Integrated Multi-Omic Data Analysis and Validation with Yeast Model Show Oxidative Phosphorylation Modulate Protein Aggregation in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic Lateral Sclerosis is a progressive, incurable amyloid aggregating neurodegenerative disease involving the motor neurons. Identification of potential biomarkers and therapeutic targets can assist in the better management of the disease. We used an integrative approach encompassing analysis of transcriptomic datasets of human and mice from GEO database. Our analysis of ALS patient datasets showed deregulation in Non-alcoholic fatty acid liver disease and oxidative phosphorylation. Transgenic mice datasets pertaining to SOD1, FUS and TDP-43 showed deregulation in oxidative phosphorylation and ribosome associated pathways. Commonality analysis between the human and mice datasets showed oxidative phosphorylation as a major deregulated pathway among the different datasets. Further, gene expression analysis of mitochondrial electron transport chain show downregulation of genes belonging to Complex I and IV. The results were then validated using the yeast model system. Inhibitor studies using metformin (complex-I inhibitor) and malonate (complex-II inhibitor) did not have any effect in mitigating the amyloids while antimycin (complex-III inhibitor) and azide (complex-IV inhibitor) reduced amyloidogenesis. Knock-out of QCR8 (complex-III) or COX8 (complex-IV) completely cleared the amyloids. Taken together, our results show a critical role for mitochondrial oxidative phosphorylation in amyloidogenesis and as a potential therapeutic target in ALS.

Introduction

Amyotrophic Lateral Sclerosis (ALS) often referred to as motor neuron disease is a neuro-muscular disease condition associated with neurodegeneration and amyloid aggregation¹. Diagnosis of ALS remains complicated and heavily relies upon clinical and electrophysiological examinations². Further, radiological methods are used negate other neurological conditions³. Riluzole is the only drug available worldwide⁴. The average age of onset of the disease is predicted to be 55–65 years⁵. However, there is a lack of appropriate statistics available on disease onset. There are around 126 genes involved in ALS, among which 118 are well curated in literature⁶. The majority of them are sporadic in nature while a some exhibit familial onset⁷.

Mutations in Super oxide dismutase-1 (SOD-1), Tar DNA binding protein (TDP-43), Fused in sarcoma (FUS), C9orf72 are known to occur in the majority of the familial forms of ALS⁸. Mutations in C9orf72 are observed in almost 34% of ALS cases⁸. Mutation in C9orf72 arises due to hexanucleotide expansion of GGGCC repeats in chromosome-9⁹. Mutations in SOD1 are associated with around 20% of familial ALS cases¹⁰. Some commonly observed SOD1 mutations are D90A, A4V, and G93A¹¹. Mutations like A4V are more lethal while few others provide a much longer life expectancy¹². FUS (Fused in Sarcoma) accounts for about 7.5% of familial ALS cases¹³. FUS gene is located on chromosome 16 and encodes a 526 amino acid protein that is involved in DNA repair, RNA splicing, transcriptional regulation, and transport of mRNA from the nucleus to the cytoplasm^{14,15}. FUS protein is extensively found in the nucleus and mutations lead to cytoplasmic aggregation¹³. The mechanism by which FUS aggregates is less understood to date¹⁶. FUS shows similarity in structure and function with TDP-43¹⁶. TDP-43 is

located in chromosome 18 and expresses ubiquitously in the nucleus and the cytoplasm ¹⁷. Two of the commonly observed mutations in patients are A382T and M337V ¹⁸.

