MITOCHONDRIAL PROTEIN INTERACTION MAPPING OF AMYOTROPHIC LATERAL SCLEROSIS IDENTIFIES POTENTIAL DRUG TARGETS

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Abstract— Amyotrophic Lateral Sclerosis is a neurodegenerative disorder involving the death of motor neurons, leading to paralysis of voluntary muscles. Oxidative stress is one of the major causes of ALS and involves several mitochondrial proteins linked in age related neurodegenerative diseases. Loss of motor neurons also gives rise to various other neurological conditions or disorders. Hence, the mitochondrial proteins directly involved in the oxidative stress pathway of ALS were identified, their interactions with other proteins were studied, and all these proteins were characterized according to other disorders they are involved in. These proteins can be used as potential drug targets to reduce the risk of related diseases in an ALS patient and improve the quality and longevity of his life.

Keywords— ALS, mitochondrial proteins, protein-protein interaction, Cytoscape, Drug target.

1. INTRODUCTION

ALS is primarily a disease of the parts of the nervous system that control voluntary muscle movement. It is one of the most common neurodegenerative disorders, with an incidence of about 1/100,000. One of the typical features of this progressive, lethal disease, occurring both sporadically and as a familial disorder, is degeneration of cortical and spinal motor neurons.

1.1 Causes of ALS

The following possible causes are being studied by ALS specialists:

1. Genetic factors- Hereditary factors and/or mutations in several genes.
2. Protein misfolding- A common feature in ALS is the presence in nerve cells of improperly folded proteins that clump together, forming "aggregates."
3. Free radicals- In ALS, free radicals may build to toxic levels and damage cells, through an attack process called "oxidative stress."
4. Excess glutamate- Evidence from studies of people with ALS points to an overabundance of glutamate in the nervous system.
5. Mitochondrial damage- Some amount of damage occurs naturally as part of the aging process, but in ALS there may be more damage to mitochondria than the average aging cell sustains.
6. Cell suicide- In ALS and other degenerative diseases, it's possible that the cell death program is activated inappropriately.
7. Immune system abnormalities- Microglia, immune system cells found in the nervous system, appear to play a role in ALS.

1.2 Symptoms of ALS

Gradual onset, painless, progressive muscle weakness is the most common initial symptom in ALS. Other early symptoms vary but can include tripping, dropping things, abnormal fatigue of the arms and/or legs, slurred speech, muscle cramps and twitches, and/or uncontrollable periods of laughingor crying. ALS-associated pain can occur as a result of tightness (spasticity) of muscles, decreased range of motion of the joints, and abnormal stresses on the muscles, bones and skin that occur as a result of immobility. It's important to note that the involuntary muscles, such as those of the heart, gastrointestinal tract, bowel and bladder, and those that regulate sexual functions are not directly affected in ALS.

1.3 Proteins involved in ALS

There are several proteins and their mutations which cause oxidative stress and are involved in ALS. After searching for these proteins by the keywords 'Oxidative Stress Pathway' and 'Amyotrophic Lateral Sclerosis' in UniProt, the following data was obtained:

Table 1: Proteins involved in ALS

S.NO.	UNIPROT ID	GENE NAME	PROTEIN NAME	GENE ORIGIN
1.	P42574	CASP3	Caspase 3	Nuclear
2.	Q00535	CDK5	Cyclindependant like kinase 5	Nuclear
3.	P10636	MAPT	Microtubule Associated Protein τ	Nuclear
4.	P12036	NEFH	Neurofilament heavy polypeptide	Nuclear
5.	Q99497	PARK7	Protein deglycase DJ-1	Nuclear
6.	P07737	PFN1	Profilin 1	Nuclear
7.	Q9UBK2	PPARGC1A	Peroxisome Poliferator Activated Receptor Gamma Coactivator 1-α	Nuclear

8.	P49768	PSEN1	Presenilin 1	Nuclear
9.	Q7Z333	SETX	Probable Helicase Senataxin	Nuclear
10.	P43004	SLC1A2	Excitatory Amino Acid Transporter 2	Nuclear
11.	P00441	SOD1	Superoxide Dismutase	Nuclear
12.	O60296	TRAK2	Trafficking Kinesin binding Protein 2	Nuclear
13.	P0CG48	UBC	Polyubiquitin- C	Nuclear
14.	Q96Q42	ALS2	Alsin	Nuclear
15.	P03897	MT-ND3	NADII-ubiquinone oxidoreductase chain 3	Mitochondrial
16.	P00395	MT-CO1	Cytochrome c oxidase subunit 1	Mitochondrial
17.	Q9NSE4	IARS2	Isoleucine-tRNA ligase, mitochondrial	Mitochondrial

1.4 Diseases involved in ALS

ALS involves the degeneration of motor neurons, which usually gives rise to various other neurological conditions or disorders. These conditions may be a cause or symptom of ALS or be a condition for which the patient may be at increased risk. These diseases include Multiple sclerosis, dementia,Parkinson's disease,Alzheimer's disease, Progressive bulbar palsy,Spinal muscular atrophy.

