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# Leigh's Syndrome

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Abreviations:
ECT – electron transport chain
MRI – magnetic resonance imaging
ATP – adenine triphosphate
ATPase – adenine triphosphate synthase
mtDNA – mitochondrial DNA
nDNA – nuclear DNA
EPR – electron paramagnetic resonance
CoQ10 – coenzyme Q10

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**Abstract**

Leigh's syndrome is a severe mitochondrial disease. It is an inherited disease that causes a disruption in cellular respiration *via* mutations in electron transport chain (ECT) components. Leigh's is a class I mitochondrial disease that involves defects directly in mitochondrial DNA (mtDNA). Leigh's patients suffer from severe neurological symptoms, muscular weakness, and social problems. Detection and diagnosis of the disease are made by classifying symptoms and characterizing mutations. There are a few treatments available to increase the quality of life, but no cure exists and pre diagnosis is very difficult. The most challenging aspect of the disease is the number of different mutations that are able to cause Leigh's. While many mutations can cause the disesase, recent results and the biology of the mutations suggest free radical production may play an important role in Leigh's.

**Introduction**

The mitochondria is the energy producing organelle of the cell. The mitochondria appears to be a primative bacteria that developed a symbiotic relationship with its host. Evidence for this lies in the fact that mitochondrial contains its own genetic information. Mitochondrial defects can cause severe symptoms in patients. This paper will focus on Leigh's syndrome. A brief overview of mitochondrial biology and the ETC will be provided to allow for a more insightful understanding of this disease. In addition, a brief overview of other mitochondrial diseases will be provided to display the many ways the mitochondria can be affected by mutation. The intricacies of Leigh's syndrome will be outlined with case studies provided to provide prospective on the severity and harshness of this disease. Lastly, a new frontier, free radical production, of the disease will be discussed.

**Mitochondrial genome**

The human mitochondria contains its own DNA which encodes a handful of proteins that are involved in the ECT. The greatest difference between mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) is mtDNA is circular while nDNA is a linear double helix. MtDNA is double stranded containing a heavy and light chain with no histone coat [1]. Each mitochondria contained in a cell may have 10 or more identical copies of its DNA. The mitochondria has its own DNA polymerase and repair machinery. The quality of this machinery is lower than in the nucleus, which equates to a higher mutational rate in the mitochondria [1]. It has been observed that mtDNA mutates

10-20 faster than nDNA due to inefficient repair and increase levels of reactive oxygen species in the mitochondria [2].

The human mitochondrial genome contains 37 gene products: 13 proteins, 2 rRNAs, and 22 tRNAs [2]. This is a modest size for a mitochondrial genome. The largest mitochondrial genome belongs to the protist *Reclinomonas americana*. Its genome consists of 97 gene products including 67 proteins [2]. The human mitochondrial gene products are all involved in oxidative phosphorylation and the ETC. The protein products include NADH dehydrogenase subunits (7) in complex I, cytochrome b of complex III, cytochrome oxidase subunits (3) of complex IV, mitochondrial ATPase subunits (2) of complex V [2]. All other mitochondrial proteins are coded for by nDNA, transcribed in the nucleus, translated in the cytoplasm and transported into the mitochondria [1].

**Electron transport chain**

The ETC passes electrons from NADH and $FADH_2$ produced from the citric acid cycle and glycolysis from complex I and II through complexes III and IV to the final electron acceptor, molecular oxygen. The molecular oxygen undergoes a 4-electron reduction and is converted to water. The overall reaction of cellular respiration (oxidative phosphorylation) is as shown below [3].

$$NADH + H^+ + \tfrac{1}{2} O_2 \longrightarrow NAD^+ + H_2O$$
$$FADH_2 + \tfrac{1}{2} O_2 \longrightarrow FAD + H_2O$$

($\Delta G^{o\prime\prime}$ values are –52.6 kcal/mol of NADH and –43.4 kcal/mol of $FADH_2$.) [3]

The mitochondria consists of a inner membrane and an outer membrane. The machinery of the ETC is integrated into the inner membrane. The machinery is step up to create a specific path for the transfer of electrons. Electrons from NADH enter the ETC at complex I when they are transferred to a flavin mononucleotide. The electron is then passed to coenzyme Q ($\Delta G^{o'}$= -16.6 kcal/mol). Coenzyme Q carries electrons from complex I and II to complex III. Electrons enter complex II *via* succinate and are then passed to coenzyme Q, which takes them to complex III. In complex III electrons are passed from cytochrome b to cytochrome c ($\Delta G^{o'}$= -10.1 kcal/mol). Ctyochrome c is a peripheral membrane protein that is bound to the outer face of the inner mitochondrial membrane. This protein carries electrons to complex IV, which is a cytochrome oxidase. The cytochrome oxidase transfers the electrons to the final electron acceptor, molecular oxygen creating water [4].

