 1, and URL: https://mseqdr.org/leigh.php). French-Canadian Type Leigh syndrome (LSFC, OMIM: 220111) is a subtype prevalent in the Saguenay-Lac-Saint-Jean region of Quebec, Canada, caused by population's founder mutations in the *LRPPRC* gene. Another Leigh syndrome subtype is Maternally Inherited Leigh Syndrome (MILS) caused by mtDNA mutations inherited from the mothers, but this designation is rarely used considering that many causative mtDNA variants are *de novo* rather than inherited.

Leigh-Like Syndrome (LLS): Some patients may not meet the strict diagnosis criteria for classic LS including the requirement to display all the hallmark symptoms. The disease name Leigh-Like Syndrome (LLS) is used for such cases whose phenotypes resemble classic Leigh syndrome but may fail to meet some of the classic diagnostic criteria such as the characteristic brain lesions are present with atypical clinical features [6,7]. Biotinidase deficiency is also regarded as LLS in cases with some LS hallmark manifestations [8].

Leigh Syndrome Spectrum (LSS): Given the overlap between LS and LLS, the Clinical Mito-GCEP Expert Panel proposed the overarching term, LSS, to be the disease entity accounting for the highly variable manifestations from LS to LLS [6]. This effort represents the first time these clinical entities have been redefined by the mitochondrial disease community in 25 years. The Mito-GCEP classified 113 genes on the basis of their LSS disease causality, classifying 31 genes as "definitive", 38 genes as "moderate", and 45 genes as "Limited" or "Disputed" to disease relationships (GDR) for LSS [6].

2. LSS Clinical Characteristics

The clinical characteristics of Leigh Syndrome Spectrum (LSS), whether caused by mutations in nuclear-encoded genes (nuclear-LSS) or mitochondrial DNA mutations (mtDNA-LSS), are largely overlapping and present as a continuum of progressive neurodegenerative disorders. Most of the common symptoms present at similar frequencies between the two causes, with the notable exceptions of muscle weakness (30% vs 75%) and dysphagia (25% vs 80% fact) which are both more common in mtDNA-LSS patients [9,10].

2.1 Neurologic Manifestations

The primary symptoms are neurologic and occur at largely similar frequencies between both nuclear-LSS and mtDNA-LSS forms. Developmental delay and/or regression are core symptoms of LSS. Motor system involvements include hypotonia and movement disorders such as dystonia and chorea, spasticity, and cerebellar ataxia. Additionally, seizures/epilepsy is a common manifestation, and peripheral neuropathy can occur in patients.

Importantly, brain stem dysfunction and lesions may lead to a variety of critical issues. These include respiratory abnormalities such as apnea, central hypoventilation or hyperventilation, and irregular respiration, as well as bulbar problems which often lead to abnormal swallowing (dysphagia)—in up to 80% mtDNA-LSS and 25% in nuclear-LSS, and speech difficulties (dysarthria). Other brain stem-related signs include ophthalmoparesis and abnormalities of thermoregulation.

2.2 Neuroradiologic Findings

Neuroimaging is crucial for LSS diagnosis and typically reveals characteristic patterns. The hallmark finding is the presence of bilateral and typically symmetric lesions in the central nervous system. On T2-weighted magnetic resonance imaging (MRI), these lesions appear as hyperintensities (bright signals). On computed tomography (CT), they may be seen as hypodensities. These lesions are most found in the basal ganglia, thalamus, and brain stem. The periaqueductal gray matter, medulla, and spinal cord can also be involved.

2.3 Non-Neurologic Manifestations

While LSS is primarily a neurologic disorder, various other systems can be affected, particularly in mtDNA-LSS. (1) Gastrointestinal manifestations that include poor weight gain and other gastrointestinal issues, (2) Cardiac involvement that may include cardiomyopathy and conduction defects, and (3) Other systems that include hepatic (liver), renal (kidney), and endocrine (hormonal) manifestations.

2.4 Disease Onset: Early-Onset vs Late-/Adult-Onset

2.4.1 Typical Onset

Leigh Syndrome Spectrum (LSS) is predominantly a group of diseases of infancy and early childhood. The onset for both nuclear-encoded (nuclear-LSS) and mitochondrial DNA-encoded (mtDNA-LSS) is typically in infancy or early childhood, often between the ages of 3 and 24 months. However, a significant minority of cases present later in childhood (late-onset) or even in adulthood (adult-onset), about 20% and 30% in two Chinese cohorts respectively [11,12]. These late-onset forms often exhibit different clinical spectrums, better prognosis, and genetic associations compared to the classic early-onset cases. LSS onset frequently follows a metabolic challenge, such as an intercurrent viral illness, surgery, prolonged fasting, or vaccinations.

2.4.2 Presentation

The disease often presents with sudden neurodevelopmental regression. This decompensation is commonly associated with elevated lactate levels in the blood and/or cerebrospinal fluid (CSF). While motor and developmental regression are hallmarks of early-onset Leigh syndrome, late-onset cases can present with a wider range of neurological symptoms, including ataxia, dystonia, seizures, and peripheral neuropathy [7,11–13].

2.5 Mode of Inheritance

Mitochondrial gene-encoded Leigh Syndrome Spectrum (mtDNA-LSS) is maternally inherited, for a minority of the patients (about 30%). About 40% of mtDNA-LSS patients have mtDNA pathogenic variants affecting complex V subunit *MT-ATP6*, with the most common variants being m.8993T>G, m.8993T>C, and m.9176T>C. Other mtDNA-LSS patients are mostly caused by variants in the complex I subunits, encoded by genes *MT-ND3*, *MT-ND5*, *MT-ND6*, *MT-ND4*, and *MT-ND1*. There is a total of 15 mtDNA genes reported, but rarely in other mtDNA genes than listed above [9].

Nuclear gene-encoded Leigh Syndrome Spectrum (nuclear-LSS) accounts for about 70% of the cases, caused by over 120 LSS-related genes reported to date. Most of the genes are associated with autosomal recessive inheritance. There are only a few genes associated with LSS in autosomal dominant modes: *DNM1L*, *OPA1*, *SLC25A4*, and *SSBP1*, which are all *de novo* pathogenic variants. Interestingly, they are all involved in mitochondrial fission/fusion (*DNM1L*, *OPA1*, *SLC25A4*) and maintenance (*SSBP1*) [6,10].

X-linked inheritance is reported for LSS caused by a heterozygous or hemizygous pathogenic variants in genes *PDHA1*, *AIFM1*, *NDUFA1*, and *HSD17B10*, where *PDHA1* is the first discovered nuclear LSS gene [5].

3. Diagnosis With Molecular Genetic Testing

Currently, the diagnosis of both mtDNA-LSS and nuclear-LSS can be established in a proband both fulfilling clinical criteria for LSS as detailed below and carrying one or two pathogenic or likely pathogenic variants. These variants in specific mtDNA or nuclear genes are listed in reference databases such as ClinVar and regional or disease society guidelines [6]. The variant genotypes and zygosity must be consistent with the expected mode of inheritance. The diagnosis of mtDNA-LSS includes identification of a heteroplasmic or homoplasmic pathogenic variant in one of the known causative mtDNA genes known to be involved in mtDNA-LSS. For nuclear-LSS, the pathogenic variants can be in more than 120 nuclear genes known to be associated with LSS under compatible autosomal recessive, autosomal dominant, or X-linked inheritance modes [6,7].

3.1 Clinical Criteria for LSS Diagnosis

Clinical criteria below for mtDNA-LSS and nuclear-LSS are adapted from the Mito-GCEP expert panel consensus [6].

Typical neuroradiologic findings: Must include the bilateral and typically symmetric hyperintensities on T2-weighted magnetic resonance imaging (MRI) or hypodensities on CT. Hyperintensities or hypodensities must be found in the brain stem and/or basal ganglia with or without bilateral. Alternatively, Hyperintensities or hypodensities must be found in the thalamus, cerebellum, subcortical white matter, and/or spinal cord.

