DIPLOMA

BIOINFORMATICS ANALYSIS OF MITOCHONDRIAL RELATIONSHIP TO NEUROLOGICAL DISEASES

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Βιοπληροφορική ανάλυση της σχέσης μεταξύ μιτοχονδρίων και νευρολογικών ασθενειών

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ABSTRACT

This thesis examined the relationships between mitochondrial functions and genes related to diseases with neurodegenerative effect like Alzheimer disease (AD), the Parkinson disease (PD) and Huntington disease (HD). Neurodegenerative diseases seem to be found in a large percentage of the general population and this raises our awareness to find the pathways that are implicated to these diseases. This research begins with the presentation of neurodegenerative diseases and their pathways under a framework of a correlation with mitochondria. Then it goes on to explain the main mitochondrial pathways mentioned in the first part of this study and continues to try to find a correlation between mitochondrial genes and genes that have been associated with some of the diseases analyzed. Finally, a comparison was made in pathways and gene level and some common findings were found, while some non-common ones could possibly be used later for further research that will help to find new possible targets for the treatment of these diseases.

ΠΕΡΙΛΗΨΗ

Σε αυτή τη πτυχιακή εργασία εξετάστηκαν οι σχέσεις που υπάρχουν μεταξύ των μιτοχονδριακών λειτουργιών και γονιδίων σχετιζόμενων με τις κύριες νευροεκφυλιστικές ασθένειες όπως το AD (νόσος του Alzheimer), το PD (νόσος του Parkinson) και το HD (νοσος του Huntington). Έγινε εστίαση σε αυτές τις ασθένειες επειδή φαίνεται να υπάρχουν σε μεγάλο ποσοστό του γενικού πληθυσμού και αυτό αυξάνει την ευαισθητοποίηση μας για να βρούμε τις οδούς που κάνουν αυτές τις ασθένειες να εμφανίζονται. Η έρευνα αυτή αρχίζει με την παρουσίαση των νευροεκφυλιστικών ασθενειών και των μονοπατιών τους, όπου έχει βρεθεί κάποια συσχέτιση με τα μιτοχόνδρια. Ακολούθως, συνεχίζει με την επεξήγηση των κύριων μιτοχονδριακών μονοπατιών τα οποία αναφέρονται στο πρώτο κομμάτι της έρευνας αυτής και συνεχίζει με την προσπάθεια εύρεσης συσχέτισης μεταξύ των μιτοχονδριακών γονιδίων και κάποιων γονιδίων τα οποία έχουν συσχετισθεί με κάποια από τις ασθένειες που αναλύθηκαν. Τέλος, έγινε μια σύγκριση (σε επίπεδο μονοπατιών και γονιδίων) και βρέθηκαν κάποια κοινά μεταξύ τους ενώ κάποια μη κοινά θα μπορούσαν να χρησιμοποιηθούν στη συνέχεια για περεταίρω έρευνα και εύρεση νέων πιθανών στόχων για αντιμετώπιση των ασθενειών αυτών.

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APP: Amyloid-beta precursor protein

ATP: Adenosine triphosphate

Drp1: Dynamin-related protein 1

ER: Endoplasmic reticulum

ETC: Electron transport chain

FIS1: Mitochondrial Fission 1 protein

HAP1A: Huntingtin-Associated Protein 1A

HK1: Hexokinase-1

HTT: Huntingtin

IMM: Inner mitochondrial membrane

InsP3R: Inositol 1, 4, 5- triphosphate receptor

LAS: Lysosomal autophagy system

MAM: Mitochondrial-associated membranes

MCU: Mitochondrial calcium uniporter

Mfn: Mitofusin

OMM: Outer mitochondrial membrane

IMM: Inner mitochondrial membrane

NFTs: Neurofibrillary tangles

NF-κB: Nuclear factor kappa- light- chain- enhancer of activated B cells

NMDAR: N- methyl- D- aspartate receptor

Nrf1/2: Nuclear respiratory factor 1/2

Opa1: Optic atrophy 1

OXPHOS: oxidative phosphorylation

PARL: MIM protease presenilin that is associated with rhomboid-like protein

PSD-95: Postsynaptic density 95

PTP: Permeability transition pore

RyRs: Ryanodine receptors

TFAM: transcription factor A, mitochondrial

VDAC: Voltage-Dependent Anion Channel

α-SYN: Alpha-Synuclein

INTRODUCTION

If we want to talk about a disease that affects the mitochondrion, at first, we have to know more about the mitochondrion and its functions that is analysed in chapter 13. It has been shown that mitochondria are derived from an organelle that was created due to the entry of an endosymbiotic alphaproteobacterium into a cell that was related to Asgard Archaea. This integration caused a big amount of changes such as the creation of many genes and a protein import system, insertion of membrane transporters, integration of metabolism and reproduction, genome reduction and also the endosymbiotic gene transfer (Roger et al., 2017).

Mitochondria are spherical organelles, consisting of a double membrane and their functions are important for the proper functioning and survival of mitochondria.

At first the OMM contains pores that allow molecules less than 5 kilodaltons to pass. Secondly the IMM contains the components of the ETC and the complex that is responsible for ATP synthesis. This membrane also has many folds that are called cristae, which increase its total area of the membrane. These two membranes creates two mitochondrial chambers, the intermembrane space that is placed in the middle of the both membranes, and the uterus which is inside the inner membrane, with the mtDNA to be inside the mitochondrial lattice (Chial et al., 2008).

The mtDNA is different from that of nuclear DNA. In humans the mitochondrial DNA includes 37 genes that are encoded by 16,569 base pairs. These 37 genes produces 13 polypeptides (ND1, ND2, ND3, ND4, ND4L, ND5, ND6, COX1, COX2, COX3, CYTB, ATP6 and ATP8), 22 tRNAs and two ribosomal RNAs. Those polypeptides are responsible for the creation of the OXPHOS complexes I, III, IV, and V, and they are critical for its activity. mtDNA also contains a noncoding region, D-loop (displacement loop), which holds all the mtDNA replication and transcription regulatory sequences (Yan et al., 2019).

ALZHEIMER DISEASE

Alzheimer, is the one of the main causes of dementia and it is characterized by the overproduction of peptide Aβ, that leads to increased fission and reduced fusion and as a result the production of mitochondria that are smaller and damaged, but also the accumulation of tau phosphorylated proteins in various brain areas. Especially in the hippocampus seems to affect the memory and the learning abilities (Zhang et al., 2019). Many studies shows that the scientific community is starting to change prospective about the factors that were given in the original amyloid hypothesis and give attention to age-related, protective, and disease-promoting factors that probably interact with the core mechanisms of the disease (Scheltens et al., 2016).

Amyloid-β peptide

Amyloid-β peptide (Aβ) is the main component of amyloid plaques that is produced by a median of 4 kilodalton fragments (Roher et al., 2017). Due to the splicing that happens to APP gene mRNA the APP protein has many isoforms. APP gene is expressed in almost all cells and a change in APP proteolysis results in accumulation of Aβ-fragments that associate in amyloid fibrils (Dovidchenko et al., 2014). Mutations in the presenilins that break down APP but also in the APP itself lead to a process called "amyloid cataract." This suggests that the disease occurs when APP is broken down by β-secretase to produce a C99 molecule, which is then broken down by γ-secretase. With the action of γ-secretase, it produces an intracellular area of 50-aa APP (AICD) as well as Ab fragments that have a median length of 40 amino acids in normal individuals, but 42 amino acids in Alzheimer, with a simultaneous increase in the ratio of Aβ42: Aβ40. Unlike Aβ40, Aβ42 is fibrinogen and you can find it inside the plaques. This disparity is lethal to cells that promote tau hyperphosphorylation resulting in AD symptoms (Area-Gomez et al., 2016).

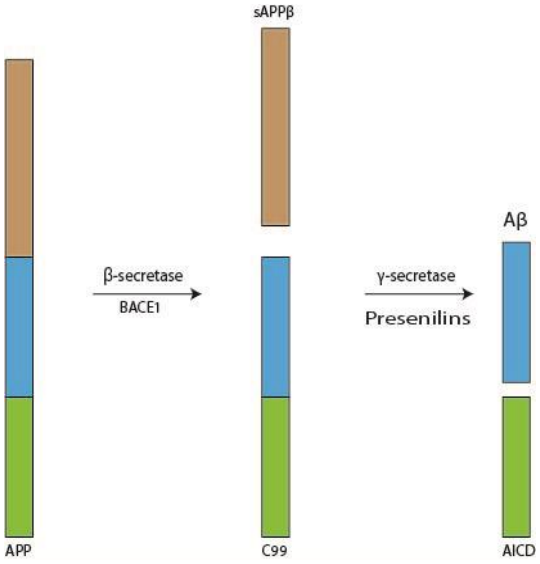


Figure 1: APP secretion by βand γ-secretase

With its production, the C99 moves to the endoplasmic reticulum, where it is broken down by the γ-secretase located in the MAM. In AD, however, this process is defective,

leading to the abnormal aggregation of C99 in the MAM, which associate with increased conversion of sphingomyelin to ceramidine from an increase of sphingomyelinase activity. Ceramidine then move to mitochondria and stops both the ETC assembly and the ATP production (Area-Gomez et al., 2019).

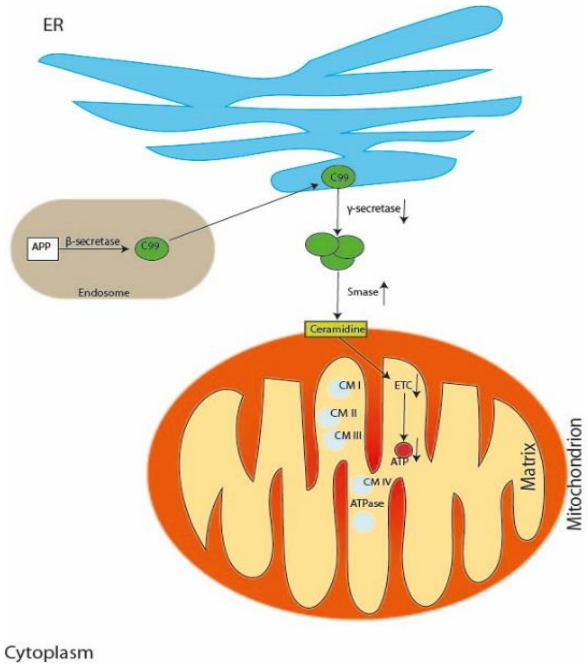


Figure 2: c99 secretion by β- and γ-secretase

Amyloid-β peptide and VDAC1

Aβ peptides can form protofibrils but also they can aggregate. The aggregated Aβ peptides interact with many receptors like the Voltage-dependent Anion Channels (plVDAC1) that is located at the mitochondrial membrane. As it was shown in cell membranes, pl-VDAC1 collaborates with Aβ oligomers in their N-terminal domain and it creates a pl-VDAC1-Aβ heterooligomer. These pores allow the peptides to get inside the cell and interfere with the mitochondrial VDAC1, causing the dissociation of the HK-I - VDAC1 complex with the formation of heteromeric Aβ-VDAC1 pores on the surface of mitochondria. The cytosolic Aβ peptide then interacts with the N-terminal domain of VDAC1 channel that is located on the mitochondria, leading to VDAC1 oligomerization and subsequent cytC release followed by the activation of the apoptotic pathway (Smilansky et al., 2015, Shoshan-Barmatz et al., 2018). Aβ peptides still have strong effects on mitochondrial processes, especially mitochondrial enzyme activity, reducing complexes III and IV activity and producing respiratory dysfunction by inhibiting

mitochondrial respiration. Aβ proteins also seems to activate and interact with signaling pathways, like NF-κB, which plays a major impact in inflammation, cell death and division (Arun et al., 2016).

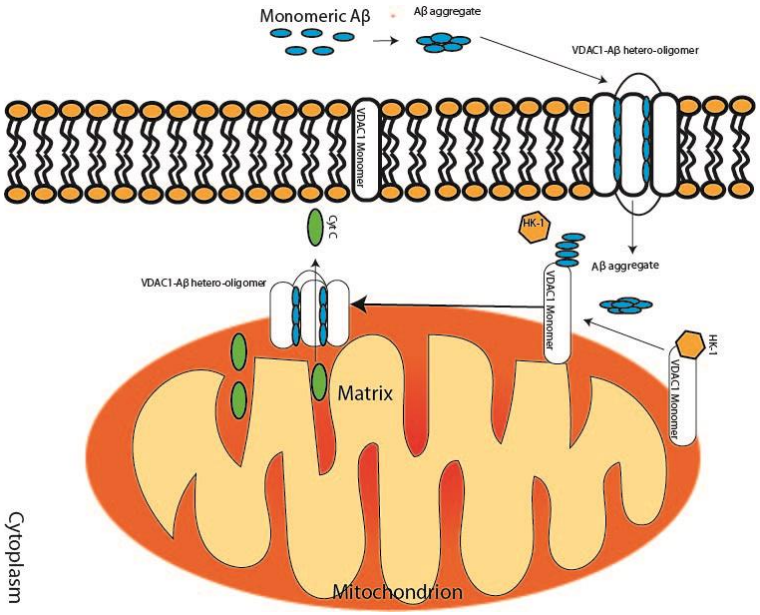


Figure 3: Amyloid-β peptide and VDAC1

Amyloid-β peptide and Ca^{2+}

Calcium movement in mitochondria is coordinated by the mitochondrial calcium uniporter, Na^{+}/ Ca^{2+} exchanger and the VDCA that was described before and the mitochondrial permeability transition pore (Sushma et al., 2019). ER calcium levels are increased in AD and in aging neurons. This increase in calcium concentration shifts the balance between calcium dependent phosphatase calcineurin (CaN) and its opponent calcium/ calmodulin-dependent protein kinase II (CaMKII), which are extremely abundant in synaptic locations and produces long-term potentiation, it is producing also long-term depression, causing synaptic loss and neurodegeneration (Pchitskaya et al., 2018). Aβ can activate NMDARs channels or create pores at the membrane of glutamatergic synapses, allowing the calcium to get inside the cell and in relationship with the activation of metabotropic receptors that release calcium from internal stores, it elevates the concentration of calcium inside the cell. After that more calcium travels inside the ER and mitochondria. Aβ triggers also the calcium to move from the ER, through the $InsP_3R$ and RyR to the plasma and with the help of VDAC (at OMM) and

MCU (at IMM) to mitochondria leading to the increase of calcium mitochondrial content. These peptides seems to reduce the entry of proteins that are encodes to the nucleus due to the interactions they have with complex TOM40, and also they create aggregates in the intramembrane space that affects the permability of both mitochondrial membranes. They also interact with the respiratory chain complexes IV and V at inner. Furthermore, Aβ may bind also to CypD promoting its translocation to the IMM, and then stops the activation of the mPTP (Naia et al., 2017).

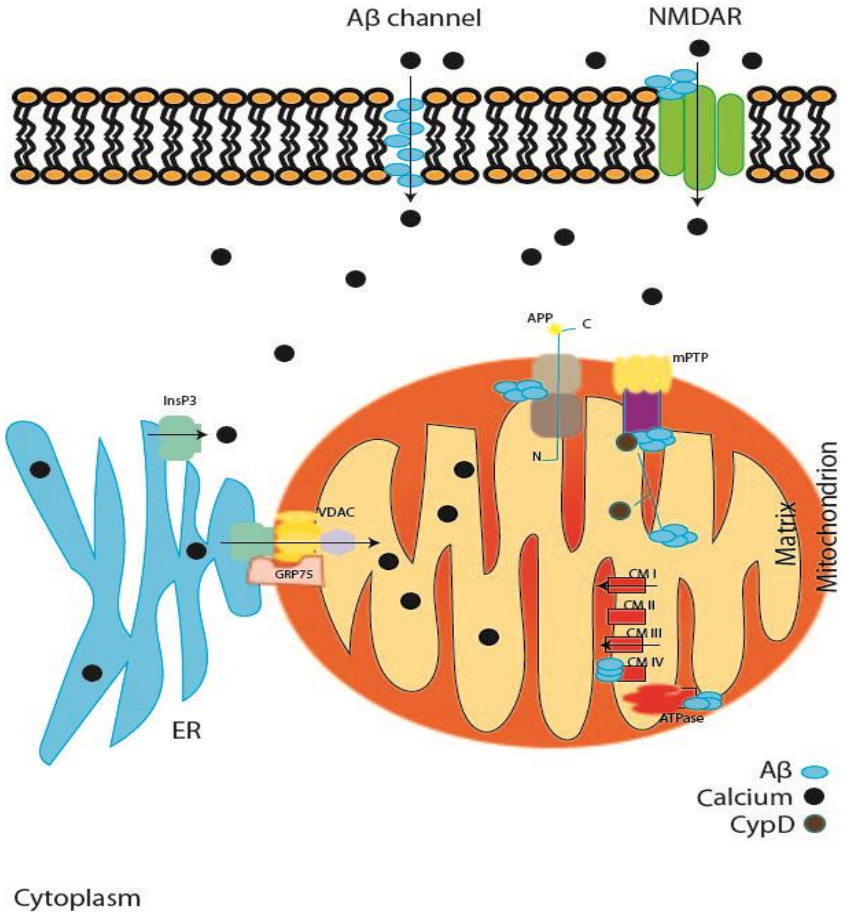


Figure 4: Amyloid-β peptide and Ca2+homeostasis

Tau Hyperphosphotylate

A second feature of AD is the accumulation of phosphorylated tau proteins in various areas of the brain. The percentage of tau protein phosphorylation also adjust microtubules stability due to kinases and the specific phosphatases that adjust the percentage of phosphorylation with the non-phosphorylated form preferentially links to microtubules

(Jouanne et al., 2017). Tau hyperphosphorylated protein appears to be afflicted by a variety of pathological factors like abnormal kinase activation, abnormal gene expression and chronic stress or disease, leading to over-aggregation and formation of neurofibrillary tangles (NFTs). NFTs causes loss of synapses, impaired axial transport, mitochondrial and cytoskeletal dysfunction and oxidative stress. (Gao Y et al., 2018).

