



Expert Panel Curation of 113 Primary Mitochondrial Disease Genes for the Leigh Syndrome Spectrum

Elizabeth M. McCormick, MS ¹, Kierstin Keller, MS,² Julie P. Taylor, PhD,³ Alison J. Coffey, PhD,³ Lishuang Shen, PhD,⁴ Danuta Krotoski, PhD,⁵ Brian Harding, DPhil, BMBCCh,^{6,7} NICHD ClinGen U24 Mitochondrial Disease Gene Curation Expert Panel, Xiaowu Gai, PhD,^{4,8} Marni J. Falk, MD,^{1,7} Zarazuela Zolkipli-Cunningham, MBChB, MRCP,^{1,7} and Shamima Rahman, FRCP, FRCPCH, PhD ⁹

Objective: Primary mitochondrial diseases (PMDs) are heterogeneous disorders caused by inherited mitochondrial dysfunction. Classically defined neuropathologically as subacute necrotizing encephalomyelopathy, Leigh syndrome spectrum (LSS) is the most frequent manifestation of PMD in children, but may also present in adults. A major challenge for accurate diagnosis of LSS in the genomic medicine era is establishing gene–disease relationships (GDRs) for this syndrome with >100 monogenic causes across both nuclear and mitochondrial genomes.

Methods: The Clinical Genome Resource (ClinGen) Mitochondrial Disease Gene Curation Expert Panel (GCEP), comprising 40 international PMD experts, met monthly for 4 years to review GDRs for LSS. The GCEP standardized gene curation for LSS by refining the phenotypic definition, modifying the ClinGen Gene–Disease Clinical Validity Curation Framework to improve interpretation for LSS, and establishing a scoring rubric for LSS.

Results: The GDR with LSS across the nuclear and mitochondrial genomes was classified as definitive for 31 of 114 GDRs curated (27%), moderate for 38 (33%), limited for 43 (38%), and disputed for 2 (2%). Ninety genes were associated with autosomal recessive inheritance, 16 were maternally inherited, 5 were autosomal dominant, and 3 were X-linked.

Interpretation: GDRs for LSS were established for genes across both nuclear and mitochondrial genomes. Establishing these GDRs will allow accurate variant interpretation, expedite genetic diagnosis of LSS, and facilitate precision medicine, multisystem organ surveillance, recurrence risk counseling, reproductive choice, natural history studies, and determination of eligibility for interventional clinical trials.

ANN NEUROL 2023;00:1–17

View this article online at [wileyonlinelibrary.com](https://www.wileyonlinelibrary.com). DOI: 10.1002/ana.26716

Received Dec 16, 2022, and in revised form May 22, 2023. Accepted for publication May 23, 2023.

Address correspondence to Dr Rahman, Mitochondrial Research Group, UCL Great Ormond Street Institute of Child Health, London, UK.

E-mail: shamima.rahman@ucl.ac.uk

Dr Falk, ClinGen Mitochondrial Disease Gene Curation Expert Panel, Children's Hospital of Philadelphia, ARC 1002c, 3615 Civic Center Blvd, Philadelphia, PA 19104, USA. E-mail: falkm@chop.edu

Xiaowu Gai, Marni J. Falk, Zarazuela Zolkipli-Cunningham, and Shamima Rahman contributed equally.

From the ¹Mitochondrial Medicine Frontier Program, Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA; ²Center for Mitochondrial and Epigenomic Medicine, Department of Pathology, Children's Hospital of Philadelphia, Philadelphia, PA; ³Illumina Clinical Services Laboratory, Illumina, San Diego, CA; ⁴Center for Personalized Medicine, Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, Los Angeles, CA; ⁵Intellectual and Developmental Disabilities Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; ⁶Department of Pathology and Laboratory Medicine (Neuropathology), Children's Hospital of Philadelphia, Philadelphia, PA; ⁷University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; ⁸Keck School of Medicine, University of Southern California, Los Angeles, CA; ⁹Mitochondrial Research Group, Genetics and Genomic Medicine, UCL Great Ormond Street Institute of Child Health, and Metabolic Unit, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

Additional supporting information can be found in the online version of this article.

Primary mitochondrial diseases (PMDs) are a group of heterogeneous disorders caused by inherited deficiencies of mitochondrial energy metabolism. Leigh syndrome (LS) is the most common PMD phenotype in children, but may rarely also present in adulthood. This progressive neurodegenerative disorder was first described in 1951 as a neuropathologic entity with characteristic brainstem, midbrain, and basal ganglia lesions,¹ and by 1977 had evolved to include impaired mitochondrial function when a link was made in some cases to mitochondrial respiratory chain complex IV deficiency. Over time, as brain imaging came into common clinical practice and genetic etiologies began to be identified, the LS diagnosis could be made prior to autopsy.² Although “Leigh-like syndrome” (LLS) began to be used to describe affected individuals who did not fulfil strict LS criteria,² this term has been interpreted and applied inconsistently. With advances in genetic understanding, it has now become apparent that LS and LLS frequently have significant clinical and biochemical overlap, resulting from pathogenic variants in the same spectrum of mitochondrial and nuclear genes.

The Clinical Genome Resource (ClinGen)³-approved Mitochondrial Disease Gene Curation Expert Panel (Mito GCEP) was formed in 2017 with grant funding from the National Institute of Child Health and Human Development at the National Institutes of Health to evaluate published evidence supporting the gene–disease relationship (GDR) for genes associated with LS, using the ClinGen framework for expert evaluation of clinical validity of GDRs.⁴ Given the overlap between LS and LLS, the Mito GCEP proposed the overarching term Leigh syndrome spectrum (LSS) to be the disease entity for expert GDR curation. Of note, this effort represents the first time these clinical entities have been redefined by the mitochondrial disease community in 25 years. Here, we describe the consensus work of this initiative, where more than three dozen global PMD experts reviewed, discussed, and agreed on the strength of evidence of GDRs for LSS that were subject to standardized curation using the ClinGen framework by a dedicated team of biocurators. Through this work, scoring recommendations were made to the ClinGen Gene Curation Standard Operating Procedure (SOP) to ensure consistency in its implementation for gene curation of nuclear and mitochondrial DNA (mtDNA) causes of LSS.

Materials and Methods

Institutional review board approval was not required, as no human subjects were involved in this project.

Mito GCEP Composition

The Mito GCEP was assembled within the ClinGen Expert Panel framework and under the umbrella of the Mitochondrial Disease Sequence Data Resource (MSeqDR).^{5–7} The panel gained ClinGen Expert Panel approval on June 20, 2018 (<https://clinicalgenome.org/affiliation/40027/>). PMD experts with a particular focus in LSS included clinical geneticists, neurologists, metabolic physicians, neuropathologists, bioinformaticians, researchers, and laboratory directors from 30 institutions (18 in the United States, 12 international) across 9 countries (Fig S1). The effort was co-led by physician–scientists with expertise in mitochondrial biology, mitochondrial disease, genetics, and LSS. Biocurators included genetic counselors and PhD-level clinical genomics scientists from both academia and diagnostic laboratories. The study coordinator, who also served as a biocurator, organized, scheduled, and moderated both small group biocurator reviews and full Mito GCEP meetings. A neuroradiologist and a neuropathologist with expertise in LSS were also invited to meetings when specific questions arose in these areas.

LSS Gene and Phenotype Prioritization

LSS was prioritized for curation as a paradigm for the approach in a major clinical subset of PMD, as no consistent syndrome is seen across all genes associated with PMD. Disease entities listed in existing sources, such as “mitochondrial complex I deficiency” or “combined oxidative phosphorylation deficiency,” are arbitrarily named based on biochemical pathway and/or disease mechanism, and represent a wide range of phenotypes, but do not have unique clinical significance. In contrast, LSS is a distinct entity, representing the most frequent presentation of PMD in childhood, and after careful consideration of ClinGen Lumping and Splitting guidelines, was chosen as the disease entity for this effort.⁸ Genes across both nuclear and mitochondrial genomes were selected for expert curation based on a prior literature review associating these genes with LSS.⁹ Additional genes were added for curation as suggested by Mito GCEP members based on having a new publication or presentation at a scientific conference. No new genes were added for Mito GCEP biocuration and expert panel review after February 11, 2021.

LSS Disease Entity Definition and Gene Curation SOP Delineation

The historic definitions of LS and LLS were reviewed and summarized by the Mito GCEP leadership. An updated overarching term, LSS, was proposed and approved by the Mito GCEP. The LSS definition had several iterations that evolved as additional gene curations were completed. Curation was performed in accordance with the ClinGen Gene Curation SOP, available at <https://clinicalgenome.org/curation-activities/gene-disease-validity/documents/>, based on the framework previously outlined by the ClinGen Gene Curation Working Group.⁴ We followed the ClinGen gene–disease validity SOP version 7 (V7) for nuclear gene curations, as this was the current version at the start of this effort. Each criterion for nuclear gene curation was first reviewed for its relevance to mitochondrial disease, and further guidance was provided, when necessary, to ensure clinical relevance to LSS and a

consistent approach to gene–disease classifications. However, it became apparent that SOP V7, which was based on Mendelian inheritance patterns, was not optimized for mtDNA gene curation, as compared to version 8 of the ClinGen SOP (V8), in which scoring was based on variant characteristics rather than inheritance pattern and which was released just prior to the Mito GCEP beginning curation of mtDNA genes. Therefore, the Mito GCEP added scoring recommendations to SOP V8 for mtDNA gene curation. Updated ClinGen Gene Curation SOPs for nuclear and mtDNA gene curation for LSS are available online (<https://clinicalgenome.org/working-groups/gene-curation/>). For curation in both genomes, when additional scoring recommendations were proposed by the Mito GCEP leadership and biocurators, expert panel consensus approval was obtained. Some scoring recommendations were specific to mitochondrial biology and/or mitochondrial disease, such as the approach to inclusion of assays of mitochondrial function, whereas other scoring recommendations could be applied more generally, such as scoring guidance for founder variants.