AND at least one of the following characteristic neurologic clinical features: Developmental regression, developmental delay, or neurobehavioral/psychiatric features.

AND at least one of the following biochemical and/or mitochondrial abnormalities: Elevated concentration of lactate and/or glycine in relevant tissues, respiratory chain enzyme activity deficiency, pyruvate dehydrogenase complex in respective tissues, or mitochondrial fission/fusion defect.

3.2 Molecular Genetic Testing

For nuclear-LSS, molecular genetic testing approaches include comprehensive whole genome (WGS) or whole exome (WES) tests, PMD-targeted multi-gene panel tests, or single-gene tests. For mtDNA-LSS, molecular genetic testing may be an integral part—either as by-product or specially augmented with mitochondrial genome content—of the comprehensive WGS or WES tests or a pure whole mtDNA genome sequencing.

Molecular testing with LSS coverage is widely available across the world, and the choice of approach may be dependent on clinical suspicion, age of presentation, and availability of genomic testing. A search of NCBI Genetic Test Registry (GTR) website returned 271 tests from 32 labs across the world, using the following keyword filters: Test purpose = "Diagnosis" and Test method = "Sequence analysis of the entire coding region" (https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=Leigh%20syndrome&filter=testpurpose:diagnosis, accessed on July 22, 2025), many of them are multi-gene panels and some of them are single LSS gene tests. Understandably, WGS and WES can also cover the test for nuclear-LSS and most mtDNA-LSS.

4. Decadal Chronicle of Molecular Genetics, Clinical Diagnosis, and Treatment Investigation

The genetic study of LS began with the first description of the disease in 1951, yet molecular genetics only truly started in the 1980s after Sanger sequencing technology became available. We searched for all the PubMed entries with "Leigh syndrome" keywork in either the title or abstract retuned about 1100 matching publications for reviewed. With a comprehensive review of historical scientific progress in understanding and treating Leigh syndrome, we can divide the period between 1951 to 2025 into 4 chronologically sequential periods, with most periods spanning roughly a decade and characterized by distinct technology status regarding LS molecular genetics, clinical investigation, and treatment innovation. We then generated a chronicle narrative summary that describes the key advancements in each era including gene discoveries, the emergence of new animal and cell line models, and the evolution of therapy development and treatment strategies.

4.1 Period 1 (1951–1999): The Clinical-Biochemical/Pre-Genomic Era

The initial decades of Leigh syndrome (LS) research was comprised mostly of clinical observations and foundational investigations in biochemistry and imaging. LS was first described as a distinct neuropathological entity as "subacute necrotizing encephalomyelopathy", characterized by symmetrical lesions in the brainstem and basal ganglia. Early research focused on establishing LS as an inborn error of metabolism, with strong evidence pointing towards defects in cellular energy production.

Studies from the 1970s through the 1990s frequently linked LS to elevated lactate and pyruvate levels, suggesting a primary defect in pyruvate metabolism. The X-linked form of Leigh syndrome was inferred from an unexplained male/female ratio in Leigh's syndrome of 1.83/1 [14]. These led to the identification of Pyruvate Dehydrogenase Complex (PDHC) deficiency as a significant cause, with mutations in the X-linked *PDHA1* gene being the first nuclear gene mutation discovered as a cause of LSS [5,15]. Deficiencies in mitochondrial respiratory chain complexes, particularly Cytochrome c Oxidase (Complex IV), were also reported [16], though the underlying genetic causes remained elusive. Functional studies were limited to biochemical assays on patient tissues, primarily muscle and fibroblasts.

Table 1. Gene discoveries first emerged by decadal period.

Decade period	Genes first discovered during the period (Year – Gene)
1990–1999, the pre-genomic era of clinical and biochemical discovery	*8 genes:* *1992 - MT-ATP6, 1993 - PDHA1, 1995 - SDHA, 1998 - NDUFS4, 1998 - NDUFS8, 1998 - SURF1, 1999 - NDUFV1, 1999 - NDUFS7*
2000–2009, the early genomics era and expansion of genetic etiologies	*19 genes:* *2000 - MT-TK, 2000 - MT-CO3, 2002 - BCS1L, 2003 - MT-ND6, 2003 - MT-ND5, 2003 - LRPPRC, 2004 - MT - ND3, 2004 - COX15, 2004 - NDUFS3, 2005 - NDUFS1, 2005 - NDUFAF2, 2006 - PDHX, 2006 - PDSS2, 2007 - SUCLA2, 2007 - MT-ND2, 2008 - NDUFA2, 2009 - MT-TW, 2009 - TACO1, 2009 – PDHB*
2010–2019, the next-generation sequencing revolution	*51 genes:* *2010 - MTRFR, 2010 - NDUFAF5, 2010 - FOXRED1, 2011 - NDUFA10, 2011 - NDUFA12, 2011 - TPK1, 2011 - MTFMT, 2012 - MT-TI, 2012 - NDUFA9, 2012 - SERAC1, 2013 - TUFM, 2013 - LIPT1, 2013 - SLC19A3, 2013 - FBXL4, 2014 - GYG2, 2014 - PET100, 2014 - IARS2, 2014 - TSFM, 2014 - ECHS1, 2015 - NDUFV2, 2015 - NARS2, 2015 - GFM2, 2015 - COX8A, 2015 - HIBCH, 2016 - GTPBP3, 2016 - CDKL5, 2016 - NDUFB8, 2016 - SLC25A46, 2016 - SCO2, 2016 - MFF, 2016 - EARS2, 2016 - PARS2, 2016 - NDUFAF6, 2016 - ADAR, 2017 - OPA1, 2017 - ACY1, 2017 - PNPT1, 2017 - NDUFAF4, 2017 - NDUFAF3, 2017 - MRPS34, 2017 - NDUFS6, 2018 - ADAMTSL2, 2018 - ATP5MK, 2019 - CRAT, 2019 - NDUFAF8, 2019 - TIMMDC1, 2019 - NDUFS2, 2019 - TIMM50, 2019 - NDUFA1, 2019 - PUS1, 2019 - PTCD3*
2020–present, the modern era of targeted therapies and sophisticated models	*38 genes:* *2020 - SUOX, 2020 - SQOR, 2020 - COQ4, 2020 - HSD17B10, 2020 - RMND1, 2020 - TRMU, 2020 - NDUFA13, 2020 - NDUFC2, 2020 - NAXE, 2020 - VPS13D, 2020 - FASTKD2, 2020 - UQCRC2, 2021 - FDXR, 2021 - HPDL, 2022 - DNAJC30, 2022 - NFU1, 2022 - MRPL38, 2022 - SLC25A19, 2022 - L2HGDH, 2022 - DLD, 2022 - DLAT, 2022 - ETHE1, 2022 - PMPCB, 2022 - PMPCA, 2022 - MTO1, 2022 - ALDH5A1, 2022 - ATP5PO, 2022 - NDUFA4, 2022 - SUCLG1, 2022 - TARS2, 2023 - MRPL39, 2023 - TMEM126B, 2024 - NDUFA3, 2024 - MRPS36, 2024 - VARS2, 2024 - RNF213, 2024 - POLG, 2025 - FASTKD5*

The first mitochondrial DNA (mtDNA) mutation linked to LS, the m.8993T>G in the *MT-ATP6* gene (Table 1, **Supplementary Table 1**), was a landmark discovery in 1992 [4]. This finding established the existence of maternally inherited LS and introduced the concept of heteroplasmy, where the percentage of mutant mtDNA correlated with disease severity. The subsequent identification of the milder severity m.8993T>C variant further solidified *MT-ATP6* genotype-disease correlation [17].