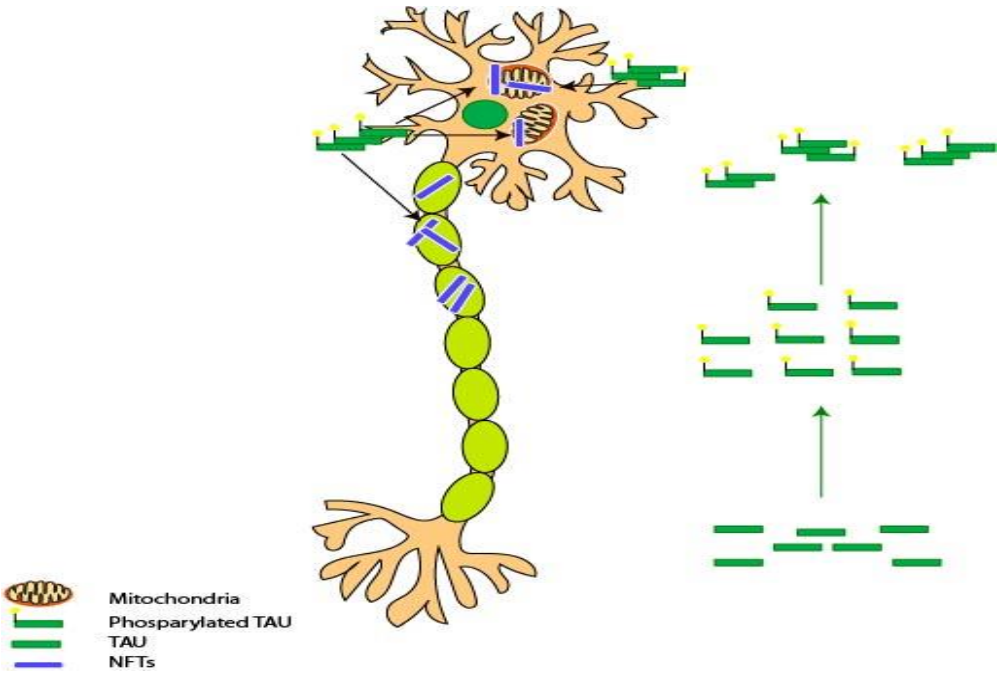


Figure 5: TAU and the interaction with neurons

Neuroinflammation in Alzheimer

It has been reported that both mitochondrial dysfunction but also neuroinflammation occur in AD. However, the relationship between them is interrelated since mitochondrial dysfunction causes inflammation, as well as inflammation leading to mitochondrial dysfunction. Mitochondrial dysfunction affects the cellular metabolism that produces the cell energy and it can release a big amount of components inside or outside of the mitochondrio. Then it triggers a number of mechanism, like apoptosis, necrosis, and inflammation. As a result, failure to produce bioenergy and combinations of mitochondrial dysfunction, neuroinflammation, and bioenergetic insufficiency can lead to the development of neurodegenerative diseases like AD. Damage-related molecular patterns (DAMPs) have been linked to sepsis in recent years. Mitochondrial DAMPs including mtDNA, formylopeptides, RNA, ATP, certain mitochondrial localized proteins

and cardiolipin are bound to the template recognition receptors (PRRs), such as toll-like receptors, formylpeptide receptors, or inflammatory NLRP3. These receptors are located in the cytosolic membrane and when they are activated they stimulate a number of inflammatory paths that are correlated with the appearance, development and outcome of many diseases. Activation of PRRs receptors leads to phosphorylation and activation of p38 mitogen-activated protein kinases (p38 MARK). These kinases then activate the NF-κB causing it to shift to the nucleus. NF-κB can initiate the transfer of APP (Amyloid precursor protein) but also facilitate the transfer of cytokines such as TNFα which can enhance inflammation by independently activating the signaling activated by p38 MARK and NFKB (Wilkins et al., 2016, Li et al., 2019). Subsequently, APP then will be cut by β and ɣ-secretases and it will cause the production of Aβ peptides that are also responsible for the appearance of amyloid plaques in AD (Roher et al., 2017).

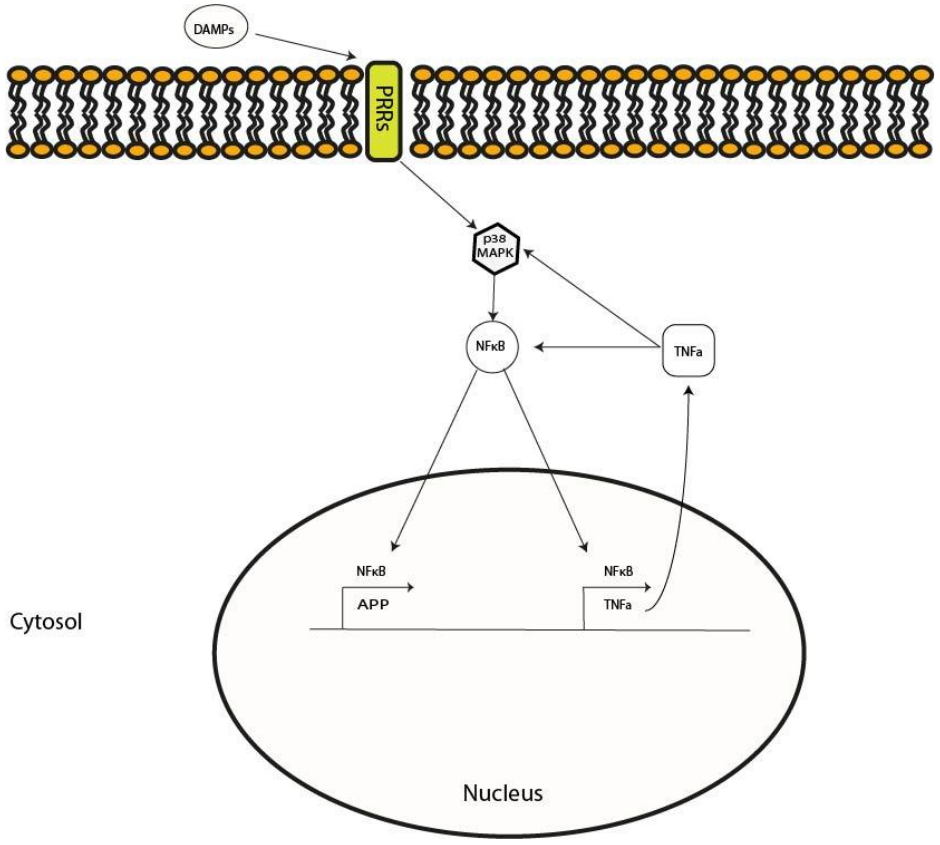


Figure 6: Neuroinflammation and AD

PARKINSON DISEASE

Parkinson's disease is associated with aging and dysfunction in mitochondria (Arun et al., 2016). It appears generally to 1 percent of persons older than 60 years, and up to 4 percent of elders with more than 80 years (Gazewood et al., 2013). Parkinson has been characterised by the classical motor features of Parkinsonism associated with Lewy bodies and loss of dopaminergic neurons in the substantia nigra (Kalia et al., 2015). This disease may appear with non-motor symptoms, like sleep disturbance, cognitive impairment and depression (Macdonald et al., 2018).

PINK1 and Parkin

PINK1 and Parkin play important part in the mitochondrial degradation pathway. Normally PINK1 can be found in mitochondria connected to the IMM, where it is cleaved and subsequently degraded with the help of PARL protein that causes the N-terminal fragmentation of PINK1. Due to that the phosphorylation of parkin stops and the mitophagy pathway stops. When mitochondrial membrane potential decreases, PINK1 accumulates in the outer membrane and then ingests parkin from the cytoplasm. This permits the activation of mitophagy or the degradation of mitochondria that have been damaged (figure 8) (Eiyama et al., 2015, Liu et al., 2019). Consequently, Parkin ubiquitinates outer mitochondrial membrane proteins that trigger selective autophagy. (Pickrell et al., 2013). Futhermore Diana Matheoud et al., 2016 in an experiment observed that the loss of function of PINK1 and Parkin showed high levels of mitochondrial antigens in macrophages and dendritic cells but also loss of mitophagy, increase of oxidative stress and neurodegeneration of the black substance (figure 7) (Barodia et al., 2016).

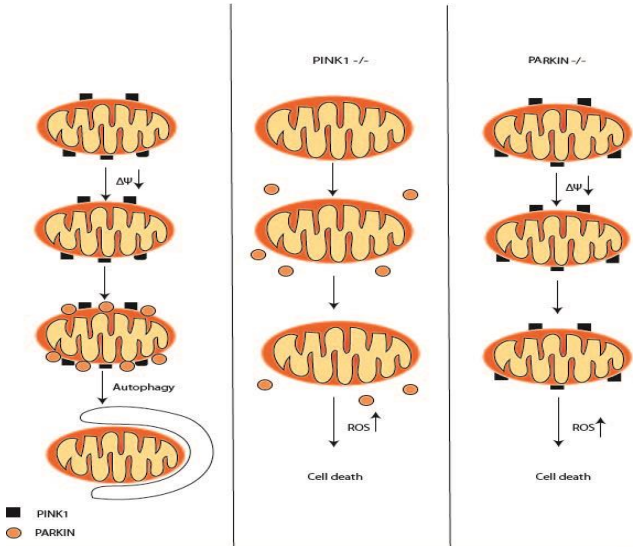


Figure 7: Loss of PINK1 or Parkin and cell death

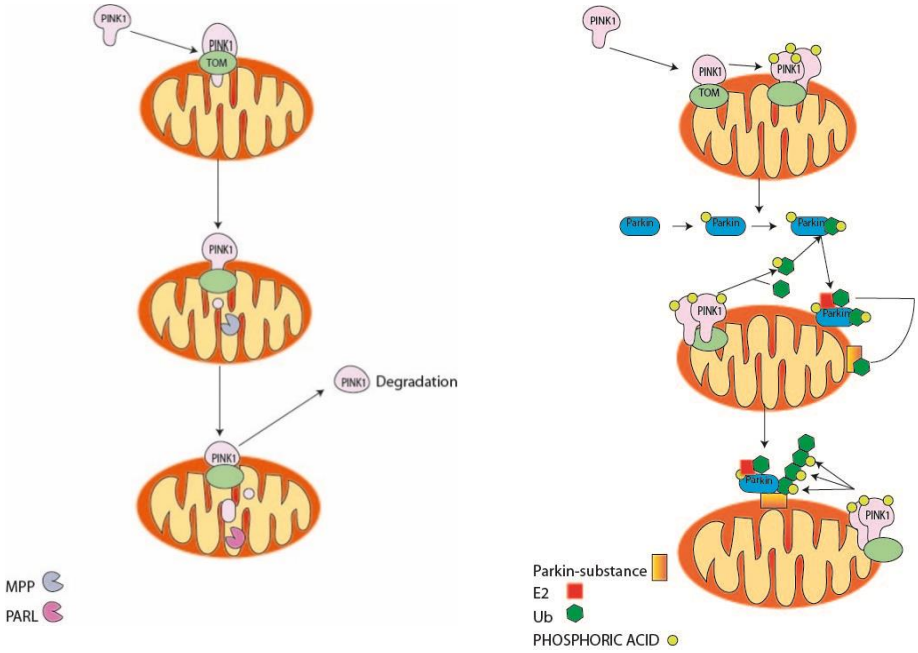


Figure 8: Pink1 and Parkin proteins actions when mitochondrial membrane potential changes. In the left one is shown the activity of Pink1 when the mitochondrial membrane potential is normal and in the right one the activity of the 2 proteins when it occurs mitochondrial membrane depolarization.

Pink1 and Fusion/Fission

Mitochondrial fusion requires the correct form of proteins Mfn and Opa1, and mitochondrial fission requires the proteins Drp1 and Fis1. Pink1 and Parkin promote fission and/or inhibit fusion, either directly or indirectly (Deng et al., 2018). It has been shown that Drp1-mediated fission is required for mitophagy and loss of Drp-1 function disturbed mitochondrial fission and mitophagy. This can lead to cell or neuron death that occur in PD (Feng et al., 2019). Mutations in PINK1 are known to alter fusion-fision machines and ultimately add to the pathogenesis of PD. In a study by Y. Yang et al. in 2008, it was indicated that PINK1 acts through Fis1 and Drp1 to regulate mitochondrial cleavage. Moreover an experiment conducted by Dadga et al. in 2009, the overexpression of the PINK1 in wild-type mitochondrial showed that less damaged and more elongated mitochondria were present. Then the loss of PINK1, led to increased mitochondrial fragmentation, showing that PINK1 and mutations in PINK1 interact with the mitochondrial fusion-fission mechanisms (Arun et al., 2016).

SNCA

Genetic anomalies in the α-SYN coding gene SNCA seems to make α-SYN a major factor in the disease pathogenesis (Guhathakurta et al., 2017). α-Syn fibrillation proceeds through oligomerisation to the formation of multiple nonfibrillar oligomer that intermediates termed protofibrils, which subsequently convert into fibrils (Paleologou et al., 2012). Improper folding of α-SYN creates aggregates, initially in a small number of cells, where it could gradually lead to the spread of the aggregates to multiple areas of the brain after a time frame has elapsed since the initial infection. With the help of neurotoxins, the aggregates can be transferred to other areas of the brain and finally released into the extracellular space. Cell experiments showed that the weakening of LAS leads to elevated secretion of α-synuclein in the extracellular space through extracorporeal cells and that endocytosis is a key mechanism of cellular α-SYN uptake. (Poewe et al., 2017). As it is shown in figure 9, the formation of toxic oligomers from α-SYN has as a result the creation of β-pleated sheets that stop the degradation of α-SYN but also produce oxidative stress to mitochondria. E.M. Rocha et al. in 2018 searched for the actions between α-SYN and the receptors of the OMM. They found out that α-SYN has a mitochondrial targeting sequence (MTS) at its N-terminal which is recognized by the displacement of the receptors on the outer membrane (TOM) of the mitochondria. These proteins, which initially target the network, are transported through the TOM complex to TIM (translocase of the inner membrane) and then to the uterus. α-SYN can still bind to the TOM20 receptor and prevent its interaction with the TOM22 co-receptor, causing inhibition of mitochondrial protein induction. The interaction between α-SYN and TOM20 was found also that takes part in mitochondrial damage and excessive ROS production.

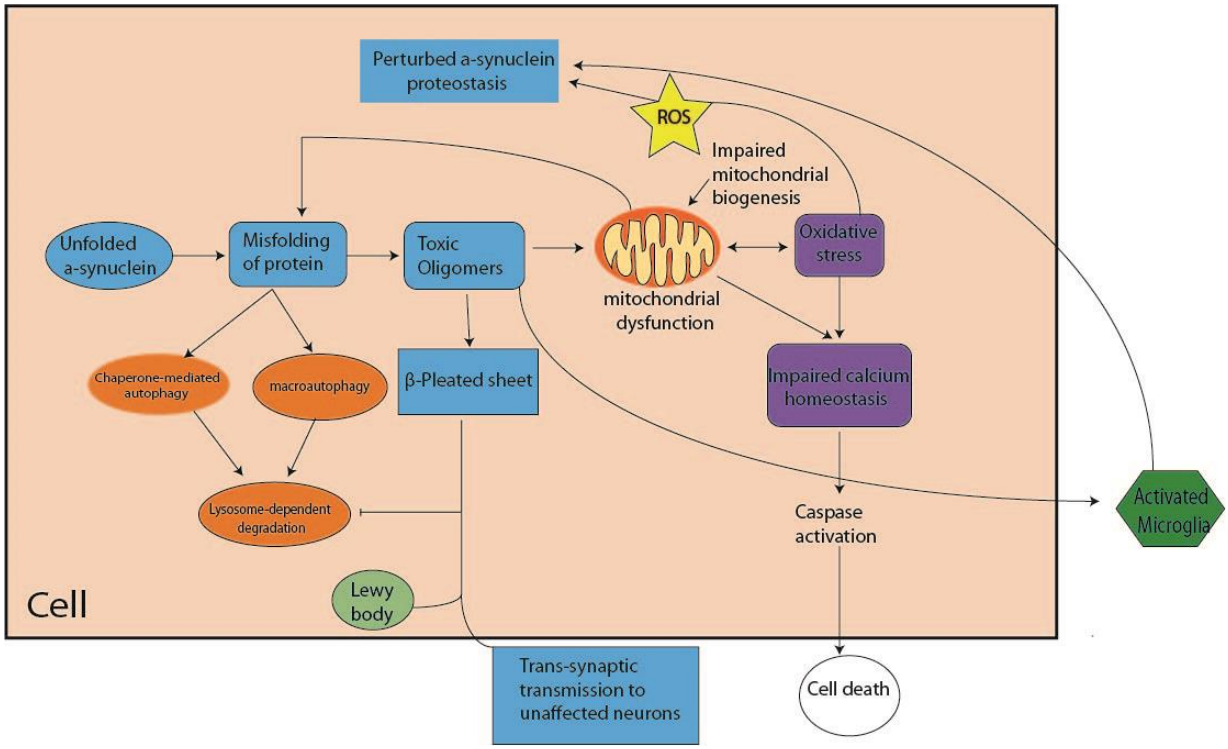


Figure 9: Mutation on SNCA

LRRK2

The mutant LRRK2 seems to appear in the majority of the Parkinson cases but also many versions of the LRRK2 gene have also been associated with sporadic PD. Genetic control of LRRK2 mutations is important for endangered populations (Sharon L et al., 2017). Mutations in LRRK2 were subsequently shown to be a relatively common genetic cause of PD worldwide. Only six of these have been identified as disease-causing: G2019S, Y1699C, I2020T and R1441C/G/H (Kluss et al., 2019). There are three mechanisms that seem to be affected from mutant LRRK2: the interaction between both LRRK2 and a-SYN that through Drp1 leads to mitophagy and cell death, the autophagic system from the decrease of Rab7 which blocks the degradation of α-SYN and at last the inflammation. The interaction between α-SYN and LRRK2 showed increased toxicity and elevated complex I inhibitors which as a result proved the effect of those two on mitochondrial respiratory chain. These complex I dysfunction caused from LRRK2 and α-SYN can lead to oxidative stress and ATP production impairments (Cresto et al., 2019).