As electrons are passed along the chain, protons are pumped out of the mitochondria into the intermembrane space. This creates a buildup of positive charge in the intermembrane space. The buildup of protons, known as the proton motive force, is used to drive the synthesis of ATP. Protons rush down the electrochemical gradient from the intermembrane space through ATPases and into the mitochondria. The proton flow powers the ATPases and ultimately drives the synthesis of ATP [4].

**Classification of mitochondrial disease**

A scheme has been developed in order to classify mitochondrial defects based on their origin and function. The scheme is based on whether the gene is encoded in the

nucleus (nDNA) or mitochondria (mtDNA) and whether or not the gene product is involved in oxidative phosphorylation (cellular respiration) [2].

Class I is defined by a primary defect in oxidative phosphorylation and a defect in mtDNA. Mutations in any mtDNA product, protein, rRNA, or tRNA, are classified as class I. Class I contains 3 subclasses: (a) – large deletions or duplications in mtDNA, (b) – point mutations and small rearrangements of protein coding mtDNA, (c) – small mutations in mtDNA encoding rRNA and tRNA [2].

Class II is defined by mutations in nDNA encoding mitochondrial proteins that are imported into the mitochondria. Class II consists of 4 subclasses: (a) – mutations in nDNA that effects mtDNA transcription, translation or replication, (b) – defects in nDNA encoding mtDNA repair machinery, (c) – defective ETC subunit import, (d) – defective ETC subunit assembly [2].

**Overview of mitochondrial diseases**

The previous section mentioned a variety of different causes of mitochondrial diseases. The mitochondria is the 'power plant' of the cell and mutations and disorders in it lead to diseases with harsh symptoms. While this paper focuses on Leigh's syndrome there are many other mitochondrial-based diseases. The most common cause of mitochondrial disease is complex I deficiency. This accounts for one third of all cases of ETC deficiencies [5]. A handful of mitochondrial diseases are outlined below:

*Leber's hereditary optic neuropathy (LHON).* Class I mitochondrial disease. LHON represents the most common cause of male adolescent blindness. This disease is caused by mutations in mtDNA encoding subunits of complex I, NADH dehydrogenase (ND) [2].

*Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE).* Class II mitochondrial disease. MNGIE is caused by mutations in nDNA that encode the enzyme thymidine phosphorylase (TP). TP is a enzyme involved in the salvage of thymine in the cell. This disease is characterized by ptosis, gastrointestinal dismotility and lactic acidosis [2].

*Mohr-Tranebhaerg syndrome/ Deafness-dystonia.* Class II mitochondrial disease. Patients with this disease suffer from deafness-dystonia. The disease is caused by mutations to deafness/dystonia peptide 1 (DDP1). The consequence of these mutations is the disruption of the import of mitochondrial proteins [2].

*Iron storage disorder/ Friedreich's ataxia (FA).* Class II mitochondrial disease. FA patients suffer from progressive spinocerebellar ataxia, neuropathy, hypertrophic cardiomyopathy, and lack of tendon reflexes [1,2]. FA affects the amount of frataxin protein that is produced. This protein is involved in iron cycling. A lack of frataxin leads to a buildup of iron that can react with hydrogen peroxide *via* the Fenton reaction to produce hydroxyl radical [1,2].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot \quad [2]$$

The hydroxyl radical will readily attack and damage anything in its path including DNA, proteins and membrane lipids.

*Hereditary spastic paraplegia (HSP).* Class II mitochondrial disease. HSP is characterized by degeneration of the long axons of the central nervous system. Patients with HSP suffer from progressive weakness of lower limbs and may be mentally retarded. The exact cause of HSP is not well understood, but a common mutation in this disease involves the protein paraplegin, which is a nuclear encoded mitochondrial metalloprotease [1,2].