Gene Curation Process

Curations were performed in the ClinGen Gene Curation Interface (GCI). Biocurators met with Mito GCEP leadership twice monthly, including a neuroradiologist and/or neuropathologist when appropriate, to review curations and ensure consistency with the phenotype as well as completeness of literature reviews (Fig 1). Unique clinical features, including characteristic magnetic resonance imaging (MRI) features and points of debate, were recorded by the coordinator for discussion during large expert panel review group calls. Curations were presented to the expert panel at monthly Mito GCEP meetings that were scheduled at staggered times to accommodate experts across time zones. Meetings were moderated by the Mito GCEP coordinator and Mito GCEP leadership. Open communication and robust discussion were encouraged, followed by expert panel voting on the final GDR clinical validity classification with a minimum of 3 experts from 3 different institutions voting. Any notable conversation points were documented in published evidence summaries in the GCI. GDRs with only one reported case were classified as limited as per the Gene Curation Validity framework, regardless of score. If a GDR had an intermediate score (6.1–6.9, between limited and moderate; 11.1–11.9, between moderate and strong/definitive), the expert panel would weigh evidence and vote upon the final classification. Recordings of Mito GCEP meetings were distributed to expert panel members unavailable at the scheduled meeting time. Following GDR classification approval by the Mito GCEP, the coordinator reviewed the curation in the GCI to ensure completeness and consistency with expert panel meeting discussion and outcome. Standardized evidence summaries were drafted by the coordinator for consistency and reviewed by the Mito GCEP leadership prior to publication on the ClinGen website (<https://search.clinicalgenome.org/kb/gene-validity>). As curations are updated periodically according to the ClinGen Gene Recuration Procedure ([\[disease_validity_recuration_procedures_v1.pdf\]\(#\)\), the most current information is available at \[clinicalgenome.org\]\(https://clinicalgenome.org\).](https://clinicalgenome.org/site/assets/files/2164/clingen_standard_gene-</p>
</div>
<div data-bbox=)

Results

Gene and Phenotype Prioritization

The Mito GCEP initially aimed to curate 90 published genes for association with LSS across both the nuclear and mitochondrial genomes. Twenty-four gene curations were added during the project period, leading to a final count of 114 gene–disease curations completed (Table 1). Thirty-one 90-minute Mito GCEP meetings held approximately once monthly were completed between November 2018 and May 2021. An average of 11 experts (range = 6–20) attended each expert panel meeting, with an average of 4 genes reviewed per meeting. In total, 113 unique genes were reviewed, as *DNM1L* was curated for both autosomal recessive and autosomal dominant inheritance in association with LSS. Two genes, *NUP62* and *MT-TL2*, were found by expert panel review to have no relationship with LSS, which is considered “disputed” under the gene curation framework.

Disease Entity Definition and Gene Curation SOP Delineation

The LSS definition, which was developed to incorporate published reports of both LS and LLS, includes consideration of neuropathologic evidence and, in the absence of neuropathologic evidence, a combination of brain imaging, neurologic, and biochemical findings (Table 2). The definition of neuropathologic evidence did not change over the course of the project period.^{1,2} Considerations for brain imaging, neurologic manifestations, and biochemical findings were reviewed extensively and updated to reflect the various molecular etiologies and associated disease mechanisms. Brain imaging findings consistent with LSS are bilateral, typically symmetric T2-weighted hyperintensities on MRI or hypodensities on computed tomography (CT) scan in the brainstem and/or basal ganglia, with or without bilateral, T2 hyperintensity on MRI or hypodensity of CT scan in the thalamus, cerebellum, subcortical white matter, and/or spinal cord. Neurologic symptoms seen in LSS include developmental regression, developmental delay, and/or psychiatric symptoms. Brain imaging and neurologic symptoms are further supported by biochemical findings such as elevated lactate in plasma and/or cerebrospinal fluid (CSF), brain magnetic resonance spectroscopy (MRS) lactate peak (in absence of acute seizures), oxidative phosphorylation (OXPHOS) enzyme activity deficiency (<30%) in affected tissue (muscle, liver, fibroblasts), pyruvate dehydrogenase complex (PDC) deficiency

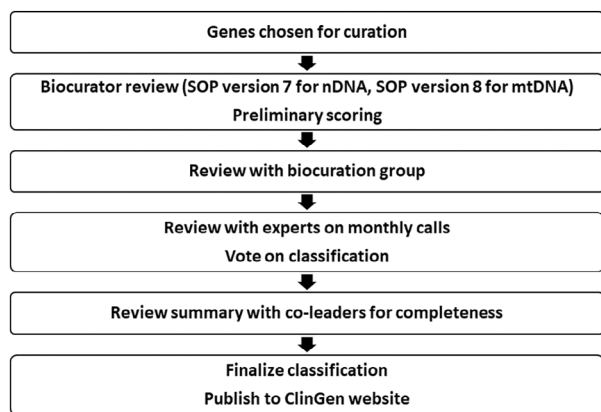


FIGURE 1: Mitochondrial Disease Gene Curation Expert Panel curation process overview. mtDNA = mitochondrial DNA; nDNA = nuclear DNA; SOP = Standard Operating Procedure.

(in fibroblasts, >2 standard deviations [SD] below mean), a mitochondrial fission/fusion defect, elevated glycine levels (if the gene is associated with a lipoic acid disorder), and/or diminished respiratory activity measured by microscale oxygraphy (eg, Oroboros or Seahorse assays), to reach a definition of LSS.

Brain imaging at first included only abnormalities seen on brain MRI but was expanded to include CT scans, as some historical cases in the literature only report CT findings. Both brain MRI and CT scans reliably detect abnormalities in the brain seen in those with LS and LLS. Cranial ultrasound imaging was discussed by the Mito GCEP, but ultimately it was decided not to consider ultrasound data for cases being reviewed for genetic-level evidence, because interpretation is subjective and operator dependent, and pinpointing relevant structures on a static ultrasound image is challenging. The areas of the brain typically affected in LS and LLS were also extensively discussed. Although areas such as the brainstem, basal ganglia, thalamus, cerebellum, and spinal cord can be affected in those with LS and LLS, isolated lesions in the thalamus, cerebellum, and spinal cord would not be consistent with these conditions. Therefore, this section of the LSS definition was refined to include, at minimum, lesions in the brainstem and/or basal ganglia, with or without additional changes in the thalamus, cerebellum, subcortical white matter, and/or spinal cord.

Neurologic features of LS and LLS were also considered. Although the classic definition of LS included neurodevelopmental regression, it is now well known that not all children with LS and LLS have normal early development followed by a regression; rather, some can have developmental delay with no period of typical

development.¹⁰ Furthermore, LS and LLS can present in adulthood with neuropsychiatric manifestations,¹¹ which were therefore added to the definition.

Lastly, the biochemical evidence classically associated with LS and LLS was reviewed. Elevated lactate, either in plasma/blood, in CSF, or as a lactate peak on brain MRS, was classically considered as part of the LS definition. However, it is well known that lactate can be normal in blood and CSF, even in those with a confirmed molecular etiology of LS, LLS, or other PMD. Therefore, this criterion was expanded to capture diverse biochemical consequences of mitochondrial dysfunction known to underlie LS and LLS. Decreased OXPHOS enzyme activities can be considered as part of the LSS phenotype when assessed in muscle, liver, or fibroblast cell cultures with activity $< 30\%$ of control mean values.¹² Decreased PDC enzyme activity measured in fibroblasts can also be considered when activity is >2 SD below the mean. Morphologic abnormalities related to defective mitochondrial fission or fusion can also be considered functional evidence of LSS. Several genes associated with lipoic acid disorders were associated with features of LSS but lacked the biochemical evidence outlined above; in these cases, elevated glycine together with biallelic pathogenic variants in a gene needed for lipoic acid biosynthesis was also considered to represent mitochondrial dysfunction. Lastly, diminished mitochondrial respiratory capacity as measured in cells or tissues by polarography or microscale oxygraphy was also included. As such, LSS is now defined as the collection of individually rare genetic diseases characterized by either typical neuropathologic findings of LS or, in the absence of neuropathology, the combination of characteristic neuroimaging findings and neurodevelopmental delay, regression, or psychiatric symptoms, further supported by evidence of mitochondrial dysfunction.

Scoring recommendations were made to the ClinGen Gene Curation SOP to be relevant for LSS curation for both nuclear and mitochondrial genes, including genetic and experimental evidence categories.

Genetic-Level Evidence Scoring Recommendations. Further guidance for applying genetic-level, or case-level, scoring was provided, as related to LSS, and initially outlined in ClinGen Gene Curation SOP V7 for nuclear genes and SOP V8 for mitochondrial genes (Fig 2). The unique features of the mitochondrial genome were carefully considered, as SOP V8 does not provide guidance for mtDNA variant consideration. Segregation evidence, as stand-alone evidence, was removed for mitochondrial gene curation, as logarithm of the odds scores cannot be calculated for the mitochondrial genome.