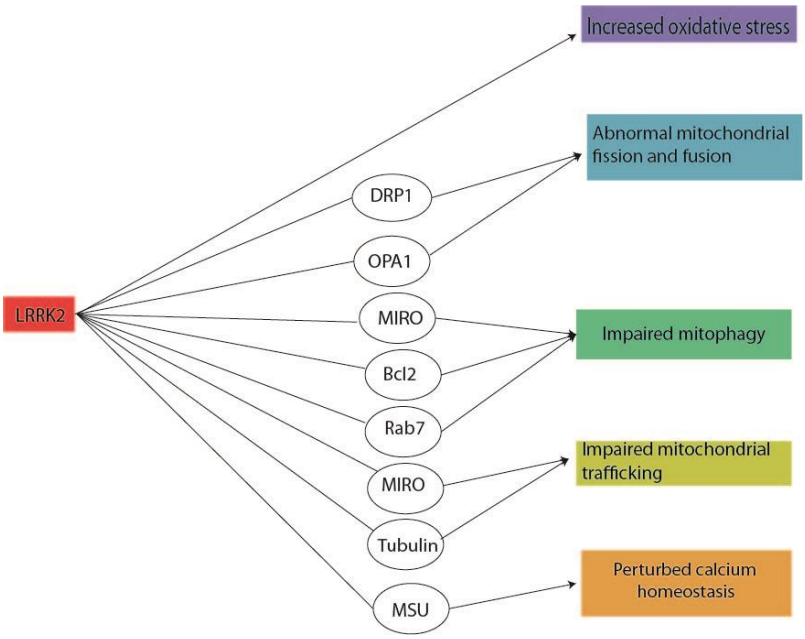


Figure 10: Mutant LRRK2 and the effect on mitochondria

HUNTINGTON DISEASE

Huntington's disease is one of the most fatal autosomal dominant neurodegenerative disorder with the average age of onset around 30 years. Key features of this disease are a combo of motor, cognitive and psychiatric symptoms, atrophy of the basal ganglia and cerebral cortex which is still observed, with the escalation of symptoms leading patients to death 5-20 years after the start of its initial symptoms. The onset of the disease appears due to the pathological increase in the number of copies of the repeated CAG in exon 1 of the Huntingtin gene that is located on chromosome 4 (Illarioshkin et al., 2018). Huntingtin has an anti-apoptotic role by affecting members of the caspase-3 family and also the family of the pro-apoptotic Bcl-2s such as BIK and BAK. Non mutant htt, seems to protect also the neurons from neurotoxins such as 3-nitropropionic acid (3-NP) which inhibits mitochondrial complex II and causes HD-like symptoms (Farshbaf et al., 2017).

HTT and the homeostasis of calcium

The interaction between the mHTT and MOM may reduce the activation of the mitochondrial PTP, which is activated by calcium, ROS or reduced adenine nucleotide levels and can cause mitochondrial swelling, depolarization and at last cell death (Carmo et al., 2017). Where mHTT is present, the normal huntingtin is no longer available to bind the PSD-95 protein, which activates NMDAR and leads to mitochondrial calcium overload. In ER, the interaction between $InsP_3R1$ with HAP_1A and mHTT increases calcium release and making mitochondria more sensitive to calcium overload. This increase of calcium, has as a result the movement of calcium inside the mitochondria through the interaction between InsP3R and VDAC and MCU. When the mitochondrial calcium storage capacity is reached, the mPTP is activated and promoting the mitochondrial depolarization and further calcium release into the cytoplasm but also mutant huntingtin may be associated with the OMM and IMM, through the direct connection to the translocase of the TIM23 that is located in IMM and the complex II of the respiratory chain, boosting energy lose and mPTP activation (Naia et al., 2017).

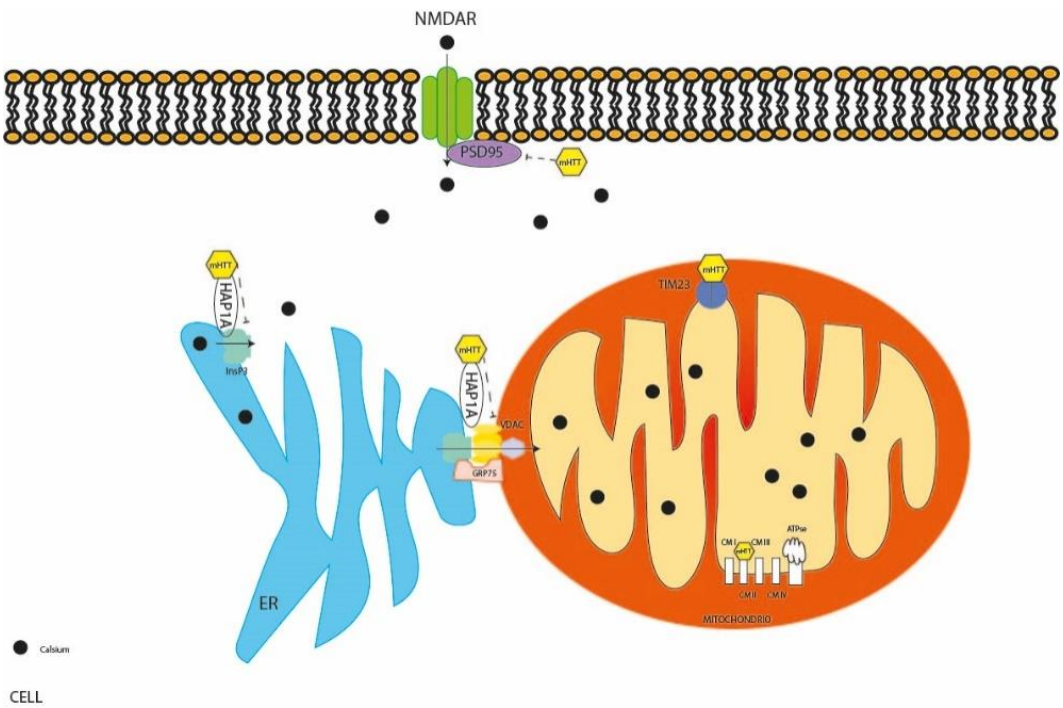


Figure 11: mHTT and calcium homeostasis

Htt and the PGC-1α pathway

The weak PGC-1α expression and function has emerged as a factor that produces the mitochondrial dysfunction in HD (Johri et al., 2013). The interactions between PGC-1a and the nuclear transcription factors NRF1 and NRF2 have a key role in mitochondrial biogenesis. The expression and the activity of these two nuclear factor increases through protein-protein interactions. NRF1 and NRF2 activates TFAM and binds to promoter regions of nuclear genes encoding subunits of the five complexes in ETC, where it increases the assembly of respiratory chain, it regulates genes that are involved in heme biosynthesis, imports nuclear encoded mitochondrial proteins, and promote mtDNA replication and transcription (Li et al, 2017). Different pathways seem to occur and control the expression of PGC-1a but also its activity. It seems that PGC-1a increases its own expression by an auto-regulatory loop but also with the phosphorylation by AMPK and p38 a, methylation by PRMT1, de-acetylation by SIRT1 and at last it is also modulated by cAMP, Ca^{2+} and CREB (Lloret et al., 2019).

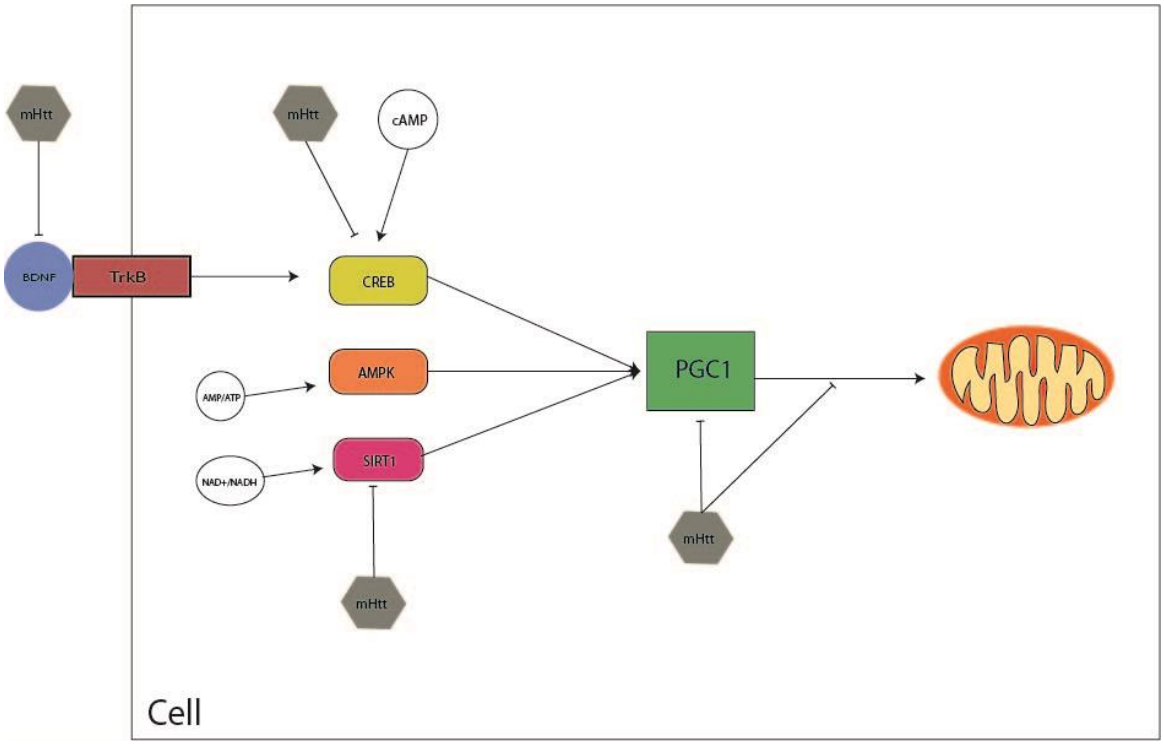


Figure 12: The suspension of PGC-1a activating pathway by mutant huntingtin

Htt and Drp1 expression

Mutant Htt reduce movement of mitochondria in neurons, which leads to loss of mitochondrial mobility and dysfunction. The increased expression of Drp1 and Fis1 but also the under-expression of mitofusins and OPA1 in cellular models of HD (Chaturvedi et al., 2011), seems to interact with Drp1 GTPase domain, propagating enzyme activity leading to disproportionate mitochondrial fission (Oliver et al., 2019). Hemachandra Reddy in 2014 revealed that some mechanisms are likely to be involved in excessive mitochondrial fission in HD-affected neurons. Extreme production of ROS can activate fission proteins and increases the Drp1 GTPase enzymatic activity, while mHtt interaction with Drp1 has as a result the increase of GTPase Drp1 enzymatic activity that increase mitochondrial fission and creates an imbalance in mitochondrial dynamics. Also the S-nitrosylation of Drp1 in mHtt improved GTPase Drp1 activity, can cause excessive mitochondrial fission. The last mechanism that was described was the phosphorylation of Drp1 at Ser 616, Ser 585 and Ser 637, sites that increases the GTPase activity and causing the elevate fission in mitochondria.

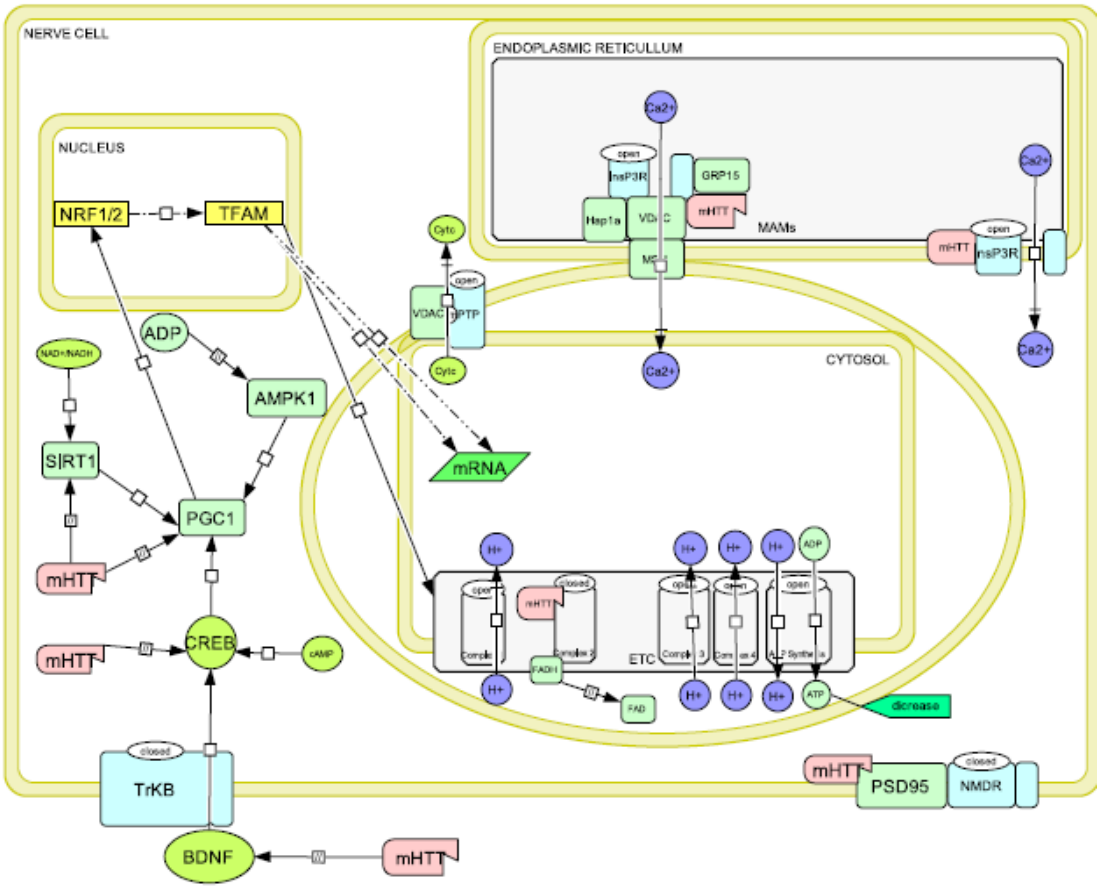


Figure 13: Metabolic Pathway of Huntington Disease

AMYOTROPHIC LATERAL SCLEROSIS

A number of cases links axial transport disorders with motor neuron disease, such as ALS, a devastating neurodegenerative disease that affects both of motor neurons (upper and lower) and causing paralysis and early death (Manfredi et al., 2009). In neurodegenerative diseases, maintaining a healthy mitochondrial network is often at stake as mitochondrial dysfunction contributes to neuronal disruption and the loss of motor neuronal. A big percentage (near 90%) of ALS cases have the sporadic form of ALS which is the most common and about the 10% have the familial form (Tadic et al., 2014). In addition, repeated GGGGCC in the first intron of the C9ORF72 gene has recently been shown to be associated with ALS. The factors that cause the disease are still unclear, although recent calcium disorders, ERstress, and mitochondrial dysfunction seems to be involved in the pathogenesis of ALS (Tadic et al., 2014). Other mechanisms that seems to be related with the ALS patient pathophysiology include: oxidative stress, protein aggregation, unregulated intracellular trafficking, reduced axial transport, neuroinflammation, and unregulated transcription, RNAic et al. (Tadic et al., 2014). Another factor is the members of the Bcl-2 family, that have role in the apoptosis like the pro- and anti-apoptotic proteins that controls the release of factors that can activate caspases from mitochondria and then activates the apoptotic signalling cascades. (Smith et al., 2017). Broken mitochondria concentrate in motor neurons affected by genetic or sporadic forms of ALS, increases and that is strongly suggesting that the failure to keep a healthy mitochondrial group plays a role in the disease progress (Manfred et al., 2018). Mitochondria are normally found in high-use ATP sites and Ca^{2+} storage requirements, like cell bodies, Ranvier nodes, and synchronous terminals. So, changes in mitochondrial transport can lead to local loss of energy, calcium imbalance and it can also cause consistent dysfunction and loss of neurons (Magrane et al., 2009).

Superoxide dismutase 1 (SOD1)

SOD1 is an antioxidant protein and when mutant SOD1 protein occurs, it promotes the apoptosis of mitochondria. Tan et.al in 2014 found that self-oligomerized SOD1 that may not be balanced by self-dissociation is a factor to the onset of the disease. That paper also found out that the oxidized form of SOD1 (OxSOD1 is open to assessment more than its wild type counterpart, just like the mutant SOD1-G93A. Also the mutant SOD1 has been

shown to increase the creation of damaging hydroxyl radicals and peroxynitrite derivatives, which stop the mitochondrial electron-transport chain resulting to a significant decrease of ATP. Mutant SOD1 can be found to the intermembrane space (IMS), where it has been shown to accumulate and decrease the ETC activity. When the mutant SOD1 starts to aggregate they moved to the surface of OMM (Tadic et al., 2014, Smith et al., 2017) and interacts with mitochondrial function that leads to cell death (Tan et al., 2014). This form also showed an increased oxidative stress and structural damage, which can cause the release of cytC.

Superoxide dismutase 1 and calcium homeostasis

In a study by Magrane and Manfredi in 2009, it was found that in G93A SOD1 mutations, there was a decrease in mitochondrial calcium capacity not only in the brain but also in spinal cord which appeared in the early stages in the course of the disease. When mitochondrial calcium uptake isn't working properly, as seen by the low calsium movement in SOD1G93A and followed by elevated calcium levels, can cause the reduced capability to limit calcium transient amplitudes in the cytosol (Jaiswal et al., 2014). This can lead to the production of ROS which eventually will cause the creation of misfolded proteins such as TDP-43, FUS and SOD1. This proteins and abnormal RNA processing can cause ER stress and increase calcium concentration in neurons. This increase of calcium, the production of ROS and the mitochondrial depolarization will activate the mPTP channel (Tadic et al., 2014). The activation of that channel will cause the increase of cytC in neuron and then the activation of the apoptotic pathway.