Table 1 lists the previously mentioned diseases and Leigh syndrome along with the mutations that are associated with the diseases.

**Table 1 [2]**

**Selected mutations associated with human mitochondrial disorders**

| Disorder | Affected protein | DNA change | Protein change | Evolutionary conservation | Structural environment |
|---|---|---|---|---|---|
| | | | | | |
| LHON | ND4 | G11778A | R340H | Absolute | N/A |
| | ND1 | G3460A | A52T | Slight | N/A |
| | ND6 | T14484C | M64V | Moderate | N/A |
| | | G14459A | A72V | High | N/A |
| Leigh syndrome | ATP6 | T8993G | L156R | Absolute | Subunit interface |
| | PDHA1 | C892G | R263G | Slight | Near active site |
| | SURF1 | G385A | G124E | Absolute | N/A |
| | | T751C | I246T | High | Predicted β-sheet |
| | SDHA | C1684T | R554W | High | Surface-exposed |
| MNGIE | TP | G1419A | G145R | High | Near active site |
| | | G1443A | G153S | Absolute | Near active site |
| | | A2744G | K222S | Absolute | Near active site |
| | | A3371 | E289A | Absolute | Not in active site |
| Deafness-dystonia | DDP1 | T151del (1 nt) | Truncation - see text | High | N/A |
| | | A183del (10 nt) | Truncation - see text | High | NA |
| | | C198G | C66W | Absolute | N/A |
| Iron-storage | ABCB7 | ATT → ATG | I400M | High | Predicted tight turn |
| HSP | Paraplegin | 784del (2 nt) | 60% truncated | High | N/A |
| | | 2228ins (1 nt = A) | 7.2% Truncated | Moderate | N/A |

ABCB7, ATP-binding cassette, subfamily B, member 7; ATP4, adenosine triphosphate synthase subunit 4 (ATP6 - subunit 6); DDP1, deafness-dystonia peptide 1, del, deletion; HSP, hereditary spastic paraplegia; ins, insertion; LHON, Leber's hereditary optic neuropathy; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; N/A, not available; ND, NADH dehydrogenase; ND1 ... NDn, ND subunits 1 ... nt, nucleotide; PDHA1, pyruvate dehydrogenase subunit E1α; SDHA, succinate dehydrogenase subunit A; SURF1, surfeit locus protein 1; TP, thymidine phosphorylase.

**Leigh's syndrome**

Leigh's syndrome is a rare inherited disorder that causes the degredation of the central nervous system, most notably the brain spinal cord and optic nerve [6]. The disease can present itself from infancy to adolescence and display a heterogenous combination of characteristics and symptoms [7].

***Symptoms*** – The symptoms of Leigh's are associated with progressive neurological deterioration. Initial symptoms appear as loss of appetite, vomiting, irritability and seizures [6]. As the disease progresses the symptoms get more extreme such as loss of motor skills, weakness, oculomotor abnormalities, ventilatory dysfunction, kidney failure and cognitive and behavioral dysfunction [7].

***Characteristics*** – Leigh's exhibits many observable disease characteristics. The disease is primarily inherited in an autosomal recessive manner, but it has also been noted that it has been inherited autosomal dominantly, X-linked recessively and mitochondrially [6]. Leigh's displays pathological characteristics such as spongiformnecrosis, myelin degeneration, vascular proliferation, and gliosis in the brainstem and spinal cord [7]. Also associated with Leigh's is developmental delay or regression, lactic acidosis, high blood lactate, and symmetrical lesions in the basal ganglia [8].

**Leigh's case studies**

Leigh's has been a well-documented disease and a variety of case studies are available. A look at the characteristics and clinical symptoms of actual patients illustrates the true destructive nature of the disease.

*Case 1* – The disease manifested at 1 month of age in this patient. He was irritable and had difficulty eating. At 3 months the patient was unresponsive to visual stimuli and suffered from seizures. The white matter of the brain displayed signs of mitochondrial energy depletion as determined by MRI. At 4 months of age the patient was blind, irritable, had difficulty swallowing and experienced increase frequency of seizures. By six months of age he was placed on a mechanical ventilator. He slipped into a coma and died days later. A 9-base pair deletion was discovered in the mitochondrial genome at the COXII – ATPase 6 junction and his mitochondrial DNA level was less than 15 percent of normal levels [7].