Hyper-metabolism is known to increase degeneration of lower motor neurons and severity of the disease ¹⁹. An Integrative analysis of clinical data and further validation by laboratory model systems will help to delineate the mechanisms, potential biomarkers and therapeutic targets associated with the disease. Here in we carry out a comparative analysis of different transcriptomic data sets from different regions of ALS brain from both patients and mice model of disease. Transcriptomic analysis of muscle from ALS patients was also carried out. Furthermore, the cell type analysis of the gene expression data sets from human post-mortem sections were carried out. Microarray and RNA sequencing datasets pertaining to brain and muscles were obtained from GEO (Gene Expression Omnibus) database. Pathway enrichment analyses pertaining to the frontal cortex, motor cortex, and cerebellum were carried out for the post-mortem sections. Our results show deregulation of similar pathways across different datasets and study settings. The results obtained from patient data were further compared with transcriptomic data sets of mice model of ALS transgenic for SOD1, FUS and TDP-43. The comparative analysis shows deregulation of similar pathways in patients and model system. Finally, yeast model has been used to validate the results using inhibitor studies, knock-out studies and sequencing-based techniques. The results of the study show a potential role for oxidative phosphorylation and reactive oxygen species (ROS) in the progression of ALS. Modulating oxidative phosphorylation and ROS amyloid load and help in better management of ALS.

Results

Gene Set Enrichment and pathway analysis of post-mortem brain sections show enrichment of oxidative phosphorylation and NAFLD (Non-Alcoholic Fatty Liver Disease):

Three different human datasets of ALS patients pertaining to cortex, cerebellum, and muscles were identified from the GEO database (**SUPPLEMENTARY-1**). GSEA was carried out for the different post-mortem sections. Enrichment of cortex sections showed oxidative phosphorylation, Alzheimer's disease, Parkinson's disease, NAFLD, etc (**FIGURE-1A**). Enrichment analysis of the cerebellum showed enrichment of NAFLD, glutamatergic synapse, oxidative phosphorylation, Parkinson's disease, etc (**FIGURE-1B**). Pathway enrichment analysis for proteomic data obtained from the literature pertaining to cortex and CSF were also carried out (**FIGURE-2A and 2B**). The results showed enrichment of nicotinate-nicotinamide metabolism, riboflavin metabolism, alanine-aspartate-glutamate metabolism, etc. in the cortex section, and pathways related to cholesterol metabolism, vitamin absorption, etc. were enriched in CSF. Finally, commonality analysis was carried out between different brain sections and proteomics of the cortex to identify common pathways that are de-regulated. Oxidative phosphorylation and NAFLD were found to be common between different cortex sections (**FIGURE-2C**).

Gene set enrichment of analysis and pathway enrichment analysis of familial mice brain datasets show enrichment of oxidative phosphorylation, vitamin metabolism, signaling pathways and axonal

conduction:

Mice datasets pertaining to common familial forms of the disease (SOD1, FUS, and TDP-43) were also identified from the GEO database (**SUPPLEMENTARY-1**). Pathway enrichment analysis and GSEA analysis of SOD1, FUS, and TDP-43 brain were carried out. The results of the individual analysis are summarized on **FIGURE-3A, FIGURE-3B, and FIGURE-3C**. Commonality analysis was performed to identify common pathways enriched between SOD1, FUS, and TDP-43 brain datasets. Oxidative phosphorylation and ribosome pathways were found to be common between different mice brain datasets (**FIGURE-4A and 4B**).

Oxidative phosphorylation was found to be common between different brain datasets:

Different brain datasets including human and mice brains showed enrichment of oxidative phosphorylation. Genes belonging to Complex-I and Complex-IV were found to be significantly enriched in the post-mortem section datasets (**FIGURE-5A**) and majority of the genes were down-regulated. Further, cell type analysis was carried out to identify the tissues associated with deregulated pathways. Astrocytes and glutamatergic neurons were found to be significantly affected cell types in post-mortem human datasets (**FIGURE-5B and FIGURE-5C**). Astrocytes are the cell types which nourish the neurons through the astrocyte-neuron Lactate shuttle ²⁰. Dysfunction of electron transport chain in astrocytes can lead to elevated levels of lactate ²¹.