TABLE 2. LSS Definition Used to Guide Case-Level Evidence Scoring for Gene Curations

Stand-alone Evidence	Combined Evidence	
Confirmed neuropathological diagnosis of Leigh syndrome	OR Neuroimaging	Bilateral, typically symmetric, T2-weighted hyperintensities on MRI or hypodensities on CT scan in: <ul style="list-style-type: none"> • Brainstem and/or • Basal ganglia
		With or without bilateral, T2 hyperintensity on MRI or hypodensity on CT scan in: <ul style="list-style-type: none"> • Thalamus • Cerebellum • Subcortical white matter • Spinal cord
		AND at least ONE of the following
	Neurologic symptoms	<ul style="list-style-type: none"> • Developmental regression • Developmental delay • Psychiatric symptoms
	Further supported by at least ONE of the following	
Biochemical and/or mitochondrial abnormality	<ul style="list-style-type: none"> • Elevated lactate in plasma and/or CSF • MRS lactate peak (in absence of acute seizures) • OXPHOS enzyme activity deficiency (<30%) in affected tissue (muscle, liver, fibroblasts) • PDC deficiency (in fibroblasts, >2 SD below mean) • Mitochondrial fission/fusion defect • Elevated glycine levels (if gene is associated with a lipoic acid disorder) • Diminished respiratory activity measured by microscale oxygraphy (eg, Oroboros or Seahorse assays) 	

Note: As a classical neuropathological diagnosis, neuropathological findings consistent with LSS alone meet criteria to establish the diagnosis. Consideration of combined criteria is recommended for living individuals.
Abbreviations: CSF = cerebrospinal fluid; CT = computed tomography; LSS = Leigh syndrome spectrum; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; OXPHOS = oxidative phosphorylation; PDC = pyruvate dehydrogenase complex; SD = standard deviation.

Experimental Evidence Scoring Recommendations. Experimental evidence scoring was carefully considered for how mitochondrial dysfunction could be considered under each category.

Evidence Category: Function. For biochemical function, evidence that gene products share a biochemical relationship or function with another gene associated with LSS was specified to group genes encoding the following categories: (1) OXPHOS subunits and assembly factors, (2) cofactor biosynthesis, (3) mtDNA maintenance, (4) mitochondrial translation, (5) mitochondrial dynamics, and (6) mitochondrial import (see Fig 2).¹³ Protein interaction evidence, or consideration of evidence of gene products that interact with

other genes associated with LSS, was specified to include genes that encode OXPHOS complex subunits and subunits of other enzymes (eg, PDC). Gene expression evidence was also considered, with emphasis on brain expression and/or disrupted expression. A baseline score was given for genes demonstrating protein expression in the brain, even if the expression pattern was largely ubiquitous across tissues, and additional points were awarded for evidence demonstrating expression in specific areas of the brain known to be impacted in LSS, such as the brainstem, basal ganglia, thalamus, cerebellum, and spinal cord.

Evidence Category: Functional Alteration. Functional alteration scoring guidance was provided for patient and

A. Genetic level evidence			B. Experimental level evidence			
AUTOSOMAL DOMINANT, X-LINKED			FUNCTION			
Variant type	Variant type (specified)	Points	Definition	Evidence type	Scoring criteria	Points
Variant is de novo	No further specification	3		Biochemical function	1 gene product	0.5
Predicted or proven null variant	Nonsense, exonic deletion, frameshift, early stop codon OR western blot showing no expression	3	Gene product shares biochemical relationship/function with another gene product associated with LS	<ul style="list-style-type: none"> Respiratory chain assembly Cofactor biosynthesis Mitochondrial maintenance Mitochondrial translation Mitochondrial dynamics Mitochondrial import 	2-5 gene products	1
	Any of the above and case has same variant as one already counted	1			6-9 gene products	1.5
Other variant type (not predicted or proven null) with some evidence of gene impact	Variant is a founder/common variant	3			10+ gene products	2.0
	Case has same variant as one already counted	1	The encoded protein interacts with another gene product associated with LS	<ul style="list-style-type: none"> Protein interaction ETC complex subunits Enzymatic subunits 	1 gene product	0.5
AUTOSOMAL RECESSIVE						
Variant type	Variant type (specified)	Points	Definition	Evidence type	Scoring criteria	Points
Two variants in trans and (1) at least one predicted or proven null; OR (2) at least one de novo	Nonsense, exonic deletion, frameshift, early stop codon OR Missense + western blot showing no expression	2			1 cell culture model	1
	Any of the above and variant is a founder/common variant	3			32 cell culture models	1.5
Two variants in trans not predicted or proven null with some evidence of gene impact	Any of the above and case has same variants as one already counted	1			Performed in neuronal cell type	+0.5
	Missense variant and variant is a founder/common variant and/or extensive functional validation of variant effect	1.5	Cells/isolated mitochondria, in which the function of the gene has been disrupted, display mitochondrial dysfunction as characterised by (including but not limited to):		Performed in cells obtained from patient meeting all criteria for LSS (neuropathology or neuroimaging, neurologic, and biochemical)	2
in trans	Missense variant with crystallography or additional evidence of gene impact (in methionine started codon)	0.5	<ul style="list-style-type: none"> OXPHOS dysfunction Decreased enzyme activity Mitochondrial, mitochondrial DNA depletion Membrane dysfunction 			
	Missense variant AND in silico analysis evidence	0.25			1 cell culture model	0.5
MITOCHONDRIAL						
Criterion	Guidance	Range	Phenotype	Example	Points	Range
De novo	> 1 tissue in mother should be assessed	0 – 0.5				
Segregation in family members	Level of heteroplasmy and disease manifestations must correlate	0 – 0.5	Neuropathological evidence	Symmetrical, bilateral lesions in basal ganglia, brainstem; spongiform lesions, neuronal loss, gliosis; proliferation of small blood vessels	3	0-4
Predicted to be deleterious	Utilize the following predictors: mRNA: APOGEE tRNA: MitotIP + HintVAR in agreement rRNA: Cannot apply, no user-friendly predictor available	0.25		Embryonic lethal – 0.5		
	Multiple respiratory chain enzyme complex deficiency (tRNA) Corresponding respiratory chain enzyme complex deficiency (mRNA)	0.5	MRI findings	Symmetrical, bilateral lesions in basal ganglia and/or brainstem, other brain areas as outlined in definition	2	0-3
Cybrids	Several factors must be considered: Biochemical deficiency must be seen in patient cell line (scored separately) and transferred to cybrids (>2 SD or <20% of control, 0-1 points) Heteroplasmy of cybrids is ≥60% (0-5 points) Correlation between heteroplasmy level and biochemical deficiency (0.5 point)	0 – 1.5	Biochemical or mitochondrial dysfunction	OXPHOS; Blue Native gel, mitochondrial depletion, elevated lactate, abnormal mitochondrial morphology	0.5	0-1
	Correlation between mutant load and OXPHOS activity	0 – 1.5				
Single fiber study	Example including but not limited to: Reduced mt-rRNA level on northern blot	0.5	Neurocognitive/developmental differences	Regression, ataxia, hypotonia, growth retardation	0.5	0-1
Other functional evidence	Case can be awarded maximum points	1.5				
Unrelated case with same variant that reached max score for cybrid or single fiber study	Confirmed status in Mitomap and/or classified as pathogenic or likely pathogenic by mtDNA Expert Panel in ClinVar	0.5				

FIGURE 2: (A) Nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) gene genetic evidence summary matrix for LSS curation. This has been amended from Figure 3 in ClinGen Gene Curation Standard Operating Procedure (SOP) version 7 (V7; for nDNA) and SOP V8 (for mtDNA). If score is 0.75, round up to 1. If score is 1.25, round up to 1.5. For mtDNA genes, to score any case, no contradictory evidence can exist such as high allele frequency, homoplasmic occurrences in mitochondrial disease-specific databases, or lack of segregation. Mitochondrial guidance is relevant for any variant type. (B) Experimental evidence summary matrix for Leigh syndrome spectrum (LSS) curation, showing functional alteration, and models – nonhuman model organism. No further scoring guidance for rescue experiments was provided, as there had been no report of rescue in a human with LSS, and other forms of rescue in models were limited. This has been amended from Figure 9 in ClinGen Gene Curation SOP V7. LS = Leigh syndrome; MRI = magnetic resonance imaging; mRNA = messenger RNA; tRNA = transfer RNA; rRNA = ribosomal RNA; OXPHOS = oxidative phosphorylation; SD = standard deviation; ETC = electron transport chain.

nonpatient cell lines that demonstrated various forms of mitochondrial dysfunction, as would be expected to occur in LSS (see Fig 2). A phenotype observed in cells that was consistent with the human phenotype in question, in cells where the gene function has been disrupted, was considered as evidence for gene–phenotype association. Careful consideration was given to features in cell lines that could be consistent with LSS. Here, the mechanisms by which mitochondrial dysfunction can be characterized in cells was specified and included studies showing decreased OXPHOS capacity or enzyme activity, mtDNA depletion, and mitochondrial membrane dysfunction. Increased scoring was suggested for evidence of more than one cell line showing alterations, studies performed in a neuronal cell line, or for cells isolated from patients with a confirmed diagnosis of LSS.

Evidence Category: Model. Nonhuman models were carefully considered for the presence of features that correlate with phenotypes seen in humans with LSS based on the refined LSS definition (see Fig 2). Neuropathologic and radiologic recapitulations were weighted higher as compared to more generalized neurologic and neurodevelopmental phenotypes and also as compared to evidence of biochemical and mitochondrial dysfunction. For models that were embryonically lethal, a minimum of 0.5 points was scored, given that LSS is a severe phenotype associated with high levels of early mortality. Cell culture models were not considered, as it is difficult to model neuropathology, radiologic findings, or neurologic alterations in vitro. Biochemical and mitochondrial dysfunction evidence in cell culture models was considered under the functional alteration category (see above).