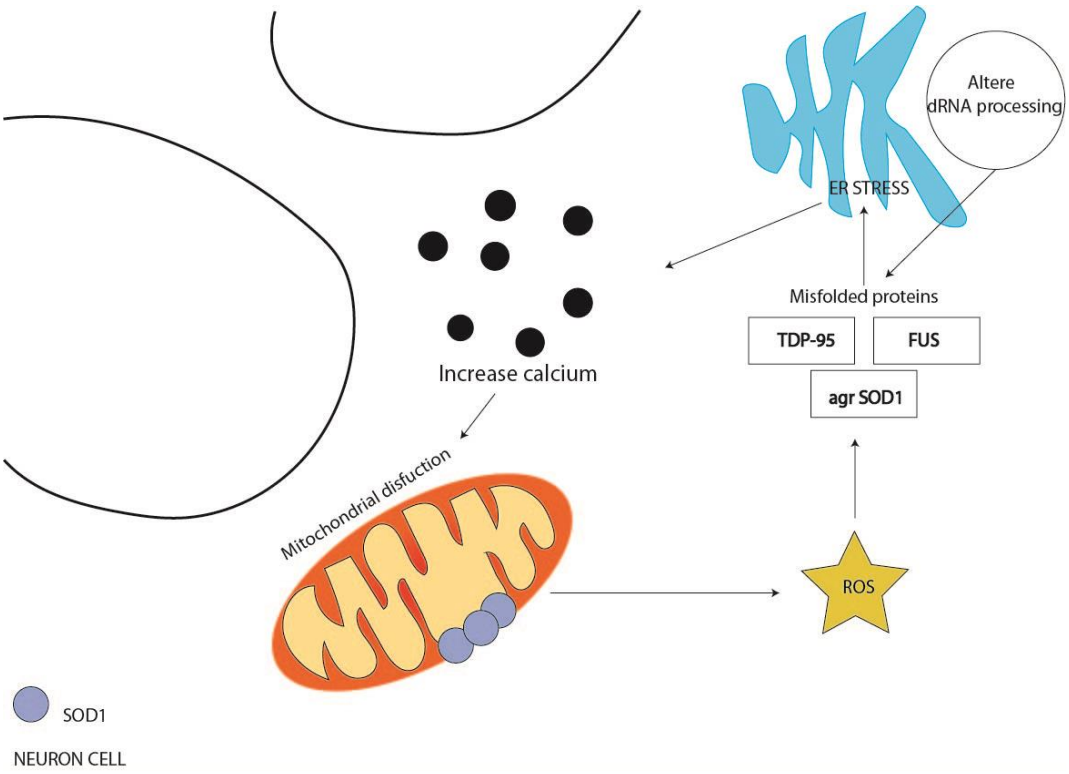


Figure 14: SOD1 and calcium homeostasis

Superoxide dismutase 1 and the apoptotic pathway

Mutant SOD1 has directly influenced apoptotic signalling by interaction with B-cell lymphoma 2 (Bcl-2). Wild-type and mutant SOD1 have been shown to bind the anti-apoptotic factor Bcl-2 in spinal cord. When bound to mutant SOD1, the BH3 domain of Bcl-2 is exposed and this causes a pro-apoptotic gain of function of the Bcl-2 protein in both cell and animal models of mutant SOD1 G93A ALS and in a mutant SOD1 patient spinal cord. The toxic mutant SOD1–Bcl-2 complex inhibits mitochondrial permeability to ADP and induces mitochondrial hyperpolarization due to reducing the interaction of SOD1 and VDAC1 (Smith et al., 2017). Another study showed that the proteins Bcl-2, Bcl-2-associated Xprotein (Bax/Bak) and the Bcl-2 interacting killer (BIK) can enhance calcium transfer from ER to mitochondria and the ensuing calcium accumulation activates apoptosis via cytC (Tadic et al., 2014). The increased calcium levels that can prevent kinesin from binding to microtubules due to the exposure to the activity of mutant SOD1 can lead to decreased anterograde axonal transport (Shi et al, 2011). The relationship between mutant SOD1 and Bcl-2 have, showed that the exposure of the BH3 domain in Bcl-2 by mutant SOD1 could potentially affect the fission and fusion machanism in mitochondria as BH3 domain only proteins like Bax and Bak can elevate mitochondrial fission (Tan et al., 2014).

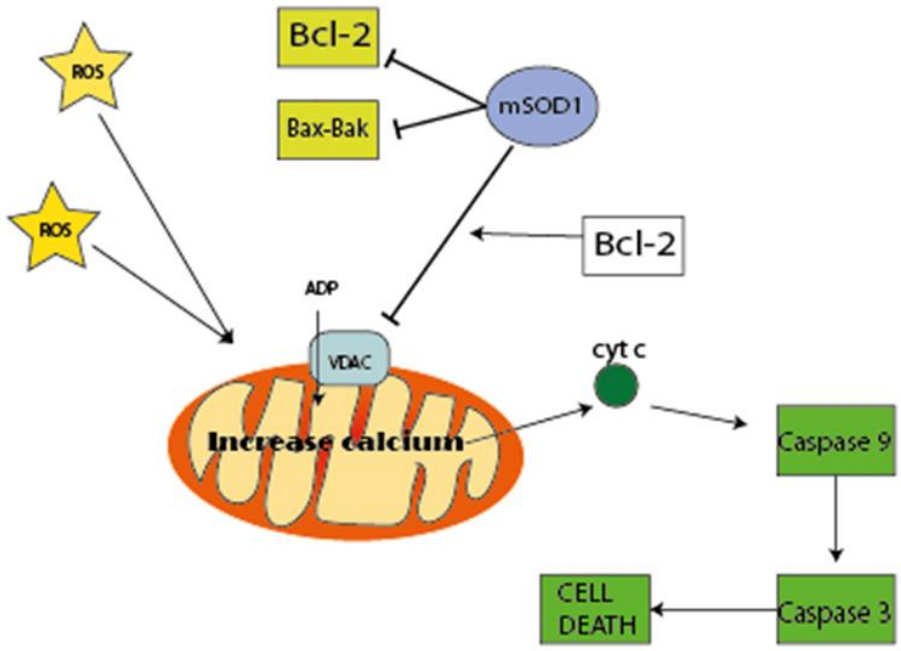


Figure 15: SOD1 and the apoptotic pathway

Superoxide dismutase 1 and axonal transport

The mutant SOD1 can also interact with factors of the axonal transport of mitochondria such as phosphorylate kinesin heavy chain and kinesin light chain, interact with the dynein-dynactin complex and may result in impairment of retrograde transport or the disturbed mitochondrial membrane potential could have an impact on mitochondrial transport (Shi et al., 2011). There was found that specific transportation of mitochondria depends on the correct motors and linkers combinations, except of SOD1 mutations. Resently have been discovered the implication in specific linkage of mitochondrial to kinesin-1 by two cargo adaptor proteins (Magrane et al., 2009)

Miro-Milton pathway

It was shown that mutations in the pathway of miro-milton but also mutations or loss of function in parkin effects the axonal movement of mitochondria and as a result they trigger neuron cell death. To begin with, Milton is an adaptor protein that interacts with kinesin heavy chain (KHC), the mitochondrial protein Miro and recruits mitochondria to the kinesin motor in microtubule-dependent transport (Shi,2009). Together all the three of

them can provide a mitochondria-certain axonal transport mechanisms (Granatiero et al., 2018). Mitochondrial rho-like GTPase Miro-1, which is found on the external side of the OMM, is connected to the membrane by a carboxy-terminal transmembrane domain. Miro then interacts to the Milton protein and then Milton connects to the kinesin-1 heavy chain (Magrane et al., 2009). Furthermore, Miro-1 carries a GTPase and calcium binding domains, and mutations in these domains trigger a negative phenotype that is described by the accumulation of mitochondria in the perinuclear region and increased apoptosis in cultured cells (Magrane et al., 2009). Veronica Granatiero et al., in 2018 showed that there are two mechanisms that have been proposed to describe the way that Miro controls the mitochondrial movement in a calcium-dependent manner. Motor-Miro binding model, shows that when the concentration of calcium is low, the C-terminal tail of kinesin is bound to the mitochondrion through its interaction with the Milton–Miro complex and then allowing the mitochondria to move. When calcium concentration starts to increase, it binds to Miro EF hands, causing a direct interaction of kinesin with Miro, which stops the mitochondrial movement. The motor-Miro binding mechanism, proposed that when calcium concentration increases, Miro stay connected to Milton and to mitochondrion, and separates from the kinesin, and as a result it stops the mitochondrial transport (Granatiero et al., 2018).

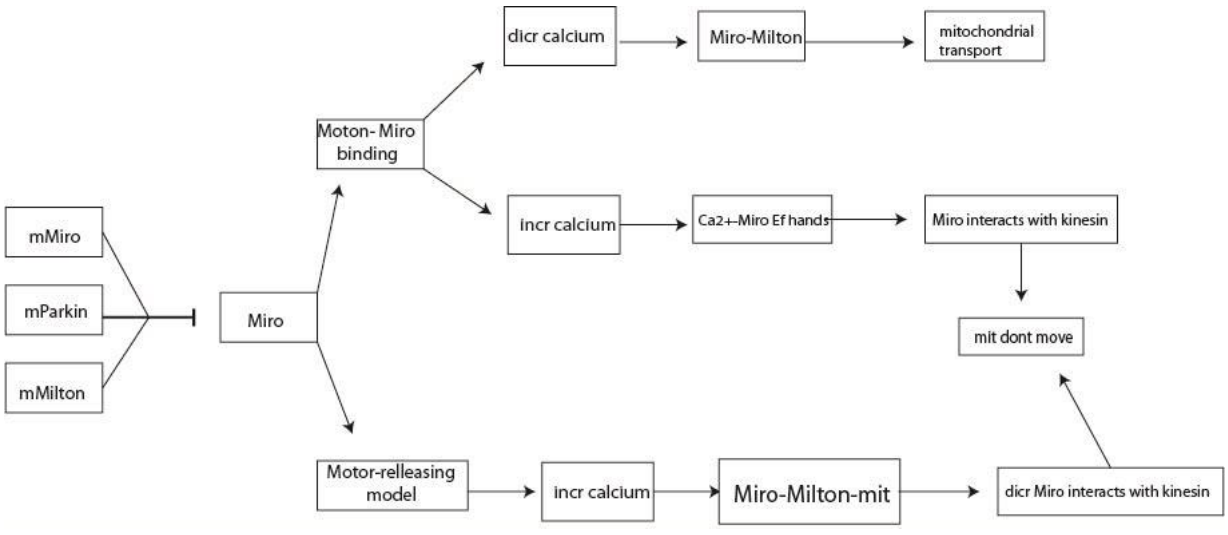


Figure 16: The Miro-Milton pathway

Optineurin mutations

Another mutated gene that was found related with ALS is *OPTN* that encodes the optineurin protein. There are three OPTN mutations that are found in ALS, first the deletion of exon 5, secondly a nonsense mutation and at last a missense mutation. This protein controls the membrane trafficking, the mitochondrial clearance impairment, protein secretion, cell division and it hosts also defence mechanism against pathogens. Optineurin normally stops NF-κB activity, but the mutant one seems to be unable to stop the activity of NF-κB. Moreover, there is a sign that incorrect NF-κB activation is the pathogenic mechanism under optineurin mutation-related ALS. (Tadic et al., 2014 & Kim et al., 2020). Optineurin also plays role in PINK1-Parkin mediated mitophagy. After Parkin is recruited to the OMM, optineurin connects to ubiquitinated mitochondria promoting the autophagosome nucleation through LC3 recruitment. The interaction between optineurin and LC3, which finalizes autophagic clearance of damaged mitochondria, depends on Tank-binding kinase that interacts with optineurin and by phosphorylating it at specific serine residues adjust its ability to connect to ubiquitinated mitochondrial proteins (Granatiero et al., 2019).

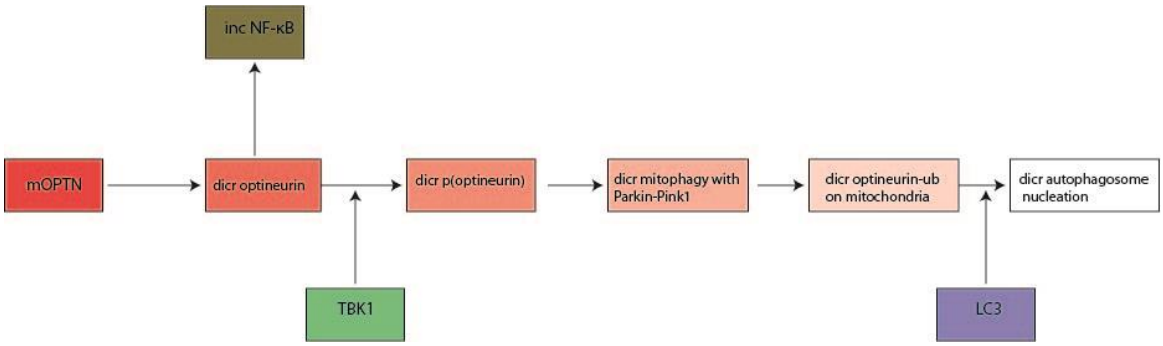


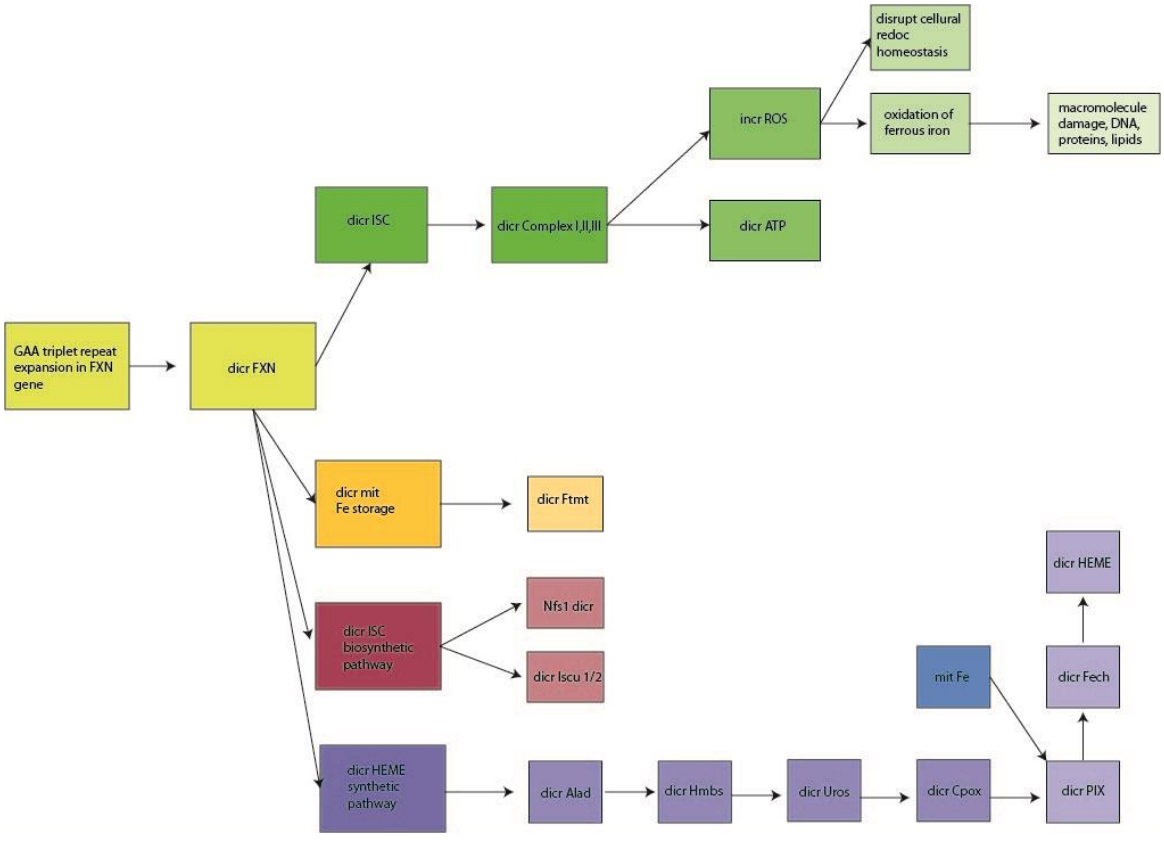
Figure 17: Mutant optineurin and ALS

FRIEDRIECH ATAXIA

Friedreich's ataxia (FRDA) is an autosomal recessive and degenerative disease which interacts with the central and the peripheral nervous systems, non-neural tissues (Liet et al., 2019), but also is considered as a cardio degenerative condition, where the loss of mitochondrial functions plays a crucial role in this disease progression (Shannon Chiang et al., 2017). FRDA is caused by a GAA triplet-repeat that is placed within an Alu sequence element in intron 1 of frataxin (FXN) gene, a protein that encodes in the mitochondria (Li et al., 2019). FXN is a nuclear-encoded protein that is transferred to mitochondria and whose function in mitochondria supports the formation of iron–sulfur

clusters, protects from oxidative stress, and maintain iron homeostasis (Jasoliya et al., 2017). It takes part in mitochondrial iron-sulfur cluster biogenesis, and in the creation of mitochondrial enzyme complexes I, II and III, other mitochondrial enzymes and therefore, a decrease in FXN might impact mitochondrial function and numbers (Jasoliya et al., 2017). As a result, frataxin loss of function leads to mitochondrial DNA damage, mitochondrial iron accumulation, ISC deficiency, perturbed heme synthesis, and oxidative damage (Li-Hsuan Huang, 2009). There are two mitoferrins MFRN1 and MFRN2 that take the responsibility to transfer the Iron across the IMM into erythroid and non-erythroid cells, which normally provides a normally homeostasis of iron. (Chiang et al., 2017).

Figure 18: The Friedriech Ataxia pathway



SPASTIC ATAXIA

Autosomal-recessive spastic ataxia of CHARLEVOIX–SAGUENAY

Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disease and is considered to be one the most usual types of autosomal-recessive ataxias in the world after Friedreich ataxia and ataxia telangiectasia (O. Bereznyakova et al., 2018). This disease is caused due to mutations in SACS, which encodes sacsin protein that is placed on the mitochondrial surface and is possibly involved in mitochondrial dynamics (Criscuolo et al., 2015). This mutation on sacsin showed altered transcript levels for OXPHOS and oxidative stress genes (Bradshaw et al., 2016). This disease at first was described as a homogeneous syndrome that was including spasticity, dysarthria, distal muscle wasting, foot deformities, truncal ataxia, absence of sensory evoked potentials in the lower limbs (O. Bereznyakova et al., 2018).