*Case 2* – A healthy woman, who had previously given birth to two healthy children, gave birth to a daughter that died in less than 5 months from Leigh's syndrome. The child displayed high blood lactate, basal ganglia lesions, severe central hypotonia and encephalopathy. While this case is similar to case 1, the notable exception is the request by the mother to have a prenatal diagnosis performed on her following pregnancy. The results and relevance of this diagnosis will be discussed in the *Detection/Diagnosis* section [9]. Case 2 is another example of the quick and cruel nature of the disease.

*Case 3* – This case sheds a ray of hope on the battle against Leigh's syndrome. Two sisters that were diagnosed with Leigh's also suffered from coenzyme Q10 (CoQ10) deficiency. In this case the sisters responded well to CoQ10 supplementation. Sister 1 was diagnosed with Leigh's at age 4. She displayed muscle wasting, became deaf and was unable to undergo puberty. At age 19 she moved with slow, uncoordinated movements, suffered from brisk knee jerks and atrophy of the leg muscles. At age 24 she

became unsocial, lost weight and became bedridden. At this time she began treatment of CoQ10 supplementation. In only 15 days she regained her appetite and her ability to walk. In the next five years she grew, gained weight and entered puberty. Her social interactions and coordination also improved. A more in depth report of the CoQ10 treatment will be discussed in the *Treatment* section [8].

Sister 2 also displayed many typical symptoms of Leigh's throughout her life. She was mentally retarded, experienced a growth delay, deafness, muscle atrophy and facial dymorphisms. She also received CoQ10 treatment at age 31. For unknown reasons her treatment was discontinued after only 6 six weeks. In the short treatment time she experienced improvements in growth and social behavior [8].

**Diagnosis/Detection**

Unlike many disease we are familiar with there is not direct test to diagnose a patient with Leigh's. Most cases are diagnosed by linking together various symptoms and characteristics of the patient. While this is common with many diseases the severity of Leigh's would favor a prenatal diagnosis or at least early enough detection to offset some of the more severe symptoms, if possible. What makes diagnosis so difficult is the wide variety of possible mutations and symptoms for any one disease. Some attempts have been noted in the literature such as prenatal exclusion, microsatellite DNA genotyping and an immunocytochemical approach.

Prenatal exclusion was mentioned in case study 2. In this case the mother requested a prenatal test for mutations in mtDNA. In this case it specifically targeted the number of T8993C mutations [9]. This is done by extracting DNA from the chorionic

villus sample or the amniotic cells, amplifying the targeted region with specifically designed primers, digesting the PCR product with HpaII, that detects T-C or T-G mutations, and running the digestion product out on an agarose gel [9]. The rational behind this test is to identify a high mutation load and terminate the pregnancy before a Leigh's baby is brought into the world. While the method may be able to identify possible Leigh's positive births, it also raises many ethical questions.

Microsatellite DNA genotyping is another type of prenatal exclusion. This method uses microsatellite markers on the flanks of punitive disease loci to identify homozygous nonsense mutations [5]. In the case of Leigh's it has been used to identify mutations in the NDUF4 gene in patients with severe complex I deficiency [5].

The immunocytochemical approach is distinctly different from the previous two methods of detection. This method makes use of monoclonal antibodies to the proteins of the ETC. Antibodies were made to specific ETC proteins and then the samples were stained to detect absences, mutations or alterations in the presence of the proteins. This method was preformed using cell lines from patients that suffered from Leigh's [10]. This method appears to be a good technique to better understand the mechanisms of Leigh's and other mitochondrial diseases.

**Treatments**

Since Leigh's is a genetic disease there is no pharmalogical or therapeutic cure. There are a few treatments available to help diminish the severity and progression of the disease. One problem with treating Leigh's is the many different mutations (discussed in *Mutations* section) and abnormalities that cause the disease. This characteristic of the

disease makes a universal treatment impossible. The only possibility for a cure lies with the advancement of gene therapy to put back or repair mutated genes. The majority of current treatments involve altering patients' diets and supplementing them with vitamins and cofactors. In addition patients undergo physical, occupational and speech therapies ease their symptoms [11]. As mentioned earlier, treatment with CoQ10 was able to induce a large improvement in sisters suffering from Leigh's. The patients were given 300mg of CoQ10 per day. Improvements were observed in growth and social behaviors and one sister entered puberty. It was shown *in vitro* that the addition of CoQ10 increased the activity of complex II and III eightfold [8]. This was an encouraging result, but a bit misleading in the overall treatment of Leigh's because not every patient is COQ10 deficient. It appears that there will be no earth shattering developments in the treatment or cure of Leigh's until molecular biological technologies are able to efficiently perform gene therapy.

