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

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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.

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Primary mitochondrial diseases (PMDs) are a group of heterogenous 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 (https://clinicalgenome.org/site/assets/files/2164/clingen_standard_gene-disease_validity_recuration_procedures_v1.pdf), the most current information is available at clinicalgenome.org.

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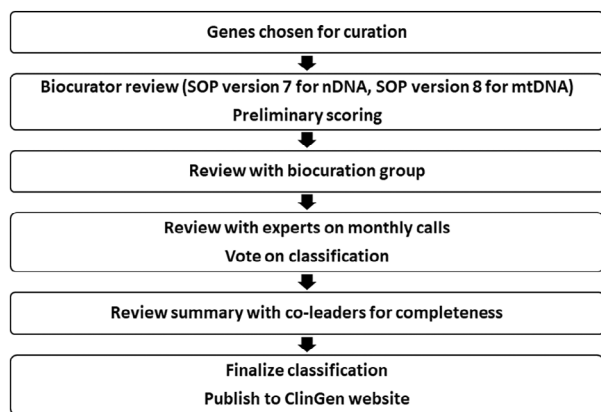
FIGURE 1: Mitochondrial Disease Gene Curation Expert Panel curation process overview. mtDNA = mitochondrial DNA; nDNA = nuclear DNA; SOP = Standard Operating Procedure.

(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 1. Genes Found to Have an Association with LSS

Disease Mechanism	Biochemical Defect	Genes with Defect (s)	Inheritance Mode
OXPHOS subunit deficiency	Complex I	*MT-ND1, MT-ND2, MT-ND3, MT-ND4, MT-ND5, MT-ND6*	Maternal/sporadic
		NDUFA1	X-linked
		NDUFA2, NDUFA9, NDUFA10, NDUFA12, NDUFA13, NDUFB8, NDUFC2, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFV1, NDUFV2	AR
	Complex II	*SDHA*	AR
	Complex III	*UQCRQ*	AR
	Complex IV	*MT-CO1, MT-CO2, MT-CO3*	Maternal/sporadic
		COX4I1, COX8A, NDUFA4	AR
	Complex V	*MT-ATP6*	Maternal/sporadic
		ATP5MD	AR
Assembly factor deficiency	Complex I	*NDUFAF2, NDUFAF4, NDUFAF5, NDUFAF6, NDUFAF8, FOXRED1, NUBPL, TIMMDC1*	AR
	Complex II	*SDHAF1*	AR
	Complex III	*BCS1L, TTC19*	AR
	Complex IV	*COX10, COX15, LRPPRC, PET100, PET117, SCO2, SURF1, TACO1*	AR
Disorders of pyruvate metabolism	Pyruvate dehydrogenase complex	*PDHA1*	X-linked
		DLAT, DLD, PDHB, PDHX	AR
Disorders of vitamin transport and metabolism	Biotin	*BTD*	AR
	Thiamine	*SLC19A3, SLC25A19, TPK1*	AR
Disorders of cofactor biosynthesis	Coenzyme Q$_{10}$	*COQ9, PDSS2*	AR
	Lipoic acid	*LIAS, LIPT1, MECR*	AR
Disorders of mtDNA maintenance	mtDNA depletion and/or multiple mtDNA deletions	*SUCLA2, SUCLG1, POLG, RNASEH1*	AR
		SLC25A4, SSBP1	De novo
Disorders of mitochondrial gene expression	Impaired mitochondrial protein synthesis	*MT-TI, MT-TK, MT-TL1, MT-TV, MT-TW*	Maternal/sporadic
		C12ORF65, EARS2, FARS2, GFM1, GFM2, GTPBP3, IARS2, MRPS34, MTFMT, NARS2, PNPT1, PTCD3, TARS2, TRMU, TSFM	AR
Disorders of mitochondrial protein quality control	Proteostasis	*CLPB, LONP1*	AR
Disorder of mitochondrial membranes	Lipid remodeling	*SERAC1*	AR
Disorders of mitochondrial dynamics	Fission	*MFF, SLC25A46, DNM1L*	AR
	Fission	*DNM1L*	AD
	Fusion	*OPA1*	AR
Disorders of mitochondrial toxicity	Sulfide metabolism	*ETHE1, SQOR*	AR
	Valine degradation	*ECHS1, HIBCH*	AR
	Detoxification	*NAXE*	AR
Disorders of autophagy and apoptosis	Mitochondrial stability, fission, clearance by mitophagy	*VPS13D*	AR
	Other	*AIFM1*	X-linked
Mechanism unclear	Not fully understood	*HPDL, FBXL4*	AR
Nonmitochondrial proteins	Other	*ADAR, MORC2, RANBP2, SLC39A8*	AR

Note: *MT-TL2* and *NUP62* were curated by the Mitochondrial Disease Gene Curation Expert Panel, and there was not convincing evidence supporting a causal role for these genes in Leigh syndrome spectrum.