Towards the end of this era, the first nuclear genes encoding respiratory chain components and assembly factors (complex II) were identified for gene *SURF1*. The study used complementation assays in patient cell lines of rodent/human rho0 hybrids (Table 2) to confirm that this nuclear gene caused LS. This led to the landmark discovery of mutations in *SURF1* as a major cause of COX-deficient LS [18]. Of note, Rho-0 (ρ0) immortalized cell lines which are devoid of mitochondrial DNA was invented in 1989 [19] and had been primarily used to create cytoplasmic hybrids (cybrids) to study the functional effects of specific mtDNA variants. But Tiranti *et al*'s work [18] used it to introduce complementing chromosomes without exogenous mtDNA to discover *SURF1*. The first reports of mutations

in nuclear-encoded Complex I subunits were *NDUFS8* [20] and *NDUFS7* [21].

Therapeutic approaches during this period were entirely supportive, focusing on managing symptoms and metabolic crises with "mitochondrial cocktails" consisting of vitamins and cofactors such as thiamine, riboflavin, and coenzyme Q10, often with limited and anecdotal success (Table 3).

The potential of sodium dichloroacetate (DCA) to lower lactate levels was explored. The lactic and pyruvic acid concentrations in serum and cerebrospinal fluid (CSF) were decreased by the oral DCA treatment in some patients but not in other patients, thus its clinical efficacy was controversial [22,23].

4.2 Period 2 (2000–2009): The Early Genomics Era and Expansion of Genetic Etiologies

The advent of Sanger sequencing technologies dramatically accelerated the pace of gene discovery. This decade saw the identification of numerous novel mutations in both mtDNA and nuclear DNA genes. The list of causative nuclear genes for Complex I deficiency expanded significantly to include *NDUFS4, NDUFS3, NDUFS2, ND-*

UFA2, and the assembly factor *NDUFAF2* (Table 1). Mutations in other respiratory chain complexes and related pathways were also identified, including *SDHA* (Complex II), *BCS1L* (Complex III), *SCO2* and *COX10* (Complex IV assembly), *SUCLA2* and *SUCLG1* (Krebs cycle), and *PDHB* (*PDHC*).

A key development was the creation of yeast [24] model and the first animal model of mouse with knockout nuclear gene *Surf1* [25]—though newer study questioned that this mode did not develop LS, rather displayed prolonged lifespan, which reflects the complexity in creating LS animal model [26] (Table 2, Ref. [19]). Cell-based models, primarily patient-derived fibroblasts, remained crucial for biochemical characterization and for confirming the pathogenicity of newly discovered genetic variants through complementation assays.

Regarding treatment options, this era remained focused on supportive care and the use of mitochondrial cocktails. However, the advancements in molecular testing and genetic mechanism study were leading to the emergence of the first targeted strategies based on specific genetic diagnoses (Table 3). For instance, thiamine and biotin supplementation were shown to be highly effective for biotin-thiamine responsive basal ganglia disease caused by mutations in the thiamine transporter gene *SLC19A3* [27].

4.3 Period 3 (2010–2019): The Next-Generation Sequencing Revolution Era

The widespread adoption of Next-Generation Sequencing (NGS), particularly Whole Exome Sequencing (WES) and large multi-gene panels designed with LSS/PMD associated genes, revolutionized the diagnostic process for LSS. This high-throughput technology enabled the rapid and cost-effective identification of causative mutations in many patients for whom previous single-gene testing had been slow and lack sensitivity. This led to an explosion in the number of novel LSS-associated genes with dozens of new genes identified (Table 1, **Supplementary Table 1**), including those involved in mitochondrial translation (*MRPS34*, *IARS2*, *NARS2*, *TSFM*), coenzyme Q10 biosynthesis (*COQ2*, *PDSS2*), and novel respiratory chain assembly factors (*NDUFAF3*, *NDUFAF4*, *NDUFAF5*, *NDUFAF6*, *ACAD9*). Biotin-responsive Basal Ganglia disease is highlighted as a treatable differential diagnosis of Leigh syndrome [28]. The genetic landscape of LSS was revealed to be far more complex than previously imagined.

This period also saw the maturation of experimental models (Table 2), most notably the $Ndufs4^{-/-}$ knockout mouse [29], which recapitulated some core features of LS, including progressive neurodegeneration and brain stem lesions. This model became a cornerstone for investigating disease pathophysiology and for preclinical testing of potential therapies. Patient-derived induced pluripotent stem cells (iPSCs) emerged as a powerful tool, allowing researchers to generate disease-relevant cell types, such

as neurons and cardiomyocytes, to study disease mechanisms in a human, patient-specific context [30]. Study on $Ndufs4^{-/-}$ knockout mouse led to the discovery that mTOR inhibition with rapamycin could significantly slow disease progression and extend lifespan [31]. This demonstrated that a mTOR signaling pathway could potentially be targeted for treating LS. Another discovery in this mouse model was that chronic hypoxia could both prevent and reverse established neurodegenerative disease [32].

These breakthroughs spurred early targeted clinical trials for LS (Table 3). The antioxidant EPI-743 (vatiquinone) was tested in a Phase 2a open-label trial, showing signs of neurological improvement in a small cohort of children [33]. These early trials, while small, marked a significant shift from purely supportive care towards incorporating mechanism-based therapeutic development. Of note, no positive results with EPI-743 have been published for Leigh Syndrome since 2012.

Piloting a new path of preventing LS occurrence, spindle-chromosome complex transfer (ST) method was used in a woman carrying the m.8993T>G mutation associated with Leigh syndrome. The transfer successfully led to the birth of a healthy child and stayed healthy in 7 months follow up assessment [34].

4.4 Period 4 (2020–Present): The Modern Era of Targeted Therapies and Sophisticated Models

The current era is characterized by a continued expansion of the genetic landscape of LSS, driven by WES, WGS, and RNA Sequencing. Adoption of the long read sequencing with PacBio and Nanopore technologies can reveal complicated variations that are previously not accessible with the short read NGS sequencing. Novel genes such as *FASTKD5*, *NDUFC2*, *SQOR*, and *TMEM126B* were shown to be linked to LS (Tables 1,4). The focus of research is now shifting from gene discovery towards understanding the complex cellular consequences of these mutations.

Experimental models have become increasingly sophisticated. Human iPSC technology is being used to create 3D cerebral organoids that model features of LS brain development and pathology, revealing defects in corticogenesis [35]. Live animal models, particularly the $Ndufs4^{-/-}$ KO mouse, are continuously used to dissect the cell-type-specific contributions to the disease [36] and to explore novel therapeutic avenues, such as acarbose which can extend mice lifespan and suggested involvement of gut microbiome in disease pathogenesis [37].

The most exciting developments are in the therapeutic arena. The advancement in molecular genetics and mechanism study along with the preclinical successes of the previous decade have paved the way for more advanced and targeted strategies (Table 3). Gene therapy is a major new focus, with AAV-based vectors being developed to deliver functional copies of defective nuclear genes. Preclinical studies using AAV9 vectors to deliver SURF1 or *NDUFS4*

Table 2. Animal and cell line model first emerged by decadal period.