Pathway and cell type analysis of ALS muscle shows deregulation of oxidative phosphorylation in myofibroblast:

ALS is a neuro-muscular disease. Hence, the understanding metabolic state of muscle from ALS patients is important in understanding the disease condition. Pathway enrichment of ALS muscle biopsies showed enrichment of Parkinson's disease, Alzheimer's disease, oxidative phosphorylation, thermogenesis etc. (**FIGURE-6A**). Further cell type analysis showed that major metabolic pathways are deregulated in fibroblast of ALS muscle (**FIGURE-6B**). Based on our earlier observations, it is evident that the mitochondrial oxidative phosphorylation pathway and Complexes-I and Complex-IV in particular might be involved in ALS disease parse. Studies from literature have shown a role of deregulated mitochondrial complexes in generating reactive oxygen species associated with FUS and TDP-43. Hence, we validated our finding from ALS patients and transgenic mice model of ALS using FUS or TDP43 expressing yeast model of ALS. *Saccharomyces cerevisiae* has been prominently used for studying disease mechanisms, protein misfolding, amyloid aggregation, and other neurodegenerative phenotypes. Further, it has been considered as the best model system for studying amyloid diseases.

Inhibitors of mitochondrial electron transport chain and knock out of genes in Complex-III and IV confirms their role in modulating aggregation of FUS and TDP-43:

Saccharomyces cerevisiae transformed with aggressive forms of ALS (FUS, TDP-43, and their mutants) were used as a model system. Transformed cells were treated with inhibitors of mitochondrial complexes

(**FIGURE-7A and 7B**). Complex-I and II inhibitors did not show anti-amyloid activity. However, inhibition of Complex-III and Complex-IV reduced amyloidogenesis. We also treated cells with Co-enzyme Q10, a modulator of complex-III that inhibits ROS production. Treatment with Coenzyme Q10 reduced amyloid aggregation in majority of mutants. Florescence quantification was carried out to quantitate the levels of amyloidogenesis and amyloid clearance (**SUPPLEMENTARY- 2**). The results obtained through quantification had a strong correlation with the imaging data. The results were further validated using knock-out studies Critical knock-outs of mitochondrial complex III and IV showed reduced amyloidogenesis in most of the mutants. Complex-IV is also known to be associated with ROS production. SOD1 and SOD2 levels are known to be directly co-related with ROS levels. Hence, SOD1 and SOD2 levels in FUS and TDP-43 transformed yeast cells were measured using the RNA sequencing technique. Our results showed that SOD1 levels were much higher in the majority of the mutants (**FIGURE-7C**). Higher ROS levels indicate greater mitochondrial dysfunction and thereby leading to neurodegeneration.

Discussion

Amyotrophic Lateral Sclerosis is a amyloid hyper-metabolic disease involving the brain, spinal cord, and muscles ²². However, the metabolic micro-environment depends on numerous extraneous variables such as tissue specificity, region specificity and, the life-style of patients. The severity of the disease is also completely dependent on the metabolic homeostasis maintained by the affected regions ²³. We performed transcriptomic analysis of GEO datasets to understand the disease biology by identifying pathways that were deregulated in the post-mortem section of ALS patients and mice model of disease. Oxidative phosphorylation and NAFLD were common between post-mortem human datasets. Further cell type analysis shows astrocytes and Glutamatergic neurons as being involved in the disease. Previous studies have shown the astrocyte-neuron lactate shuttle is affected in many neurodegenerative diseases ²¹. The astrocytes also exhibit an impaired ability to take up glutamate and convert it into glutamine, which is further released back to be taken up by the neurons ²⁴. This impaired ability to take up glutamate by astrocytes is often ascribed to glutamate mediated excitotoxicity in many neurodegenerative diseases ²⁵. The proteomic data sets from the brain also showed enrichment of oxidative phosphorylation. Cell type analysis of ALS muscle shows enrichment of myofibroblasts. Myofibroblasts are known to contribute to the disproportionate deposition of connective matrix proteins which delay tissue repair and thereby contribute to scarring and fibrosis of muscles ²⁶.