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; mtDNA = mitochondrial DNA; OXPHOS = oxidative phosphorylation.

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: • Brainstem and/or • Basal ganglia
			With or without bilateral, T2 hyperintensity on MRI or hypodensity on CT scan in: • Thalamus • Cerebellum • Subcortical white matter • Spinal cord
		AND at least ONE of the following	
		Neurologic symptoms	• Developmental regression • Developmental delay • Psychiatric symptoms
		Further supported by at least ONE of the following	
		Biochemical and/or mitochondrial abnormality	• 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

AUTOSOMAL DOMINANT, X-LINKED

Variant type	Variant type (specified)	Points
Variant is de novo	No further specification	3
Predicted or proven null variant	Nonsense, exonic deletion, frameshift, early stop codon OR western blot showing no expression	3
	Any of the above and case has same variant as one already counted	1
Other variant type (not predicted or proven null) with some evidence of gene impact	Variant is a founder/common variant	3
	Case has same variant as one already counted	1

AUTOSOMAL RECESSIVE

Variant type	Variant type (specified)	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
	Any of the above and variant is a founder/common variant	3
	Any of the above and case has same variants as one already counted	1
Two variants in trans not predicted or proven null with some evidence of gene impact in trans	Missense variant, small in-frame insertions and deletion AND Western blot or other evidence showing reduced expression	1
	Missense variant and variant is a founder/common variant and/or extensive functional validation of variant effect	1.5
	Missense variant and case has same genotype as one already counted	0.5
	Missense variant with crystallography or additional evidence of gene impact (in methionine started codon)	0.5
	Missense variant AND in silico analysis evidence	0.25

MITOCHONDRIAL

Criterion	Guidance	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
Predicted to be deleterious	Utilize the following predictors: mRNA: APOGEE tRNA: MitoTIP + HmtVAR in agreement rRNA: Cannot apply, no user-friendly predictor available	0.25
Corresponding biochemical deficiency noted in subject	Multiple respiratory chain enzyme complex deficiency (tRNA) Corresponding respiratory chain enzyme complex deficiency (mRNA)	0.5
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 point) Correlation between heteroplasmy level and biochemical deficiency (0.5 point)	0 – 1.5
Single fiber study	Correlation between mutant load and OXPHOS activity	0 – 1.5
Other functional evidence	Example including but not limited to: Reduced mt-tRNA level on northern blot	0.5
Unrelated case with same variant that reached max score (for cybrid or single fiber study)	Case can be awarded maximum points	1.5
Case with universally accepted pathogenic variant	Confirmed status in Mitomap and/or classified as pathogenic or likely pathogenic by mtDNA Expert Panel in ClinVar	0.5

B. Experimental level evidence

FUNCTION

Evidence type	Definition	Scoring criteria	Points
Biochemical function • Respiratory chain assembly • Cofactor biosynthesis • Mitochondrial maintenance • Mitochondrial translation • Mitochondrial dynamics • Mitochondrial import	Gene product shares biochemical relationship/function with another gene product associated with LS	1 gene product	0.5
		2-5 gene products	1
		6-9 gene products	1.5
		10+ gene products	2.0
Protein interaction • ETC complex subunits • Enzymatic subunits	The encoded protein interacts with another gene product associated with LS	1 gene product	0.5
		2-5 gene products	1
		6-9 gene products	1.5
		10+ gene products	2.0
Expression	Evidence showing expression present or is disrupted in the following areas	Any tissue or cell type; and/or in brain	0.5
		Brainstem, basal ganglia, thalamus, cerebellum, spinal cord	1

