Leigh Syndrome

Referral

A 6-month-old female infant presented with a two-week history of irritation, crying spells, poor feeding, respiratory difficulties, and decreased muscle tone (phenomizer). Pregnancy and prenatal history were uncomplicated, with age-appropriate growth up to 3 months (Ruhoy and Saneto, 2014). No notable family history of neurological disorders. Upon examination, the patient exhibits significant developmental delays and poor strength in all extremities. Lab tests for full blood count (FBC), basic metabolic panel, liver enzymes, thyroid function tests, lactate ammonia, and calcium/potassium levels were tested and showed slightly elevated lactate and ammonia. Brain/spine MRI reveals basal ganglia and brainstem atrophy and demyelination. These preliminary and radiological findings suggest a genetic/ metabolic neurological disorder (Bonfante *et al.*, 2016). For a conclusive diagnosis, an electroretinogram is necessary (orpha.net) and further genetic analysis will include human whole exome scanning which is shown in **Table 1**.

Table 1 – This table shows the genes used in the mitochondrial genome sequencing to assess genes related to mitochondrial disorders (e.g., Leigh syndrome/ NARP). It also shows the genes used in the next-generation sequencing of a leukodystrophy panel. (Loeffen *et al.*, 1998; Mao *et al.*, 2015)

Genetic analysis	**Genes**					
Mitochondrial exome Sequencing	MT-ATP 6	SLC19A3	MT-ND5	SURF1	SCO2	PDHA1
Next generation sequencing	SPG11	ASAH1	GJB1	POLR3A	TUBB4A	

Aetiology

This 7-month-old female likely suffers from Leigh syndrome (LS) which involves neurodegeneration due to defects in mitochondrial metabolism and unmet ATP demands in the central nervous system (CNS). This leads to neural loss/death in subcortical regions. Generally, MRI scans show bilateral symmetric necrotic lesions typically extending from the basal ganglia and thalamus through the brain stem structures to the posterior columns of the spinal cord (Rahman, 2023). In LS symptoms start showing in infancy where the initial signs appear to be dysphagia, vomiting and diarrhoea. However, 6 months onwards symptoms such as neurological regression (E.G. intellectual disability, hypotonia, weakness), movement abnormalities (E.G. ataxia, dystonia), and they fail to thrive by age 7.

In most cases LS is inherited as an autosomal recessive trait however, in mitochondrial DNA mutation transmission its associated as a X-linked recessive and maternal inheritance (Thorburn, Rahman and Rahman, 1993). In these cases, LS can present itself through direct or low-level maternal mosaicism, where <5% of the variant is in the patient's tissues as mutation has occurred post-fertilisation (Ashhad and Feldman, 2020). Mosaicism will allow transmission to the embryo as a de novo mutation. There have been multiple studies to determine the prevalence of LS mutations in both hereditary and de novo cases, however large cohort case studies are difficult to construct with a substantial participation rate that can be representative of all populations globally.

Pathology

LS has more than 75 monogenic causes with the most common genes presented in **Table 1.** It is a disease that stems from the mitochondria specifically the process of oxidative phosphorylation (Ox Phos). This case study is looking at a patient with a mutation in MT-ATP 6 located between bases 8527 and 9207. The gene encodes ATP synthase F0 subunit 6 comprising of a proton channel also known as complex V. This is responsible for the hydrogen ion (H+) influx into the inner membrane of the mitochondria creating an electrochemical gradient. Conformational changes induced by the proton movements causes rotation of the associated c-ring and central stalk components (Jonckheere, Smeitink and Rodenburg, 2012). This mechanical rotation is coupled to

conformational changes in ATP synthase F1 subunit that catalyses ATP from ADP and inorganic phosphate at the matrix of the mitochondria. The most common mutations to date are found in m.8993T>G/C and m.9176T>G/C which covers a spectrum that varies between ataxia, NARP, bilateral striatal necrosis, to leigh or leigh like syndromes (Jonckheere, Smeitink and Rodenburg, 2012).

