Title: Leigh Syndrome: One disorder, more than 75 monogenic causes

Running head: Leigh Syndrome: One disorder, many genes

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Abstract

Leigh syndrome is the most common pediatric presentation of mitochondrial disease. This neurodegenerative disorder is genetically heterogeneous, and to date pathogenic mutations in more than 75 genes have been identified, encoded by two genomes (mitochondrial and nuclear). More than a third of these disease genes have been characterized in the last 5 years alone, reflecting the significant advances made in understanding its etiological basis. We review the diverse biochemical and genetic etiology of Leigh syndrome and associated clinical, neuroradiological and metabolic features that can provide clues for diagnosis. We discuss the emergence of genotype-phenotype correlations, insights gleaned into the molecular basis of disease and available therapeutic options.

Introduction

Disorders of mitochondrial energy generation are the most frequent group of inherited metabolic disorders, with an estimated incidence of at least 1 in 5000 live births.[1] The most common clinical presentation of mitochondrial disease in children is a progressive neurodegenerative disorder known as Leigh syndrome (MIM 25600, LS). LS has an estimated prevalence of ~1 per 40,000 live births,[2] however much higher incidences have been observed in specific populations owing to founder mutations.[3,4]

Since the identification of the first pathogenic mutation in a LS patient in 1991,[5] more than 75 disease genes have been identified, highlighting the remarkable heterogeneity underlying this disorder. Nearly 30 of these LS disease genes have been characterized in the last 5 years alone, reflecting how the introduction and greater utility of

massively parallel sequencing (MPS) technology has transformed our ability to identify the genetic basis of disease. Beyond the identification of novel disease genes, MPS has also facilitated the identification of patients with mutations in known disease genes, enabling the emergence of genotype-phenotype relationships for this rare disorder. Advances in characterizing the genetic basis of LS have consolidated our understanding of the molecular basis of disease, revealing a diverse biochemical etiology consistent with the annotation of LS as a disorder of energy generation.[2]

Clinical and neuroradiological features

LS is clinically heterogeneous, with significant variation between patients with respect to age of onset, age of death and symptomatology. Generally, onset occurs by 2 years of age,[6] with symptoms often presenting during infection or illness after an initial period of normal development. Patients develop neurological symptoms including developmental delay and regression, hypotonia, ataxia, dystonia, and ophthalmological abnormalities including nystagmus and optic atrophy.[2,6-8] The presentation can also be multisystemic; cardiac, hepatic, gastrointestinal and renal tubular dysfunction have been observed.[9-12] Progression is often episodic, and typically results in death by 3 years of age.[2,7,8,13] Adult-onset LS has been infrequently reported.[14]

Neurological decline in LS patients is associated with the development of bilateral symmetrical lesions within the brain stem and basal ganglia structures, which characterize the disease.[13,15,16] These lesions are observed as regions of focal hyperintensity on T2-weighted magnetic resonance imaging (MRI), and elevated lactate may be detected by MR spectroscopy. Additional neuroradiological

abnormalities such as white matter involvement and cerebral atrophy may also be observed.[17] In 1996, we defined criteria for a stringent diagnosis of LS, requiring that characteristic neuropathology or neuroradiology must be accompanied by progressive neurodegeneration with (1) clinical evidence of brainstem and or basal ganglia dysfunction, (2) intellectual and motor developmental delay and (3) elevated serum or CSF lactate indicating abnormal energy metabolism.[2] In 2015, it would seem prudent to amend criterion (3) to 'abnormal energy metabolism indicated by a severe defect in OXPHOS or PDHc activity, a molecular diagnosis in a gene related to mitochondrial energy generation or elevated serum or CSF lactate'. Where patients do not fulfill these stringent criteria, a diagnosis of Leigh-like syndrome can be considered, particularly in patients with atypical neuroradiology or normal lactate levels.[2]

The development of our understanding of the etiological basis of Leigh syndrome

Since Denis Leigh's first description of LS in 1951,[18] there have been several key research milestones that have contributed to our understanding of the etiological basis of LS (Fig 1). The similarity of the neuropathology to Wernicke's encephalopathy, a condition of thiamine deficiency, and findings of elevated blood lactate and pyruvate suggested that a metabolic abnormality was the underlying disease etiology.[19,20] Before the first genetic mutation was identified in 1991,[5] 40 years of clinical and biochemical investigation provided evidence that deficiency of the pyruvate dehydrogenase complex (PDHc), and of NADH:ubiquinone oxidoreductase (complex I) and cytochrome *c* oxidase (complex IV) within the oxidative phosphorylation (OXPHOS) pathway, could cause LS.[21-23] Together with the finding of abnormal mitochondrial morphology in skeletal muscle tissue from patients, these abnormalities

suggested a common theme of defective mitochondrial energy metabolism.[22] Subsequently LS was re-annotated as a mitochondrial encephalopathy.

It has since been established that multiple other enzymatic deficiencies can underlie LS, mostly linked directly to OXPHOS or broader pathways of energy generation. The OXPHOS pathway of electron transfer, proton transport, and ATP synthesis comprises five multi-protein complexes located in the mitochondrial inner membrane (Fig 2). Biochemical defects in each of the five OXPHOS complexes, and the electron carrier coenzyme Q_{10} (CoQ_{10}), have been observed in LS patients. PDHc enables the generation of electron donors for OXPHOS; hence PDHc deficiency also represents a disorder of energy generation. Complex I deficiency is the most common biochemical cause of LS with nearly a third of all LS disease genes associated with complex I deficiency. Isolated complex IV deficiency and multiple OXPHOS defects are also frequently observed, whereas defects of complexes II, III, V or of CoQ_{10} are relatively rare.[2,6,7] Measurement of OXPHOS and PDHc enzymes in a patient biopsy or cell line is often undertaken to provide evidence of a biochemical defect in these pathways to support a clinical diagnosis of LS.

The heterogeneous genetic basis of Leigh syndrome

To date, pathogenic mutations in more than 75 genes have been identified in affected patients (Table 1, and see SupplementaryTable for more detailed information). Most of these disease genes encode structural components of the OXPHOS complexes, or proteins required for their assembly, stability and activity. As is the case for other mitochondrial diseases, there are several possible modes of inheritance including maternal (for mutations in mitochondrial DNA, mtDNA) and autosomal recessive or

X-linked (for nuclear-encoded genes). Despite the remarkable number of established disease genes, many LS patients remain without a genetic diagnosis, indicating that there are still more disease genes to be identified.

The aim of this article is not to provide an in-depth review of all known forms of Leigh and Leigh-like syndrome, but rather to discuss the most relevant biochemical and genetic etiologies, associated clinical and biochemical features relevant for diagnosis, and therapeutic options (where available, summarized in Table 2). We focus on the more common nuclear-encoded genetic causes, as well as describing types of Leigh and Leigh-like syndrome caused by mtDNA mutations.