FUNCTIONAL ALTERATION

Evidence type	Definition	Scoring criteria	Points
Patient cells	Cells/isolated mitochondria, in which the function of the gene has been disrupted, display mitochondrial dysfunction as characterized by (including but not limited to): • OXPHOS dysfunction • Decreased enzyme activity • Mitochondrial, mitochondrial DNA depletion • Membrane dysfunction	1 cell culture model	1
		≥2 cell culture models	1.5
		Performed in neuronal cell type	+0.5
		Performed in cells obtained from patient meeting all criteria for LS (neuropathology or neuroimaging, neurologic, and biochemical)	2
Non-patient cells		1 cell culture model	0.5
		≥2 cell culture models	1
		Performed in neuronal cell type	+0.5

MODELS, NON-HUMAN MODEL ORGANISM

Phenotype	Example	Points	Range
Neuropathological evidence	Symmetrical, bilateral lesions in basal ganglia, brainstem; spongiform lesions, neuronal loss, gliosis; proliferation of small blood vessels *embryonic lethal – 0.5	3	0–4
MRI findings	Symmetrical, bilateral lesions in basal ganglia and/or brainstem, other brain areas as outlined in definition	2	0–3
Biochemical or mitochondrial dysfunction	OXPHOS, Blue Native gel, mitochondrial depletion, elevated lactate, abnormal mitochondrial morphology	0.5	0–1
Neurocognitive/ developmental differences	Regression, ataxia, hypotonia, growth retardation	0.5	0–1

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 function, functional alteration, and models – nonhuman model organism. No further scoring guidance for rescue experiments was provided. 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; rRNA = ribosomal RNA; tRNA = transfer RNA; SD = standard deviation; OXPHOS = oxidative phosphorylation; 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 *BCS1L* (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 (*BCS1L* 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.
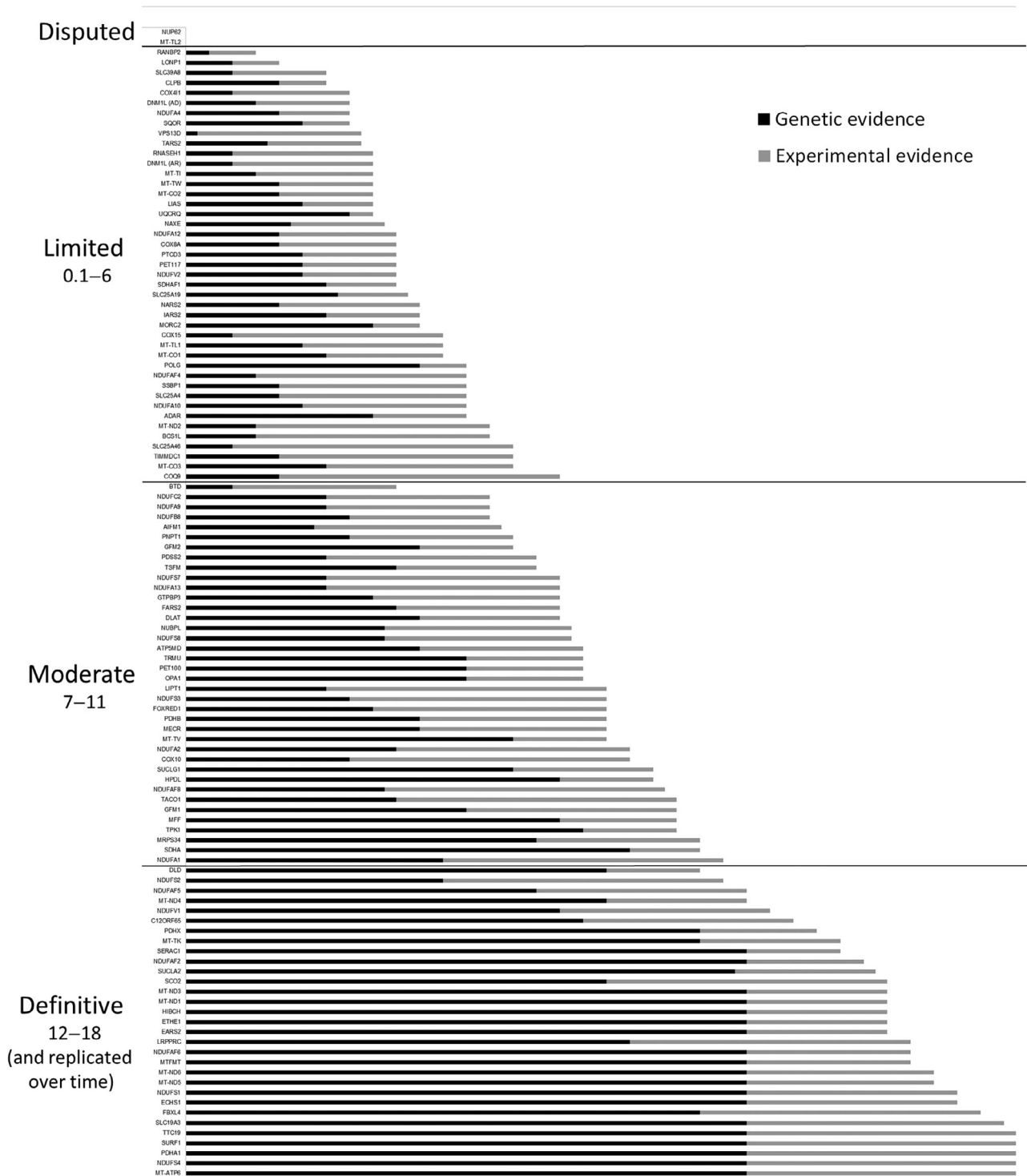
FIGURE 3: Scores for each curated gene–disease relationship with Leigh syndrome spectrum by the Mitochondrial Disease Gene Curation Expert Panel. The default scoring range for a definitive classification is 12–18, moderate is 7–11, and limited is 0.1–6.