Decade period	Models first reported	Model details	Model species/type	Citation
1989*–1999	Patient-derived Fibroblasts, Complementation Hybrids	Patient skin biopsies cultured to study biochemical defects. Cell fusion techniques (patient cells + mtDNA-less rho0 cells) used to distinguish nuclear vs. mitochondrial genetic origin.	Human cell lines	(King *et al.*, PMID: 2814477, 1989; Miranda *et al.*, PMID: 2540452, 1989)
2000–2009	*Surf1* Knockout Mouse, *E. coli* model, *Yarrowia lipolytica* (yeast) model	*Surf1* KO mice developed LS-like phenotype with COX deficiency, but not reduced survival of LS; Yeast models were created to study the functional consequences of specific human mutations (e.g., *MT-ATP6*, *NDUFS7/8*).	Mouse, Yeast	(Agostino *et al.*, PMID: 12566387, 2003; Dell'agnello *et al.*, PMID: 17210671, 2007; Kucharczyk *et al.*, PMID: 19454486, 2009; Ahlers *et al.*, PMID: 11004438, 2000)
2010–2019	*Ndufs4*$^{-/-}$ Knockout Mouse, Patient-derived iPSCs, *Drosophila melanogaster* model	*Ndufs4*$^{-/-}$ KO mouse became the cornerstone model for preclinical therapy testing; Patient-derived iPSCs were generated, allowing differentiation into disease-relevant cell types like neurons; *Drosophila* models were used to study genetic interactions and pathophysiology.	Mouse, Human iPSCs, Fruit Fly	(Quintana *et al.*, PMID: 20534480, 2010; Galera-Monge *et al.*, PMID: 27346203, 2016; Loewen *et al.*, PMID: 29496745, 2018)
2020–Present	iPSC-derived Cerebral Organoids, Conditional Knockout Mice, Zebrafish Models	iPSC-derived neural progenitor cells (NPCs) from LS patients for drug repurposing prioritized sildenafil and shown as effective treatment in 6 patients; 3D brain organoids created from patient iPSCs to model neurodevelopmental aspects; Conditional KOs (*Ndufs4*$^{-/-}$ in specific neurons) used to dissect cell-type-specific disease contributions; CRISPR/Cas9-generated *surf1* knockout zebrafish models developed for high-throughput screening.	Human organoids, Mouse, Zebrafish	(Zink *et al.*, preprint, 2025; Romero-Morales *et al.*, PMID: 35792828, 2022; Gella *et al.*, PMID: 32850799, 2020; Haroon *et al.*, PMID: 36795052, 2023; Hyslop *et al.*, 2025, PMID: 40673696 and McFarland *et al.*, PMID: 40689593, 2025)

*: The decade is extended by one year to 1989 to include the first rho-zero cell model [19].

to mouse models have shown significant biochemical and clinical improvements [38,39]. A double administration of self-complementary AAV9NDUFS4 prevents Leigh syndrome in *Ndufs4*$^{-/-}$ mice, leading to 9 months' heathy lifespan [40]. An optimized mitoBEs single-base-editing could precisely edit and introduce LS-causing mtDNA variant *mt-Atp6* T8591C into mouse and observed LS symptoms [41]. Another study used engineered mtDNA adenine base editor (eTd-mtABE), first introducing the pathogenic mtDNA variant into rat zygotes and later correcting the introduced variant at high efficiency—to 53% wild type allele—in a rat Leigh syndrome model [42].

5. Current and Future Therapeutic Landscape

A review of publications and clinical trial registries confirms multiple exciting avenues of development: several targeted therapies are in active clinical development, a few effective small molecule drugs are promising in compassionate prescription to patients, and a preventive treatment program using mitochondria replacement (MRT) achieved initial success in clinical practice. Further, mitochondrial transplantation preclinical studies successfully delivered healthy mitochondria to affected live animal model of Leigh syndrome and improved morbidity and mortality.

5.1 Three-Person IVF by Mitochondrial Replacement Therapy (MRT)—Preventing the Transmission of mtDNA Diseases

Mitochondrial replacement/donation therapy (MRT) with meiotic spindle transfer or pronuclear transfer has been developed. In such so-called three-person IVF techniques, the nuclear DNA from an oocyte or a zygote (the first two persons) carrying a pathogenic mtDNA variant is transferred into an enucleated donor cell (the third person) that provides wild-type mtDNA background.

In one early human trial, spindle-chromosome complex transfer (ST) method was used on a female carrier of

Table 3. Selected treatment innovation first emerged by decadal period.

Decade period	First reported treatments/Therapies	Therapy details	Citation
1990–1999	Ketogenic Diet, Thiamine (Vitamin B1), Dichloroacetate (DCA)	Supportive/Targeted: Ketogenic diet improved lesions and stabilized a *PDHC*-deficient patient; High-dose thiamine used for thiamine-responsive PDH deficiency; DCA investigated to lower lactate levels, with transient effects.	(Wijburg *et al*., PMID: 1641082, 1992; Naito *et al*., PMID: 9266390, 1997; Fujii, Kymura *et al*., PMID: 9440797, 1997)
2000–2009	Coenzyme Q10, Idebenone -a derivative of coenzyme Q-10	Supportive/Targeted: CoQ10 showed a dramatic response in a CoQ10 deficiency case; Idebenone, a derivative of coenzyme Q-10 improved respiratory function.	(Van Maldergem *et al*., PMID: 12447928, 2002; Haginoya *et al*., PMID: 19101701, 2009)
2010–2019	EPI-743 (Vatiquinone), Rapamycin (mTOR inhibition), Hypoxia, Gene Therapy (preclinical)	Targeted: EPI-743 (antioxidant) entered Phase 2A clinical trial (NCT01370447), showing neurologic improvement; Rapamycin and hypoxia showed dramatic rescue in $Ndufs4^{-/-}$ mouse models; First AAV-based gene therapy concepts tested preclinically.	(Martinelli *et al*., PMID: 23010433, 2012; Johnson *et al*., PMID: 24231806, 2013; Ferrari *et al*., PMID: 28483998, 2017, Di Meo *et al*., PMID: 28753212, 2017)
2020–Present	Small molecule drugs sildenafil and HypoxyStat, Gene Therapy (clinical trials), Leukocyte Depletion, Acarbose, Mitochondrial Transfer, Mitochondrial Replacement	Targeted: Human off-label compassionate treatment with sildenafil in six LS patients showed improvements in motor function and resistance to metabolic crises; HypoxyStat is well-tolerated and effective and improved neurologic outcomes in the LS mouse; Preclinical work showed mice gene therapy on *SURF1* KO mice *with human AAV9/hSURF1* is safe and effective; CSF1R inhibitors (leukocyte depletion), acarbose (modulating gut microbiome), and mitochondrial transfer therapy are effective in mouse models.	(Zink *et al*., preprint, 2025; Blume *et al*., PMID: 39965572, 2025; Ling *et al*., PMID: 34703839, 2021; Bitto *et al*., PMID: 37365290, 2023; Nakai *et al*., PMID: 39223312, 2024)

Leigh syndrome (mtDNA mutation 8993T>G), with a long history of pregnancy losses and deaths of offspring. The treatment successfully led to the birth of a healthy boy with neonatal mtDNA mutation load of 2.36–9.23%, who stayed healthy at 7 months' follow up assessment [34].

In two recent back-to-back publications, the studies used mitochondrial donation through pronuclear transfer on 22 patients carrying pathogenic mtDNA variants with homoplasmy or elevated heteroplasmy. There have since been 8 live births from 22 patients (36%), with all infants reported as being healthy at birth, with no or low levels of mtDNA heteroplasmy in blood, being 77% to 100% lower than levels of the maternal pathogenic mtDNA variants [43,44] (ClinicalTrials.gov number: NCT04113447).

Put together, mitochondrial donation through meiotic spindle transfer or pronuclear transfer was effective in reducing the transmission of homoplasmic and heteroplasmic pathogenic mtDNA variants. MRT is currently only approved in the United Kingdom. Despite regulatory concerns about the probability of mitochondrion—nuclear incompatibility after introduction of mitochondria from a different origin into an embryo and concerns about a low probability of pathogenic variant heteroplasmy reversion, these precision therapies represent a new frontier in the treatment of Leigh syndrome, offering real and proven hope for disease-preventing interventions against mtDNA-LSS.

5.2 Mitochondrial Augmentation/Transplantation

The mitochondrial transplantation approach delivers healthy mitochondria to affected cells. While still in early stages, preclinical studies have shown its promise in LS mouse models. The recent study by Nakai *et al*. [45] demonstrated that both bone marrow transplantation (which releases mitochondria into circulation) and direct administration of isolated healthy mitochondria could significantly improve morbidity and mortality in the $Ndufs4^{-/-}$ LS mouse model. Further, even cross-species administration of human mitochondria to $Ndufs4^{-/-}$ mice improves LS symptoms [45]. This pre-clinical success provides a strong rationale for exploring this strategy in humans to treat more mtDNA-caused mitochondrial diseases including mtDNA-LSS. Clinical trials for mitochondrial transplantation are underway for more diseases, and the positive preclinical data in LS mouse models make this a compelling research direction.