The pathways mitophagy, riboflavin metabolism, nicotinate nicotinamide metabolism, alanine, aspartate, and glutamate metabolism, etc. were also enriched in different data sets. Previous studies have shown impaired electron transport chain function and oxidative phosphorylation in ALS ²⁷.

The function of mitochondrial Complex I is known to be compromised in ALS ²⁸. Similarly compromised complex III and complex VI function has been reported in ALS ^{29,30}. The compromised ETC lead to elevated ROS which in turn results in damage to other mitochondria and causes cell death ³¹. In addition,

mitophagy was found to be compromised in ALS³². Mitophagy is a process that helps to maintain mitochondrial quality by selectively damaged mitochondria. In ALS, the compromised mitophagy leads to the accumulation of damaged mitochondria resulting in elevated ROS³².

Further, our analysis also shows a compromised riboflavin pathway. Studies have shown a role for riboflavin in FMN biosynthesis³². FMN addition was shown to rescue the yeast model of Alzheimer's Disease³². Overall, the results show improved mitochondrial quality control might mitigate amyloidogenesis in ALS.

Experimental mice datasets of different familial forms of ALS also showed similar results. Having observed the common and conserved oxidative phosphorylation pathway to different data sets as well as mice models, we looked for the deregulation of genes in different ETC complexes. Our analysis shows that genes belonging to complex I and Complex IV are downregulated in all the data sets. Complex I, II, and III in ETC contribute to ROS generation³³. In addition, the enzymes of TCA cycle aconitase, α -ketoglutarate dehydrogenase, pyruvate dehydrogenase, glycerol-3-phosphate dehydrogenase, dihydroorotate dehydrogenase, the monoamine oxidase A and B, and cytochrome B5 reductase also contribute to ROS³⁴. Studies have shown that compromised function of complex I lead to elevated levels of ROS³⁵. complex I related diseases mutations lead to increased ROS production and oxidative stress³⁶. Supplementation of NAD⁺ or intermediates in its biosynthesis was shown to mitigate symptoms and was found to be beneficial in mitochondrial and neurodegenerative diseases³⁷. Increased complex II activity can lead to reverse electron transfer from complex II resulting in increased ROS production³⁸. The major production of ROS in mitochondria takes place in Complex III, followed by complex I and II as well as to a lesser extent in complex IV³⁹. The site production in complex I is the Q reduction site while in the case of complex III it is the QH₂ –cytochrome c reductase site⁴⁰. Further complex IV is also capable of producing ROS and complex IV dysfunction is associated with many diseases⁴¹. To reiterate the importance of mitochondrial ETC in amyloidogenesis we employed inhibitors and modulators of ETC as well as gene knock-out studies. Our results showed that Azide an inhibitor of complex IV attenuated amyloidogenesis in both FUS and TDP 43 yeast models of ALS. The results were validated by inhibitor and knock-out studies that were carried out using our model systems. Yeast knockout of COX-8 (Knock-out of cytochrome C oxidase), that is a part of complex IV was used for the study. COX-8 knock-out showed a reduction in amyloids. Dysregulation of complex IV is observed in several of neurodegenerative diseases. Dysfunction of complex IV genes leads to the production of excessive quantities of ROS which in turn can lead to progression of neurodegeneration in ALS patients. QCR8 is involved in the electron transfer from ubiquinol to cytochrome c. The ubiquinol binding site in complex III is a major ROS-producing site⁴². Knock-out of QCR8 leads to attenuation of ETC function⁴³. QCR8 knock-out cells also have increased ubiquinone. The increased ubiquinone could be reduced by complex I and II⁴⁴. Knock-out of COX8 in yeast was shown to reduces the activity of complex IV. The reduced activity of COX4 is shown to reduce the production of ROS with potentially favourable consequences⁴⁵. Knock-out of COX5A or 5B is also known to significantly drop oxygen consumption⁴⁶. Previous studies have shown