II. MATERIALS

The following databases/software's were used:

2.1 UniProt

Universal Protein resource is a primary protein database containing relevant information regarding a query protein. It is a primary database because it takes maximum of its data from the genome sequencing projects.

2.2 STRING

Search Tool for the Retrieval of Interacting Genes/Proteins is a biological database of ExPaSy which gives us information about protein-protein interaction. It contains information from different sources like experiments, computational predictions, scholars, and other databases like KEGG..

2.3 GeneMANIA

With the help of a large set of functional association data, GeneMANIA manages to find other set of related genes. This database can also be used to find new members of a pathway or complex or any additional gene with a specific function.

2.4 Cytoscape

It is a project to build open-source network visualization and analysis software. This software provides basic functionality to layout and query the network to viually integrate the network with state data.

2.5 GeneCards

It is a database of human genes can has all the relevant information of the known or predicted structures of human proteins such as their functions, disease(s) in which they are involved, related pathways etc.

III. METHODOLOGY

The following steps were taken to conduct the study:

Step 1: Information regarding ALS, oxidative stress pathway and the proteins involved in ALS was studied via various research papers and journals, websites and databases.

Step 2: The list of the proteins (query proteins) involved in the oxidative stress pathway of ALS was collected from UniProt. The keywords used for the search of proteins were 'Oxidative Stress Pathway' and 'Amyotrophic Lateral Sclerosis'.

Step 3: The interactions of these query proteins with other proteins were checked in STRING and GeneMANIA databases. Data of all the experimentally determined interactions was collected and compiled in an excel worksheet.

Step 4: This excel worksheet, after being thoroughly checked for any repeating interactions, was then uploaded on Cytoscape v_3.3.0 and a network of proteins involved in ALS was obtained.

Step 5: All the mitochondrial proteins in this network were then highlighted.

Step 6: The interactions of these proteins with other mitochondrial proteins were again checked in STRING and GeneMANIA databases. Data of all the experimentally determined interactions was collected and compiled in an excel worksheet.

Step 7: This excel worksheet, after being thoroughly checked for any repeating interactions, was uploaded in Cytoscape v_3.3.0 and a network of the mitochondrial proteins involved in ALS was obtained.

Step 8: All these mitochondrial proteins were then checked for whether they are involved in any diseases other than ALS. This information was collected using UniProt and GeneCards databases.

Step 9: The proteins in the mitochondrial network were then highlighted with different colours according to the diseases they are involved in other than ALS.

Step 10: The proteins which are not involved in any disease were taken and their interactions with the query proteins were observed.

IV. RESULT

The network of proteins involved in ALS, as obtained in Cytoscape v_3.3.0 is shown by figure 1. Figure 1

indicates the network of main/query proteins with its interacting proteins. These interactions were checked in STRING and GeneMANIA databases and the data of only experimentally determined interactions was taken. The proteins highlighted in blue are coded by genes present in the nuclear genome and the proteins highlighted in green are coded by genes present in the mitochondrial genome of humans.