Table 4. Status of 144 LSS gene classification (July, 2025).

Biochemical function and complex	Genes	Gene names***	Gene Classification by MITO_GCEP as LSS (* Phase I)
Complex I	35	*DNAJC30, FOXRED1, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6, N-DUFA1, NDUFA10, NDUFA12, NDUFA13, NDUFA2, NDUFA3, NDUFA9, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUF-AF6, NDUFAF8, NDUFB8, NDUFC2, ND-UFS1, NDUFS2, NDUFS3, NDUFS4, ND-UFS6, NDUFS7, NDUFS8, NDUFV1, ND-UFV2, NUBPL, TIMMDC1, TMEM126B*	Definitive/Moderate (26): *FOXRED1, MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6, NDUFA1, NDUFA13, NDUFA2, NDUFA9, NDUFAF2, NDUFAF5, NDUFAF6, NDUFAF8, NDUFB8, NDUFC2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NUBPL* *Limited evidence (6):* ** *MT-ND2, NDUFA10, NDUFA12, NDUFAF4, NDUFV2, TIMMDC1*
Complex II	3	*SDHA, SDHAF1, SDHB*	Definitive/Moderate (1): *SDHA* *Limited evidence (1):* *SDHAF1*
Complex III	4	*BCS1L, TTC19, UQCRC2, UQCRQ*	Definitive/Moderate (1): *TTC19* *Limited evidence (2):* *BCS1L, UQCRQ*
Complex IV	15	*COA6, COX10, COX15, COX4I1, COX8A, LRPPRC, MT-CO1, MT-CO2, MT-CO3, N-DUFA4, PET100, PET117, SCO2, SURF1, TACO1*	Definitive/Moderate (8): *COX10, LRPPRC, MT-CO1, MT-CO3, PET100, SCO2, SURF1, TACO1* *Limited evidence (8):* *COX15, COX4I1, COX8A, MT-CO1, MT-CO2, MT-CO3, NDUFA4, PET117*
Complex V	3	*ATP5MK, ATP5PO, MT-ATP6*	Definitive/Moderate (1): MT-ATP6
Biotin/Thiamine	4	*BTD, SLC19A3, SLC25A19, TPK1*	Definitive/Moderate (3): *BTD, SLC19A3, TPK1* *Limited evidence (1):* *SLC25A19*
Coenzyme Q10	5	*COQ4, COQ7, COQ9, HPDL, PDSS2*	Definitive/Moderate (2): *HPDL, PDSS2* *Limited evidence (1):* *COQ9*
Defect of mitochondrial gene expression	2	*MRPL3, MRPL39*	Definitive/Moderate (0):
Detoxification	1	*NAXE*	*Limited evidence (1):* *NAXE*
Disorders of mitochondrial toxicity	1	*L2HGDH*	
Disorders of mitochondrial toxicity/Valine degradation	1	*HIBCH*	Definitive/Moderate (1): *HIBCH*
Fission	4	*DNM1L, MFF, MFN2, SLC25A46*	Definitive/Moderate (1): *MFF* *Limited evidence (2):* *DNM1L, SLC25A46*
Fusion/Fission	1	*OPA1*	Definitive/Moderate (1): *OPA1*
Impaired mitochondrial protein synthesis	2	*FARS2, PTCD3*	Definitive/Moderate (1): *FARS2* *Limited evidence (1):* *PTCD3*

Table 4. Continued.

Biochemical function and complex	Genes	Gene names***	Gene Classification by MITO_GCEP as LSS (* Phase I)
Lipid remodeling	2	*SERAC1, TOMM7*	Definitive/Moderate (1): *SERAC1*
Lipoic acid	3	*LIAS, LIPT1, MECR*	Definitive/Moderate (2): *LIPT1, MECR* Limited evidence (1): *LIAS*
Mitochondrial DNA maintenance	1	*FBXL4*	Definitive/Moderate (1): *FBXL4*
Mitochondrial gene expression	1	*MTRFR*	
Mitochondrial stability, fission, clearance by mitophagy	1	*VPS13D*	Definitive/Moderate (-) Limited evidence (1): *VPS13D*
Mitochondrial translation	19	*EARS2, GFM1, GFM2, GTPBP3, IARS2, MRPS34, MT-TI, MT-TK, MT-TL1, MT-TL-2, MT-TV, MT-TW, MTFMT, MTRFR, NA-RS2, PNPT1, TARS2, TRMU, TSFM*	Definitive/Moderate (15): *ARS2, GFM1, GFM2, GTPBP3, MRPS34, MT-TI, MT-TK, MT-TL1, MT-TL2, MT-TV, MT-TW, MTFMT, PNPT1, TRMU, TSFM* Limited evidence (7): *IARS2, MT-TI, MT-TL1, MT-TL2, MT-TW, NARS2, TARS2*
mtDNA depletion and/or multiple mtDNA deletions	9	*MPV17, POLG, RNASEH1, RRM2B, SLC-25A4, SSBP1, SUCLA2, SUCLG1, TWNK*	Definitive/Moderate (2): *SUCLA2, SUCLG1* Limited evidence (4): *POLG, RNASEH1, SLC25A4, SSBP1*
OXPHOS ± PDHc enzyme deficiency	5	*ADAR, AIFM1, MORC2, RANBP2, SLC39-A8*	Definitive/Moderate (1): *AIFM1* Limited evidence (4): *ADAR, MORC2, RANBP2, SLC39A8*
OXPHOS ± PDHc enzyme deficiency/Disorders of mitochondrial toxicity	1	*ECHS1*	Definitive/Moderate (1): *ECHS1*
Proteostasis	2	*CLPB, LONP1*	Definitive/Moderate (0) Limited evidence (2): *CLPB, LONP1*
Proteostasis/Defect of mitochondrial protein quality control	4	*HTRA2, PMPCA, PMPCB, SPG7*	Definitive/Moderate (1): *PMPCB*
Pyruvate dehydrogenase complex	6	*DLAT, DLD, MPC1, PDHA1, PDHB, PD-HX*	Definitive/Moderate (5): *DLAT, DLD, PDHA1, PDHB, PDHX*
Sulfide metabolism/Disorders of mitochondrial toxicity	2	*ETHE1, SQOR*	Definitive/Moderate (1): *ETHE1* Limited evidence (1): *SQOR*
To be assigned by function and complex	8	*CRAT, FASTKD2, FASTKD5, GYG2, HSD-17B10, KGD4, MTO1, NUP62*	Definitive/Moderate (1): *MTO1* Limited evidence (1): *NUP62*

Note: *: MITO_GCEP Phase I (McCormick *et al.* [6] 2023, PMID: 37255483); ** Italic font: Genes classified in Phase I as "Limited_Evidence" for LSS, MITO_GCEP Phase II is on-going to add more published cases and evidences to update the classification (Shen L, personal communication, July 2025). ***: Gene names include the 113 genes reviewed by Mito-GCEP, other LSS- causative genes reported in publications, and reported at ClinVar and HGMD reference databases.

5.3 Small Molecule and Repurposed Drugs

To date, no FDA-approved small molecule therapies exist for Leigh Syndrome Spectrum (LSS) or other primary mitochondrial diseases [46]. Nevertheless, several promising candidates are emerging, with early evidence drawn from preclinical *in vivo* studies in animal or organoid models, compassionate use in affected patients, and ongoing clinical trials.

5.3.1 Sildenafil

iPSC-derived neural progenitor cells (NPCs) from LS patients was used for drug repurposing which prioritized sildenafil as an effective off-label compassionate treatment in 6 patients with outcome improvements [47].