Evidence Category: Rescue. No further scoring guidance for rescue experiments was provided from the existing ClinGen Gene Curation SOP, as there had been no report of rescue in a human with LSS, and other forms of rescue in models were limited. Complementation assays demonstrating biochemical and/or mitochondrial dysfunction rescue were either awarded baseline points under this category or used to support case-level evidence (eg, yeast complementation assays to support variant pathogenicity).

LSS Gene Curation Results. A total of 114 GDRs were carefully reviewed, curated, debated, and had a clinical validity classification ultimately agreed on by consensus voting of expert panel members of the Mito GCEP (Figs 3 and 4A). Thirty-one of the 114 GDRs curated were classified as having a definitive GDR with LSS (27%, including 24 nuclear genes and 7 mtDNA genes); none was classified as strong; 38 were classified as moderate (33%, including 37 nuclear

genes and 1 mtDNA gene); 43 were classified as limited (38%, including 36 nuclear genes and 7 mtDNA genes; 30 of these had only one published case meeting LSS criteria); and 2 were classified as disputed (2%, including one nuclear gene, *NUP62*, and one mtDNA gene, *MT-TL2*). Although *BCSIL* (total score = 6.5), *COQ9* (total score = 8), *MT-CO3* (total score = 7), *MT-ND2* (total score = 6.5), *SLC25A46* (total score = 7), and *TIMMDC1* (total score = 7) score >6 points and thus could be considered for a "moderate" classification, only one case with LSS was reported for each of these genes, thus leaving these at a "limited" classification. Six genes had final GDR scores between 6.1 and 6.9, leaving the final classification to be decided by the expert panel. Two genes (*BCSIL* and *MT-ND2*) scored 6.5 but only had one case with LSS reported; therefore, these were classified as limited. The expert panel decided to classify the other 4 GDRs (*NDUFA9*, *NDUFB3*, *NDUFC2*, and *AIFM1*) as moderate, given the abundance of evidence reported. Two genes (*NDUFS2* and *NDUFA1*) scored between 11.1 and 11.9, leaving the final classification of moderate or definitive (strong would not apply, as >3 years had passed since the initial report) to be decided upon by the expert panel. *NDUFS2* was deemed to have a definitive GDR for LSS and a moderate GDR was agreed for *NDUFA1*. *DLD* scored at the upper range of moderate (score = 11) but was classified as "definitive," as the experts knew of numerous other cases not reported in the medical literature that would have otherwise increased the scoring to definitive. Most genes curated are associated with autosomal recessive inheritance (n = 90), followed by maternal (n = 16), autosomal dominant (n = 5), and X-linked (n = 3; see Fig 4B). Average genetic- and experimental-level evidence scores for each strength classification are listed in Figure 4C. Gene defects were reported to be associated with LSS beginning in 1992 (Fig 4D). Twenty-four gene defects were associated with LSS for the first time during this project period (2017 onward). Trends among genes sharing a common biochemical function were assessed (Fig 4F).

Evidence Category: Model. Sixty-three of 114 gene curations had at least one experimental model scored (see Fig 4E, Table S1). Models considered for scoring included bacteria, yeast, fungus, cellular slime mold, nematode, drosophila, zebrafish, mouse, hamster, dog, pig, and cow. Three genes had 3 experimental models scored: *AIFM1* (mouse, nematode, drosophila), *SLC25A46* (zebrafish, mouse, cow), and *SURF1* (drosophila, mouse, pig). Experimental models were scored for 47 genes for mouse, 13 for drosophila, 6 for zebrafish, 5 for yeast, 3 for nematode, and 1 each for cellular slime mold, fungus, hamster, dog, cow, and pig.

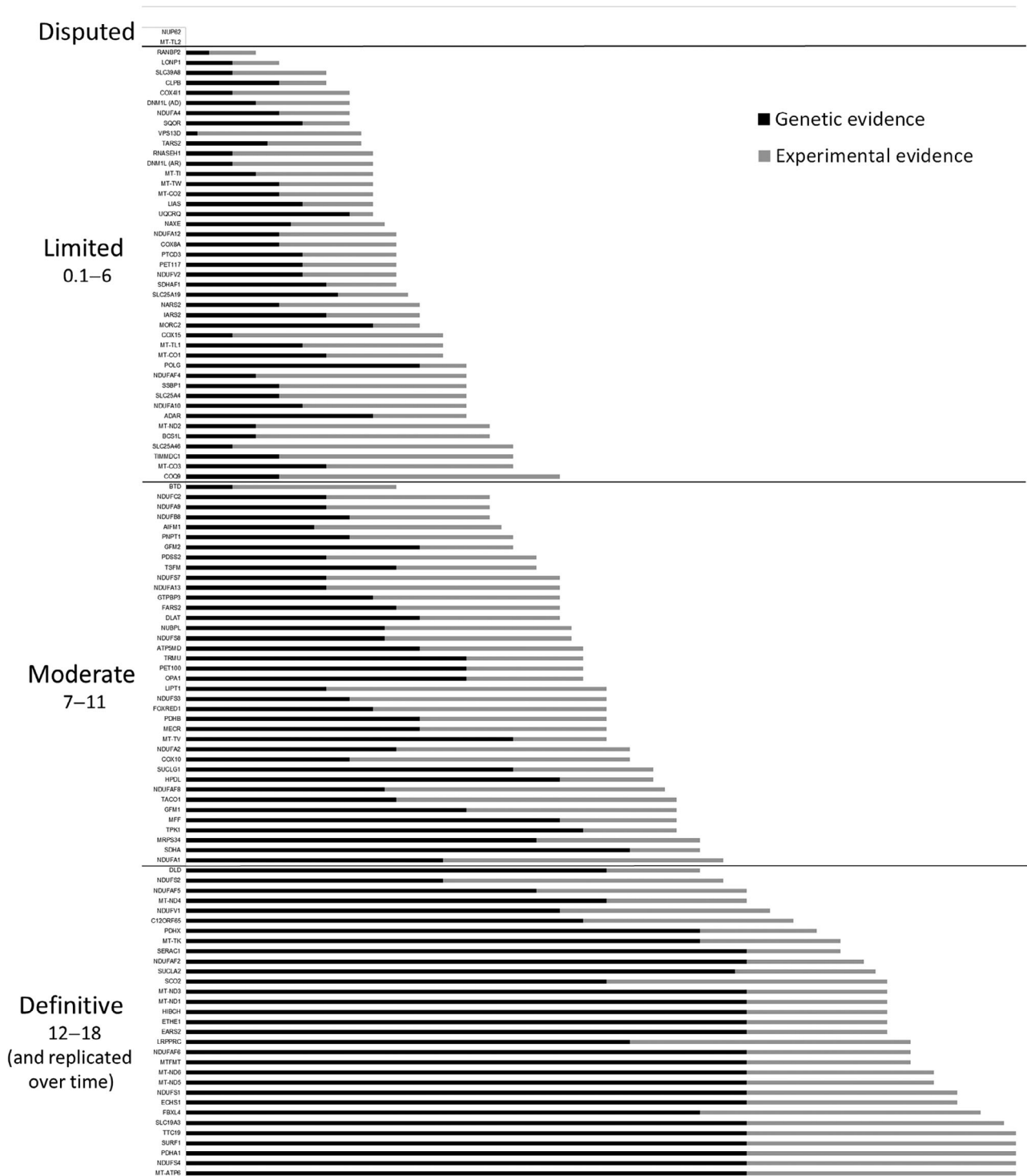


FIGURE 3: Scores for each curated gene–disease relationship with Leigh syndrome spectrum by the Mitochondrial Disease Gene Curation Expert Panel. The default scoring range for a definitive classification is 12–18, moderate is 7–11, and limited is 0.1–6.

Discussion

We report here the work of the ClinGen Mito GCEP, a global collaboration of PMD experts assembled to review systematically and agree on the consensus expert panel definition of, and strength of association for, 113 genes with LSS.

The first step was to gain expert consensus on the LS phenotype, which was expanded to encompass LLS in an overarching entity now referred to as LSS. The gene curation process aimed not to revisit and amend diagnoses of historic cases, but rather to streamline how this disease