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

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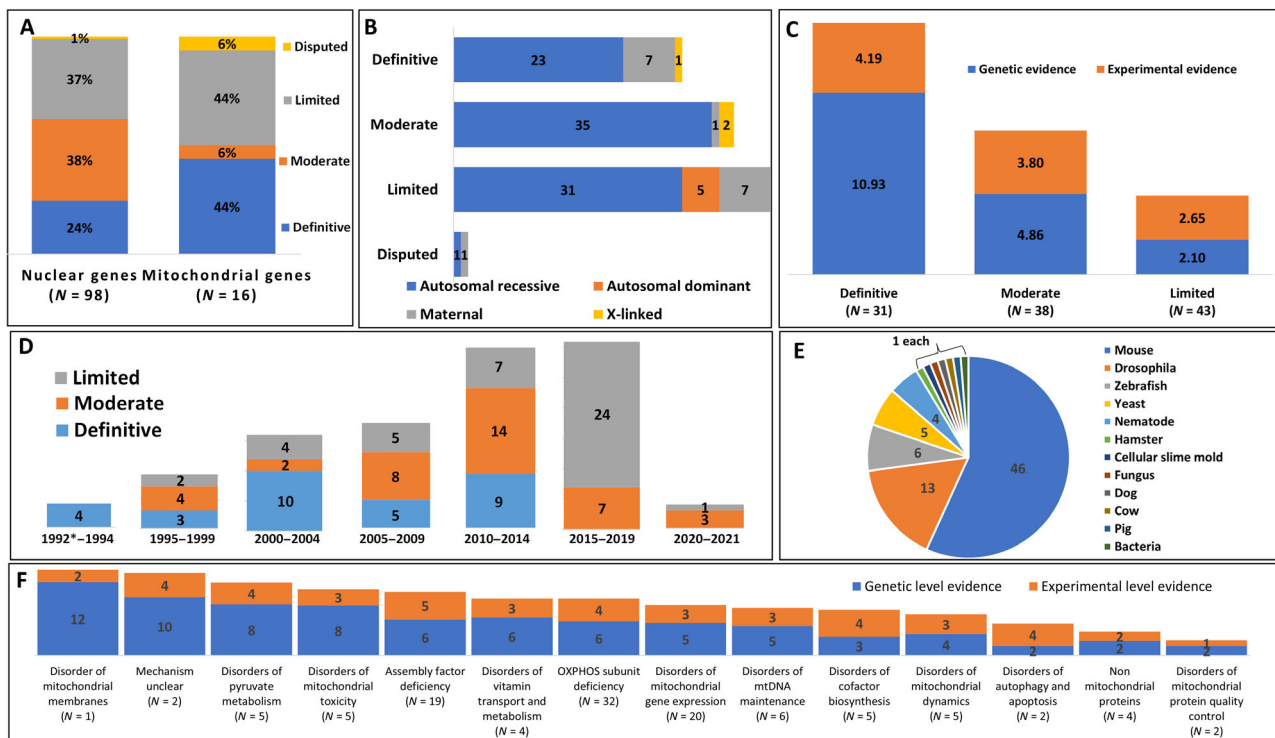
FIGURE 4: Leigh syndrome spectrum (LSS) classification overview by associated genome, association strength, score, time since discovery, gene class, and experimental models. (A) Classifications of nuclear genes (n = 98) and mitochondrial DNA (mtDNA) genes (n = 16) curated for association with LSS. (B) 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. OXPHOS = 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 heterogenous 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 hypoxemic 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 • *GFM1*, fatal infantile hepatopathy • *NDUFAF5*, early lethality before MRI could be performed	Coenen et al, 2004[20] Smits et al, 2011[21] Sugiana et al, 2008[22]
		Increased utilization of NBS led to treatment before developing LSS • *BTD* 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 • For example, biotinidase deficiency	Mitchell et al, 1986[23] Baumgartner et al, 1989[24]
	Cases reported in cohorts with minimal phenotypic details provided	Missing phenotypic information • *TSFM*, no brain imaging data for 6 cases • *TPK1*, 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 • *RANBP2*, no lactate or OXPHOS measurements in many cases Limited knowledge at time of report • *HPDL*, 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	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]
High allele frequencies in healthy population databases	Uncertainty regarding pathogenic nature of variants and/or phasing	Hypomorphic alleles • *NDUFS2*, 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 *trans*	-