5.3.2 HypoxyStat

A small-molecule form of hypoxia therapy that increases oxygen-hemoglobin affinity, HypoxyStat is well-tolerated and effective for pre-symptomatic and advanced disease treatment, improving neurologic outcomes in the LS mouse model [48].

5.3.3 KL1333

An orally available small molecule that acts as an NAD+ modulator. One pre-clinical trial result indicates KL1333 is safe and well tolerated, supporting the efficacy of KL1333 in improving fatigue, functional strength, endurance [49]. A clinical trial is currently recruiting adult primary mitochondrial disease patients (NCT05650229) to evaluate drug efficacy (FALCON).

5.3.4 mTOR Inhibitors (e.g., Rapamycin)

The striking preclinical success in the $Ndufs4^{-/-}$ mouse model has generated significant interest. Clinical trials exploring mTOR inhibitors for various mitochondrial diseases, including LS, are being considered and are listed in registries (e.g., NCT06843811, Sirolimus for Leigh syndrome for MELAS).

5.3.5 Vatiquinone (EPI-743)

Despite promising early phase results, PTC Therapeutics announced in 2023 that the Phase 3 MIT-E trial for mitochondrial epilepsy did not meet their primary endpoints. Its future in Leigh syndrome treatment is uncertain (Press release: https://ir.ptcbio.com/news-releases/news-release-details/ptc-therapeutics-announces-results-mit-e-clinical-trial).

5.3.6 Cannabidiol (CBD)

Daily CBD In $Ndufs4^{-/-}$ Leigh Syndrome mouse models significantly delayed neurological and motor decline and extended the median lifespan from 57 to 70 days. CBD also improved phenotypes in patient-derived fibroblasts with reduced $PPAR\gamma$ expression [50]. Further mechanistic studies identified peroxisome proliferator-activated receptor gamma ($PPAR\gamma$) as the mediator of CBD's effects.

6. Informatics Resources for LSS Case and Molecular Genetics

Over the past 4 decades of molecular genetic studies, over 120 LSS genes have been discovered and critically assessed for their GDRs [6], and several dozens of additional genes reported causative for LSS but remain pending systematic assessment with standard ClinGen framework (Table 4, Ref. [6]). Table 4 compiled 144 candidate LSS genes from publications and reference databases including ClinVar and the Human Gene Mutation Database (HGMD®) and organized them into 29 biochemical and functional categories. Over 1000 pathogenic variants from the nuclear and mitochondrial genomes have been reported in patients according to a recent search of the ClinVar and HGMD databases (data not shown). This wealth of rich clinical and genomics data underscores the need for comprehensive and regularly updated database-driven resources to disseminate the data and support ongoing research, improve genetic diagnosis, and aid in patient and provider education.

6.1 Systematically Assessed LSS Gene-Disease Relationships (GDRs)

The diagnostic criteria for LSS, the approaches to gene discovery, and individual cases diagnosis practices by different clinicians have evolved over decades, which occasionally leads to inconsistencies across time and between healthcare centers. To standardize the evidence assessment for the LSS gene–disease relationship (GDR), the international ClinGen Mito-GCEP Expert Panel was established in 2017, with over 30 geneticists, neurologists, metabolic physicians, neuropathologists, bioinformaticians, researchers, and laboratory directors from 9 countries. Using a modified ClinGen framework for Gene–Disease Clinical Validity Curation, this multidisciplinary panel systematically evaluated 113 LSS genes for GDR. In the first phase, the panel classified 31 GDRs as "Definitive", 38 as "Moderate" and 45 as "Limited" or "Disputed" [6]. The renewed funding supported the phase 2 work of expanding to all PMD genes, which continuously updated clinical cases and functional evidence thus leading to GDR upgrading 16 of the 43 "Limited" GDRs to "Definitive" or "Moderate" (Shen L, personal communication, July 2025).

These 114 curated LSS GDRs represent one of the largest resources systematically evaluated by expert consensus and serves as a reference dataset through the Leigh Syndrome Spectrum portal on the Mitochondrial Disease Sequence Data Resource Consortium (MSeqDR) website ([51]; Portal URL: https://mseqdr.org/leigh.php). This GDR resource supports accurate variant interpretation, facilitates genetic diagnosis of LSS, and advances precision medicine efforts.

6.2 Web-Based Reference Resources for LSS: Case-Level Data, and Gene and Variant to Disease Relationships

Because LSS is a rare disease, case-level, genetic, and mutational data are often sparse and dispersed across various database resources. This creates a challenge for the disease community due to a lack of comprehensive and centralized resources. This section describes several web-based, comprehensive resources that aggregate heterogeneous data for LSS genetics and molecular diagnosis.

6.2.1 ClinVar—The FDA-Recognized Reference Database for Genotype-Disease Relationships

ClinVar is a freely accessible public archive of interpretations of clinically relevant human genetic variations [52]. The U.S. FDA recognizes variant classifications submitted to ClinVar by ClinGen's Variant Curation Expert Panels (VCEPs), which follow a standardized protocol. Mito-VCEP actively contributes expert-assessed variant-disease relationships for LSS and other PMDs. Other ClinGen expert panels and worldwide genetic testing providers also contribute pathogenicity assessments to ClinVar. LSS-related variant data can be searched and browsed on the ClinVar website and downloaded from its FTP site. As of July 20, 2025, there are 804 Leigh syndrome-associated entries at ClinVar, with ACMG classifications ranging from "Pathogenic" to "Uncertain Significance" or "Benign" (data not shown here).

6.2.2 Leigh Syndrome Spectrum (LSS) Knowledge Portal by MSeqDR

The MSeqDR Consortium's website resource (https://mseqdr.org) supports LSS gene and variant curation projects by the Mito-GCEP and Mito-VCEP expert panels and stores the curation results from these projects [51]. The MSeqDR Consortium is a global effort focused on collecting and sharing data for rare mitochondrial diseases, including rich data for LSS cases, genes, and variants. Three types of LSS data are available through the "Leigh Syndrome Spectrum Resource" Portal (URL: https://mseqdr.org/leigh.php).

6.2.2.1 LSS GDR Results from Mito-GCEP.
These 114 curated LSS GDRs from section 6.1 serve as a reference dataset to facilitates genetic diagnosis of LSS, through the Leigh portal on the MSeqDR Consortium website.

6.2.2.2 LSS Case-Level Clinical and Genetic Data.
The "LSS Virtual Registry" link directs visitors to a virtual registry of over 2000 published cases established through literature mining [53]. A demo subset includes 440 pseudo-cases that were expertly annotated for GDR classification by the Mito-GCEP expert panel and is accessible using a "guest" account without pre-registration.

6.2.2.3 Disease-to-Phenotype-to-Variant Associations.
The MSeqDR platform provides integrated views of disease-phenotype-variant associations. Starting from the Leigh "Disease Ontology Tree" node, users can navigate to interconnected data from OMIM, the MONDO disease ontology, the Human Phenotype Ontology (HPO), and ClinVar. This data is integrated with analysis tools available on the MSeqDR website.

7. Conclusion

Leigh Syndrome Spectrum (LSS) research is moving rapidly from genetic discovery towards precise and highly personalized medicine including genetics-guided, point mutation-targeted treatments such as the successful human MRT correction on m.8993T>G mutation for healthy births from carrier mothers. We are witnessing concurrent progress in comprehensive genetic diagnosis through WES/WGS genotyping with well-defined clinical criteria; the increasingly sophisticated animal models, iPSC models, and organoid models; the intriguing experiments testing patient-specific drug responses; and the growing pipeline of developing targeted therapies. Together they offer unprecedented opportunities to provide precision therapies for LSS patients. MRT, mitochondrial transplantation, and gene therapy solutions are rapidly moving from purely theoretical concepts to entering clinical practice with successful results in human beings. While significant technical, regulatory governance, and ethical challenges remain, the collaborative efforts of scientists, clinicians, governments, and patient communities will lead to the future where Leigh Syndrome Spectrum is no longer an untreatable disease but a condition that can be precisely managed, prevented, and potentially cured.