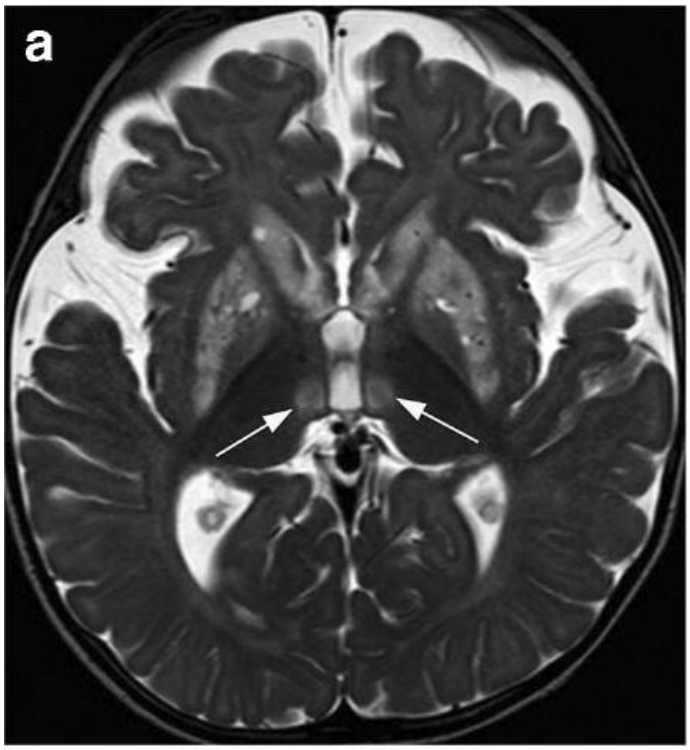

Figure 1 shows a 6-year old girls brain with leigh syndrome in acute phase, it's an axial T2 weighted image at the basal ganglia level that shows focal thalamic lesions at the arrows (Van Cauter *et al.*, 2020).

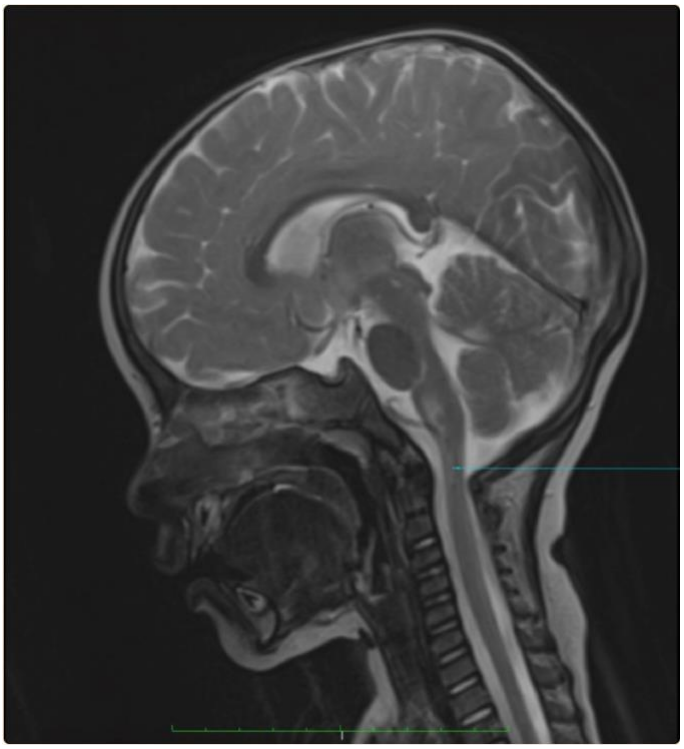

Figure 2 shows a 2-year-old presented with leigh syndrome, this is a T2W sagittal image showing the hyper intensity in the brain stem mainly seen in the posterior aspect of pons and anterior aspect of the medulla (Rahman, 2023).

Mutations the mutations that occur in ATP synthase subunit a cause the influx of H+ ions to declined which affects the ATP supply of the cell causing premature cell apoptosis. LS is a disease commonly found in the brain therefore neural cell death is irreversible. The apoptosis cause lesions and complications in the phenotype arise as disease symptoms. In this case study this happens as basal ganglia and brainstem bilateral lesions develop, which is similar to the lesion found in a 6-year-old female (Van Cauter *et al.*, 2020) shown in **Figure 1** where the child has focal thalamic lesions and 2-year-old MRI scans where the child has hyperintensity in the brainstem (pons and medulla) shown in **Figure 2.** Although these studies cannot be applied to the population that is affected by the disease the information provided applies to this patient. It's fair to conclude that the female patients' symptoms are acute however can advance quite rapidly.