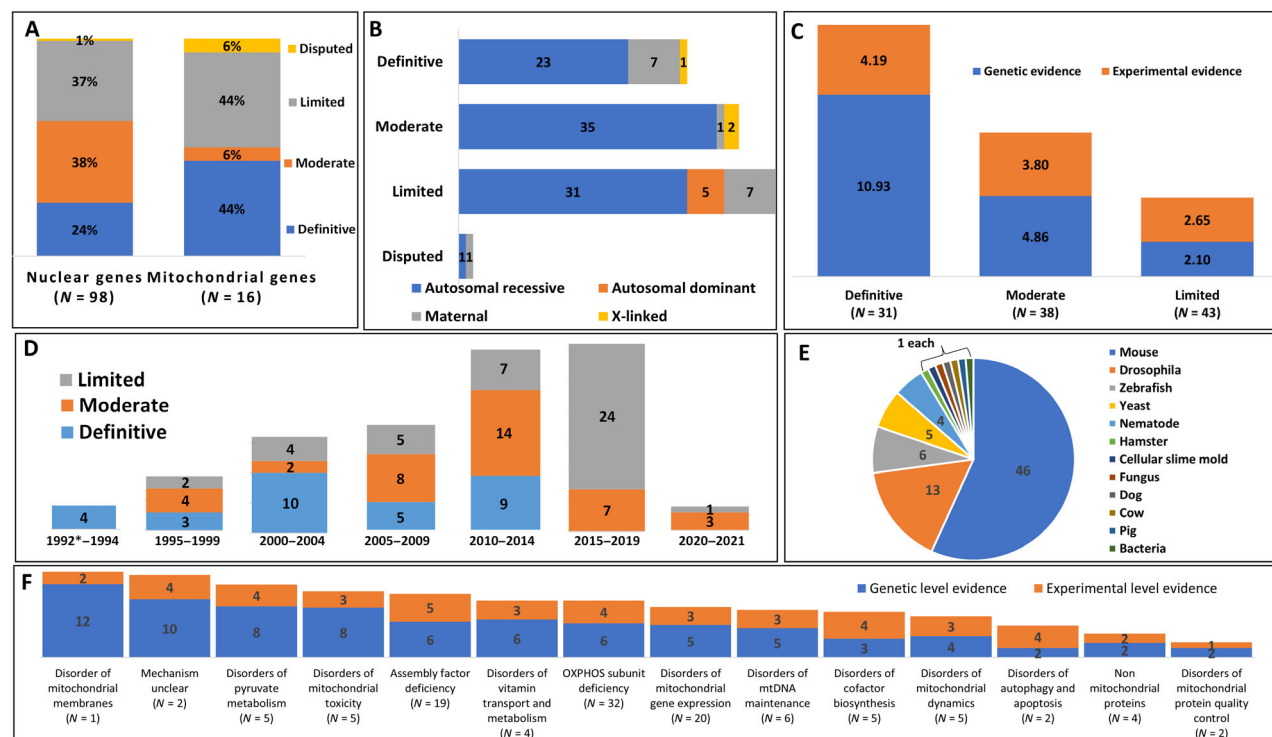


FIGURE 4: Leigh syndrome spectrum (LSS) classification overview by associated genome, association strength, score, time since discovery, gene class, and experimental models. (A) Classifications of nuclear genes ($n = 98$) and mitochondrial DNA (mtDNA) genes ($n = 16$) curated for association with LSS. (B) Number of genes reaching definitive, moderate, limited, and disputed classifications for LSS by inheritance pattern. (C) Average scores for each clinical validity classification for LSS. The default scoring range for a definitive classification is 12–18, moderate is 7–11, and limited is 0.1–6. (D) Number of genes first reported to be associated with LSS by year. Genes with a “disputed” classification are excluded. *DNM1L* is included twice (once for first association with autosomal dominant disease and once for first association with autosomal recessive disease). *The first gene-disease association was reported in 1992. (E) Numbers of experimental models curated. (F) Average curation scores for genes associated with LSS by gene class. OXPPOS = oxidative phosphorylation.

entity is considered moving forward based on current genomic, clinical, and biochemical understanding. It is hoped this revised definition will facilitate inclusive clinical trials aimed at treating LSS by providing a curated minimum gene set of 111 genes now associated with LSS. Importantly, we recognize that a distinction exists between reviewing and comparing reported cases in the published literature to set criteria as was completed here for LSS by the Mito GCEP, and the prospective clinical challenge of diagnosing individual cases with features concerning for LSS as new variants and genes are discovered. Refining the classical definitions reflects the expanding landscape of PMD and LSS pathogenic mechanisms, as it is now known that PMD and LSS may be caused by a wide variety of insults to mitochondrial function. Additionally, this updated definition now captures the increasingly recognized heterogeneous nature of LSS, including the neurologic presentation and asymmetric brain imaging changes in some cases.¹⁴

Scoring recommendations were made to the ClinGen Gene Curation SOP to account for LSS clinical

presentations, hallmark findings of mitochondrial dysfunction, and the unique features of the mitochondrial genome. Although this guidance was developed for LSS, it can be applicable for other mitochondrial and possibly other metabolic conditions. The mtDNA genes required additional guidance that was based largely on the published mtDNA variant American College of Medical Genetics (ACMG) and Association of Molecular Pathology (AMP) specifications.¹⁵ Guidance was also provided for review of experimental evidence relevant for curation of gene relationships with LSS. We based experimental model curation guidance on the *Ndufs4*^{-/-} mouse, a model that the Mito GCEP considered a gold standard knockout mouse model of PMD, recapitulating major findings of LSS.^{16,17}

For the neuroradiologic aspects of the LSS definition, the Mito GCEP concluded after extensive debate that isolated thalamic lesions could not be considered diagnostic of LSS, because they might simply reflect hypoxic ischemic encephalopathy. Two neuroradiologic terms with overlap with LSS are striatal necrosis and

necrotizing encephalopathy. Some cases under consideration had classic features of these entities, which would be consistent with LSS, but had no biochemical testing to fulfill the other necessary criteria of the LSS definition. For example, *NUP62* defects are associated with infantile bilateral striatal necrosis; pathogenic variants were reported in a large kindred with brain imaging resembling LSS, but no biochemical evidence was documented.¹⁸ *NUP62* encodes a nucleoporin, a component of the nuclear pore complex. However, we cannot exclude that a GDR with LSS could have been established had biochemical testing been performed. *RANBP2* defects cause acute necrotizing encephalopathy.¹⁹ Some cases in the literature had MRI findings consistent with LSS, but missing mitochondrial assessments or biochemical tests in many cases meant they failed to meet Mito GCEP criteria for association with LSS, likely contributing to the "limited" classification for this GDR.

Pathogenic variants in several genes associated with LSS were also associated with clinical syndromes other than LSS; these phenotypes were not curated as part of this initiative. They may be curated by other ClinGen Gene Curation Expert Panels in the future. When genes were associated with clinically heterogeneous mitochondrial disease, the relationship specifically with LSS tended to be classified as moderate. Several of these genes were involved in mitochondrial translation, including *TRMU*, where at least 7 reported cases with lactic acidosis and liver disease could not be scored for LSS, as they lacked characteristic brain lesions, and *PNPT1*, where 12 reported cases not meeting LSS criteria had other phenotypes, including isolated hearing loss or choroidoretinal disease.

During this curation, several barriers were faced, as summarized in Table 3. The biggest barrier was that many genetic causes of LSS are rare and/or recently discovered, and for several genes, there was a paucity of published cases available for expert panel curation. Other challenges included cases that met the criteria for LSS but were not described as LSS in the publication, which complicated literature review, curation, and discussion of these cases; patients dying of comorbidities before developing clinical features of LSS; increased utilization of newborn screening leading to cases being detected early and treated before developing LSS; inability to score some cases due to only minimal details being provided in the literature of the results of clinical and biochemical assessments (this was especially true for publications reporting large cohorts of patients who had received a genetic diagnosis of PMD through exome sequencing); and finding high allele frequencies in control databases such as gnomAD,³⁶ raising questions regarding whether these variants were truly pathogenic, hypomorphic, or even benign. Lastly,

although LS was historically a neuropathologic diagnosis, only 54 cases reviewed for curation across all 113 genes had neuropathology findings reported. Collectively, Mito GCEP review identified 24 cases with neuropathologic confirmation of LS that were associated with defects in 19 different genes. Other cases had neuropathological findings reported but not in enough detail to be diagnostic of LSS, and a third group had neuropathologic findings described that were not consistent with LSS (Table 4). Furthermore, some recurrent variants were observed in specific ethnic groups (Table S2).

Experimental models were scored as experimental evidence for 64 genes associated with LSS. Model organisms ranged from bacteria to canine, porcine, and bovine models. Most were genetically engineered, but some were naturally occurring, such as *SLC19A3* variants identified in Alaskan Husky encephalopathy with neuropathologic changes that were consistent with LSS,⁷⁸ and *SLC25A46* variants in French Rouge des Prés cattle that caused poor balance and neuropathologic changes reminiscent of LSS (turning calves syndrome).⁷⁹ The gold standard experimental animal used to guide scoring was the *Ndufs4* knockout mouse model, which has brain MRI lesions consistent with human LSS.⁸⁰ Twelve knockout mouse models were reported to be embryonically lethal and were more prevalent in the genes scored as moderate or limited. As embryonic lethality remains a significant hurdle in the creation of whole-body knockout mice, the complementary approach of tissue-specific knockout mice, like neuronal-specific knockouts, may be considered as a more feasible option for creating future LSS models. For example, the *MECR* Purkinje cell knockout mouse presented with a biochemical, neurodevelopmental, and neuropathologic phenotype.⁸¹ Consideration of experimental models as they relate to LSS is especially important, as efficacy of novel therapies can be tested in these models as they arise.