Complex I deficiency

The most frequent clinical presentation of complex I deficiency is LS,[24] and complex I deficiency is the leading biochemical basis of LS.[2,6,7] Mutations in the NADH Dehydrogenase (Ubiquinone) Fe-Sulfur protein 4 (NDUFS4) subunit are the most frequent autosomal recessive cause of complex I-associated LS, with more than 20 cases reported.[24] Interestingly, almost all patients reported with *NDUFS4* mutations had LS, in contrast to mutations in other nuclear-encoded complex I subunits, which may be associated with heterogeneous phenotypes. Patients with *NDUFS4* mutations display a characteristic LS presentation, experiencing onset and demise typically within the first 8 months of life and no later than 30 months. Neuroradiological features in these infants included bilateral symmetrical basal ganglia lesions in almost all cases, often extending down to the cerebral peduncles, pons and medulla, and frequently with associated cerebral atrophy.[24,25] Approximately a third of all reported cases also had hypertrophic cardiomyopathy.

Mutations in NADH Dehydrogenase (Ubiquinone) Flavoprotein 1 (*NDUFV1*) and *NDUFS1* have also been widely reported to cause LS. *NDUFS1* mutations appear to be associated with an early age of death, with all reported patients presenting by 8.5 months and succumbing by 4 years.[24] Most patients with *NDUFV1* mutations also died in early childhood, although a few cases were alive in late childhood.[26,27] Thus, *NDUFS4*-, *NDUFV1*- and *NDUFS1*-mediated LS appear to be particularly severe forms of the disease. While all reported *NDUFS4* mutations except one predict complete loss-of-function alleles, no patients have been described with two null alleles of *NDUFV1* or *NDUFS1*, suggesting that complete loss of these two proteins may be incompatible with life.

Complex II, Complex III and Coenzyme Q_{10} deficiencies

Deficiencies of complex II, complex III or CoQ_{10} (ubiquinone) are rare causes of LS, and collectively underlie fewer than 10% of all cases.[2,6,28] Mutations in the complex II subunit SDHA were the first nuclear mutations identified to cause OXPHOS disease.[29] Some patients with *SDHA* mutations have a severe infantile presentation of LS with rapid demise,[29] but other patients may experience a mild LS course, with survival into late childhood years, preservation of cognitive abilities and an 'almost normal school life'.[28,30] Nearly all patients with mutations in the complex III assembly factor TTC19 develop neuroimaging abnormalities consistent with LS.[31] Occasional patients with LS and CoQ_{10} deficiency have been reported.[9,32] Prompt recognition of this subgroup of LS is imperative, since these patients may respond to CoQ_{10} supplementation and survive into adulthood,[32] although this has not been consistently observed.[33] One report described improvement of hepatic but not neurological

symptoms,[34] and another patient with CoQ_{10} deficient LS due to *PDSS2* mutations did not respond to treatment.[9]

Complex IV deficiency

Complex IV deficiency underlies ~15% of LS cases.[2,6,7] Mutations in *SURF1*, encoding a complex IV assembly factor, are the most common cause of complex IV deficient LS and are one of the most frequently reported causes of LS, with more than 200 cases described to date in the literature.[35] Our recent natural history study of a *SURF1* patient cohort highlighted the relatively homogenous clinical and biochemical presentation associated with SURF1 deficiency.[35] However, a minority of patients with *SURF1* mutations lack the neuroradiological hallmarks of LS or show atypical features such as leukodystrophy and atrophy.[35,36] Survival analysis of 142 *SURF1* patients revealed a median age of death at 5.4 years,[35] indicating longer survival in *SURF1*-associated LS than is observed for LS patients due to mutations in other complex IV disease genes *SCO2*, *LRPPRC*, and *ETHE1*.[37-39]

LRPPRC mutations were originally reported in an isolated French-Canadian population.[37,40] Characteristic clinical features associated with French-Canadian LS (LSFC, MIM 220111) include mild facial dysmorphism, liver pathology and a clinical course punctuated by episodes of acute metabolic decompensation that contribute significantly to mortality.[3,37] Fifty-five of 56 reported cases inherited homozygous *LRPPRC* p.A354V mutations,[37] reflecting a founder effect that has resulted in an incidence of LSFC of ~1 in 2000 live births (Table 3).[3] Broader effects of LRPPRC dysfunction on mitochondrial post-transcriptional and translation processes have recently been recognized, including deficiency of ATP synthase.[41] Despite presenting

as an isolated complex IV deficiency, the pathogenesis of *LRPPRC* mutations may therefore be attributable to a global defect of mitochondrial translation.

Mutations in *ETHE1* cause ethylmalonic encephalopathy (EE) (MIM 602473), a presentation that resembles LS clinically and neuroradiologically.[38,42] The characterization of a role for ETHE1 in sulfide detoxification revealed EE as a manifestation of sulfide toxicity, whereby the associated isolated complex IV deficiency is a consequence of sulfide accumulation.[43] Accordingly, therapeutic strategies that reduce sulfide accumulation resulted in clinical, biochemical and neuroradiological improvement in patients with *ETHE1* mutations.[44]

The only complex IV subunit with mutations causing LS is the recently reassigned subunit NDUFA4, which was previously thought to be a subunit of complex I. *NDUFA4* mutations have been reported to cause childhood-onset LS with prominent epilepsy, learning difficulties and survival into adulthood.[45]

Combined OXPHOS deficiencies

Since mtDNA encodes structural components of complexes I, III, IV and V, a molecular defect that impairs mtDNA replication or translation can cause combined OXPHOS deficiency. Thus, mutations in various disease genes associated with mtDNA depletion or defective translation have been identified in patients with LS (see Table 1, see Supplementary Table, A for more detailed information). The predominant causes of LS associated with mtDNA depletion are mutations in *SUCLA2* or *SUCLG1*, encoding subunits of succinyl-CoA synthetase which catalyzes the conversion of succinyl-CoA to succinate within the citric acid cycle.