Epidemiology

Primary mitochondrial disease (MD) is the most inherited metabolic disorder where the estimated prevalence is 1.6 per 5000 worldwide (Schlieben and Prokisch, 2020). The most common MD in children is LS where the estimated prevalence is ~1 in 40,000 (Alves *et al.*, 2020; Hong *et al.*, 2020; Lim *et al.*, 2022). However, there is no clear evidence in which ethnic or regional population LS is prevalent. While cases have been reported globally, certain mutations of the disease may cluster based on immigration patterns and ancestral mitochondrial lineages, but the data is limited. The rates of incidences differ marginally for autosomal recessive subtypes based on carrier frequencies studies in east Asia (Stenton *et al.*, 2022). A study conducted on 209 patients of Chinese ethnic backgrounds across 30 Beijing children hospitals showed that mutations in the gene MT-ATP6 were the most frequent. In **Figure 3** >25 patients presented with MT-ATP6 mutations than any other causational transmission in LS. The findings of this study were cross-referenced with large LS cohort studies conducted in Europe which include studies of UK (Lim *et al.*, 2022), Norway, Sweden, Finland (Sofou *et al.*, 2018), Italy (Stenton *et al.*, 2021). The comparison led to the conclusion that MT-ATP6 is a high-risk causal factor in LS making up 18% of all cohorts combined in not only East Asia but also in parts of Europe. Furthermore, age of onset has proven to have important implications for disease manifestations with late onset of LS generally seen in MT-ATP6 (m.9176T>C and m.8993T>C) are associated with disorders in movement and coordination and in earlier onsets (m.8993T>G) a trend in poor survival rates were observed however no significant association was calculated. A Russian study also concluded that out of the 219 patients that were involved in the study 25 (36.8%) presented in the MT-ATP6 gene (OMIM*516060) specifically in the variant m.8993T>C/G (Kistol *et al.*, 2023). Therefore, it seems likely that the patient may be presented with the early onset MT-ATP6 variant - m.8993T>G.

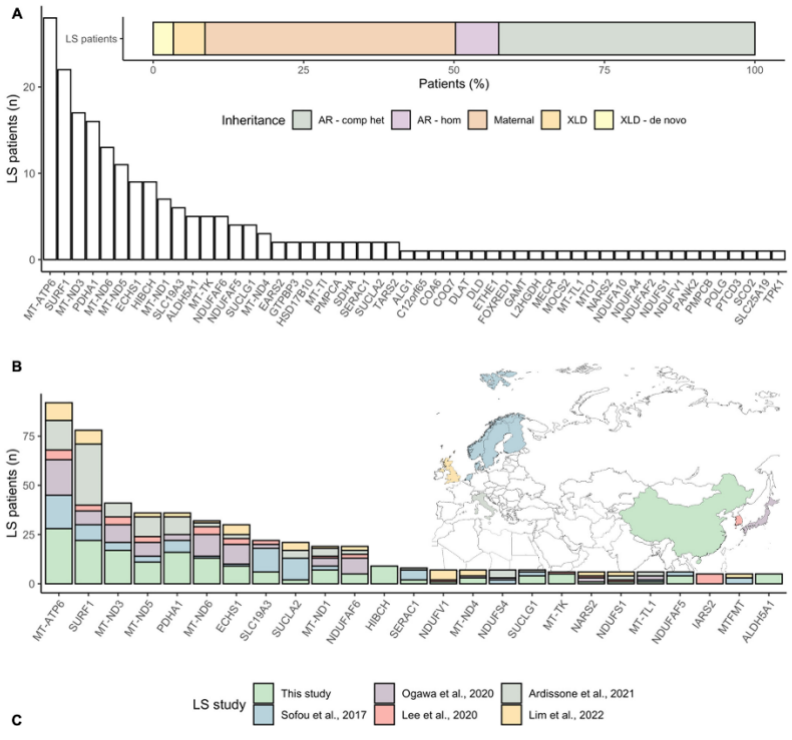

Figure 3 shows the frequency of molecular diagnosis in the cohort of (Stenton *et al.*, 2022) indicating the proportion of diagnosis by modes of inheritance. It also shows frequency of molecular genetic diagnosis in patients with LS reported by 6 most recent cohort studies across Europe and east Asia (Lim *et al.*, 2022; Sofou *et al.*, 2018; Stenton *et al.*, 2021).