Spastic Ataxia 5

The ATPase family 3-like gene (AFG3L2) encodes a subunit of the m-AAA class of mitochondrial proteases (Pierson et al., 2011). AFG3L2 also encodes an ATP-dependent proteolytic complex of the IMM that degrades misfolded proteins and regulates ribosome assembly (Dosi et al., 2020). These ATP-dependent metallopeptidases assemble into large proteolytic complexes in the IMM and function to ensure mitochondrial protein quality control through the degradation of misfolded proteins and the maturation of essential proteins (Pierson et al., 2011). In mitochondria, the *m*-AAA protease is having 2 highly homologous subunits, the paraplegin that is encoded by the *SPG7* gene and AFG3L2, which assemble to form hetero-oligomeric and, in the case of AFG3L2 only, homo-oligomeric functional complexes (Almajan et al., 2012). *m*-AAA proteases adjust the creation of the MCU and the reduce of its subunit EMRE in AFG3L2-deficient mitochondria results are the unregulated MCU complexes, the increase mitochondrial calcium concentration and promote the calcium related nevron death (Patron et al., 2018).

Spastic Ataxia 7

The SPG7-AFG3L2 complex take part in many pathways that are critical for mitochondrial function, including mitochondrial protein quality control and homeostasis (Dosi et al., 2020). Mutant paraplegin, can cause an autosomal recessive form of

hereditary spastic paraplegia (HSP) (Atorino et al., 2003). Also the complex impairment can lead to dysfunctions in mitochondrial protein synthesis, respiration, mitochondrial integrity and networking, axonal transport, as well as dysfunctions in Ca^{2+} flow and Ca^{2+} - related cell death (Dosi et al., 2020). The reduced complex SPG7-AFG3L2 promotes the impairment of the complex I activity and an elevated sensitivity to oxidant stress that can be altered with the grand of exogenous expression of wild-type paraplegin (Atorino et al., 2003).

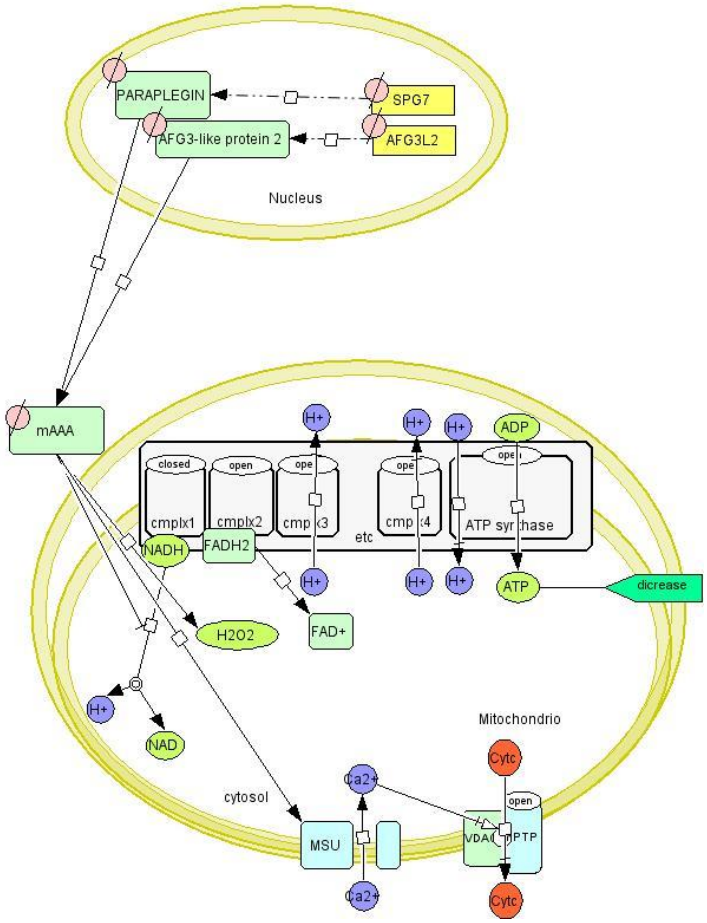


Figure 19: Metabolic Pathway of Spastic Ataxia 5 and 7

BULBOSPINAL MUSCULAR ATROPHY

Bulbospinal muscular atrophy (BSMA) disease, also known as Kennedy's disease, is a rare, adult-onset, X-linked, recessive trinucleotide and polyglutamine (poly-G) disorder, that is caused by expansion of an unstable CAG-tandem repeat in exon 1 of the androgen-receptor (AR) gene on chromosome Xq11-12 (Finsterer et al., 2010). BSMA is characterized by selective degeneration of lower motor neurons (Finsterer et al, 2016). The number of CAG repeats often exceeds 40, compared with 17-26 repeats for healthy controls (KM Au et al., 2003). Several findings suggest that the main factor in BSMA is the creation of polyQ-AR, likely due to defects in mitochondrial trafficking but also the interference it has with the PGC-1α, a master regulator of mitochondrial biogenesis and function that is decrease during the disease. At last, polyQ-AR N-terminal fragments can regulate a mitochondrial-originating Bax-dependent apoptotic cascade, potentially connecting mitochondrial damage with neuronal death (J. Cortes et al., 2018).

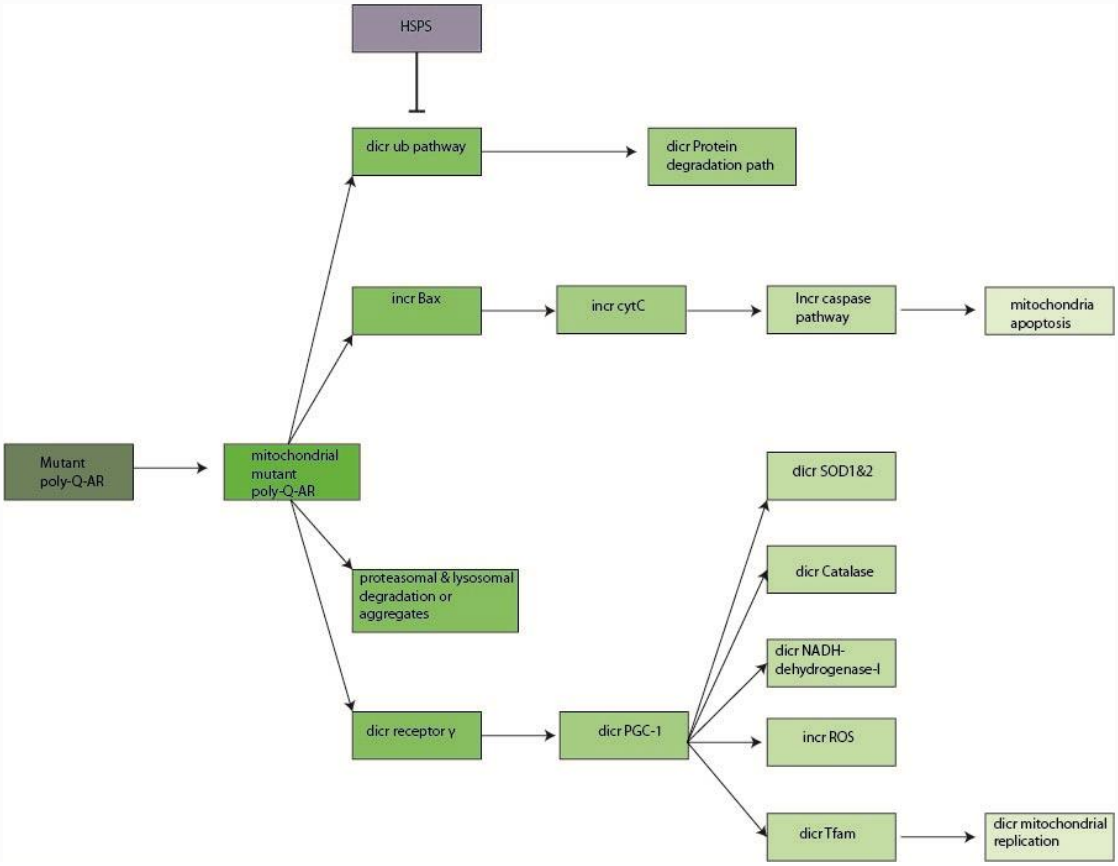


Figure 20: Metabolic Pathway of Bulbospinal muscular atrophy

Neurodegenerative Diseases and Mitochondrial functions

The next step is to discuss the mitochondrial processes that are affected by the neurodegenerative diseases that was discussed above.

Fusion and Fission

There are two mechanisms that control the mitochondrial dynamics: fusion and fission, and the mitochondrial biogenesis and degradation, but also processes like intracellular transport that can sustain mitochondrial homeostasis, adjust the mitochondrial form, and take part in cellular stress response (Meyer et al., 2017). Both processes fission and fusion can be controlled by GTPases (Youle et al., 2012). The mitochondrial dynamics include dynamin-related/-like protein 1 (Drp1/DLP1), Mfn, and OPA1. Drp1/DLP1 controls mitochondrial fission, where Mfn and OPA1 control the fusion of outer and inner membranes, respectively and in case of mitochondrial fusion the inner membrane dynamin OPA1 is required for not only IMM fusion, but also maintenance of cristae structure. The OMM fusion protein Mfn has two isoforms: Mfn1 and Mfn2. Mfn2 also can be found to the ER membranes where it interacts with proteins Mfn1 or Mfn2 that are localized in mitochondria to tether two organelles or to keep the critical distance (Lee et al., 2016).

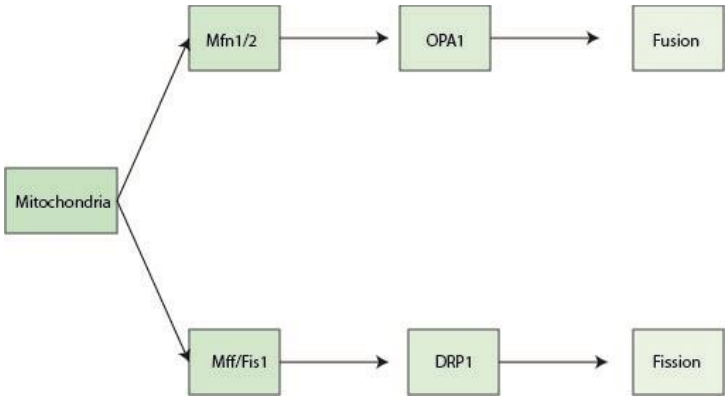


Figure 21: Fusion and Fission pathway

Apoptosis

The process which controls the regulation form of cell death is called Apoptosis. It is also responsible for the mechanism that controls the maintenance of cell numbers, and also it acts as a defence mechanism to remove dangerous and unwanted cells (Estaquier et al., 2011). Proteins of the Bcl-2 family have either pro- or anti-apoptotic jobs and can regulate the mitochondrial pathway of apoptosis by controlling the permeabilization of the OMM so in response to many types of stress or damage, members of the Bcl-2 family (like BH3-only proteins) are activated (Joslyn K et al., 2009). This allows the release of mitochondrial intermembrane space proteins like BAX and BAK that activate cytC which binds to apoptotic peptidase activating factor 1 (APAF1), forming a heptameric structure called the apoptosome. Then the apoptosome recruits and activates the initiator caspase 9, and from that activation caspase 9 activates the caspase 3 and caspase 7 (Bock et al., 2020). As a result this activation produce the apoptosis of nerve cells and neurodegeneration.

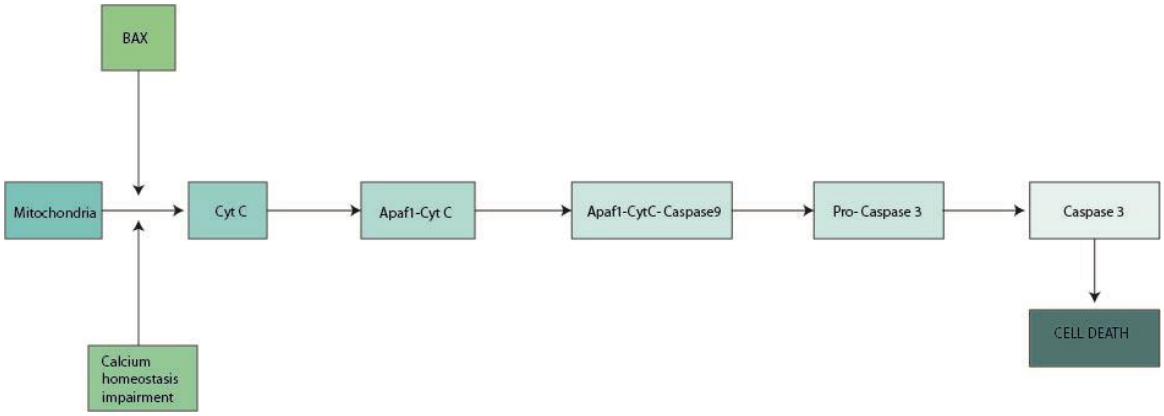


Figure 22: The apoptotic pathway

Oxidative phosphorylation

Respiration is one of the most important mechanisms of living organisms and is completed by respiratory chain complexes located on the MIM (Guo et al., 2017). In the ETC, the electrons go through complexes that elevate the mitochondrial membrane potential and produce a release in energy that can be released as heat or utilized to pump hydrogen ions (H^+) from the mitochondrial matrix to the intermembrane space and create a proton gradient. The OXPHOS is composed of five enzymes complexes and all of them except for Complex II, have subunits encoded both in the mitochondrial genome and the

nuclear genome (Signes et al., 2018). Complex I (NADH: ubiquinone oxidoreductase) plays a central role in cellular metabolism. By oxidizing NADH to NAD^+ in the mitochondrial matrix, it supplies reducing equivalents to support the Krebs cycle and the β-oxidation of fatty acids (Sousa et al., 2018). The proton pumps that is located in Complex I, transfers two electrons to ubiquinone and then tho to cytochrome c oxidoreductase but also moves four protons across the membrane (Sousa et al., 2018). Complex II, also known as succinate dehydrogenase, takes electrons from succinate and acts as a second entry point to the ETC. When succinate approaches the Complex II it oxidizes to fumarate, 2 electrons are accepted by FAD within Complex II. Then FAD passes the 2 electrons to Fe-S clusters and then to coenzyme Q, that is similar to Complex I without protons moving across the membrane and as a result less ATP production with this pathway. Complex III (CIII) or Q-cytochrome c oxidoreductase, catalyzes the transfer of electrons from ubiquinol to cytC coupling this redox reaction to the translocation of protons across the IMM (Cogliati et al., 2018). Complex IV is also known as cytC oxidase or COX that terminates the flow of electrons through the ETC, reducing oxygen to water and it's activity can be regulated by signals including the mitochondrial ATP/ADP ratio (Van der Bliek et al., 2017). Complex V or ATPase is responsible for the generation of ATP through phosphorylation of ADP by using electrochemical energy generated by the process that was described earlier (Neupane et al., 2019).

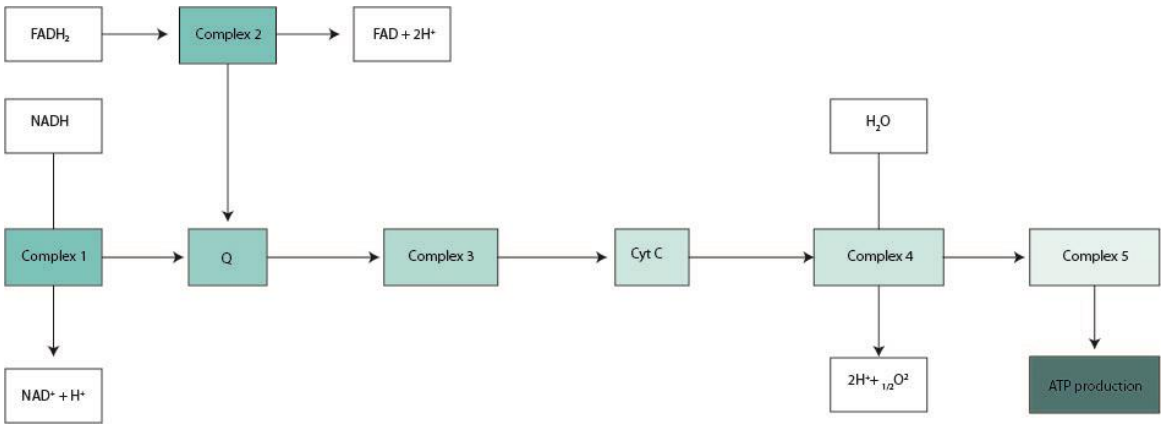


Figure 23: The Oxidative phosphorylation

Mitophagy

Mitophagy is the selective removal and degradation of dysfunctional mitochondria to prevent damages or the promotion of cell death (V´asquez-Trincado et al., 2017). PINK1-Parkin-dependent mitophagy is initiated when a decrease in mitochondrial membrane potential caused by mitochondrial damage leads to the stabilization of the ubiquitin kinase (PTEN)-induced kinase 1 (PINK1) on the OMM (Elayne M et al, 2017). In normal conditions, where mitochondria are polarized, PINK1 is maintained in low basal levels and for that is imported into IMM and processed by mitochondrial peptidases, such as the protease presenilin-associated rhomboid-like protein (Moreira et al., 2017). Accumulated PINK1 is autophosphorylated and activated, and then it phosphorylates ubiquitin on serine 65, which recruits Parkin and when Parkin is phosphorylated and activated by PINK1, (Yoo et al., 2017) it polyubiquitinates mitochondrial proteins (such as VDAC1, MFN 1/2 and Miro 1), leading to their association with the ubiquitin-binding domains of autophagy receptors and the formation of the autophagosome (Fivenson et al., 2017).