This global PMD expert consensus work holds important implications for clinicians, diagnostic laboratories, and patients. Determining the pathogenicity of a variant requires establishing the strength of the relationship between the gene and the disease.⁸² Evaluating the clinical validity for the GDR through gene curation is considered an essential first step for variant classification and clinical reporting.⁸³ One of the biggest areas of impact of gene curation in rare disease is in the confident reporting of variants of uncertain significance in genes of uncertain significance, such as in those having a GDR classified as limited. Careful consideration of GDR is also valuable in triaging variants to help with the identification of candidate variants, especially in exome and genome screening, thereby preventing variants with the potential of being

TABLE 3. Challenges and Limitations to Genetic-Level Data Curation

Challenge/ Limitation	Explanation	Example	Reference (s)
Paucity of published cases	Many genetic causes of LSS are rare and/or recently discovered	30/43 genes reaching limited classification only had one case reported Mito GCEP members knew of additional cases not reported in the medical literature	- -
Exclusion of potentially scorable cases	Phenotype modified by comorbidities or treatments	Cases died of comorbidities before developing LSS <ul style="list-style-type: none"> • <i>GFM1</i>, fatal infantile hepatopathy • <i>NDUFAF5</i>, early lethality before MRI could be performed 	Coenen et al, 2004 ²⁰ Smits et al, 2011 ²¹ Sugiana et al, 2008 ²²
	Cases reported in cohorts with minimal phenotypic details provided	Increased utilization of NBS led to treatment before developing LSS <ul style="list-style-type: none"> • <i>BTB</i> associated with biotinidase deficiency, treated from birth following NBS in many countries, affected individuals do not develop LSS Historical cases diagnosed biochemically did not routinely undergo genetic testing to confirm the genetic etiology <ul style="list-style-type: none"> • For example, biotinidase deficiency 	Mitchell et al, 1986 ²³ Baumgartner et al, 1989 ²⁴
High allele frequencies in healthy population databases	Uncertainty regarding pathogenic nature of variants and/or phasing	Missing phenotypic information <ul style="list-style-type: none"> • <i>TSMF</i>, no brain imaging data for 6 cases • <i>TPK1</i>, 4 cases had clinical features and imaging changes suggestive of LSS but could not be scored, as no lactate levels or other biochemical parameters were reported • <i>RANBP2</i>, no lactate or OXPHOS measurements in many cases 	Smeitink et al, 2006 ²⁵ Banka et al, 2014 ²⁶ Mahajan et al, 2017 ²⁷ Ortigoza-Escobar et al, 2017 ²⁸ Hu et al, 2020 ²⁹ Chow et al, 2020 ³⁰ Legati et al, 2016 ³¹ Kelly et al, 2019 ³² Husain et al, 2020 ³³
		Limited knowledge at time of report <ul style="list-style-type: none"> • <i>HPDL</i>, not yet associated with mitochondrial dysfunction at time of initial reports, therefore screening investigations typically performed in individuals with suspected mitochondrial disease were not performed 	
Variant phasing	Lack of parental testing	Hypomorphic alleles <ul style="list-style-type: none"> • <i>NDUFS2</i>, c.875 T > C (p.Met292Thr) is a founder variant, had a high allele frequency (gnomAD, 28/6136, 0.004563) and several homozygous occurrences in gnomAD (v2.1.2) • Functional validation demonstrated a deleterious effect of this variant • Mito GCEP agreed that this likely was a hypomorphic allele as well as a founder variant, could be scored as disease-causing in the compound heterozygous state 	Karczewski et al, 2020 ³⁴ Tuppen et al, 2010 ³⁵
Variant phasing	Lack of parental testing	Lack of parental testing limited case scoring, as SOP states that variants need to be confirmed in <i>trans</i>	-

Abbreviations: LSS = Leigh syndrome spectrum; Mito GCEP = Mitochondrial Disease Gene Curation Expert Panel; MRI = magnetic resonance imaging; NBS = newborn screening; OXPHOS = oxidative phosphorylation; SOP = Standard Operating Procedure.

research candidates from being excluded or overlooked, as well as preventing harm that may relate to returning variants to families in genes with no established relationship to disease. As clinical diagnostic laboratories utilize ClinGen Gene Curation Expert Panel work to inform panel development, establishing accurate GDRs can facilitate reliable interpretation of relevance for variants

identified on genomic sequencing tests. Although mitochondrial disease genetic etiologies are phenotypically heterogeneous, we have shown here a relationship between 111 single genes and LSS with varying levels of evidence, and disputed 2 genes as having a GDR with LSS. It is important to note that a limited classification for a specific gene with a given disease does not mean there is no

TABLE 4. Neuropathology Reported for Leigh Syndrome Spectrum Curated Genes

Gene	Leigh	Possible Leigh	Not Leigh ^a	Comments	References
<i>AIFM1</i>			✓	Occipital lesion, hepatopathy, Alpers	Morton et al, 2017 ³⁷
<i>ATP5MD</i>	✓ (2)				Barca et al, 2018 ³⁸
<i>CLPB</i>			✓	“Nonspecific lesions”	Capo-Chichi et al, 2015 ³⁹
<i>COQ9</i>			✓ (2)	Global ischemia in one case, calcification in GP and olivary dysplasia in the other	Smith et al, 2018 ⁴⁰
<i>DNM1L</i>	✓				Zaha et al, 2016 ⁴¹
<i>ECHS1</i>			✓	Poorly preserved brain on macroscopy with BG cavitation, microscopy statement	Haack et al, 2015 ⁴²
<i>FARS2</i>			✓	Alpers neuropathology	Elo et al, 2012 ⁴³
<i>FARS2</i>			✓	Alpers neuropathology	Walker et al, 2016 ⁴⁴
<i>GFM1</i>			✓	WM lesion, BG, hypoplastic CC, hepatopathy	Coenen et al, 2004 ²⁰
<i>GFM1</i>			✓	Bilateral porencephaly, microcephaly, dysgenesis of cingulate gyri, hepatopathy	Antonicka et al, 2006 ⁴⁵
<i>FM1</i>			✓	Polymicrogyria and hepatopathy	Ravn et al, 2015 ⁴⁶
<i>LRPPRC</i>		✓ (7)		Statement only: “lesions typical of Leighs”	Morin et al, 1993 ⁴⁷
<i>MRPS34</i>	✓				Lake et al, 2017 ⁴⁸
<i>MT-ATP6</i>	✓				Tatuch et al, 1992 ⁴⁹
<i>MT-ND3</i>		✓			McFarland et al, 2004 ⁵⁰
<i>MT-ND4</i>	✓				Hadzsiev et al, 2010 ⁵¹
<i>MT-ND5</i>	✓				Taylor et al, 2002 ⁵² Morris et al, 1996 ⁵³
<i>MT-ND5</i>	✓ (2)			Leigh plus MELAS	Ng et al, 2018 ⁵⁴
<i>MT-ND6</i>		✓		Statement only	Ugalde et al, 2003 ⁵⁵
<i>MT-ND6</i>		✓ (2)		Statement only	Naess et al, 2009 ⁵⁶
<i>MT-TI</i>	✓ (2)				Limongelli et al, 2004 ⁵⁷
<i>MT-TK</i>		✓		Statement only	Silvestri et al, 1993 ⁵⁸
<i>MT-TK</i>	✓				Sweeney et al, 1994 ⁵⁹
<i>MT-TK</i>				Statement only	Santorelli et al, 1998 ⁶⁰
<i>MT-TK</i>	✓				Pronicki et al, 2007 ⁶¹
<i>MT-TL1</i>	✓				Koga et al, 2000 ⁶²
<i>MT-TW</i>				Statement only	Santorelli et al, 1997 ⁶³
<i>NARS2</i>	✓			Leigh plus MELAS	Simon et al, 2015 ⁶⁴
<i>NAXE</i>		✓ (3)			Kremer et al, 2016 ⁶⁵

TABLE 4. Continued

Gene	Leigh	Possible Leigh	Not Leigh ^a	Comments	References
<i>NDUFA10</i>	✓			Limited description, no pictures	Hoefs et al, 2011 ⁶⁶
<i>NDUFAF2</i>			✓	Resembles vanishing white matter disease	Ogilvie et al, 2005 ⁶⁷
<i>NDUFAF2</i>	✓				Herzer et al, 2010 ⁶⁸
<i>NDUFAF2</i>		✓		Statement only	Calvo et al, 2010 ⁶⁹
<i>NDUFS8</i>	✓				Loeffen et al, 1998 ⁷⁰
<i>NDUFV1</i>		✓		Minimal description	Benit et al, 2001 ⁷¹
<i>PDHB</i>	✓				Quintana et al, 2009 ⁷²
<i>PDHB</i>			✓	Developmental abnormalities, PMG, pachygyria, dentato-olivary dysplasia	Pirot et al, 2016 ⁷³
<i>PNPT</i>	✓ (2)				Matilainen et al, 2017 ⁷⁴
<i>SCO2</i>	✓ (2)				Papadopoulou et al, 1999 ⁷⁵
<i>SDHAF1</i>	✓				Brockmann et al, 2002 ⁷⁶
<i>TRMU</i>		✓		Brief description, hepatopathy	Sala-Coromina et al, 2021 ⁷⁷

Note. Numbers in parentheses refer to number of cases with neuropathology.
Abbreviations: BG = basal ganglia; CC = corpus callosum; GP = globus pallidus; MELAS = mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes; PMG = polymicrogyria; WM = white matter.
^aCase not scored because of incomplete description or other neuropathology, for example, Alpers.

disease association. In the case of LSS, this was frequently due to a lack of reported cases, reflecting rare or newly identified etiologies. New evidence is likely to emerge over time, and recuration standards have been proposed. These include how much time should pass before a GDR is revisited and depend on whether the classification is limited (3 years from GCEP classification approval date), moderate (2 years from GCEP classification approval date), strong (3 years from discovery date), or definitive (no set requirement; https://clinicalgenome.org/site/assets/files/2164/clingen_standard_gene-disease_validity_recuration_procedures_v1.pdf). Furthermore, there will continue to be discovery of novel genes associated with LSS. The GDRs for these genes with LSS could be evaluated; however, the scope of this project can also be expanded to a broader PMD phenotype, capturing the full spectrum of features associated with a gene but also highlighting LSS as an associated phenotype.

Some LSS genes curated as part of this work cause treatable conditions when pathogenic variants are present, for example, biotin and/or thiamine for LSS

associated with pathogenic variants in *BTD*, *SLC19A3*, and *TPK1*, coenzyme Q₁₀ supplementation for disorders affecting its biosynthesis, and ketogenic diet for gene defects causing PDC deficiency.⁸⁴ Expediting clinical diagnosis for individual cases is critical for natural history study and clinical drug trial eligibility and enrollment, particularly because emerging clinical drug trials for LSS consider genetic diagnosis in inclusion and exclusion criteria.

In conclusion, it is our hope that the extensive curations for LSS reported here by the Mito GCEP will facilitate improved diagnostic accuracy and future therapeutic development for the heterogeneous group of LSS disorders.