**Mutations that lead to Leigh's syndrome**

The most challenging aspect of Leigh's syndrome is the numerous mutations that cause the disease. Some of the more common mutatins are overviewed below.

***cytochrome c oxidase*** **–** The most common defect in Leigh's is cytochrome c oxidase (COX) deficiency [12]. COX is apart of complex IV and is responsible for transferring electrons to the final acceptor, molecular oxygen. A published case study of 100 patients with a clinical diagnosis of Leigh's revealed that 21 of the cases had specific enzyme defects. Of this group of 21, 15 involved COX, 4 involved the pyruvate dehydrogenase complex, one involved complex I and one involved complex II [13].

While COX defects are most common, this nuclear encoded gene has not been found to be mutated. Instead mutations have been identified in the SURF1 gene. SURF1 consists of 9 exons approximately 1kb in length and is located on chromosome 9p34 [12,14]. It encodes a 300 amino acid protein that is involved in COX biogenesis. Over 30 different mutations in this gene have been observed in Leigh's patients with the most frequent being a 312-321 deletion [12]. SURF1 mutate patients typically have COX activity reduced to 20 percent of normal levels [14]. All types of mutations (insertion, deletion, missense and nonsense) of SURF1 have been shown to induce COX deficiency and cause Leigh's syndrome [12].

***ATPase 6*** – The mitochondria is known as the 'powerhouse' of the cell. The cells primary source of energy, ATP, is produced by ATPases. Two of the subunits for this enzyme are coded for by mtDNA. Leigh's patients have recently been observed to carry a mutation in the ATPase 6 gene. Two different point mutations have been reported, T9176C and T8993G [15,16]. For a patient to show symptoms more than 60 percent of the mtDNA must be mutated [15]. When the mutation load is above 80 percent, ATPase activity has been shown to decrease over 70 percent [15]. Patients with this mutation do not suffer from symptoms as severe as COX defect patients. While the COX defects are directly involved in the transfer of electrons, ATPase mutations are involved in energy production and the flux of protons [15]. It appears that mutations in the ETC lead to more severe cases of Leigh's. This may suggest that the disruption of the flow of the ETC leads to the production of free radicals that produce additional, more severe damage in the mitochondria, while mutations in the ATPase are limited to energy depletion toxicity.

***SDH (complex II) –*** Mutations in succinate dehydrogenase (SDH) are a minor cause of Leigh's. While it is a minor cause the disease it can be linked to current research involving SDHC mutant cell lines and free radical production. At least three different mutations have been reported in the flavoprotein (Fp) subunit of complex II [17]. An Arg554Trp substitution, a one bp deletion the causes a frameshift, and an A to C transition that changes the initial methionine to leucine all have been identified in Leigh's patients [17,18]. These patients directly correlate with my current research with complex II mutants. My research has shown a five-fold increase in the production of superoxide radicals in the SDHC mutants. It has previously been noted in this paper that mutations that disrupt the flow of electrons produce more severe cases of Leigh's, than mutations only in energy production machinery. This strongly suggests that free radicals play a critical role in Leigh's syndrome.

**$O_2^{\bullet-}$ and SOD**

While there has been little work done to investigate the role of free radicals in Leigh's syndrome, a small study has been published supporting the role of free radicals in this disease. Samples were taken from normal patients and patients diagnosed with mitochondrial defects, including Leigh's. The investigators used the luminometric probe lucigenin to measure the amount of superoxide radicals produced [19]. Enzymatic activity assays were also performed to determine the activity of superoxide dismutase (SOD). The results of the experiment indicated that the level of superoxide radicals is slightly elevated in Leigh's. The SOD assay indicated that the level of MnSOD was not altered in Leigh's disease. The investigators also performed a northern blot to determine

mRNA levels of MnSOD. This experiment also indicated that the levels are not altered in Leigh's disease [19]. This experiment simply scratches the surface of what can be done to discover the role of free radicals in Leigh's disease. The increase in superoxide radicals that was observed is an encouraging result to build on.