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 heterogenous, 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
AIFM1			✓	Occipital lesion, hepatopathy, Alpers	Morton et al, 2017[37]
ATP5MD	✓ (2)				Barca et al, 2018[38]
CLPB			✓	"Nonspecific lesions"	Capo-Chichi et al, 2015[39]
COQ9			✓ (2)	Global ischemia in one case, calcification in GP and olivary dysplasia in the other	Smith et al, 2018[40]
DNM1L	✓				Zaha et al, 2016[41]
ECHS1			✓	Poorly preserved brain on macroscopy with BG cavitation, microscopy statement	Haack et al, 2015[42]
FARS2			✓	Alpers neuropathology	Elo et al, 2012[43]
FARS2			✓	Alpers neuropathology	Walker et al, 2016[44]
GFM1			✓	WM lesion, BG, hypoplastic CC, hepatopathy	Coenen et al, 2004[20]
GFM1			✓	Bilateral porencephaly, microcephaly, dysgenesis of cingulate gyri, hepatopathy	Antonicka et al, 2006[45]
FM1			✓	Polymicrogyria and hepatopathy	Ravn et al, 2015[46]
LRPPRC		✓ (7)		Statement only: "lesions typical of Leighs"	Morin et al, 1993[47]
MRPS34	✓				Lake et al, 2017[48]
MT-ATP6	✓				Tatuch et al, 1992[49]
MT-ND3		✓			McFarland et al, 2004[50]
MT-ND4	✓				Hadzsiev et al, 2010[51]
MT-ND5	✓				Taylor et al, 2002[52] Morris et al, 1996[53]
MT-ND5	✓ (2)			Leigh plus MELAS	Ng et al, 2018[54]
MT-ND6		✓		Statement only	Ugalde et al, 2003[55]
MT-ND6		✓ (2)		Statement only	Naess et al, 2009[56]
MT-TI	✓ (2)				Limongelli et al, 2004[57]
MT-TK		✓		Statement only	Silvestri et al, 1993[58]
MT-TK	✓				Sweeney et al, 1994[59]
MT-TK				Statement only	Santorelli et al, 1998[60]
MT-TK	✓				Pronicki et al, 2007[61]
MT-TL1	✓				Koga et al, 2000[62]
MT-TW				Statement only	Santorelli et al, 1997[63]
NARS2	✓			Leigh plus MELAS	Simon et al, 2015[64]
NAXE		✓ (3)			Kremer et al, 2016[65]

TABLE 4. Continued

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

[a]Case 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 Q_{10} 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.

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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/.

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