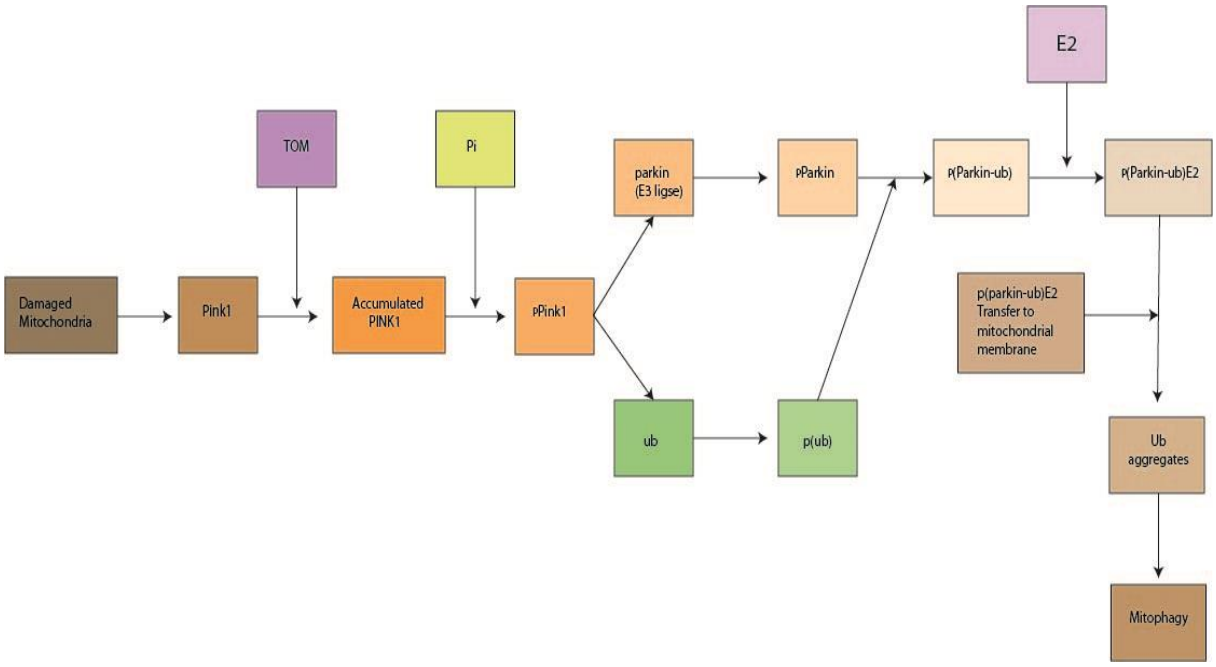


Figure 24: The Mitophagy Pathway

PGC-1 pathway

Members of the PGC-1 family have been shown to have role in the regulation of energy metabolism. All the members of the PGC-1 family such as PGC-1a, PGC-1b and PRC can interfere in mitochondrial biogenesis by regulating overlapping gene expression programs (Villena et al., 2014). The lack of energy, that is produced due to the decreased amount of the ATP/AMP and NADH/NAD^+ ratios, activates PGC-1α because of the phosphorylation by the AMP activated protein kinase (AMPK) or by sirtuin (SIRT1) mediated deacetylation. Also it has been found that cellular energy status also signals to PGC-1α through mammalian target of rapamycin (mTOR) and YY1, and intriguingly, the regulation of PGC-1α activity by exercise and cold exposure is also under the control of stress signalling via the MAPK pathway, as well as through cellular calcium and cAMP signalling (Jones et al., 2011). The PGC-1 family members interact and strength the activity of a diverse set of transcription factors, exemplified by PPARs, nuclear respiratory factor-1/2 (NRF1/2), yin yang 1 (YY1), and estrogen-related receptors (ERRs), which collectively control expression of a large number of proteins involved in mitochondrial biology (Luo et al., 2017). The NRF1 was identified through its binding to a palindromic sequence in the cytC promoter (Scarpulla et al, 2012), and has been associated with the expression of many genes that are crucial for the correct synthesis and mechanism of the respiratory chain. Similarly, NRF2 was identified as a multi subunit activator of cytochrome oxidase expression (Scarpulla et al., 2011).

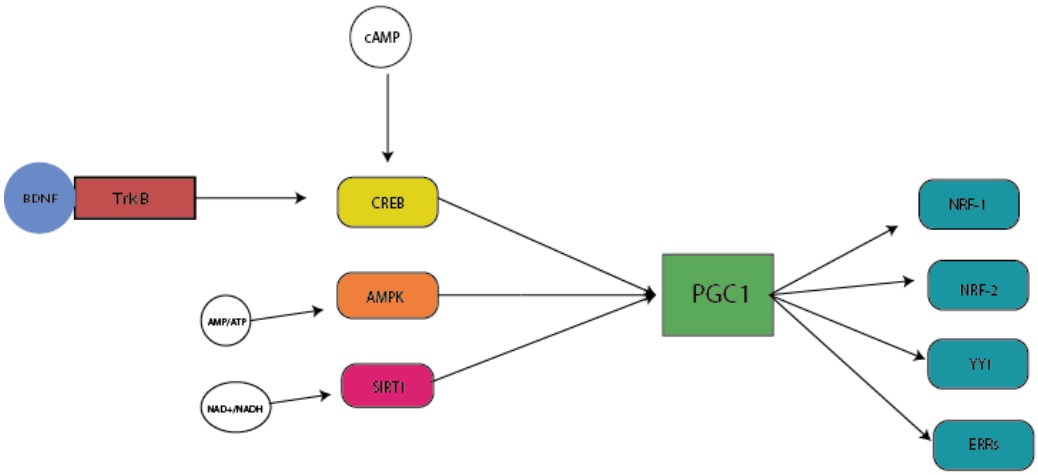


Figure 25 The PGC-1 pathway

Mitochondrial Movement

There are 2 models which are considered to be used for the axial transport of mitochondria which had been described earlier (figure 16). One model proposes that increased calcium homeostasis promotes the binding of Miro1 to the motor domain of kinesin-1, thereby sterically inhibiting access to the microtubule and the second one suggests that dicreased calcium levels cause the dissociation of kinesin-1 from mitochondria and the Miro/TRAK complex (Maday et al., 2014).

BIOINFORMATICS ANALYSIS

According to all above, it has been observed that among the neurodegenerative diseases studied there are common biochemical pathways and mutations. Thus the Bioinformatics approach of this work aimed to explore the relathionship between mitochondrial metabolic pathways and previously studied diseases. The results are shown in the table and the network below. As shown in the table below, diseases such as AD and PD have common paths where they seem to affect such as Oxidative Phosphorylation and Apoptosis but it was noticed that there are some paths that they do not have common but which are common with some other diseases.

Table 1: Diseases and Common Pathways

	MOVEMENT	PGC1 PATH	Mitophagy	Oxidative phosphorylation	Apoptosis	Fusion and Fission	NFTs
AD	-	-	-	×	×	-	x
PD	×	-	×	×	×	×	-
ALS	×	-	×	-	×	×	-
BSMA	-	×	×	x	×	-	-
FRIEDRIECH ATAXIA	-	-	-	×	-	-	-
SPASTIC ATAXIA	-	-	-	×	×	-	-
HUNTIGTON	-	×	-	×	×	×	-

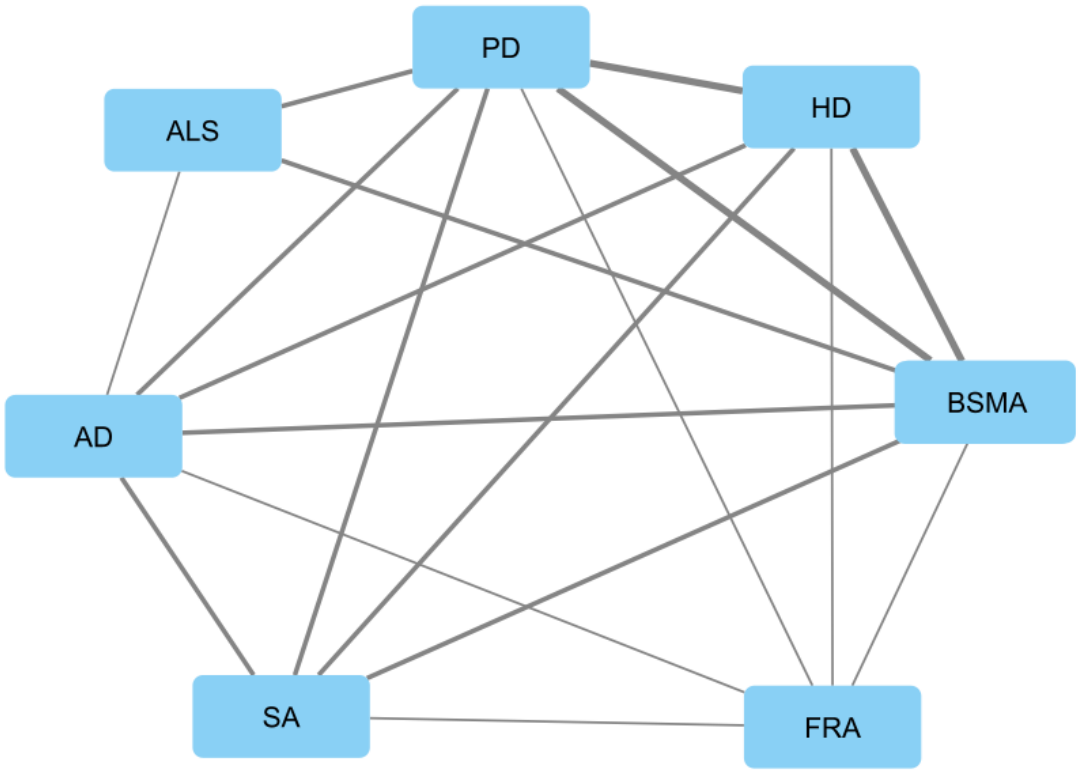


Figure 26: Disease-to-Disease Network based on their common mitochondrial mechanism

The first stage started with the use of UCSC genome browser and the use of mitochondrial DNA that showed the expression of 37 genes. This genes showed that were encoding for 13 proteins (polypeptides). These 13 proteins were put in the STRING database (https://string-db.org/), a network was generated and then first and second neighbours with a score of over 0.400 were added to these network. STRING is a database that hosts known and predicted protein-protein interactions including physical and functional associations. It forms networks where it connects genes or proteins with neighboring genes / proteins allowing the user to select the types of interactions he wants to see but and what score the neighbours want to appear using filtering.

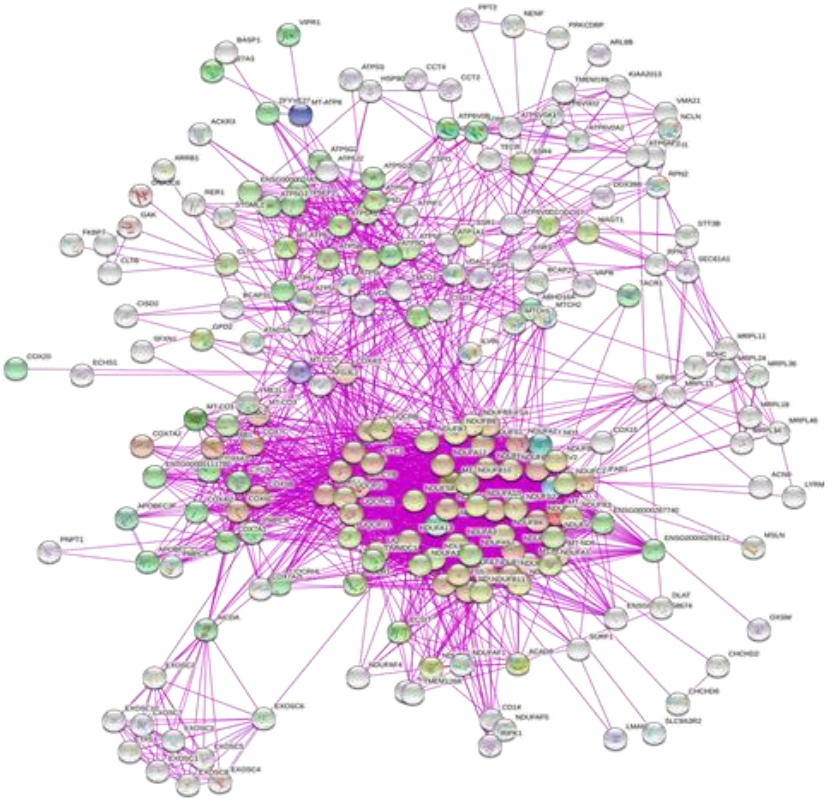
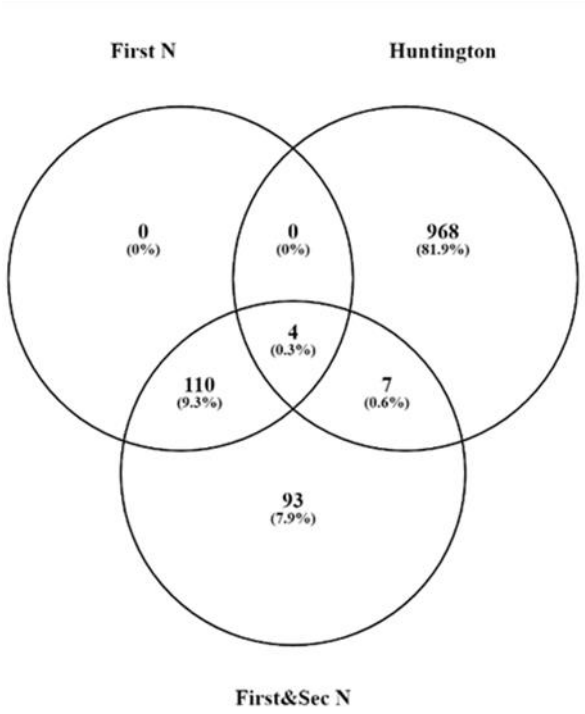
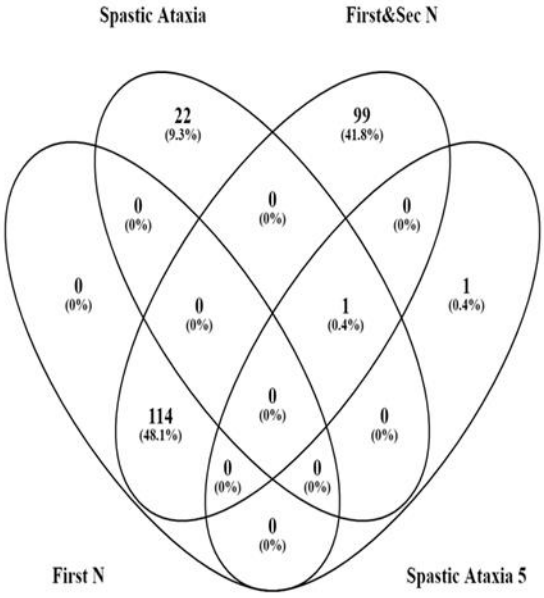
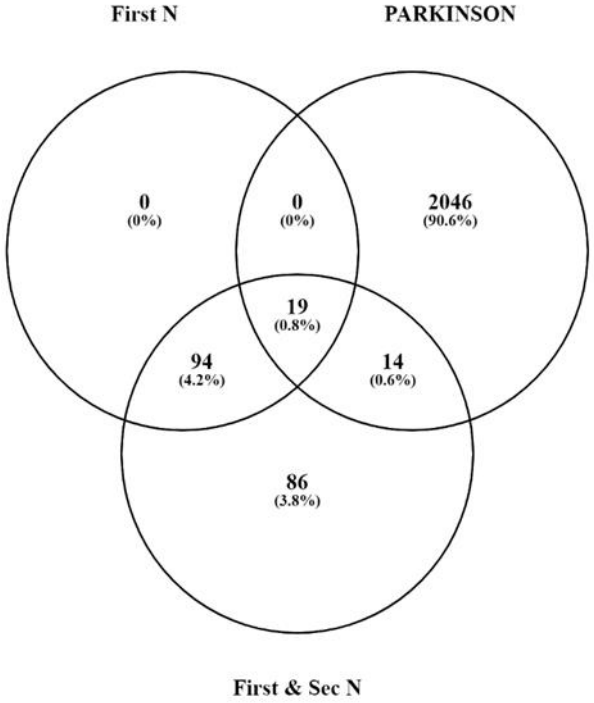


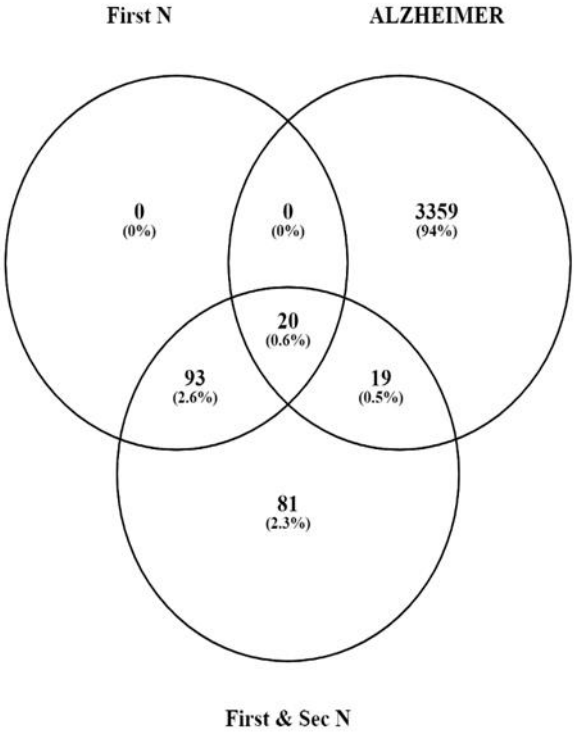
Figure 27: String Network of the 13 mitochondrial proteins with first & second neighbours

The second stage, continued with the help of DisGeNET (https://www.disgenet.org/), a database from which data on the relationship of diseases to various genes are derived. DisGeNET located the genes associated with the diseases in question, and then, with the VENNY tool (https://bioinfogp.cnb.csic.es/tools/venny/), common genes between mitochondrial proteins/protein interactions and disease-related genes were discovered. This is achieved by using the first and second neighbours of the mitochondrial genes found through the STRING and the genes designated by DisGeNET as registrations.