Methylmalonic aciduria is frequently observed in these patients due to an accumulation of succinyl-CoA, and can be used to screen for these two defects in LS patients.[46,47] Symptoms generally present in early infancy, and patients can survive into teenage years and young adulthood.[48,49] Although patients with mutations in *SUCLG1* and *SUCLA2* manifest similarly, they can be differentiated by liver involvement (*SUCLG1*) or deafness (more common with *SUCLA2* mutations).[49] The *SUCLA2* c.534+1G>A mutation has a high carrier frequency in the Faroe Islands owing to a founder effect, with an estimated LS incidence of 1 in 1700 in that population (Table 3).[4] An interaction between succinyl-CoA synthetase and nucleoside diphosphate kinase, an enzyme involved in regulating mitochondrial nucleotide supply, has been hypothesized to account for mtDNA depletion in patients with *SUCLA2* and *SUCLG1* mutations.[46]

Nuclear encoded mt-tRNA modifying enzymes are required for efficient mitochondrial translation, and mutations in these proteins represent a relatively new class of LS disease genes. Mitochondrial methionyl-tRNA formyltransferase (*MTFMT*) was the first LS disease gene of this type to be reported. *MTFMT* mutations were initially identified in two unrelated LS patients through targeted exome sequencing of the "MitoExome",[50] and subsequently identified in multiple other LS patients.[51] Most cases carry at least one copy of the c.626C>T mutation, which has a carrier frequency of ~1 in 100 in the European population and so may account for a significant proportion of LS within this population.[51]

Mitochondrial DNA mutations causing Leigh syndrome associated with Complex I, Complex IV, Complex V and combined OXPHOS deficiencies

Cohort studies have suggested that mtDNA mutations underlie approximately 10-20% of LS cases.[2,6,7] To date, mutations in 13 of the 37 mtDNA-encoded genes have been described to cause LS, with the majority in genes encoding subunits of complexes I or V, or in mitochondrial tRNAs (see Table 1, and Supplementary Table, A for more detailed information). LS can also be caused by large-scale mtDNA deletions.[2] The percentage of mtDNA molecules that carry the pathogenic mutation, known as the level of heteroplasmy, is an important determinant of the clinical presentation. As a severe manifestation of mitochondrial disease, LS is typically associated with high mutant loads of ≥ 90%, although *MTND5* mutations associated with complex I deficiency are notable for their propensity to cause LS even when the mutant load is <50% in tissues including brain, where patients have been observed to survive into adulthood.[52-54]

Mutations in complex I subunits MTND3 and MTND5, and in the complex V subunit MTATP6, are the most frequent mtDNA causes of LS. *MTATP6* mutations represent the only established genetic basis of complex V-mediated LS, and mutations in this gene are estimated to underlie ~10% of all LS cases.[2,6,7,55] The majority of these patients carry either the *MTATP6* m.8993T>G or the less severe m.8993T>C mutation, which were among the first LS mutations described.[56,57] Although uncommon, mutations in five mt-tRNAs have been identified in LS patients, including the well-known m.3243A>G *MTTL1* mutation that is more commonly associated with the syndromes of mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) and maternally inherited diabetes and deafness

(MIDD), and the m.8344A>G in *MTTK* that typically causes myoclonic epilepsy with ragged red fibers (MERRF).[58,59]

Although mtDNA is inherited maternally, many patients with mtDNA mutations lack any maternal family history suggesting mtDNA disease. This can be because the heteroplasmic mutant load in other family members is below the pathogenic threshold for that mutation. *De novo* mutations in mtDNA are also relatively common, so a mutation present at high levels of heteroplasmy in the proband can be undetectable in the mother and other maternal relatives.[60,61]

Pyruvate dehydrogenase complex deficiency

Evidence of elevated pyruvate and a lactate:pyruvate ratio of <20 can indicate PDHc deficiency in LS patients, with agenesis of the corpus callosum another common finding.[62] Mutations in *PDHA1*, encoding the E1 alpha subunit, are the predominant cause of PDHc-associated LS.[62,63] *PDHA1* is an X-chromosome gene, but approximately equal numbers of boys and girls are diagnosed with *PDHA1* mutations.[62] This pseudo-dominant inheritance is not surprising given that random X-inactivation means that typically ~50% of cells will express the mutant allele. Skewed X-inactivation means that the biochemical diagnosis of PDHc deficiency in females can be challenging.[64] Cohort studies of PDHc deficient individuals support previous observations that *PDHA1* likely has a high *de novo* mutation rate.[62,63]

Mutations in genes related to the PDHc co-factors lipoic acid and thiamine pyrophosphate (TPP) have more recently been described to cause LS. The *LIPT1* and *LIAS* genes encode enzymes responsible for synthesis and transfer of the lipoic acid

cofactor, and the *DLD* gene encodes the PDHc subunit E3 dihydrolipoamide dehydrogenase. Patients with PDHc deficiency due to mutations in these genes present with a recognizable metabolic profile that reflects additional deficiency of α-ketoglutarate dehydrogenase and branched-chain α-keto acid dehydrogenase.[65-67] PDHc deficiency due to mutations in genes associated with TPP availability (*TPK1*, *SLC19A3* and *SLC25A19*) can be missed because of the presence of TPP in routine PDHc enzymatic assays, but may be identifiable when measuring PDHc activity in the absence of TPP.[68,69] Mutations in the thiamine transporter *SLC19A3* are well known to cause biotin/thiamine-responsive basal ganglia disease (BTBGD) (MIM 607483), which phenocopies LS as a progressive encephalopathy with similar neuroimaging and episodic decline.[70] Unlike typical LS, patients with BTBGD can show significant clinical and neuroradiological improvement following administration of thiamine and high dose biotin, particularly when commenced early in the disease course.[71] Indeed patients with *SLC19A3* mutations have been described with 'treatable' or 'reversible' LS.[71,72]

Disease genes associated with Leigh-like syndrome

A diagnosis of Leigh-like syndrome usually refers to patients presenting with atypical neuropathology or neuroradiology, but can also reflect an atypical clinical presentation or course.[2] Leigh-like presentations can display overlap with other mitochondrial encephalopathies, and many cases are caused by mutations in the same genes associated with LS. *MTND5* and *MTND3* mutations have been described to underlie a rare clinical presentation of a LS/MELAS overlap syndrome where patients simultaneously display neuroradiological and clinical characteristics of both syndromes,[36,73] while large-scale mtDNA deletions can cause Pearson syndrome with

Leigh-like neuropathology.[74,75] Furthermore, Leigh-like patients with *POLG* mutations generally lie on a spectrum between Alpers-Huttenlocher syndrome (MIM 203700) and LS, where the neuropathology exhibits features characteristic of both syndromes and hepatic dysfunction is present.[76,77] Disease genes that predominantly underlie a Leigh-like presentation that may not fit a stringent LS diagnosis also include *SERAC1* which causes 3-methylglutaconic aciduria with deafness, encephalopathy and Leigh-like syndrome (MEGDEL) associated with isolated or combined OXPHOS enzyme deficiency.[78] These patients often present with atypical neuroradiology, or with unusual additional symptoms including abnormal behavior.[79]

The emergence of genotype-phenotype relationships

Historically, strong correlations between a molecular defect and clinical features could not be identified among LS patients,[2] in part reflecting the low numbers of patients for most disease genes. However the increasing utilization of MPS technology has facilitated the identification of multiple LS patients with mutations in the same gene, enabling the recognition of emerging genotype-phenotype correlations (see Table 3 for examples). The presence of these signature features in combination with a biochemical deficiency and knowledge of the patient's ethnicity may suggest a specific genetic basis. Beyond having characteristic symptoms, different LS disease genes can underlie distinct disease courses, where differences in median age of onset, and length of disease course, can be observed (illustrated in Fig 3). For example, *NDUFS4* mutations appear to be associated with early onset and death, whereas patients with mutations in *SUCLA2* or the complex IV assembly factor PET100 experience early onset but a more variable lifespan including survival into adolescence.