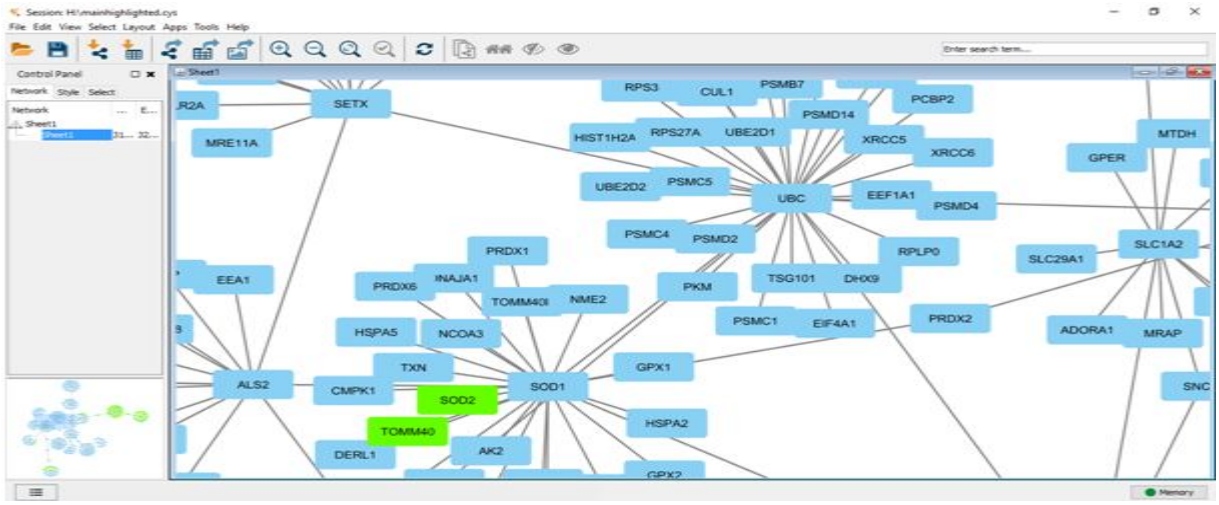

Figure 1: This figure indicates the network of proteins involved in ALS. The proteins highlighted in green are mitochondrial proteins.

From this network, all the mitochondrial proteins were taken and a new network was created using Cytoscape.

The network of mitochondrial proteins involved in ALS, as obtained by Cytoscape v_3.3.0, is shown by figure 2. Figure 2 indicates the network of all mitochondrial protein interactions. These interactions were checked in STRING and GeneMANIA databases and only the experimentally determined interactions were taken. These proteins were highlighted with different colours in the network, according to the diseases they are involved in. The proteins highlighted in violet are involved in Leigh's Syndrome. The proteins highlighted in deep blue are involved in blood disorders. The proteins highlighted in light blue are involved in Combined Oxidative Phosphorylation Deficiency (COPD). The proteins highlighted in green are involved in Leber Hereditary Optic Neuropathy (LHON). The proteins highlighted in yellow are involved in Alzheimer's disease. The proteins highlighted in orange are involved in Charcot Marie Tooth Disease. The proteins highlighted in pink are involved in skin defects. The proteins highlighted in brown are involved in deafness. The proteins highlighted in red are involved in other diseases. It is important to note that the proteins which are not highlighted with any colour (white) are the proteins which are not involved in any disease.

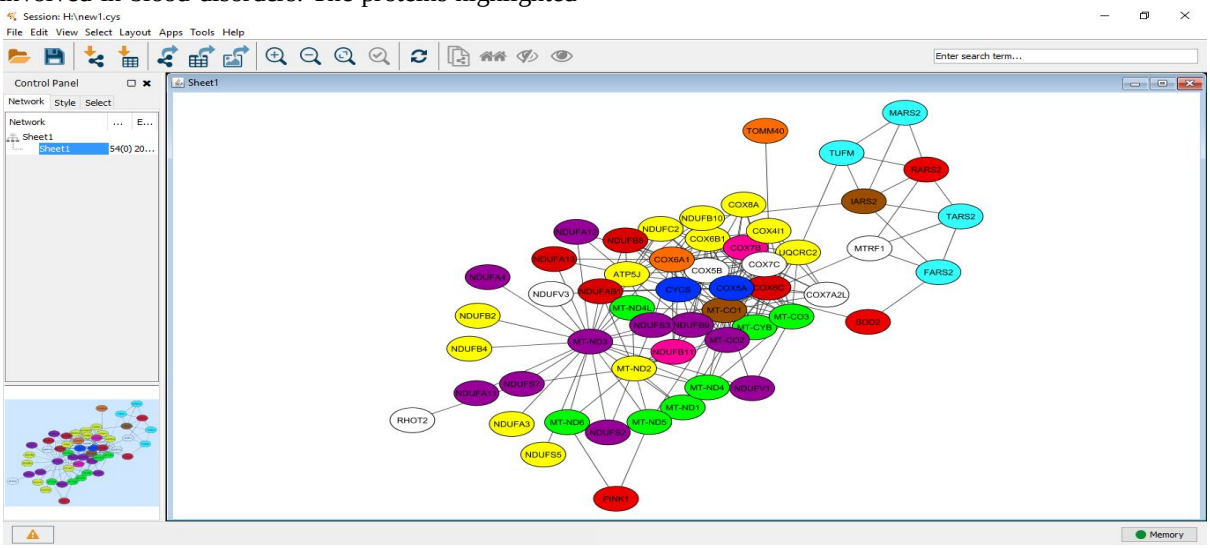

Figure 2: This figure indicates the network of mitochondrial proteins involved in ALS. These proteins are highlighted with different colours according to the diseases they are involved in.