Author Contributions

LS is the sole author, with contributions to all the manuscript work: the design, the literature and data collection, the data analysis, data summarization, manuscript drafting, editing, and submission. LS has prepared, read and approved the final manuscript, and agrees to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The author declares no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

Gemini-2.5 AI was used in grammar checking, spelling checking, making formatting, and expression suggestions, which are all human reviewed based on author's domain knowledge relevant to this review article's topic.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBS45427.

References

[1] Leigh D. Subacute necrotizing encephalomyelopathy in an infant. Journal of Neurology, Neurosurgery, and Psychiatry. 1951; 14: 216–221. https://doi.org/10.1136/jnnp.14.3.216.

[2] Rahman S, Blok RB, Dahl HH, Danks DM, Kirby DM, Chow CW, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. Annals of Neurology. 1996; 39: 343–351. https://doi.org/10.1002/ana.410390311.

[3] Schubert Baldo M, Vilarinho L. Molecular basis of Leigh syndrome: a current look. Orphanet Journal of Rare Diseases. 2020; 15: 31. https://doi.org/10.1186/s13023-020-1297-9.

[4] Tatuch Y, Christodoulou J, Feigenbaum A, Clarke JT, Wherret J, Smith C, et al. Heteroplasmic mtDNA mutation (T----G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. American Journal of Human Genetics. 1992; 50: 852–858.

[5] Matthews PM, Marchington DR, Squier M, Land J, Brown RM, Brown GK. Molecular genetic characterization of an X-linked form of Leigh's syndrome. Annals of Neurology. 1993; 33: 652–655. https://doi.org/10.1002/ana.410330616.

[6] McCormick EM, Keller K, Taylor JP, Coffey AJ, Shen L, Krotoski D, et al. Expert Panel Curation of 113 Primary Mitochondrial Disease Genes for the Leigh Syndrome Spectrum. Annals of Neurology. 2023; 94: 696–712. https://doi.org/10.1002/ana.26716.

[7] Lake NJ, Compton AG, Rahman S, Thorburn DR. Leigh syndrome: One disorder, more than 75 monogenic causes. Annals of Neurology. 2016; 79: 190–203. https://doi.org/10.1002/ana.24551.

[8] Baumgartner ER, Suormala TM, Wick H, Probst A, Blauenstein U, Bachmann C, et al. Biotinidase deficiency: a cause of subacute necrotizing encephalomyelopathy (Leigh syndrome). Report of a case with lethal outcome. Pediatric Research. 1989; 26: 260–266. https://doi.org/10.1203/00006450-198909000-00021.

[9] Ball M, Thorburn DR, Rahman S. Mitochondrial DNA-Associated Leigh Syndrome Spectrum. In Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A (eds.) GeneReviews® [Internet]. University of Washington, Seattle: Seattle (WA). 2024.

[10] Rahman S, Thorburn DR, Ball M. Nuclear Gene-Encoded Leigh Syndrome Spectrum Overview. In: Adam MP, Bick S, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews® [Internet]. University of Washington, Seattle: Seattle (WA). 2025.

[11] Hong CM, Na JH, Park S, Lee YM. Clinical Characteristics of Early-Onset and Late-Onset Leigh Syndrome. Frontiers in Neurology. 2020; 11: 267. https://doi.org/10.3389/fneur.2020.00267.

[12] 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. Annals of Neurology. 2022; 91: 466–482. https://doi.org/10.1002/ana.26313.

[13] Incecik F, Herguner OM, Besen S, Bozdoğan ST, Mungan NO. Late-Onset Leigh Syndrome due to NDUFV1 Mutation in a 10-Year-Old Boy Initially Presenting with Ataxia. Journal of Pediatric Neurosciences. 2018; 13: 205–207. https://doi.org/10.4103/jpn.JPN_138_17.

[14] Benke PJ, Parker JC, Jr, Lubs ML, Benkendorf J, Feuer AE. X-linked Leigh's syndrome. Human Genetics. 1982; 62: 52–59. https://doi.org/10.1007/BF00295603.

[15] De Meirleir L, Lissens W, Benelli C, Ponsot G, Desguerre I, Marsac C, et al. Aberrant splicing of exon 6 in the pyruvate dehydrogenase-E1 alpha mRNA linked to a silent mutation in a large family with Leigh's encephalomyelopathy. Pediatric Research. 1994; 36: 707–712. https://doi.org/10.1203/00006450-199412000-00004.

[16] DiMauro S, Servidei S, Zeviani M, DiRocco M, DeVivo DC, DiDonato S, et al. Cytochrome c oxidase deficiency in Leigh syndrome. Annals of Neurology. 1987; 22: 498–506. https://doi.org/10.1002/ana.410220409.

[17] Santorelli FM, Shanske S, Jain KD, Tick D, Schon EA, DiMauro S. A T->C mutation at nt 8993 of mitochondrial DNA in a child with Leigh syndrome. Neurology. 1994; 44: 972–974. https://doi.org/10.1212/wnl.44.5.972.

[18] Tiranti V, Hoertnagel K, Carrozzo R, Galimberti C, Munaro M, Granatiero M, et al. Mutations of SURF-1 in Leigh disease associated with cytochrome c oxidase deficiency. American Journal of Human Genetics. 1998; 63: 1609–1621. https://doi.org/10.1086/302150.

[19] King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. Science (New York, N.Y.). 1989; 246: 500–503. https://doi.org/10.1126/science.2814477.

[20] Loeffen J, Smeitink J, Triepels R, Smeets R, Schuelke M, Sengers R, et al. The first nuclear-encoded complex I mutation in a patient with Leigh syndrome. American Journal of Human Genetics. 1998; 63: 1598–1608. https://doi.org/10.1086/302154.

[21] Triepels RH, van den Heuvel LP, Loeffen JL, Buskens CA, Smeets RJ, Rubio Gozalbo ME, et al. Leigh syndrome associated with a mutation in the NDUFS7 (PSST) nuclear encoded subunit of complex I. Annals of Neurology. 1999; 45: 787–790. https://doi.org/10.1002/1531-8249(199906)45:6<787::aid-ana13>3.0.co;2-6.

[22] Kimura S, Osaka H, Saitou K, Ohtuki N, Kobayashi T, Nezu A. Improvement of lesions shown on MRI and CT scan by administration of dichloroacetate in patients with Leigh syndrome. Journal of the Neurological Sciences. 1995; 134: 103–107. https://doi.org/10.1016/0022-510x(95)00218-8.

[23] Takanashi J, Sugita K, Tanabe Y, Maemoto T, Niimi H. Dichloroacetate treatment in Leigh syndrome caused by mitochondrial DNA mutation. Journal of the Neurological Sciences. 1997; 145: 83–86. https://doi.org/10.1016/s0022-510x(96)00248-1.

[24] Ahlers PM, Garofano A, Kerscher SJ, Brandt U. Application of the obligate aerobic yeast Yarrowia lipolytica as a eucaryotic model to analyse Leigh syndrome mutations in the complex I

core subunits PSST and TYKY. Biochimica et Biophysica Acta. 2000; 1459: 258–265. https://doi.org/10.1016/s0005-2728(00)00160-2.

[25] Agostino A, Invernizzi F, Tiveron C, Fagiolari G, Prelle A, Lamantea E, et al. Constitutive knockout of Surf1 is associated with high embryonic lethality, mitochondrial disease and cytochrome c oxidase deficiency in mice. Human Molecular Genetics. 2003; 12: 399–413. https://doi.org/10.1093/hmg/ddg038.

[26] Dell'agnello C, Leo S, Agostino A, Szabadkai G, Tiveron C, Zulian A, et al. Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Human Molecular Genetics. 2007; 16: 431–444. https://doi.org/10.1093/hmg/ddl477.