The accumulation of additional patients with an established genetic basis of disease and thorough phenotyping may reveal more LS genotype-phenotype correlations. More research is required to determine the molecular basis of such relationships, although they may be expected to in part reflect tissue-specific effects relating to expression levels, function, and or responses to the genetic defect. Being able to recognize the genetic or biochemical basis of disease has important utility for guiding treatment options, and in some cases can enable life-saving intervention for the genetic forms that are most responsive to treatment such as *SLC19A3* or *BTD* mutations (outlined in Table 2).

Established molecular defects indicate a common disorder of energy generation

Our current understanding of the genetic basis of LS supports impairment in mitochondrial energy generation being the key to pathogenesis. However, it appears that the specific enzyme or process that is disrupted is not as relevant to molecular pathogenesis, given the remarkably diverse range of molecular defects causing LS. These include defects of catalytic activity, stability or assembly of individual OXPHOS complexes, of mtDNA maintenance and expression, and of PDHc activity and cofactors required for enzyme activity (see Table 1, and Supplementary Table, A for more detailed information).

The identification of several disease genes that are not known to encode proteins with a direct role in OXPHOS or PDHc activity raises new questions about the pathogenesis of LS (see Table 1, and Supplementary Table, B for more detailed information). Some of these defects appear to cause secondary defects of PDHc or

OXPHOS enzymes, which may show variability across patients and tissues.[78,80-82] Such secondary defects have not been reported in patients with biotinidase deficiency due to *BTD* mutations, a treatable condition which can manifest as LS (Table 2),[83,84] although there is evidence of ATP deficiency and complex IV inhibition from animal and cellular models.[85,86]

Mutations in the phospholipid remodeling protein SERAC1 and apoptosis-inducing factor AIFM1 may impair OXPHOS by reducing the stability of the mitochondrial membrane.[78,82] However, the mechanism by which mutations in enzymes involved in valine catabolism (ECHS1, HIBCH) and biotin recycling (BTD) impair OXPHOS and PDHc activity remains to be determined. These enzymes facilitate pathways that can produce substrates for the citric acid cycle,[81,87] an important source of electrons for OXPHOS. However the relevance of this to pathogenesis is unclear, as is the contribution of primary effects of these disorders such as perturbed valine catabolism.[80,81] Instead, an accumulation of toxic reactive metabolites has been suggested to be the mechanism by which *ECHS1* and *HIBCH* mutations impair OXPHOS and PDHc activity,[81,88] similar to the toxic accumulation of sulfide due to *ETHE1* mutations described above.[43] Although it may not be the primary consequence of mutation, an indirect effect on mitochondrial energy generation can therefore be sufficient to cause the development of LS.

Our understanding of cerebral energy metabolism further supports the central role of a diminished energy supply to the pathogenesis of LS. Brain metabolism accounts for ~20–25% of resting energy expenditure in adults.[89] The metabolic requirements of the brain increase during infancy and peak in early childhood, where it uses glucose at a

rate of up to 66% of the body's resting metabolism.[90] Therefore it is not surprising that defects in OXPHOS and energy generation can cause early onset neurodegeneration in the form of LS. Studies indicating that the brain's metabolic requirements are highest at ~5 years of age[90] are relevant to observations suggesting that survival past periods of increased vulnerability in infancy and early childhood may predict a life span into and beyond late childhood for patients with LS. This is supported by the cohort study of *LRPPRC* patients, where no metabolic crises were observed in patients after the age of 7,[37] and suggested by the notably later median age at last report relative to the median age of death observed across patients with either *PET100*, *NDUFV1* or *MTFMT* mutations in Figure 3.

The particular vulnerability of the basal ganglia and brain stem structures to neurodegeneration in LS patients is unexplained. Positron emission tomography studies examining the cerebral metabolic rate for glucose in infants and children suggested that the brainstem and basal ganglia/thalamus do not necessarily have the highest energy requirement of all examined brain structures in conditions of basal metabolism.[91] Furthermore the capacity for patients with inherited mitochondrial energy generation disorders to develop neuropathological abnormalities distinct from LS, including in infancy and early childhood,[92] suggests that other factors beyond a reduced energy supply must contribute to pathogenesis. Previous studies demonstrating differences in mitochondrial protein expression across mouse tissues, including between different brain regions,[93] imply that cell- and tissue-specific responses to a molecular defect of mitochondrial energy generation will also contribute to pathogenesis. This is supported by transcriptomic studies of cells and

tissues from mitochondrial disease patients demonstrating tissue-specific patterns of transcriptional dysregulation.[94]

Updating diagnostic strategies to incorporate new knowledge of molecular etiologies

Detailed approaches to diagnosis of Leigh syndrome have been published elsewhere [18,55]. The initial steps remain a detailed medical and family history, physical examination including determining extent of multi-organ involvement, measurement of laboratory parameters such as lactic acid in blood and CSF, and imaging (MRI and magnetic resonance spectroscopy) [18]. In patients such as those described in Table 3, the phenotype may prompt investigation of a specific gene, such as *SURF1* in a child with hypertrichosis and relative cognitive sparing [35]. However, in most patients the next step has traditionally been to measure OXPHOS and PDHc enzyme activities in a muscle biopsy and cultured skin fibroblasts respectively, the results of which can guide subsequent gene-by-gene analysis. While enzyme testing may often still be required for diagnosis, the diagnostic paradigm is increasingly shifting to using MPS first, in order to potentially avoid the need for muscle biopsy. Testing gene panels of perhaps a few hundred genes is a popular approach, although the rate of discovery of novel disease genes means that such panels can become outdated quite quickly. Whole exome sequencing can potentially detect mutations in any nuclear gene but typically is not a sensitive test for mtDNA mutations, so may need to be accompanied by a separate test for mtDNA mutations. Whole genome sequencing can detect mutations in nuclear and mtDNA genes, but is less widely available than exome sequencing. Each of these approaches has specific advantages and disadvantages [95 96] but in our experience they can enable molecular diagnosis of LS in more than 60% of

patients with a stringent LS diagnosis, as defined by the original criteria of Rahman et al[2].