Variant

This patient has presented with a de novo missense variant of NC_012920.1(MTATP6): m.8993T>G (p. Leu156Arg) in the gene MT-ATP6 (Uittenbogaard *et al.*, 2018; Richards *et al.*, 2015). However, as this is a very rare disease and only presents itself as often as 1 in 12,000 cases models to understand the disease have been developed, such as the use of yeast (Srivastava *et al.*, 2018), fruit flies (Scialo *et al.*, 2016), and human-induced pluripotent stem cells (hiPSC) to generate patient-specific stem cells for disease modelling (Meshrkey *et al.*, 2023). Studies have used yeast as a model showing the m.8993 T > G mutation that converts leucine at position 156 of human subunit a with arginine (aL156R) by mutating position 173 instead. Showing that the change from leucine to arginine causes a 90% drop in ATP production. The study's purpose was to revert the mutation and see the effects, although the effect was a positive one seen in a Saccharomyces cerevisiae model, it may not correlate itself to humans exactly (Su *et al.*, 2021). The mutation was also induced at a different position than m.8993T>G which could cause other effects that were not accounted for in this study. As this variant is in a highly conserved, hydrophobic, and non-polar area. This is because of the amino acid leucine at position 156 that causes these biochemical phenotypes to present however, when mutated to arginine which is a positively charged, hydrophilic amino acid, this transition is described as moderate radical with a Grantham score of 102. Additional salt bridges and hydrogen bonds can also form disrupting transmembrane proton channel function in the F0 subunit hindering the flow of protons across the mitochondria. (Senior, Nadanaciva and Weber, 2002). This then stops the rotation of F1 subunits required for ATP synthesis and causes a biochemical abnormality as blood lactate levels start to increase. Many papers and bioinformatic tools (**Table 2**) have identified a similar variant that is mainly associated with the late onset of LS also known as NARP (Ganetzky *et al.*, 2019).

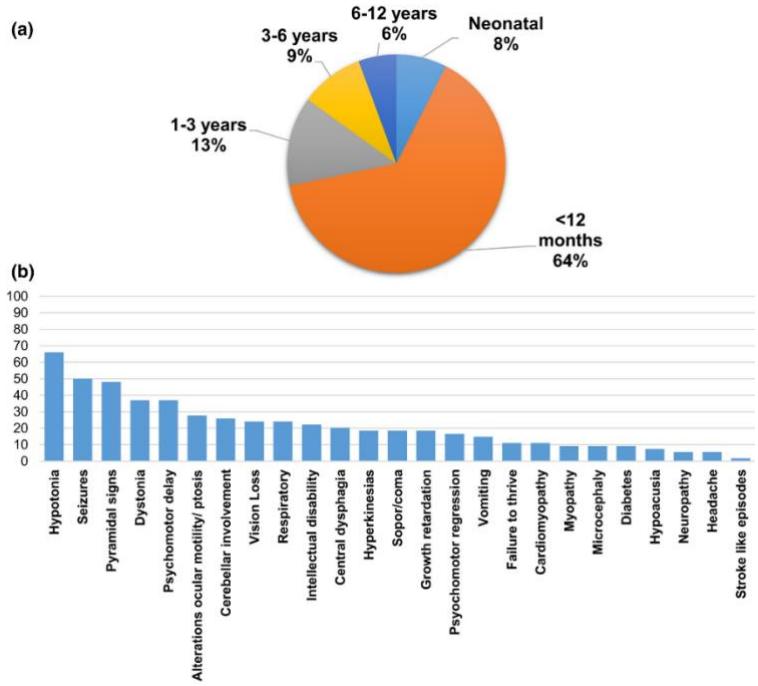

Figure 3 shows the age at onset distribution (a). Clinical findings of the study done by (Ardissone *et al.*, 2023) showing the number of patients that presented with each symptom associated with Leigh Syndrome (b).