[27] Zeng WQ, Al-Yamani E, Acierno JS, Jr, Slaugenhaupt S, Gillis T, MacDonald ME, et al. Biotin-responsive basal ganglia disease maps to 2q36.3 and is due to mutations in SLC19A3. American Journal of Human Genetics. 2005; 77: 16–26. https://doi.org/10.1086/431216.

[28] Distelmaier F, Huppke P, Pieperhoff P, Amunts K, Schaper J, Morava E, et al. Biotin-responsive Basal Ganglia disease: a treatable differential diagnosis of leigh syndrome. JIMD Reports. 2014; 13: 53–57. https://doi.org/10.1007/8904_2013_271.

[29] Quintana A, Kruse SE, Kapur RP, Sanz E, Palmiter RD. Complex I deficiency due to loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107: 10996–11001. https://doi.org/10.1073/pnas.1006214107.

[30] Galera-Monge T, Zurita-Díaz F, González-Páramos C, Moreno-Izquierdo A, Fraga MF, Fernández AF, et al. Generation of a human iPSC line from a patient with Leigh syndrome caused by a mutation in the MT-ATP6 gene. Stem Cell Research. 2016; 16: 766–769. https://doi.org/10.1016/j.scr.2016.04.012.

[31] Johnson SC, Yanos ME, Kayser EB, Quintana A, Sangesland M, Castanza A, et al. mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science (New York, N.Y.). 2013; 342: 1524–1528. https://doi.org/10.1126/science.1244360.

[32] Ferrari M, Jain IH, Goldberger O, Rezoagli E, Thoonen R, Cheng KH, et al. Hypoxia treatment reverses neurodegenerative disease in a mouse model of Leigh syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2017; 114: E4241–E4250. https://doi.org/10.1073/pnas.1621511114.

[33] Martinelli D, Catteruccia M, Piemonte F, Pastore A, Tozzi G, Dionisi-Vici C, et al. EPI-743 reverses the progression of the pediatric mitochondrial disease–genetically defined Leigh Syndrome. Molecular Genetics and Metabolism. 2012; 107: 383–388. https://doi.org/10.1016/j.ymgme.2012.09.007.

[34] Zhang J, Liu H, Luo S, Lu Z, Chávez-Badiola A, Liu Z, et al. Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reproductive Biomedicine Online. 2017; 34: 361–368. https://doi.org/10.1016/j.rbmo.2017.01.013.

[35] Romero-Morales AI, Robertson GL, Rastogi A, Rasmussen ML, Temuri H, McElroy GS, et al. Human iPSC-derived cerebral organoids model features of Leigh syndrome and reveal abnormal corticogenesis. Development (Cambridge, England). 2022; 149: dev199914. https://doi.org/10.1242/dev.199914.

[36] Stokes JC, Bornstein RL, James K, Park KY, Spencer KA, Vo K, et al. Leukocytes mediate disease pathogenesis in the Ndufs4(KO) mouse model of Leigh syndrome. JCI Insight. 2022; 7: e156522. https://doi.org/10.1172/jci.insight.156522.

[37] Bitto A, Grillo AS, Ito TK, Stanaway IB, Nguyen BMG, Ying K, et al. Acarbose suppresses symptoms of mitochondrial disease

[38] Reynaud-Dulaurier R, Benegiamo G, Marrocco E, Al-Tannir R, Surace EM, Auwerx J, et al. Gene replacement therapy provides benefit in an adult mouse model of Leigh syndrome. Brain: a Journal of Neurology. 2020; 143: 1686–1696. https://doi.org/10.1093/brain/awaa105.

[39] Ling Q, Rioux M, Hu Y, Lee M, Gray SJ. Adeno-associated viral vector serotype 9-based gene replacement therapy for SURF1-related Leigh syndrome. Molecular Therapy. Methods & Clinical Development. 2021; 23: 158–168. https://doi.org/10.1016/j.omtm.2021.09.001.

[40] Corrà S, Cerutti R, Balmaceda V, Viscomi C, Zeviani M. Double administration of self-complementary AAV9NDUFS4 prevents Leigh disease in Ndufs4-/- mice. Brain: a Journal of Neurology. 2022; 145: 3405–3414. https://doi.org/10.1093/brain/awac182.

[41] Zhang X, Zhang X, Ren J, Li J, Wei X, Yu Y, et al. Precise modelling of mitochondrial diseases using optimized mitoBEs. Nature. 2025; 639: 735–745. https://doi.org/10.1038/s41586-024-08469-8.

[42] Chen L, Hong M, Luan C, Yuan M, Wang Y, Guo X, et al. Efficient mitochondrial A-to-G base editors for the generation of mitochondrial disease models. Nature Biotechnology. 2025. https://doi.org/10.1038/s41587-025-02685-x. (online ahead of print)

[43] Hyslop LA, Blakely EL, Aushev M, Marley J, Takeda Y, Pyle A, et al. Mitochondrial Donation and Preimplantation Genetic Testing for mtDNA Disease. The New England Journal of Medicine. 2025; 393: 438–449. https://doi.org/10.1056/NEJMoa2415539.

[44] McFarland R, Hyslop LA, Feeney C, Pillai RN, Blakely EL, Moody E, et al. Mitochondrial Donation in a Reproductive Care Pathway for mtDNA Disease. The New England Journal of Medicine. 2025; 393: 461–468. https://doi.org/10.1056/NEJMoa2503658.

[45] Nakai R, Varnum S, Field RL, Shi H, Giwa R, Jia W, et al. Mitochondria transfer-based therapies reduce the morbidity and mortality of Leigh syndrome. Nature Metabolism. 2024; 6: 1886–1896. https://doi.org/10.1038/s42255-024-01125-5.

[46] Magro G, Laterza V, Tosto F. Leigh Syndrome: A Comprehensive Review of the Disease and Present and Future Treatments. Biomedicines. 2025; 13: 733. https://doi.org/10.3390/biomedicines13030733.

[47] Zink A, Dai DF, Wittich A, Henke MT, Pedrotti G, Heiduschka S, et al. Pluripotent stem cell-based drug discovery uncovers sildenafil as a treatment for mitochondrial disease. medRxiv. 2025. https://doi.org/10.1101/2025.05.15.25325571. (preprint)

[48] Blume SY, Garg A, Martí-Mateos Y, Midha AD, Chew BTL, Lin B, et al. HypoxyStat, a small-molecule form of hypoxia therapy that increases oxygen-hemoglobin affinity. Cell. 2025; 188: 1580–1588.e11. https://doi.org/10.1016/j.cell.2025.01.029.

[49] Pizzamiglio C, Stefanetti RJ, McFarland R, Thomas N, Ransley G, Hugerth M, et al. Optimizing rare disorder trials: a phase 1a/1b randomized study of KL1333 in adults with mitochondrial disease. Brain: a Journal of Neurology. 2025; 148: 39–46. https://doi.org/10.1093/brain/awae308.

[50] Puighermanal E, Luna-Sánchez M, Gella A, van der Walt G, Urpi A, Royo M, et al. Cannabidiol ameliorates mitochondrial disease via PPARγ activation in preclinical models. Nature Communications. 2024; 15: 7730. https://doi.org/10.1038/s41467-024-51884-8.

[51] Shen L, Diroma MA, Gonzalez M, Navarro-Gomez D, Leipzig J, Lott MT, et al. MSeqDR: A Centralized Knowledge Repository and Bioinformatics Web Resource to Facilitate Genomic Investigations in Mitochondrial Disease. Human Mutation. 2016; 37: 540–548. https://doi.org/10.1002/humu.22974.

[52] Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipi-

ralla S, *et al*. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Research. 2018; 46: D1062–D1067. https://doi.org/10.1093/nar/gkx1153.

[53] Shen L, Lott M, McCormick E, Muraresku C, Keller K, Wallace D, *et al*. P704: A virtual registry of 9K Leigh syndrome and primary mitochondrial disease cases constructed through semi-automated literature mining and expert curation. Genetics in Medicine Open. 2025; 3: 103073. https://doi.org/10.1016/j.gimo.2025.103073.