Insights into broader cellular dysfunction: an emerging area for therapeutic intervention

There is a growing body of evidence supporting the contribution of broader cellular dysfunction in disorders of mitochondrial energy generation. Recent studies have demonstrated that PDHc and OXPHOS defects can induce transcriptional dysregulation of multiple cellular pathways including those that regulate RNA and protein metabolism.[94] These disturbances in cellular metabolism appear to be primarily mediated by effects on pathways responsible for sensing and responding to nutrient availability, including effects on the expression and activity of regulator of cellular growth mTORC1.[94] Treatment with rapamycin, an inhibitor of mTORC1, improved lifespan and attenuated neurodegeneration in the *Ndufs4* knockout mouse model of LS,[97] supporting the notion that dysregulation of these pathways contributes to pathogenesis. More recently, improvement in mitochondrial cristae morphology was observed to modestly but significantly improve motor performance, lifespan and complex I activity in *Ndufs4* knockout mice,[98] suggesting that effects on mitochondrial morphology may additionally contribute to disease pathology.

Molecular defects of PDHc and OXPHOS may furthermore impair normal cell function through mechanisms distinct from energy generation *per se*. PDHc and OXPHOS enzyme defects can produce a cellular environment more conducive for the production of reactive oxygen species (ROS). Increased ROS have been observed in cellular models of PDHc and OXPHOS enzyme deficiencies,[99,100] and there is

evidence of increased oxidative damage to proteins in affected brain regions of the *Ndufs4* knockout mice.[101] However these results have been inconsistent,[102,103] and increases in ROS production do not always correlate with neurodegeneration.[104] A possible alternative explanation could be that elevated ROS production is simply reflecting a highly reduced state of the respiratory chain. This typically leads to a partial block in NADH oxidation, with a concomitant decrease in the NAD^+/NADH ratio.[105] This redox imbalance may lead to dysregulation of the signaling pathways related to nutrient sensing referred to earlier.[94,105] NAD metabolism varies in different brain regions[106] and could potentially contribute to different susceptibility of brainstem and basal ganglia in conditions such as LS. Drugs such as nicotinamide riboside that can boost NAD synthesis or PARP inhibitors that can block NAD degradation are thus promising agents for treating mitochondrial diseases.[107,108]

Alleviation of oxidative stress has therefore emerged as a promising avenue of therapy for LS and other mitochondrial diseases, including the development of antioxidants EPI-743 and RP103, which are currently being examined in clinical trials.[109] Increased ROS are also implicated in the activation of inflammatory pathways which may in turn alter mitochondrial function and produce a state of chronic stress that further compromises cellular function.[110,111] The contribution of oxidative stress to disease pathogenesis may therefore be multifaceted.

Another relevant consequence of the molecular defects observed to cause LS is elevation of lactate.[2] Increased lactate levels reflect a greater utilization of glycolysis for energy generation, and may contribute to lesion development in LS patients, including through alterations of pH when lactic acidosis ensues.[16] An observed

correlation between CSF lactate levels and disease severity in LS patients[6] supports the pathological significance of lactate. Interestingly, attenuation of lesion development and neurological decline in *Ndufs4* knockout mice following rapamycin treatment was suggested to in part reflect a reduction in toxic glycolytic metabolites within the brain.[97] Significantly, this study also demonstrated that neurological improvement and an increase in lifespan could be achieved independently of an effect on the primary defect in complex I assembly. This supports the role of broader cellular dysfunction in disease pathogenesis, and the validity of targeting these pathways therapeutically. Characterization of the precise molecular mechanisms driving disease pathology could therefore have great utility for the development of new therapies.

Conclusion

Like other mitochondrial diseases, there is currently no effective treatment for LS as a group.[112] However there are several genetic and biochemical forms of LS that can benefit from therapeutic intervention, emphasizing the importance of determining the molecular basis of disease in LS patients. Furthermore, confirmation of the genetic basis of disease enables access to accurate genetic counseling and assisted reproductive technologies. MPS has transformed the approach for determining the genetic basis of LS, and therefore has the potential to markedly improve diagnostic and clinical outcomes for patients. Consolidating our understanding of the cellular mechanisms underlying disease pathology is important for enabling the development of novel therapeutics.

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Author contributions

All authors contributed to reviewing publications on the genetics, clinical and pathological features of Leigh syndrome and on mechanisms of disease causation. All authors contributed to drafting and review of the manuscript.

Potential Conflicts of Interest

Nothing to report.

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Figure 1: The development of our understanding of Leigh syndrome. Timeline depicting significant advances in the understanding of the biochemical and genetic basis of Leigh syndrome. The graph represents the number of new LS disease genes identified in each year relative to the timeline. The coloring represents the location of the gene, either on the X-chromosome (green), autosome (red), or mitochondrial DNA (blue). Abbreviations: CI, CII, CIII, CIV and CV refer to Oxidative Phosphorylation Complexes I, II, III, IV and V, respectively; CoQ_{10}, Coenzyme Q_{10}; LS, Leigh syndrome; MPS, Massively Parallel Sequencing; PDHc, Pyruvate Dehydrogenase Complex.

Figure 2: The biochemical basis of Leigh syndrome.

Leigh syndrome (LS) is caused by a genetic impairment of the mitochondrial pathways of energy generation. Briefly, pyruvate is metabolized by pyruvate dehydrogenase (PDHc) to produce acetyl coenzyme A (acetyl CoA), which is utilized by the citric acid cycle (TCA) to produce electron donors for the pathway of oxidative phosphorylation (OXPHOS). OXPHOS is performed within the mitochondrial inner membrane by five multi-protein complexes, known as complexes I to V, and electron carriers coenzyme Q (CoQ_{10}) and cytochrome *c* (Cyt *c*). Complexes I-IV, often referred to as the respiratory chain or electron transfer chain, direct the flow of electrons provided by the TCA cycle through the multi-protein complexes. CoQ_{10} mediates electron transfer between complex I and complex II to complex III, while Cyt *c* transfers electrons from complex III to complex IV. The respiratory chain utilizes the energy produced by this electron transfer to pump protons (H^+) through complexes I, III and IV into the intermembrane space, generating a proton gradient

that can be harnessed by ATP synthase (complex V) to drive the synthesis of ATP. Deficiency of PDHc, complexes I-V, and CoQ_{10}, can cause LS.

Figure 3: Genotype-phenotype correlations with respect to disease course. Box and whisker plot representation of the ages of onset, death or last report for patients with mutations in *NDUFS4, NDUFV1* (complex I deficiency)*, SURF1, PET100* (complex IV deficiency)*, SUCLA2* and *MTFMT* (combined OXPHOS deficiency) as reported in the literature. Genotype-phenotype differences in disease course can be observed between the genetic and biochemical forms of disease. Data points are represented as: error bars depicting minimum and maximum, the box edges depicting the 1st and 3rd quartiles, with the line within the box representing the median. Number of patients for which information was available: *NDUFS4*- 21, *NDUFV1*- 12, *SURF1*- 21, *PET100*- 10, *SUCLA2*- 31, and *MTFMT*- 12.

Table 1: Genes in which mutations cause Leigh syndrome or Leigh-like syndrome. ΔmtDNA = mtDNA deletion. X-linked inheritance indicated by [#], maternal inheritance by [*], ^indicates sporadic inheritance; all other genes are associated with autosomal recessive inheritance. Supplementary Table 1 contains additional information on the role of each gene product.