Acknowledgments

We thank the ClinGen Gene Curation Working Group and Clinical Domain Working Group Oversight Committee for their detailed review, suggestions, and approval of this Mito GCEP. We are grateful to the United

Mitochondrial Disease Foundation for their organizational and administrative support, their partnership in MSeqDR Consortium activities, and funding of MSeqDR. This work was supported by NIH grant U24-HD093483 (to M.J.F. and X.G.), as well as Great Ormond Street Hospital Children's Charity, the Lily Foundation, and the National Institute of Health Research (NIHR) Great Ormond Street Hospital Biomedical Research Centre (to S.R.). The views expressed are those of the authors and not necessarily those of the funding agencies, including the NIH, National Health Service, and NIHR. The research conducted at the Murdoch Children's Research Institute was supported by the Victorian government's operational infrastructure support program. The Chair in Genomic Medicine awarded to J.C. is generously supported by the Royal Children's Hospital Foundation. We are grateful to the Crane, Perkins, and Miller families for their generous financial support. We thank Dr. James Nurse for his contribution to the visual abstract.

Author Contributions

M.J.F., X.G., and D.K. contributed to the conception and design of the study. E.M.M., K.K., J.P.T., A.J.C., L.S., B.H., Z.Z.-C., and S.R. contributed to the acquisition and analysis of data. E.M.M., K.K., J.P.T., A.J.C., B.H., X.G., M.J.F., Z.Z.-C., and S.R. contributed to drafting the text or preparing the figures. All authors wrote, critically reviewed, and revised the manuscript.

Potential Conflicts of Interest

Nothing to report. Detailed forms are on file with the journal.

Data Availability Statement

Gene curation scoring and outcomes are available at <https://clinicalgenome.org/affiliation/40027/>.

References

1. Leigh D. Subacute necrotizing encephalomyelopathy in an infant. *J Neurol Neurosurg Psychiatry* 1951;14:216–221.
2. Rahman S, Blok RB, Dahl HH, et al. Leigh syndrome: clinical features and biochemical and DNA abnormalities. *Ann Neurol* 1996;39:343–351.
3. Rehm HL, Berg JS, Brooks LD, et al. ClinGen—the clinical genome resource. *N Engl J Med* 2015;372:2235–2242.
4. Strande NT, Riggs ER, Buchanan AH, et al. Evaluating the clinical validity of gene-disease associations: an evidence-based framework developed by the clinical genome resource. *Am J Hum Genet* 2017;100:895–906.
5. Falk MJ, Shen L, Gonzalez M, et al. Mitochondrial disease sequence data resource (MSeqDR): a global grass-roots consortium to facilitate deposition, curation, annotation, and integrated analysis of genomic data for the mitochondrial disease clinical and research communities. *Mol Genet Metab* 2015;114:388–396.
6. Shen L, Diroma MA, Gonzalez M, et al. MSeqDR: a centralized knowledge repository and bioinformatics web resource to facilitate genomic investigations in mitochondrial disease. *Hum Mutat* 2016;37:540–548.
7. Falk MJ, Shen L, Gai X. From case studies to community knowledge base: MSeqDR provides a platform for the curation and genomic analysis of mitochondrial diseases. *Mol Case Stud* 2016;2:a001065.
8. Thaxton C, Goldstein J, DiStefano M, et al. Lumping versus splitting: how to approach defining a disease to enable accurate genomic curation. *Cell Genom* 2022;2:100131.
9. Rahman J, Noronha A, Thiele I, Rahman S. Leigh map: a novel computational diagnostic resource for mitochondrial disease. *Ann Neurol* 2017;81:9–16.
10. Tinker RJ, Falk MJ, Goldstein A, et al. Early developmental delay in Leigh syndrome spectrum disorders is associated with poor clinical prognosis. *Mol Genet Metab* 2022;135:342–349.
11. Nogueira C, Barros J, Sá MJ, et al. Novel TTC19 mutation in a family with severe psychiatric manifestations and complex III deficiency. *Neurogenetics* 2013;14:153–160.
12. Bemier FP, Boneh A, Dennett X, et al. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* 2002;59:1406–1411.
13. Ferreira CR, Rahman S, Keller M, et al. An international classification of inherited metabolic disorders (ICIMD). *J Inher Metab Dis* 2021;44:164–177.
14. Alves C, Teixeira SR, Martin-Saavedra JS, et al. Pediatric leigh syndrome: neuroimaging features and genetic correlations. *Ann Neurol* 2020;88:218–232.
15. McCormick EM, Lott MT, Dulik MC, et al. Specifications of the ACMG/AMP standards and guidelines for mitochondrial DNA variant interpretation. *Hum Mutat* 2020;41:2028–2057.
16. Kruse SE, Watt WC, Marcinek DJ, et al. Mice with mitochondrial complex I deficiency develop a fatal encephalomyopathy. *Cell Metab* 2008;7:312–320.
17. Quintana A, Zanella S, Koch H, et al. Fatal breathing dysfunction in a mouse model of Leigh syndrome. *J Clin Invest* 2012;122:2359–2368.
18. Basel-Vanagaite L, Muncher L, Straussberg R, et al. Mutated nup62 causes autosomal recessive infantile bilateral striatal necrosis. *Ann Neurol* 2006;60:214–222.
19. Neilson DE, Adams MD, Orr CM, et al. Infection-triggered familial or recurrent cases of acute necrotizing encephalopathy caused by mutations in a component of the nuclear pore, RANBP2. *Am J Hum Genet* 2009;84:44–51.
20. Coenen MJ, Antonicka H, Ugalde C, et al. Mutant mitochondrial elongation factor G1 and combined oxidative phosphorylation deficiency. *N Engl J Med*. 2004;351:2080–2086.
21. Smits P, Antonicka H, van Hasselt PM, et al. Mutation in subdomain G' of mitochondrial elongation factor G1 is associated with combined OXPHOS deficiency in fibroblasts but not in muscle. *Eur J Hum Genet* 2011;19:275–279.
22. Sugiana C, Pagliarini DJ, McKenzie M, et al. Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease. *Am J Hum Genet* 2008;83:468–478.
23. Mitchell G, Ogier H, Munnich A, et al. Neurological deterioration and lactic acidemia in biotinidase deficiency. A treatable condition mimicking Leigh's disease. *Neuropediatrics* 1986;17:129–131.
24. Baumgartner ER, Suomalainen TM, Wick H, et al. Biotinidase deficiency: a cause of subacute necrotizing encephalomyelopathy (Leigh syndrome). Report of a case with lethal outcome. *Pediatr Res* 1989;26:260–266.
25. Smeitink JA, Elpeleg O, Antonicka H, et al. Distinct clinical phenotypes associated with a mutation in the mitochondrial translation elongation factor EFTs. *Am J Hum Genet* 2006;79:869–877.