First N
Huntington
0
(0%)
0
(0%)
968
(81.9%)
4
(0.3%)
110
(9.3%)
7
(0.6%)
93
(7.9%)
First&Sec N


Spastic Ataxia
First&Sec N
22
(9.3%)
99
(41.8%)
0
(0%)
0
(0%)
0
(0%)
0
(0%)
0
(0%)
1
(0.4%)
1
(0.4%)
0
(0%)
114
(48.1%)
0
(0%)
0
(0%)
0
(0%)
0
(0%)
0
(0%)
First N
Spastic Ataxia 5


First N
PARKINSON
0
(0%)
0
(0%)
2046
(90.6%)
19
(0.8%)
94
(4.2%)
14
(0.6%)
86
(3.8%)
First & Sec N


First N
ALZHEIMER
0
(0%)
0
(0%)
3359
(94%)
20
(0.6%)
93
(2.6%)
19
(0.5%)
81
(2.3%)
First & Sec N

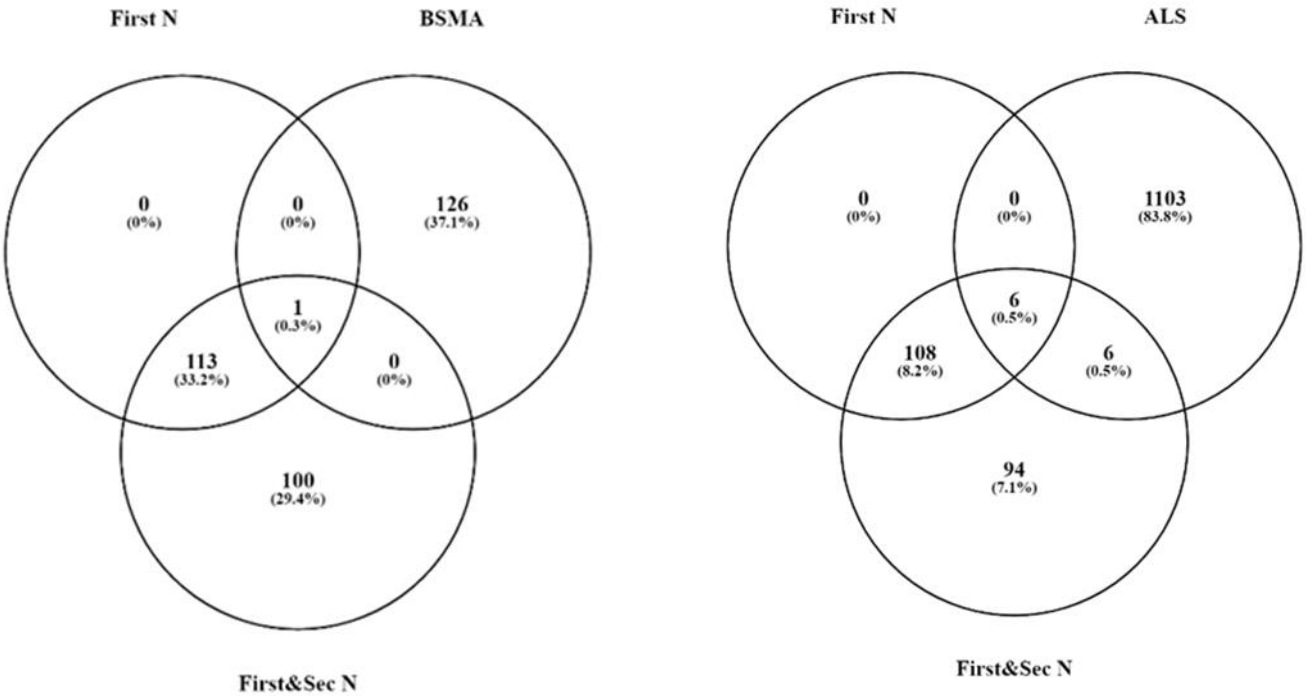


Figure 28: Common genes between mitochondrial proteins/protein interactors and disease-related genes were discovered through VENNY diagram for each disease

Then the third stage included the collection of the scores from DisGeNET, as well as from another database, Expression Atlas (https://www.ebi.ac.uk/gxa/home), from which expression data for these genes were collected using the diseases studied as records. Once these data were found, we used them as input to the Enrichr database (https://maayanlab.cloud/Enrichr/) that facilitates multi-database functional analysis. We proceeded with the cases where the number of genes was sufficient to obtain data from the database to find the pathways where these genes appear in each of these diseases. As entries to Enrichr database we used the related genes for each disease and pathways for KEGG 2019 HUMAN and GO Biological Process 2018 databases were collected. The findings from Enrichr proved that the genes that we used have a relationship with not only mitochondrial functions but also with the other diseases that were analysed before. For example GO Biological Process 2018 showed that AD has a connection with ETC and also with the synthesis of ATP and as it was described before AD can reduce the synthesis of ETC, stop the activity of complex IV and as a result the ATP concentration is reduced.

Table 2: Alzheimer disease result in Enrichr database

ALZHEIMER DISEASE				
KEGG 2019 HUMAN				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
Oxidative phosphorylation	5.99E-37	1.85E-34	65.25748	5442.805723
Parkinson disease	3.05E-36	4.70E-34	61.12145	4998.291496
Non-alcoholic fatty liver disease (NAFLD)	1.16E-33	1.20E-31	55.71736	4225.234117
Huntington disease	5.54E-33	4.27E-31	44.97018	3340.065784
Alzheimer disease	2.90E-32	1.79E-30	48.54905	3525.474926
Thermogenesis	2.90E-29	1.49E-27	35.9389	2361.573401
Retrograde endocannabinoid signaling	2.60E-20	1.14E-18	38.24579	1724.806922
Cardiac muscle contraction	1.96E-06	7.56E-05	24.18965	317.8667418
Cholesterol metabolism	0.007781	0.26629	15.09434	73.29876387
Necroptosis	0.009087	0.279884	6.98812	32.85040982
Legionellosis	0.009354	0.261899	13.72213	64.10978617
Influenza A	0.010521	0.270048	6.620324	30.15126382
NOD-like receptor signaling pathway	0.011723	0.277739	6.359975	28.2778749
Tuberculosis	0.011901	0.261812	6.324444	28.02468332
NF-κB pathway	0.026296	0.539954	7.944389	28.90424104
Toll-like receptor pathway	0.031073	0.598154	7.256894	25.19171792
MAPK signaling pathway	0.043339	0.785205	3.837544	12.04488744
Apoptosis	0.055201	0.944557	5.277741	15.28838538
Phagosome	0.061471	0.996481	4.965243	13.84898495
Hepatitis C	0.063613	0.979646	4.869142	13.41415217
Collecting duct acid secretion	0.069182	1	13.97624	37.3307721
Protein processing in endoplasmic reticulum	0.070933	0.993061	4.574042	12.10300942
Citrate cycle (TCA cycle)	0.076573	1	12.57862	32.32096116
Primary immunodeficiency	0.093594	1	10.19888	24.15894704
Epstein-Barr virus infection	0.099319	1	3.754811	8.671438542
Porphyrin and chlorophyll metabolism	0.105564	1	8.984726	20.20159948
Human immunodeficiency virus 1 infection	0.108543	1	3.559986	7.905322259
Human T-cell leukemia virus 1 infection	0.11453	1	3.446196	7.46763178
Hedgehog signaling	0.117378	1	8.028904	17.20073816

pathway				
Intestinal immune network for IgA production	0.119723	1	7.861635	16.68691304
Human cytomegalovirus infection	0.119728	1	3.354298	7.11960001
Vibrio cholerae infection	0.124393	1	7.54717	15.73060673
Amyotrophic lateral sclerosis (ALS)	0.12672	1	7.399186	15.28507586
GO Biological Process 2018				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
respiratory electron transport chain	5.88E-48	3.00E-44	104.3758	11351.09
mitochondrial ATP synthesis coupled electron transport	8.49E-47	2.17E-43	110.9878	11773.85
mitochondrial electron transport, NADH to ubiquinone	4.27E-36	7.26E-33	147.662	12025.96
NADH dehydrogenase complex assembly	2.25E-35	2.87E-32	112.0283	8937.499
mitochondrial respiratory chain complex I biogenesis	2.25E-35	2.30E-32	112.0283	8937.499
mitochondrial respiratory chain complex I assembly	2.25E-35	1.91E-32	112.0283	8937.499
mitochondrial respiratory chain complex assembly	1.14E-33	8.31E-31	77.80587	5901.902
cellular respiration	2.57E-14	1.64E-11	59.58292	1864.429
mitochondrial electron transport, cytochrome c to oxygen	2.12E-09	1.20E-06	89.84726	1794.355
aerobic respiration	1.01E-08	5.16E-06	67.38544	1240.591
ATP synthesis coupled electron transport	1.46E-06	6.77E-04	125.7862	1690.249
mitochondrion organization	5.14E-06	0.002185	13.55779	165.1162
mitochondrial electron transport, ubiquinol to cytochrome c	6.27E-06	0.002459	80.86253	968.774
cytochrome complex assembly	3.06E-04	0.111488	75.4717	610.7444
energy coupled proton transmembrane transport, against electrochemical gradient	3.06E-04	0.104056	75.4717	610.7444
oxidative phosphorylation	3.73E-04	0.119028	68.61063	541.5705
DNA deamination	4.47E-04	0.134204	62.89308	485.0796
necroptotic process	6.14E-04	0.174166	53.90836	398.6499

protein stabilization	7.03E-04	0.188818	9.930487	72.0965
heme biosynthetic process	8.07E-04	0.206003	47.16981	335.9301
programmed necrotic cell death	8.07E-04	0.196193	47.16981	335.9301
chaperone-mediated protein complex assembly	9.13E-04	0.211885	44.39512	310.6882
porphyrin-containing compound biosynthetic process	0.001026	0.227621	41.92872	288.5604
mitochondrial calcium ion transmembrane transport	0.001401	0.297884	35.9389	236.1395
positive regulation of ERK1 and ERK2 cascade	0.001973	0.402778	7.509622	46.7705
respiratory chain complex IV assembly	0.001988	0.390159	30.18868	187.7943
hydrogen ion transmembrane transport	0.002492	0.470994	26.95418	161.5811
negative regulation of response to DNA damage stimulus	0.002492	0.454172	26.95418	161.5811
TRIF-dependent toll-like receptor signaling pathway	0.002672	0.470197	26.02472	154.1937

Table 3: Parkinson disease result in Enrichr database

PARKINSON DISEASE				
KEGG 2019 Human				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
Parkinson disease	7.41E-18	2.28E-15	48.28974	1904.715
Oxidative phosphorylation	2.69E-16	4.14E-14	47.26101	1694.438
Huntington disease	3.17E-16	3.26E-14	35.52924	1267.918
Non-alcoholic fatty liver disease (NAFLD)	9.65E-16	7.43E-14	42.186	1458.544
Alzheimer disease	4.50E-15	2.77E-13	36.75856	1214.277
Thermogenesis	1.26E-13	6.45E-12	27.21088	808.3097
Retrograde endocannabinoid signaling	1.48E-10	6.50E-09	30.88803	699.1815
Cholesterol metabolism	9.09E-05	0.003501	34.28571	319.0369
Cardiac muscle contraction	3.41E-04	0.011682	21.97802	175.4411
NF-kappa B signaling pathway	0.011997	0.369511	12.03008	53.2101
Toll-like receptor signaling pathway	0.014251	0.39902	10.98901	46.71372
Neuroactive ligand-receptor interaction	0.021006	0.539142	5.071851	19.59241
Necroptosis	0.03258	0.771906	7.054674	24.1555
NOD-like receptor	0.038681	0.850977	6.420546	20.88226

signaling pathway				
Renin-angiotensin system	0.039506	0.811184	24.84472	80.281
Calcium signaling pathway	0.042698	0.821944	6.079027	19.17078
Human T-cell leukemia virus 1 infection	0.05608	1	5.218526	15.03445
Endocytosis	0.067802	1	4.683841	12.60497
Hedgehog signaling pathway	0.079112	1	12.15805	30.84369
Pathogenic Escherichia coli infection	0.091958	1	10.38961	24.79404
Legionellosis	0.091958	1	10.38961	24.79404
MAPK signaling pathway	0.093915	1	3.874092	9.163645
Cytosolic DNA-sensing pathway	0.10463	1	9.070295	20.47464
Acute myeloid leukemia	0.109337	1	8.658009	19.16294
RIG-I-like receptor signaling pathway	0.115576	1	8.163265	17.61489
Pertussis	0.124856	1	7.518797	15.64358
RNA degradation	0.12946	1	7.233273	14.78759
Salmonella infection	0.140112	1	6.644518	13.05858
Morphine addiction	0.147642	1	6.279435	12.01232
GO Biological Process 2018				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
mitochondrial ATP synthesis coupled electron transport	2.19E-27	1.12E-23	100.8403	6190.167
respiratory electron transport chain	1.12E-26	2.86E-23	91.18541	5448.481
NADH dehydrogenase complex assembly	1.40E-24	2.39E-21	116.0714	6375.066
mitochondrial respiratory chain complex I biogenesis	1.40E-24	1.79E-21	116.0714	6375.066
mitochondrial respiratory chain complex I assembly	1.40E-24	1.43E-21	116.0714	6375.066
mitochondrial electron transport, NADH to ubiquinone	3.67E-24	3.12E-21	149.0683	8044.034
mitochondrial respiratory chain complex assembly	4.81E-22	3.51E-19	76.58321	3759.136
cellular respiration	6.59E-12	4.20E-09	70.17544	1806.714
apoptotic process	4.82E-05	0.027307	12.36858	122.9557
mitochondrion organization	2.01E-04	0.102377	13.68691	116.5317
mitochondrial electron transport, ubiquinol to cytochrome c	2.67E-04	0.123947	81.63265	671.6401
necroptotic process	2.67E-04	0.113618	81.63265	671.6401
programmed necrotic cell death	3.52E-04	0.137997	71.42857	568.0825

regulation of protein phosphorylation	0.00108	0.393484	8.757526	59.82477
aerobic respiration	0.001093	0.371802	40.81633	278.3236
TRIF-dependent toll-like receptor signaling pathway	0.001173	0.373973	39.40887	265.9533
synaptic vesicle endocytosis	0.001173	0.351975	39.40887	265.9533
MyD88-independent toll-like receptor signaling pathway	0.001255	0.355774	38.09524	254.5017
positive regulation of tumor necrosis factor superfamily cytokine production	0.001428	0.383471	35.71429	233.987
endoplasmic reticulum organization	0.001611	0.411136	33.61345	216.1574
regulation of interleukin-8 production	0.001611	0.391558	33.61345	216.1574
clathrin-dependent endocytosis	0.002117	0.490983	29.30403	180.4509
positive regulation of interleukin-8 production	0.002688	0.596285	25.97403	153.7435
positive regulation of tumor necrosis factor production	0.003061	0.650845	24.31611	140.7663
ATP metabolic process	0.004318	0.881381	20.40816	111.1219
anion transport	0.004318	0.847482	20.40816	111.1219
positive regulation of cell death	0.00447	0.844845	20.05013	108.4782
I-kappaB kinase/NF-kappaBsignaling	0.00447	0.814672	20.05013	108.4782
regulation of tumor necrosis factor production	0.005103	0.897966	18.73536	98.88358

Table 4: Huntington disease results in Enrichr database

HUNTINGTON DISEASE				
KEGG 2019 HUMAN				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
Sphingolipid metabolism	0.025555	1	38.68472	141.8546
Cholesterol metabolism	0.027165	1	36.36364	131.1205
Pathogenic Escherichia coli infection	0.029845	1	33.05785	116.0909
Legionellosis	0.029845	1	33.05785	116.0909
Acute myeloid leukemia	0.035716	1	27.54821	91.79532
Pertussis	0.041025	1	23.92344	76.4015
Salmonella infection	0.046307	1	21.14165	64.95684
NF-kappa B signaling pathway	0.051039	1	19.13876	56.94104

Amoebiasis	0.051563	1	18.93939	56.15429
Hematopoietic cell lineage	0.052087	1	18.74414	55.38581
Toll-like receptor signaling pathway	0.055749	1	17.48252	50.47018
Sphingolipid signaling pathway	0.063552	1	15.27884	42.10682
Oxidative phosphorylation	0.070782	1	13.67054	36.20155
Parkinson disease	0.075404	1	12.8041	33.09732
Retrograde endocannabinoid signaling	0.078473	1	12.28501	31.26541
Non-alcoholic fatty liver disease	0.078983	1	12.20256	30.97643
Phagosome	0.080514	1	11.96172	30.13551
Alzheimer disease	0.090152	1	10.63264	25.58488
Tuberculosis	0.094183	1	10.15744	23.99714
Transcriptional misregulation in cancer	0.097696	1	9.775171	22.73599
Huntington disease	0.101197	1	9.420631	21.57966
Human T-cell leukemia virus 1 infection	0.114094	1	8.3022	18.02186
Thermogenesis	0.119989	1	7.870917	16.68912
Cytokine-cytokine receptor interaction	0.150359	1	6.184292	11.71758
MAPK signaling pathway	0.150833	1	6.163328	11.65845
Neuroactive ligand-receptor interaction	0.171	1	5.379236	9.500212
GO Biological Process 2018				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
mitochondrion organization	8.98E-05	0.458318	32.66195	304.3368
mitochondrial electron transport, NADH to ubiquinone	2.81E-04	0.716708	79.05138	646.4446
mitochondrial respiratory chain complex I biogenesis	5.44E-04	0.925673	56.81818	427.0572
NADH dehydrogenase complex assembly	5.44E-04	0.694255	56.81818	427.0572
mitochondrial respiratory chain complex I assembly	5.44E-04	0.555404	56.81818	427.0572
mitochondrial ATP synthesis coupled electron transport	9.58E-04	0.814461	42.78075	297.3713
respiratory electron transport chain	0.001169	0.85244	38.68472	261.1732
mitochondrial respiratory chain complex assembly	0.001244	0.793804	37.48828	250.7614
neutrophil degranulation	0.001952	1	11.38736	71.04653

neutrophil activation involved in immune response	0.001999	1	11.29305	70.1895
neutrophil mediated immunity	0.002046	0.94932	11.2003	69.34887
cellular response to lipoteichoic acid	0.003296	1	303.0303	1731.848
cellular response to bacterial lipopeptide	0.003296	1	303.0303	1731.848
response to lipoteichoic acid	0.003296	1	303.0303	1731.848
regulation of sterol transport	0.003844	1	259.7403	1444.467
negative regulation of MyD88-independent toll-like receptor signaling pathway	0.003844	1	259.7403	1444.467
positive regulation of protein localization to Cajal body	0.004392	1	227.2727	1233.617
regulation of protein localization to Cajal body	0.004392	1	227.2727	1233.617
regulation of MyD88-independent toll-like receptor signaling pathway	0.004392	1	227.2727	1233.617
membrane lipid catabolic process	0.004392	1	227.2727	1233.617
positive regulation of establishment of protein localization to telomere	0.00494	1	202.0202	1072.804
regulation of establishment of protein localization to telomere	0.005488	1	181.8182	946.4128
sphingolipid catabolic process	0.006035	1	165.2893	844.6627
magnesium ion transport	0.006035	1	165.2893	844.6627
positive regulation of protein localization to chromosome, telomeric region	0.006035	1	165.2893	844.6627
positive regulation of establishment of protein localization	0.006582	1	151.5152	761.1284
apoptotic process	0.006821	1	15.74183	78.51745
positive regulation of interleukin-8 secretion	0.007675	1	129.8701	632.441
necroptotic process	0.007675	1	129.8701	632.441