**Identifying Free Radicals in Leigh's syndrome**

Free radicals are species with an unpaired electron. Leigh's disease is a good candidate to involve free radicals because of the disruption of the ETC. Defects in complex I or COX for example inhibits the flow of electrons. This creates electron leaks in the ETC. The leakage of free electrons into the mitochoindria creates free radicals. The free electrons react with nearby molecules giving them an extra electron and in most cases (excluding metals and molecules in need of electrons) producing a free radical. These free radicals can react to damage the cell themselves or produce products that are harmful to the cell.

There are many simple and straightforward experiments that can be performed to determine the involvement of free radicals in Leigh's. Tissues sample can be taken from patients to determine levels of SOD, catalase, gutathione peroxidase, NAD/ NADPH ratio and oxidized and reduced glutathione. An assay of these antioxidant proteins gives a good background to the oxidation state of the cell and may help to explain later free radical results and propose a mechanism to cell damage.

The measurement of superoxide radical can be done by dihydrethidine (DHE) staining. When oxidized DHE is converted to ethidium and intercalates into the DNA and fluoresces red at 585nm [20]. One nice feature of DHE is when it is oxidized it is not

able to enter the cell. This characteristic reduces background and produces more accurate measurements. Tissue samples can be harvested and snap frozen, and then treated with DHE for 30 min [20]. Images can be obtained using a laser scanning confocal fluorescence microscope [20]. DHE has been reported to be somewhat specific for the detection of superoxide radical [20]. While it is not completely specific, we have had encouraging results confirming the detection of superoxide. In cultured cells we have transfected MnSOD to suppress the amount of superoxide and the DHE signal has decreased, indicating that DHE is detecting superoxide radical. Leigh's patients can be compared to age, sex and ethnic matched normal patients to determine if superoxide radical levels are higher in Leigh's patients. This is a simple initial experiment that gives easily interpretable results. In order to convincingly determine that superoxide is present and being measured, some of the tissue sample must be examined by EPR. EPR is the only method in which one can directly identify the presence of a free radical. While EPR is not quantitative, it can confirm that superoxide radical exists in the sample.

Another experiment that can confirm the presence of free radicals in the measurement of 8-oxo-deoxy-guanosine (8-OHdG). 8-OHdG is an indicator of DNA damage due to the hydroxyl radical [21]. The hydroxyl radical oxidizes the guanine bases present in DNA at the eighth position [21]. Again this radical may be highly prevalent in Leigh's patients when compared to normal controls because of defects in the ETC and free leaking electrons. The most difficult aspect of this experiment is not oxidizing the samples while obtaining them. This is very important to the accuracy of the experiment. This experiment not only suggests the presence of free radicals, but also gives a assessment of the damage done by free radicals [21]. Once again the only way to

definitively determine the presence of free radicals is to perform EPR. Taking an EPR of this sample can confirm the presence of hydroxyl radical and strengthen the claim of free radical involvement.

There is strong evidence that free radicals are involved in this disease. The disruption of the ETC and leakage of electrons must produce free radicals. Plus the degenerative nature of the disease suggests harsh conditions, such as hydroxyl radicals, exist to damage the cell. Another proponent of free radicals involvement is the loss of mtDNA. Hydroxyl radicals are known to cause DNA breaks and damage. With further research it appears free radicals will come to the forefront of Leigh's syndrome.

**Summary**

Leigh's syndrome is an inherited mitochondrial disease. Its symptoms can be quite severe and degenerative such as various neurological symptoms, muscular weakness, and social problems. Detection and diagnosis of the disease can be made by characterizing symptoms and mutation pattern, however no cure exists. Very few successful treatments are available, but some such as CoQ10 have shown promise. The wide range of mutations and causes of the disease make it difficult to treat and nearly impossible to cure. Attempts have been made to identify the disease prenatally in order to identify Leigh's patients before birth. The role of free radicals has yet to be explored, but may prove critical in treatment of the disease. If the production of harmful free radicals can be identified, treatments can be designed to target and reverse such effects.

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