The variant m.8993T>C (OMIM: 256000) has been shown in the gnomAD data based on the analyses of 141,456 unrelated individuals through a unified pipeline containing variants from 125,748 exomes and 15,708 genomes. ClinVar has suggested that this mutation changes the leucine to proline which affects the formation and stability of the overall protein structure of ATP synthase subunit a. Many studies and genetic sequences found in databases such as dbSNP and Clinvar have several accessions that evidence the pathogenicity of m.8993T>C/G mutations in patients aged from infancy to adulthood. These studies are from de novo and maternally inherited cases showed disease development and showed how the mutants for NARP and LS are pathogenic (Saneto, Patrick and Perez, 2021). Others meta-analysis studies haves showed that is both mutations heteroplasmy of the genes is a common determinant across ages and whether you have LS or NARP. Development of the disease early on would result in the heteroplasmy of >90% whereas for late onset of NARP heteroplasmy between 70-90% were commonly seen among the patients (Stendel *et al.*, 2020). Although there is a definite trend and relation to heteroplasmy that alone cannot predict the disease severity as there are other factors that affect the expression of the mutation and how it will present itself and where. As this prediction cannot be made treatment has proven difficult and so LS cannot be treated currently. However it may be that the diagnostic and identification of the gene mutations may need to be question.

Figure 4 shows all mutations that occur in homo sapiens, protein ATP synthase subunit a, gene MT-ATP6 generated by UniProt showing variant m.8993T>G pathogenicity (red dot on the yellow guideline). This is supported by ClinGen, Ensemble and dbSNP (Mishmar *et al.*, 2003; Lamminen *et al.*, 1995)

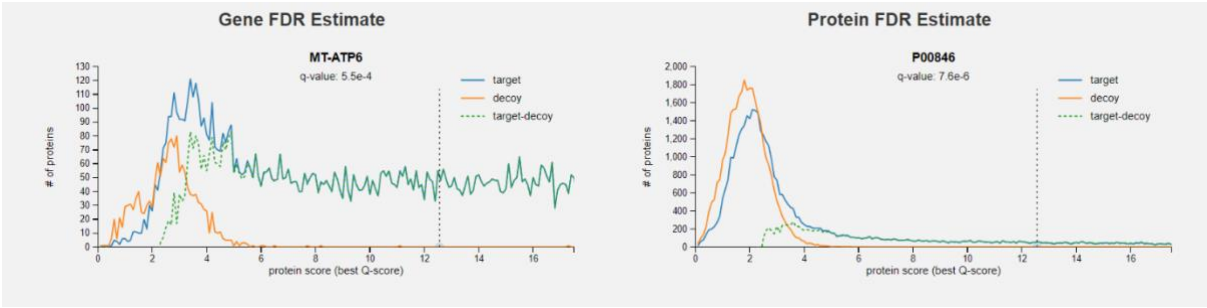

Figure 5 shows the false discovery rate (FDR) of the gene MT-ATP6 and the protein ATP synthase subunit a where the Q-score is <50 for target and decoy. FDR is shown to be low. Sourced from proteonomicsdb

The incidence and prevalence of LS is very low as it's a very rare disease that is also difficult to diagnose early as there is no screening test for this specific variant. There is also an issue that there are many variants that can cause different types of LS. Therefore proteomics dB was used to calculate and present the Q value of false discovery

rate (FDR) for the protein ATP synthase subunit a, and gene MT-ATP6 related diseases shown in **Figure 5**. MT- ATP6 and ATP synthase have a q-value of 5.5e-4 and 7.6e-6 showing a high statistically significance for the identification of this gene to be current and a low chance of false positive results to be observed. However, although the Q-values and scores shows there is a low FDR, there are no details on the exact computation that result in these score nor a description of the scale used for the target, decoy and target decoy genes and proteins used to determine these values. Therefore, all that can be concluded is that false results are low and all cases that have been diagnosed thus far are correctly identified and pathogenic as described in literature.

Although the FDA is statistically significant the cases of the variant are insignificant and many pathogenic effects of the disease variant m.8993 T > G are still yet to be discovered. Recent development has now led to using hiPSC's to correctly model the patient's mutation. This study specifically models MT-ATP6 mutations showing m.8993T>G to have 34% reduced maximum respiration than healthy controls. This models specificity to the mutation position can be used with CRISPER cas9 as genetic therapy as its predicted to have a moderate to high, 63-92%, Doench and Moreno-Mateos efficiency scores, suggesting good on-target editing efficiency. However, the Bae out of frame score of 54 implies that there may be a These models have translational value as any therapies that can improve the energy production and respiratory capacity in hiPSCs is likely to do the same in the patient. In addition these models that mimic the disease can differentiated into cell type models for LS pathology and serve as a drug testing (Meshrkey *et al.*, 2023). However, as there is variability in such models the results can be confound, additionally bioenergy profiles of hiPSCs may not fully reflect mature cell types that are affected in LS. This may cause problem these are used as genetic therapy tools.