Biochemical deficiency	Genes
Pyruvate dehydrogenase	*PDHA1*[#], *PDHB*, *PDHX* , *DLAT*, *DLD*, *LIPT1*, *LIAS*, *TPK1*, *SLC19A3*, *SLC25A19*
Complex I	*MTND1*[*], *MTND2*[*], *MTND3*[*], *MTND4*[*], *MTND5*[*], *MTND6**, *NDUF1V, NDUFV2, NDUFS1*, *NDUFS2*, *NDUFS3*, *NDUFS4*, *NDUFS7*, *NDUFS8*, *NDUFA1*[#], *NDUFA2*, *NDUFA9*, *NDUFA10*, *NDUFA12*, *NDUFAF2*, *NDUFAF5*, *NDUFAF6*, *FOXRED1*
Complex II	*SDHA*, *SDHAF1*
Coenzyme Q_{10}	*PDSS2*
Complex III	*UQCRQ, BCS1L, TTC19*
Complex IV	*MTCO3*[*], *NDUFA4*, *SURF1*, *COX10*, *COX15*, *SCO2*, *PET100*, *LRPPRC*, *TACO1, ETHE1*
Complex V	*MTATP6*[*]
OXPHOS ± PDHc	*HIBCH, ECHS1, SERAC1, AIFM1*[#]
Biotinidase	*BTD*
Combined OXPHOS defects - Pathway affected	
Mitochondrial DNA maintenance	*FBXL4*, *POLG*, *SUCLA2*, *SUCLG1*
Mitochondrial translation	ΔmtDNA[^], *MTTI*[*], *MTTK*[*], *MTTL1*[*], *MTTV*[*], *MTTW*[*], *MTFMT, GTPBP3*, *TRMU, EARS2*, *FARS2*, *IARS2, NARS2*, *GFM1*, *GFM2*, *TSFM, C12orf65, PNPT1*

Table 2: Available therapeutic options for Leigh syndrome patients, and the genetic and biochemical forms that can respond to treatment. Apart from targeted therapies, all LS patients can be offered treatment for symptoms such as acidosis, seizures, dystonia or cardiomyopathy. It is also important to ensure good nutrition, aggressive management of intercurrent illnesses and caution with anesthesia.[113]

Gene or biochemical form	**Treatment**
Coenzyme Q_{10} deficiency (*PDSS2*)	Coenzyme Q_{10}
Ethylmalonic encephalopathy (*ETHE1)*	Metronidazole and N-acetylcysteine
Biotin/thiamine-responsive basal ganglia disease (*SLC19A3)*	Thiamine and high dose biotin
TPK deficiency (*TPK1*)	Thiamine
Biotinidase deficiency (*BTD*)	Biotin
Pyruvate dehydrogenase deficiency (*PDHA1*)	Thiamine and high-fat diet

Table 3: Characteristic Leigh syndrome disease features, affected ethnicities and founder genotypes associated with the most common nuclear disease genes (all with ≥ 10 patients reported).				
Gene and biochemical defect		**Characteristic disease features**	**Ethnicities notably affected**	**Founder / frequent genotypes**
NDUFS4	CI	'Typical' LS disease course with death by 3 years	Ashkenazi Jews	c.462delA with an estimated frequency of 1:1000[25,113]
SURF1	CIV	Hypertrichosis, cognitive sparing	(Pan-ethnic)	c.311_312insATdel10 in Caucasian Europeans[35]
LRPPRC	CIV	Mild facial dysmorphism, liver pathology and a course punctuated by episodes of acute metabolic decompensation	Saguenay-Lac-Saint-Jean region of Quebec, Canada	c.1119C>T, p.A354V with an estimated carrier frequency of 1:23[3,37]
SCO2	CIV	Hypertrophic cardiomyopathy, spinal muscular atrophy pattern of histopathology in skeletal muscle	(Pan-ethnic)	c.1541G>A, p.E140K[39]
PET100	CIV	Seizures	Lebanese	c.3G>C[114]
ETHE1	CIV	Ethylmalonic aciduria, acrocyanosis, petechiae, gastrointestinal involvement	Mediterranean basin and Arabian peninsula regions	c.487C>T, p.R163W[38]
SUCLA2	CI, III, IV &V	Methylmalonic aciduria, dystonia, deafness, often Leigh-like presentation,	Faroese Islanders	c.534+1G>A with an estimated carrier

		cerebral atrophy, onset in early infancy with long disease duration		frequency of ~1:33 people[4,115]
MTFMT	CI, III, IV &V	Microcephaly frequently observed	Europeans	c.626C>T with an estimated carrier frequency of 1:100 in Europeans[51]
SERAC1	Multiple	Hypoglycemia, liver involvement, behavioral disturbance	(Pan-ethnic)	-
PDHA1	PDHc	Lactate:pyruvate ratio <20, agenesis of corpus callosum frequently observed	(Pan-ethnic)	-
PDHX	PDHc	Lactic acidosis crisis in newborn or in early infancy, cerebral palsy-like presentation	Roma children from Bulgaria	c.1336C>T, p.R446*[116]

Supplementary Table A: Leigh syndrome disease genes that directly affect the structure, assembly, stability or activity of the pyruvate dehydrogenase complex (PDHc) and OXPHOS enzymes. Pathogenic mutations in these genes have been described in patients diagnosed with Leigh or Leigh-like syndrome, or in patients with a reported presentation that is compatible with Leigh or Leigh-like syndrome. Mitochondrial DNA-encoded genes are denoted with the MT- prefix. [#]with additional deficiency of alpha-ketoglutarate dehydrogenase (α-KGDH) and branched chain alpha-ketoacid dehydrogenase (BCKDH) and *additional deficiency of the glycine cleavage enzyme system. [†]PDHc deficiency only observed in absence of thiamine pyrophosphate (TPP) in assay medium. AR = autosomal recessive. Appropriate references can be obtained from the OMIM entry for each gene.