26. Banka S, de Goede C, Yue WW, et al. Expanding the clinical and molecular spectrum of thiamine pyrophosphokinase deficiency: a treatable neurological disorder caused by TPK1 mutations. *Mol Genet Metab* 2014;113:301–306.
27. Mahajan A, Sidiropoulos C. TPK1 mutation induced childhood onset idiopathic generalized dystonia: report of a rare mutation and effect of deep brain stimulation. *J Neurol Sci* 2017;376:42–43.
28. Ortigoza-Escobar JD, Alfadhel M, Molero-Luis M, et al. Thiamine deficiency in childhood with attention to genetic causes: survival and outcome predictors. *Ann Neurol* 2017;82:317–330.
29. Hu C, Li X, Zhao L, et al. Clinical and molecular characterization of pediatric mitochondrial disorders in south of China. *Eur J Med Genet* 2020;63:103898.
30. Chow CK, Ma CKL. Presentation and outcome of acute necrotizing encephalopathy of childhood: a 10-year single-center retrospective study from Hong Kong. *J Child Neurol* 2020;35:674–680.
31. Legati A, Reyes A, Nasca A, et al. New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies. *Biochim Biophys Acta* 2016;1857:1326–1335.
32. Kelly E, Harvey J, Brion K, et al. Relapsing necrotising encephalomyelopathy due to RANBP2 mutation. *Pract Neurol* 2019;19:360–363.
33. Husain RA, Grimm M, Wagner M, et al. Bi-allelic HPDL variants cause a neurodegenerative disease ranging from neonatal encephalopathy to adolescent-onset Spastic paraplegia. *Am J Hum Genet* 2020;107:364–373.
34. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020;581:434–443.
35. Tuppen HA, Hogan VE, He L, et al. The p.M292T NDUFS2 mutation causes complex I-deficient Leigh syndrome in multiple families. *Brain* 2010;133:2952–2963.
36. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 2020;581:434–443.
37. Morton SU, Prabhu SP, Lidov HGW, et al. AIFM1 mutation presenting with fatal encephalomyopathy and mitochondrial disease in an infant. *Cold Spring Harb Mol Case Stud* 2017;3:a001560.
38. Barca E, Ganetzky RD, Potluri P, et al. USMG5 Ashkenazi Jewish founder mutation impairs mitochondrial complex V dimerization and ATP synthesis. *Hum Mol Genet* 2018;27:3305–3312.
39. Capo-Chichi JM, Boissel S, Brustein E, et al. Disruption of CLPB is associated with congenital microcephaly, severe encephalopathy and 3-methylglutaconic aciduria. *J Med Genet* 2015;52:303–311.
40. Smith AC, Ito Y, Ahmed A, et al. A family segregating lethal neonatal coenzyme Q10 deficiency caused by mutations in COQ9. *J Inher Metab Dis* 2018;41:719–729.
41. Zaha K, Matsumoto H, Itoh M, et al. DNM1L-related encephalopathy in infancy with Leigh syndrome-like phenotype and suppression-burst. *Clin Genet* 2016;90:472–474.
42. Haack TB, Jackson CB, Murayama K, et al. Deficiency of ECHS1 causes mitochondrial encephalopathy with cardiac involvement. *Ann Clin Transl Neurol* 2015;2:492–509.
43. Elo JM, Yadavalli SS, Euro L, et al. Mitochondrial phenylalanyl-tRNA synthetase mutations underlie fatal infantile Alpers encephalopathy. *Hum Mol Genet* 2012;21:4521–4529.
44. Walker MA, Mohler KP, Hopkins KW, et al. Novel Compound Heterozygous Mutations Expand the Recognized Phenotypes of FARS2-Linked Disease. *J Child Neurol* 2016;31:1127–1137.
45. Antonicka H, Sasarman F, Kennaway NG, et al. The molecular basis for tissue specificity of the oxidative phosphorylation deficiencies in patients with mutations in the mitochondrial translation factor EFG1. *Hum Mol Genet* 2006;15:1835–1846.
46. Ravn K, Schönewolf-Greulich B, Hansen RM, et al. Neonatal mitochondrial hepatoencephalopathy caused by novel GFM1 mutations. *Mol Genet Metab Rep* 2015;20:5–10.
47. Morin C, Mitchell G, Larochelle J, et al. Clinical, metabolic, and genetic aspects of cytochrome C oxidase deficiency in Saguenay-Lac-Saint-Jean. *Am J Hum Genet* 1993;53:488–496.
48. Lake NJ, Webb BD, Stroud DA, et al. Biallelic Mutations in MRPS34 Lead to Instability of the Small Mitochondrial Subunit and Leigh Syndrome. *Am J Hum Genet* 2017;101:239–254.
49. Tatuch Y, Christodoulou J, Feigenbaum A, et al. Heteroplasmic mt DNA mutation (T---G) at 8993 can cause Leigh disease when the percentage of abnormal mt DNA is high. *Am J Hum Genet* 1992;50:852–858.
50. McFarland R, Kirby DM, Fowler KJ, et al. De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency. *Ann Neurol* 2004;55:58–64.
51. Hadzsiev K, Maasz A, Kisfali P, et al. Mitochondrial DNA 11777C>A mutation associated Leigh syndrome: case report with a review of the previously described pedigrees. *Neuromolecular Med* 2010;12:277–284.
52. Taylor RW, Morris AA, Hutchinson M, et al. Leigh disease associated with a novel mitochondrial DNA ND5 mutation. *Eur J Hum Genet* 2002;10:141–144.
53. Morris AA, Leonard JV, Brown GK, et al. Deficiency of respiratory chain complex I is a common cause of Leigh disease. *Ann Neurol* 1996;40:25–30.
54. Ng YS, Lax NZ, Maddison P, et al. MT-ND5 Mutation Exhibits Highly Variable Neurological Manifestations at Low Mutant Load. *EBio Medicine* 2018;30:86–93.
55. Ugalde C, Triepels RH, Coenen MJ, et al. Impaired complex I assembly in a Leigh syndrome patient with a novel missense mutation in the ND6 gene. *Ann Neurol* 2003;54:665–669.
56. Naess K, Freyer C, Bruhn H, et al. Mt DNA mutations are a common cause of severe disease phenotypes in children with Leigh syndrome. *Biochim Biophys Acta* 2009;1787:484–490.
57. Limongelli A, Schaefer J, Jackson S, et al. Variable penetrance of a familial progressive necrotising encephalopathy due to a novel tRNA (Ile) homoplasmic mutation in the mitochondrial genome. *J Med Genet* 2004;41:342–349.
58. Silvestri G, Ciafaloni E, Santorelli FM, et al. Clinical features associated with the A->G transition at nucleotide 8344 of mt DNA ("MERRF mutation"). *Neurology* 1993;43:1200.
59. Sweeney MG, Hammans SR, Duchon LW, et al. Mitochondrial DNA mutation underlying Leigh's syndrome: clinical, pathological, biochemical, and genetic studies of a patient presenting with progressive myoclonic epilepsy. *J Neurol Sci* 1994;121:57–65.
60. Santorelli FM, Tanji K, Shanske S, et al. The mitochondrial DNA A8344G mutation in Leigh syndrome revealed by analysis in paraffin-embedded sections: revisiting the past. *Ann Neurol* 1998;44:962–964.
61. Pronicki M, Sykut-Cegielska J, Matyja E, et al. G8363A mitochondrial DNA mutation is not a rare cause of Leigh syndrome - clinical, biochemical and pathological study of an affected child. *Folia Neuro-pathol* 2007;45:187–191.
62. Koga Y, Akita Y, Takane N, et al. Heterogeneous presentation in A3243G mutation in the mitochondrial tRNA (Leu (UUR)) gene. *Arch Dis Child* 2000;82:407–411. <https://doi.org/10.1136/adc.82.5.407>.
63. Santorelli FM, Tanji K, Sano M, et al. Maternally inherited encephalopathy associated with a single-base insertion in the mitochondrial tRNATrp gene. *Ann Neurol* 1997;42:256–260.
64. Simon M, Richard EM, Wang X, et al. Mutations of human NARS2, encoding the mitochondrial asparaginyl-tRNA synthetase, cause

- nonsyndromic deafness and Leigh syndrome. *PLoS Genet* 2015;11:e1005097.
65. Kremer LS, Danhauser K, Herebian D. NAXE Mutations Disrupt the Cellular NAD (P) HX Repair System and Cause a Lethal Neuro-metabolic Disorder of Early Childhood. *Am J Hum Genet* 2016;99:894–902.
 66. Hoefs SJ, van Spronsen FJ, Lenssen EW, et al. NDUFA10 mutations cause complex I deficiency in a patient with Leigh disease. *Eur J Hum Genet* 2011;19:270–274.
 67. Ogilvie I, Kennaway NG, Shoubridge EA. A molecular chaperone for mitochondrial complex I assembly is mutated in a progressive encephalopathy. *J Clin Invest* 2005;115:2784–2792.
 68. Herzer M, Koch J, Prokisch H, et al. Leigh disease with brainstem involvement in complex I deficiency due to assembly factor NDUFAF2 defect. *Neuro pediatrics* 2010;41:30–34.
 69. Calvo SE, Tucker EJ, Compton AG, et al. High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. *Nat Genet* 2010;42:851–858.
 70. Loeffen J, Smeitink J, Triepels R, et al. The first nuclear-encoded complex I mutation in a patient with Leigh syndrome. *Am J Hum Genet* 1998;63:1598–1608.
 71. Bénit P, Chretien D, Kadhon M, et al. Large-scale deletion and point mutations of the nuclear NDUFV1 and NDUFS1 genes in mitochondrial complex I deficiency. *Am J Hum Genet* 2001;68:1344–1352.
 72. Quintana E, Mayr JA, García Silva MT, et al. PDH E1 α deficiency with novel mutations in two patients with Leigh syndrome. *J Inherit Metab Dis* 2009;32:S339–S343.
 73. Pirot N, Crahes M, Adle-Biassette H, et al. Phenotypic and Neuro-pathological Characterization of Fetal Pyruvate Dehydrogenase Deficiency. *J Neuropathol Exp Neurol* 2016;75:227–238.
 74. Matilainen S, Carroll CJ, Richter U, et al. Defective mitochondrial RNA processing due to PNPT1 variants causes Leigh syndrome. *Hum Mol Genet* 2017;26:3352–3361.
 75. Papadopoulou LC, Sue CM, Davidson MM, et al. Fatal infantile cardioencephalomyopathy with COX deficiency and mutations in SCO2, a COX assembly gene. *Nat Genet* 1999;23:333–337.
 76. Brockmann K, Bjornstad A, Dechent P, et al. Succinate in dystrophic white matter: a proton magnetic resonance spectroscopy finding characteristic for complex II deficiency. *Ann Neurol* 2002;52:38–46.
 77. Sala-Coromina J, Miguel LD, de Las HJ, et al. Leigh syndrome associated with TRMU gene mutations. *Mol Genet Metab Rep* 2020;15:100690.
 78. Vernau KM, Runstadler JA, Brown EA, et al. Genome-wide association analysis identifies a mutation in the thiamine transporter 2 (SLC19A3) gene associated with Alaskan Husky encephalopathy. *PLoS One* 2013;8:e57195.
 79. Duchesne A, Vaiman A, Castille J, et al. Bovine and murine models highlight novel roles for SLC25A46 in mitochondrial dynamics and metabolism, with implications for human and animal health. *PLoS Genet* 2017;13:e1006597.
 80. van de Wal MAE, Adjobo-Hermans MJW, Keijer J, et al. Ndufs4 knockout mouse models of Leigh syndrome: pathophysiology and intervention. *Brain* 2022;145:45–63.
 81. Nair RR, Koivisto H, Jokivarsi K, et al. Impaired mitochondrial fatty acid synthesis leads to neurodegeneration in mice. *J Neurosci* 2018;38:9781–9800.
 82. Bean LJH, Funke B, Carlston CM, et al. Diagnostic gene sequencing panels: from design to report-a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2020;22:453–461.
 83. Thaxton C, Good ME, DiStefano MT, et al. Utilizing ClinGen gene-disease validity and dosage sensitivity curations to inform variant classification. *Hum Mutat* 2022;43:1031–1040.
 84. Barcelos I, Shadiack E, Ganetzky RD, Falk MJ. Mitochondrial medicine therapies: rationale, evidence, and dosing guidelines. *Curr Opin Pediatr* 2020;32:707–718.