Table 5: ALS disease results in Enrichr database

ALS				
KEGG 2019 Human				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**
Cholesterol metabolism	3.18E-06	9.80E-04	100	1265.759
NF-kappa B signaling pathway	0.001429	0.219991	35.08772	229.8639
Toll-like receptor signaling pathway	0.001708	0.1754	32.05128	204.2363
Necroptosis	0.00408	0.314174	20.57613	113.2019
NOD-like receptor signaling pathway	0.004902	0.301994	18.72659	99.5882
Calcium signaling pathway	0.005452	0.279883	17.7305	92.40645
Human T-cell leukemia virus 1 infection	0.007328	0.322439	15.2207	74.82545
Neuroactive ligand-receptor interaction	0.016805	0.646988	9.861933	40.29671
Pathogenic Escherichia coli infection	0.032514	1	30.30303	103.8207
Legionellosis	0.032514	1	30.30303	103.8207
Cytosolic DNA-sensing pathway	0.037162	1	26.45503	87.1025
Acute myeloid leukemia	0.038899	0.998418	25.25253	81.9893
RIG-I-like receptor signaling pathway	0.041212	0.976402	23.80952	75.92933
Pertussis	0.044671	0.982756	21.92982	68.16747
Salmonella infection	0.05041	1	19.37984	57.89845
Amoebiasis	0.056118	1	17.36111	50.00512
Hematopoietic cell lineage	0.056687	1	17.18213	49.31625
TNF signaling pathway	0.064056	1	15.15152	41.63621
Parkinson disease	0.081972	1	11.73709	29.35892
Apoptosis	0.082527	1	11.65501	29.075
Phagosome	0.087506	1	10.96491	26.71111
Hepatitis C	0.08916	1	10.75269	25.99275
Cellular senescence	0.091911	1	10.41667	24.86396
cGMP-PKG signaling pathway	0.095201	1	10.04016	23.61205
Influenza A	0.097935	1	9.746589	22.64567
Tuberculosis	0.102294	1	9.310987	21.22813
Transcriptional misregulation in cancer	0.106092	1	8.960573	20.10256
Huntington disease	0.109876	1	8.635579	19.07086
Epstein-Barr virus infection	0.114182	1	8.291874	17.99308
Human immunodeficiency virus 1 infection	0.120071	1	7.861635	16.6641
GO Biological Process 2018				
Term	**P-value**	**Adjusted P-value**	**Odds Ratio**	**Combined Score**

apoptotic process	7.98E-06	0.040725	28.86003	338.7733
necroptotic process	2.99E-05	0.076315	238.0952	2480.313
programmed necrotic cell death	3.94E-05	0.067046	208.3333	2112.781
mitochondrion organization	1.19E-04	0.151836	29.94012	270.5462
TRIF-dependent toll-like receptor signaling pathway (GO:0035666)	1.33E-04	0.135516	114.9425	1026.07
MyD88-independent toll-like receptor signaling pathway	1.42E-04	0.120956	111.1111	984.2389
positive regulation of tumor necrosis factor superfamily cytokine production	1.62E-04	0.118137	104.1667	909.1235
positive regulation of interleukin-8 production	3.08E-04	0.196367	75.75758	612.5693
positive regulation of tumor necrosis factor production	3.51E-04	0.199258	70.92199	564.079
anion transport	4.99E-04	0.254713	59.52381	452.5367
positive regulation of cell death	5.17E-04	0.239898	58.47953	442.5281
I-kappaB kinase/NF-kappaBsignaling	5.17E-04	0.219906	58.47953	442.5281
regulation of tumor necrosis factor production	5.92E-04	0.232442	54.64481	406.1065
toll-like receptor signaling pathway	0.001173	0.427513	38.75969	261.5619
mitochondrial transport	0.002856	0.971444	24.69136	144.6544
cellular response to lipoteichoic acid	0.003595	1	277.7778	1563.392
peptidyl-serine autophosphorylation	0.003595	1	277.7778	1563.392
positive regulation of oxidative stress-induced cell death	0.003595	1	277.7778	1563.392
response to lipoteichoic acid	0.003595	0.965541	277.7778	1563.392
positive regulation of humoral immune response	0.003595	0.917264	277.7778	1563.392
cellular response to bacterial lipopeptide	0.003595	0.873585	277.7778	1563.392
negative regulation of glucocorticoid receptor signaling pathway	0.003595	0.833876	277.7778	1563.392
positive regulation of flagellated sperm motility	0.003595	0.797621	277.7778	1563.392
regulation of glucocorticoid receptor signaling pathway	0.004193	0.89154	238.0952	1303.413
regulation of sterol transport	0.004193	0.855878	238.0952	1303.413
modification by symbiont of host morphology or	0.004193	0.82296	238.0952	1303.413

physiology				
activation of signaling protein activity involved in unfolded protein response	0.004193	0.79248	238.0952	1303.413
progesterone receptor signaling pathway	0.004193	0.764177	238.0952	1303.413
death-inducing signaling complex assembly	0.004193	0.737826	238.0952	1303.413

Table 6: Alzheimer related genes scores

Alzheimer			
Genes	DisGeNET score	Expression Atlas	
		Adjusted p-value	log2- fold change
ACKR3	0.04	1.0077*10^-3	-2
AICDA	0.12		
BCAP31	0.01		
ATP6V0D1	0.01	4.8418*10^-5	-2.5
CD14	0.1		
CHCHD2	0.03		
CISD2	0.02	1.5461*10^-4	-2
ECSIT	0.01		
GLP1R	0.1		
EXOSC6	0.01		
HSP90AB1	0.01	1.1237*10^-4	-2.4
MAGT1	0.01		
MTCH2	0.01		
PHB	0.01		
PHB2	0.02		
RER1	0.01		
SURF1	0.01		
TSPO	0.1		
VDAC1	0.09	8.074*10^-4	-2.3
MT-COX3	0.02		
COX4I1		4.1644*10^-5	-2.3
COX5A	0.05		
COX6B1	0.01		
CYC1		2.3746*10^-4	-2.1
MT-COX1	0.07		
MT-COX2	0.1		
MT-CYTB	0.01		
NDUFA10		1.0681*10^-5	-2
NDUFA12	0.1		
NDUFA3		3.2933*10^-6	-2
NDUFA5	0.01	3.0802*10^-5	-2.1
NDUFA6	0.01		
NDUFA9	0.01		
NDUFB4		3.9179*10^-5	-2.6
NDUFB5		8.4793*10^-5	-2
NDUFB7		2.9288*10^-6	-3.2
NDUFB8	0.01		
NDUFS3		8.2845*10^-5	-2.1

NDUFS7		2.1843*10^-6	-2.6
NDUFS8		1.1146*10^-6	-2.9
NDUFV1		3.6436*10^-5	-2.2
NDUFV3		3.0725*10^-5	-2.3
SDHB		1.8228*10^-4	-2.1
UQCRC1	0.01	1.0077*10^-3	-2
MT-ND1	0.01		
MT-ND2	0.04		
MT-ND4	0.02		
RIPK1	0.07		
COX15	0.01		
ARRB1	0.01	3.7897*10^-7	2.8
CCT2	0.01	7.4928*10^-6	-2.2
DDX39B	0.01		
CYCS	0.1		

Table 7: Parkinson related genes scores

Parkinson			
Genes	DisGeNET score	Expression Atlas	
		Adjusted p-value	log2- fold change
NDUFA5		3.3022*10^-2	-1.3
NDUFS1		8.9174*10^-4	-1.2
ACAD9	0.01		
ARRB1	0.01		
CD14	0.01		
COX5A	0.01		
EXOSC6	0.01		
FKBP7	0.01		
NDUFS7	0.01		
PHB	0.01		
RIPK1	0.02		
TACR1	0.01		
VIPR1	0.01		
MT-CYTB	0.02		
MT-ND5	0.02		
NDUFA1	0.02		
NDUFA2	0.02		
NDUFS3	0.02		
NDUFS4	0.02		
NDUFV2	0.02		
VDAC1	0.03		
RER1	0.01		
DNAJC6	0.05	3.3022*10^-2	-1.2
MT-COX2	0.05		
TSPO	0.07		
CHCHD2	0.1		
MT-ND1	0.2		
MT-ND2	0.2		
MT-ND3	0.21		
GAK	0.5		

ATP6AP2	0.02		
CISD1	0.02		
VAPB	0.01		
UQCRH	0.01		
CYC1	0.01		

Table 8: Huntington related genes scores

Huntington			
Genes	DisGeNET score	Adjusted p-value	log2- fold change
ACKR3	0.02		
ATAD3A	0.01		
CD14	0.01		
CHCHD2	0.01		
MAGT1	0.01		
MT-CYTB	0.01		
MT-ND3	0.2		
NDUFA1	0.01		
SGPL1	0.01		
TSPO	0.02		
CCT2	0.01		

Table 9: ALS related genes scores

ALS			
Genes	DisGeNET score	Adjusted p-value	log2- fold change
MT-ND5	0.01		
MT-COX2	0.03		
MT-ATP6	0.01		
TACR1	0.01		
CD14	0.01		
CHCHD2	0.04		
PHB	0.01		
TSPO	0.02		
VAPB	0.5		
VDAC1	0.04		
ZFYVE27	0.01		
RIPK1	0.07		

Table 10: BSMA and Spastic Ataxia related genes score

BSMA			
Genes	DisGeNET score	Adjusted p-value	log2- fold change
COX5B	0.01		
SPASTIC ATAXIA			
Genes	DisGeNET score	Adjusted p-value	log2- fold change
AFG3L2	0.01		
SPASTIC ATAXIA TYPE 5			
Genes	DisGeNET score	Adjusted p-value	log2- fold change
AFG3L2	0.7		

At last with the help of Cytoscape (https://cytoscape.org/), an application that accepts data from tables in order to form networks according to the data and features provided by the user. Thus a network was created using the diseases that were presented above, the genes that were found to have a relationship with them, but also the point of the ETC that those genes seem to have a connection. With red colour are presented the diseases, with green the complexes of the ETC, with blue the genes that were used and lastly with pink the 13 mitochondrial proteins that were discussed above. Also the scores that were collected form DisGeNET were used to create the width of the edges in the following network in order to show the strength of the relationship between the diseases and the genes that were found.

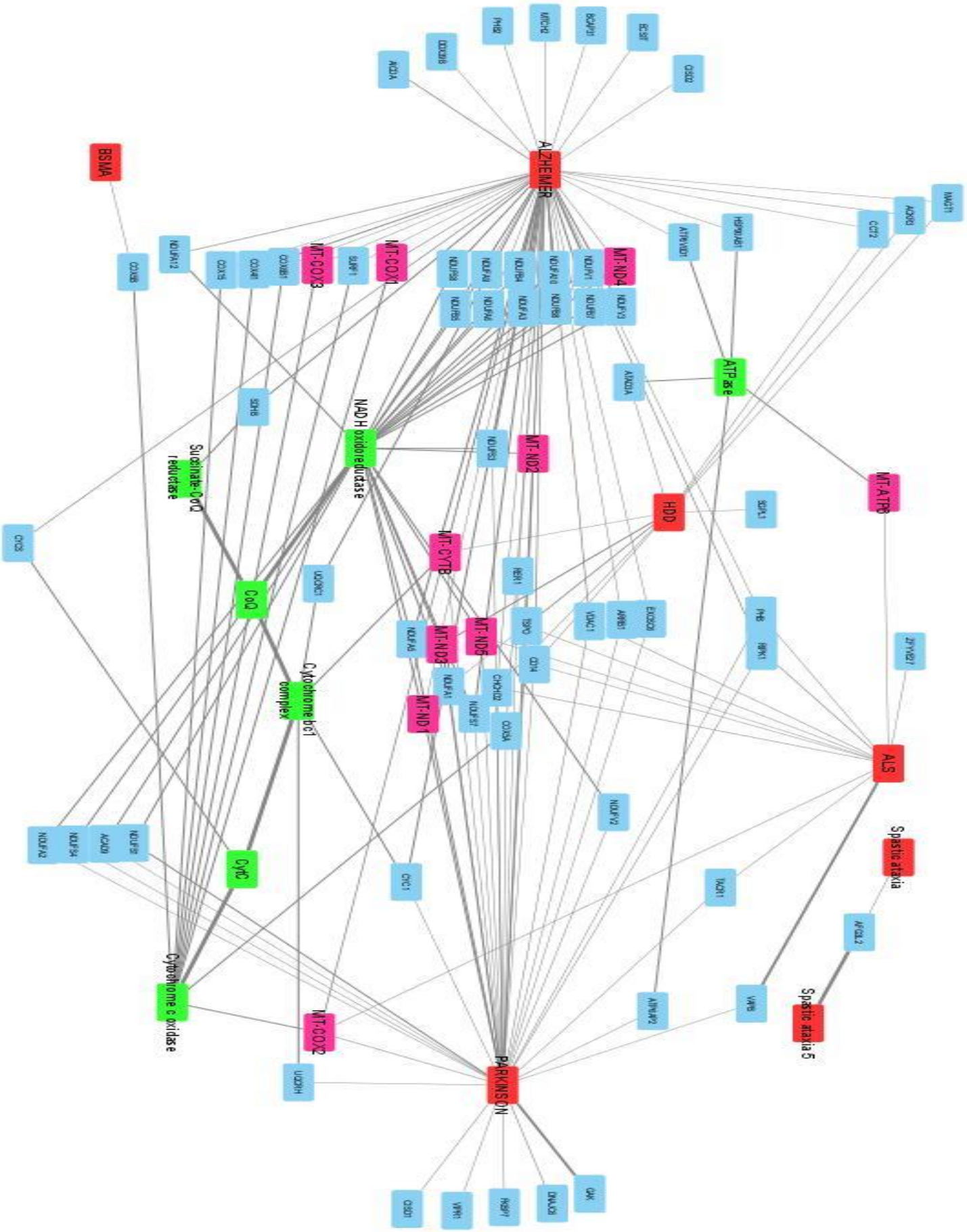


Figure 29 Interaction between the diseases and ETC

CONCLUSION

With the help of these tools, it was observed that there is a direct implication of the mitochondrial function in the examined neurodegenerative diseases. The examination of those mitochondrial function showed that some genes may involve in the pathogenesis of those diseases. After finding the related genes, those diseases were put in some databases and scores (Table 6-10) but also pathways (Table 3-5) were collected. From the scores it became clear that the down-regulation of those genes seems to have a relationship with the diseases that are related. Furthermore the pathways that was found from the Enrichr database verified the findings of the theoretical aspect of this work. As an example Alzheimer disease seems to stop complex V and the production of ATP in the mitochondria but also genes that are related to complex V like HSP90AB1 and ATP6V0D1 which were found to trigger the AD. According to this network and all the above in the theoretical aspect of this thesis, it is concluded that a lot of those diseases seem to affect the same mitochondrial complexes and also be triggered by the same mitochondrial genes or neighbors of those genes. The table below shows the relationship between the theoretical and the bioinformatics aspect of this work.

Table 11: Comparison of theoretical and bioinformation aspect

	COMPLEX	THEORETICAL	BIOINFORMATICS
ALZHEIMER	*NADH-Q oxidoreductase*		*
ALZHEIMER	*Succinate dehydrogenase*		*
ALZHEIMER	*Q-cytochrome* c *oxidoreductase*		*
ALZHEIMER	Cytochrome ***c*** oxidase	*	*
ALZHEIMER	ATP synthase	*	*
ALZHEIMER	CYT C	*	*
PARKINSON	*NADH-Q oxidoreductase*	*	*
PARKINSON	*Q-cytochrome* c *oxidoreductase*		*
PARKINSON	Cytochrome ***c*** oxidase		*
PARKINSON	ATP synthase		*
HUNTINGTON	*NADH-Q oxidoreductase*		*
HUNTINGTON	*Succinate dehydrogenase*	*	
HUNTINGTON	*Q-cytochrome* c *oxidoreductase*		*
HUNTINGTON	ATP synthase		*
HUNTINGTON	CYTC	*	
ALS	*NADH-Q oxidoreductase*	*	*
ALS	Cytochrome ***c*** oxidase		*
ALS	ATP synthase		*
ALS	CYTC	*	
BSMA	*NADH-Q oxidoreductase*	*	
BSMA	Cytochrome ***c*** oxidase		*
SPASTIC ATAXIA	*NADH-Q oxidoreductase*	*	*
FRDA	*NADH-Q oxidoreductase*	*	
FRDA	*Q-cytochrome c oxidoreductase*	*	
FRDA	*Succinate dehydrogenase*	*	

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