Biochemical deficiency	Gene	Protein function	Inheritance
Pyruvate dehydrogenase	*PDHA1*	*Subunit*	X-linked
	PDHB	*Subunit*	AR
	PDHX	*Subunit binding protein*	AR
	DLAT	*Subunit*	AR
	DLD[#]	*Subunit*	AR
	LIPT1[#]	*Co-factor attachment*	AR
	*LIAS**	*Co-factor synthesis*	AR
	TPK1[†]	*Co-factor synthesis*	AR
	SLC19A3[†]	*Co-factor transporter*	AR
	SLC25A19[†]	*Co-factor transporter*	AR
Complex I	*MTND1*	*Subunit*	Maternal
	MTND2	*Subunit*	Maternal
	MTND3	*Subunit*	Maternal
	MTND4	*Subunit*	Maternal

	MTND5	*Subunit*	Maternal
	MTND6	*Subunit*	Maternal
	NDUFV1	*Subunit*	AR
	NDUFV2	*Subunit*	AR
	NDUFS1	*Subunit*	AR
	NDUFS2	*Subunit*	AR
	NDUFS3	*Subunit*	AR
	NDUFS4	*Subunit*	AR
	NDUFS7	*Subunit*	AR
	NDUFS8	*Subunit*	AR
	NDUFA1	*Subunit*	X-linked
	NDUFA2	*Subunit*	AR
	NDUFA9	*Subunit*	AR
	NDUFA10	*Subunit*	AR
	NDUFA12	*Subunit*	AR
	NDUFAF2	*Assembly factor*	AR
	NDUFAF5	*Assembly factor*	AR
	NDUFAF6	*Assembly factor*	AR
	FOXRED1	*Assembly factor*	AR
Complex II	*SDHA*	*Subunit*	AR
	SDHAF1	*Assembly factor*	AR
Coenzyme Q_{10}	*PDSS2*	*CoQ_{10} synthesis*	AR
Complex III	*UQCRQ*	*Subunit*	AR
	BCS1L	*Assembly factor*	AR
	TTC19	*Assembly factor*	AR
Complex IV	*MTCO3*	*Subunit*	Maternal
	NDUFA4	*Subunit*	AR
	SURF1	*Assembly factor*	AR
	COX10	*Assembly factor*	AR
	COX15	*Assembly factor*	AR

	SCO2	*Assembly factor*	AR
	PET100	*Assembly factor*	AR
	LRPPRC	*mRNA stability*	AR
	TACO1	*Translational activator*	AR
	ETHE1	*Sulfide detoxification*	AR
Complex V	*MTATP6*	*Subunit*	Maternal

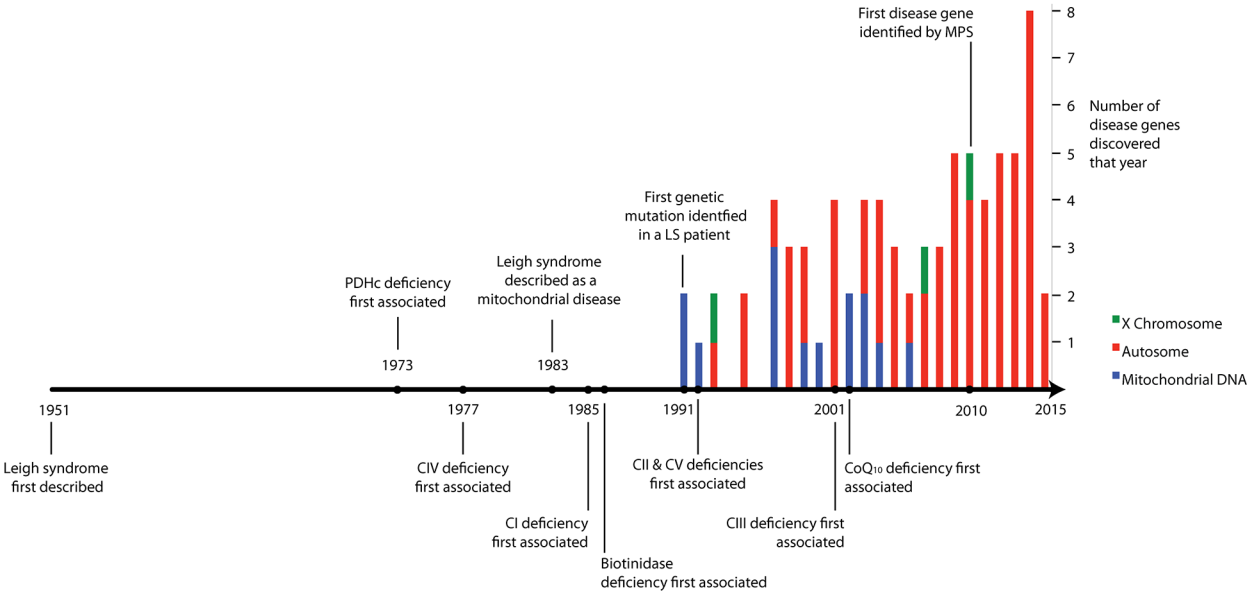
Supplementary Table A continued:

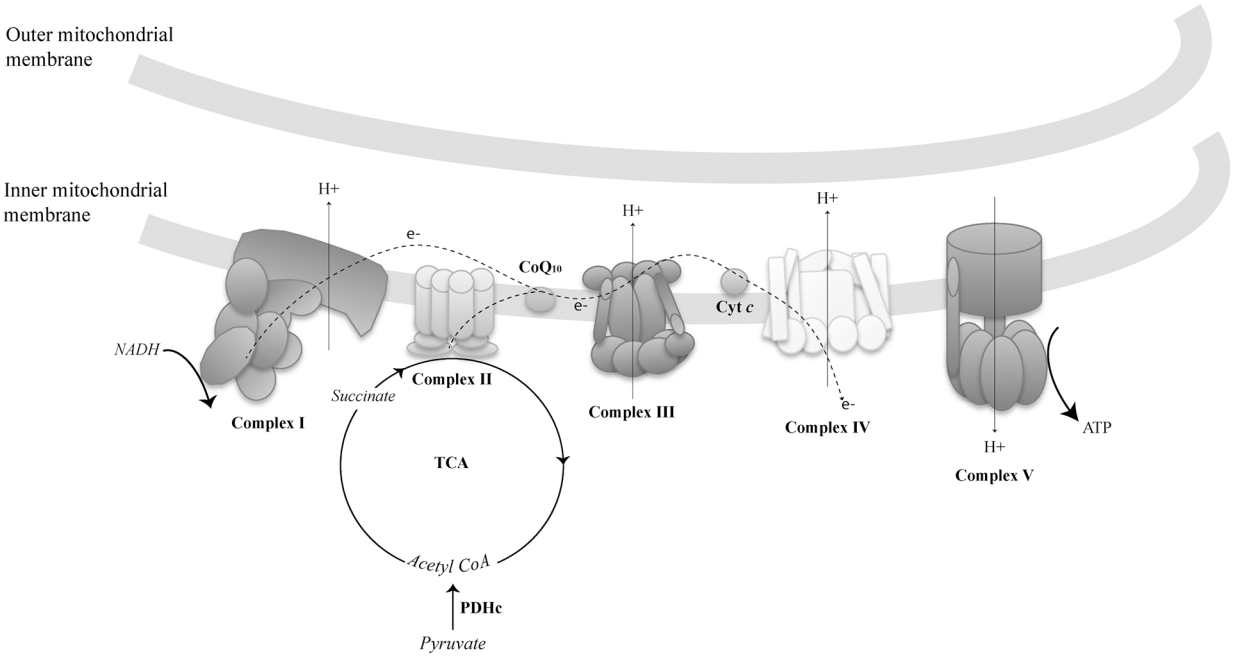
Biochemical deficiency			
Complexes I, III, IV &V			
Pathway affected	**Gene**	**Protein function**	**Inheritance**
Mitochondrial DNA maintenance	*FBXL4*	*(Unclear)*	AR
	POLG	*mtDNA replication*	AR
	SUCLA2	*Nucleotide synthesis*	AR
	SUCLG1	*Nucleotide synthesis*	AR
Mitochondrial translation	*mtDNA deletion*	-	Sporadic
	MTTI	*Mitochondrial tRNA*	Maternal
	MTTK	*Mitochondrial tRNA*	Maternal
	MTTL1	*Mitochondrial tRNA*	Maternal
	MTTV	*Mitochondrial tRNA*	Maternal
	MTTW	*Mitochondrial tRNA*	Maternal
	MTFMT	*tRNA modification*	AR
	GTPBP3	*tRNA modification*	AR
	TRMU	*tRNA modification*	AR
	EARS2	*tRNA aminoacylation*	AR
	FARS2	*tRNA aminoacylation*	AR
	IARS2	*tRNA aminoacylation*	AR
	NARS2	*tRNA aminoacylation*	AR