Leigh's syndrome
Blood disorders
Combined Oxidative Phosphorylation Deficiency
Leber Hereditary Optic Neuropathy
Alzheimer's disease
Charcot Marie Tooth Disease
Skin Defects
Deafness
Other diseases
No involvement in any disease

From the mitochondrial protein network, those proteins which are not involved in any disease were identified. These proteins are COX5B, COX7C, COX7A2L, NDUFV3, MTRF1 and RHOT2. The interactions of these proteins from the major/query proteins were observed and a new network of mitochondrial proteins was obtained using Cytoscape v_3.3.0, as shown in figure 3. Figure 3 indicates the interactions of the proteins which are not involved in any disease with the main/query proteins. The (query) proteins highlighted in brown are involved in deafness (apart from ALS) and the (query) proteins highlighted in violet are involved in Leigh's syndrome (apart from ALS).

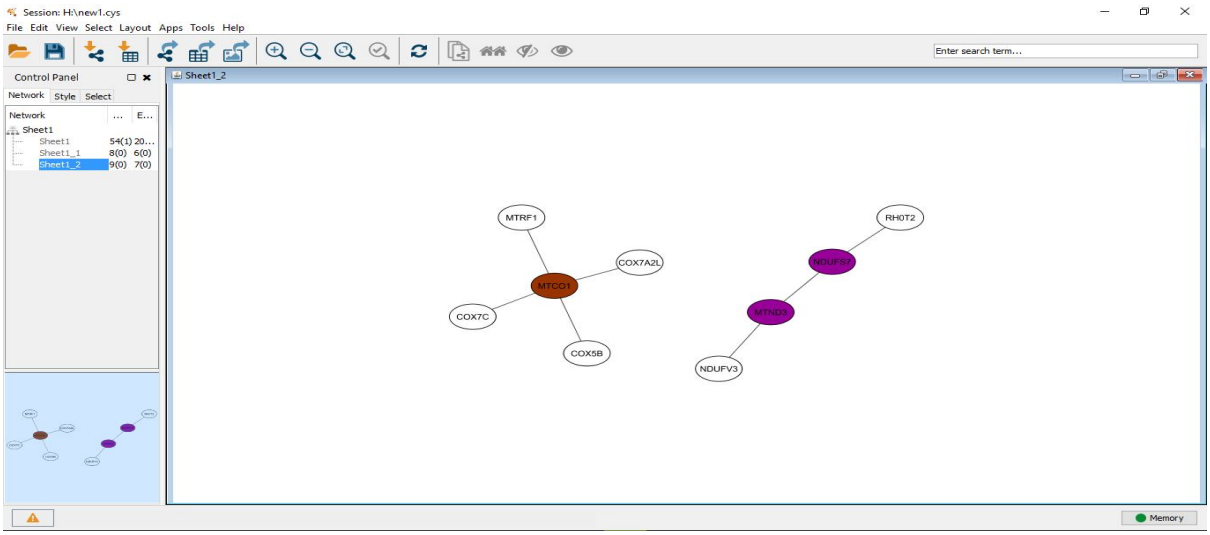

Figure 3: This figure shows interactions of ALS proteins with proteins not involved in any disease. The proteins are highlighted with different colours according to the diseases they are involved in.

Leigh's syndrome
Deafness
No involvement in any disease

The results are summarized in the table below:

Table 2: Interactions of proteins (not involved in any disease) with the query proteins.

S.no.	PROTEIN (NOT INVOLVED IN ANY DISEASE)	INTERACTION WITH QUERY PROTEIN	DISEASE IN WHICH THE QUERY PROTEIN IS INVOLVED (APART FROM ALS)
1.	COX5B	MT-CO1	Deafness
2.	COX7C	MT-CO1	Deafness
3.	COX7A2L	MT-CO1	Deafness
4.	MTRF1	MT-CO1	Deafness
5.	NDUFV3	MT-ND3	Leigh's syndrome
6.	RHOT2	MT-ND3 (indirect interaction via NDUFS7)	Leigh's syndrome

CONCLUSION AND DISCUSSION

It can be seen that COX5B, COX7C, COX7A2L and MTRF1 were directly interacting with MT-CO1 (query protein) in the protein interactions network obtained from Cytoscape v_3.3.0, hence, it can be concluded that COX5B, COX7C, COX7A2L and MTRF1 might be involved in sensorineural deafness. Also, NDUFV3 is seen to directly interact with MT-ND3 in the protein interactions network, hence, it can be concluded that NDUFV3 might be involved in Leigh's syndrome.RHOT2, however, does not directly interact with any of the query proteins, but it indirectly interacts with MT-ND3 via NDUFS7, both of which are involved in Leigh's syndrome. The work of this report can be extended to target these, and potentially other mitochondrial proteins via protein/drug targeting to reduce the risk of ALS and other related diseases in people prone to neurodegenerative diseases. Also, targeting these proteins can reduce the risk of other related diseases in a person already suffering from ALS, and hence improve the quality and longevity of his life.

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