The variant m.8993 T > G is complex and extremely understudied compared to other MT-ATP6 variants such and SURF1 which is abundant with treatments. in LS mitochondrial disease affected complex V the symptoms are managed and treated accordance to reducing acidosis and genetic counselling is recommended.

Table 2 shows the all-bioinformatics tools that were used to determine the variants classification. The links to those tools and descriptions (Richards *et al.*, 2015).

Bioinformatics tool	Link	Description
Genome browser – PhastCons package	hg38 100 vertebrates Basewise Conservati PhyloP (phyloP100way) (ucsc.edu)	Based on a hidden Markov model which estimates the likely hood that each nucleotide belongs to a conserved element by considering the entire alignment including neighbouring columns.
(gnomAD)	hg38 Genome Aggregation Database (gno Genome Variants (19e342bdbf0fe71a934f171571b7ccd1) (ucsc.edu)	gnomAD versions 2 and 3 have been used to map gnomes and exomes to the GRCh37/hg19 reference sequence and lifted to the GRCh38/hg38 this track package provides annotations, predicted pathogenicity metrics and measures of mutation constraints.
OMIM		Data base of human genes and genetic Conditions that also contains a representative sampling of disease – associated genetic variants.
ClinVar		Database of assertions about clinical significance and phenotype relationship of human variations.
Phenomizer	https://compbio.charite.de/phenomizer/	Used to help diagnose genetic diseases And cellular defects based on patients Observable characteristics.
Orphanet	https://www.orpha.net/consor/cgi-bin/index	Comprehensive information about rare Genetic diseases and orphan drugs for

		Improved diagnosis, care and Treatment.
Proteonomicsdb	Proteomics DB	Allows access to mass spectrometry Proteomics datasets. Allows for Comparisons and spectrum plotting
UniProt	https://www.uniprot.org/uniprotkb/P00846/	Provides details abouts protein names Taxonomy, sequence, function, Interactions, location, amino acid modifications and links to specialised Databases
SIFT (Sorting Intolerant From Tolerant)		Prediction of whether an amino acid Substitution affects protein function Based on sequence homology
PolyPhen-2 (Polymorphism Phenotyping v2)		Predictions based on analysis of Sequence conservation, structural Effects and evaluation of substation Against empirical rules coded by tool Developers.
VEP (Variant Effect Predictor)		Annotation from ensemble databases Enable rich contextual variant Interpretation. Assesses consequence type, impact Classifications and region data

Recommendations

Although the research on m.T8993G is limited the approach for treatment in LS patients is to upregulate mitochondrial biogenesis or preventing oxidative damage (Lightowlers, Taylor and Turnbull, 2015; Povea-Cabello *et al.*, 2021). These approaches mainly target AMP-activated protein kinase (AMPK), Sirtuins (Sirt1), and mammalian target of rapamycin complex 1 (mTORC1) pathways (Viscomi and Zeviani, 2020). These targets activate transcription factors with enhance mitochondrial biogenesis. Therefore, nicotinamide riboside (vitamin B3) is recommended to boost levels of NAD+, bezafibrate is recommended to be avoided as patient has muscle weakness and the medication can worsen the symptom. Vitamin B3 dosage will need adjusted.

The patient will be put on a ketogenic diet with intention to shift metabolism towards beta oxidation and ketone body production. Another intention is to increase transcription of OxPhos and glycolysis genes (Bough *et al.*, 2006). Sodium citrate should also be administered to clear the build-up of lactic acid. this may need to be given as an IV as well as essential nutrient for proper muscle development.

Conclusion

The patient has a rare neurodegenerative disease of leigh syndrome with a mutation of m.T8993G which hasn't been studied as often as other variants in this disease. There are many models that are currently being used to allow for better understanding of the disease however, currently with the lack of treatments the available to the patients have caused infant that develop the disease as early as 3 months old present with failure to survive although the false discovery rate of the disease is significantly low. Overall, treating the symptoms and managing diet to up regulate biogenesis and minimise any side effects is all the treatment medical research can offer at this time.

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