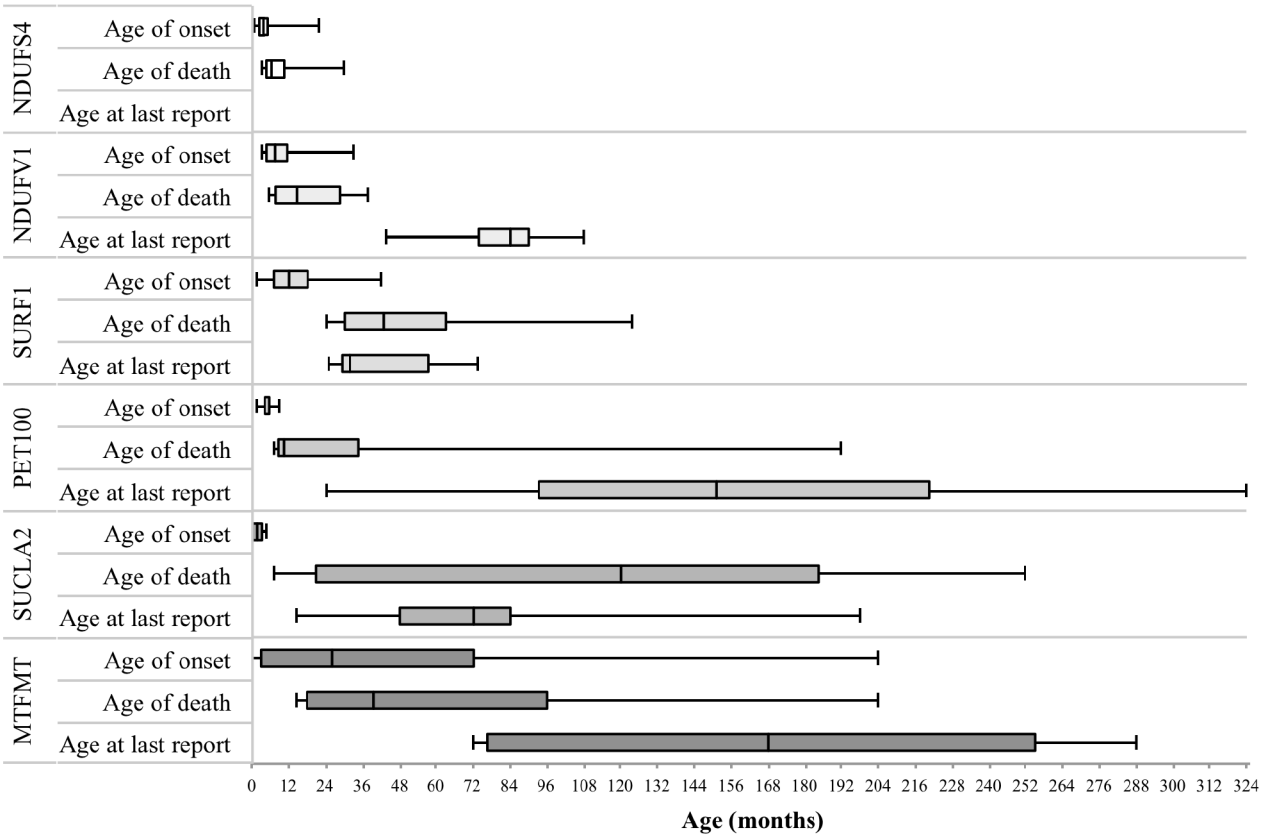
	GFM1	*Translation elongation*	AR
	GFM2	*Ribosome recycling*	AR
	TSFM	*Translation elongation*	AR
	C12orf65	*Mitochondrial peptide release*	AR
	PNPT1	*Mitochondrial RNA import*	AR

Supplementary Table B: LS disease genes that cause secondary impairment of mitochondrial energy generation.

Associated biochemical deficiency	**Genes**	**Protein function**	**Inheritance**
OXPHOS ± PDHc enzyme deficiency	*HIBCH*	*Valine catabolism*	AR
	ECHS1	*Valine catabolism*	AR
	SERAC1	*Phospholipid remodeling*	AR
	AIFM1	*Assembly factor/apoptosis*	X-linked
Biotinidase deficiency	*BTD*	*Biotin recycling*	AR


First disease gene identified by MPS
8
7
6
5
4
3
2
1
Number of disease genes discovered that year
First genetic mutation identfied in a LS patient
Leigh syndrome described as a mitochondrial disease
PDHc deficiency first associated
1973
1983
X Chromosome
Autosome
Mitochondrial DNA
1951
1977
1985
1991
2001
2010
2015
Leigh syndrome first described
CIV deficiency first associated
CI deficiency first associated
Biotinidase deficiency first associated
CII & CV deficiencies first associated
CIII deficiency first associated
CoQ10 deficiency first associated


Outer mitochondrial membrane
Inner mitochondrial membrane
H+
e-
CoQ10
e-
H+
Cyt c
H+
e-
NADH
Complex I
Succinate
Complex II
TCA
Acetyl CoA
PDHc
Pyruvate
Complex III
Complex IV
Complex V
H+
ATP


NDUFS4
Age of onset
Age of death
Age at last report
NDUFV1
Age of onset
Age of death
Age at last report
SURF1
Age of onset
Age of death
Age at last report
PET100
Age of onset
Age of death
Age at last report
SUCLA2
Age of onset
Age of death
Age at last report
MTFMT
Age of onset
Age of death
Age at last report
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180 192 204 216 228 240 252 264 276 288 300 312 324
Age (months)