that TDP-43 induced cell death through apoptosis or necrosis in the yeast model of ALS is dependent on ETC function ⁴⁷. Co-enzyme Q10 is known to mitigate disease severity and increase life span in the mice model of ALS ⁴⁸. Consistent with this our study also shows coenzyme Q10 reduced amyloid load in yeast model. However, Coenzyme Q10 was not found to be effective in clinical trials ⁴⁹. Our analysis shows a potential role for Co-enzyme Q₁₀, ROS scavengers like glutathione, and metabolic pathways associated with the disease.

ALS is associated with oxidative stress ⁵⁰. The increase ROS production leads to activation of pathways to neutralize it. This in turn leads to the activation of glutathione and other pathways involved in ROS scavenging ⁵¹. Consistent with this observation, analysis of SOD1 expression levels in transcriptomic datasets of majority of disease mutants yeast model of ALS was significantly higher. SOD1 levels are often correlated with ROS levels in literature. Therefore, scavengers of ROS can be potential therapeutic agents in ALS disease biology. Oxidative phosphorylation was also enriched in fibroblasts of ALS muscle. Our studies conclusively pinpoint the role of oxidative phosphorylation and ROS in ALS brain and muscle **(FIGURE-8)**. The above finding can have implications for understanding mechanisms associated with disease, potential biomarkers associated progression and prognosis as well as potential therapeutic targets in ALS.

Conclusion

Our results of transcriptomic data sets from ALS patients and transgenic mice models of ALS (SOD1, TDP43 and FUS) shows deregulation of oxidative phosphorylation. Gene expression analysis of GEO datasets show downregulation of genes belonging to mitochondrial complex III and IV. FUS and TDP-43 expressing yeast show changes in expression levels of oxidative stress marker (SOD1). Mitochondrial complex I inhibitor (metformin) did not have any effect on protein aggregation, while inhibitor of complex II (malonate) significantly increased amyloidogenesis. Inhibitor of complex III (Antimycin) and complex IV (Azide) significantly reduced amyloid levels. Knock out of QCR8 and COX8 that are subunits of complex III and IV respectively significantly reduced amyloid levels. Overall, our results show a critical role for oxidative phosphorylation involving complex III and IV in protein aggregation. Mitochondria might emerge as potential therapeutic target in ALS. Further, reactive oxygen levels can be a major contributor in the progression of the disease condition.

Materials And Methods

Post-Mortem Sections and muscle biopsy section analysis

Post mortem cortex samples were obtained from GEO datasets GSE124439⁵² and GSE67196^{53,54} containing gene expression data of ALS patients and control samples (146 samples + 16 controls). GSE124439 was analyzed using Agilent Genespring and Network Analyst (www.networkanalyst.ca)⁵⁵⁻⁵⁸. Differential gene expression for GSE67196 (10 samples + 8 controls) was carried using GREIN

(www.ilincs.org/apps/grein)⁵⁹. Gene set enrichment analysis was carried out using the Kegg database and Network analyst. GEO dataset (GSE41414⁶⁰) containing muscle biopsies of ALS patients was used for the study. Analysis was carried out using the GEO2R tool that utilizes bio-conductor packages GEO Query and Limma. Pathway enrichment analysis was carried out using Enrichr (maayanlab.cloud/Enrichr)^{61–63}. Further, cell type analysis was carried out using Enrichr and Azimuth cell type database. Finally, commonality analysis was carried out to identify common pathways. Common pathways between different datasets were identified using Venny (bioinfogp.cnb.csic.es/tools/venny)⁶⁴ and draw custom Venn (bioinformatics.psb.ugent.be/webtools/Venn).

Pathway Enrichment Analysis of Proteomics datasets

Proteomic datasets pertaining to the cortex and cerebrospinal fluid were obtained from the literature^{65–68}. Enrichment analysis was carried out using Enrichr and KEGG database. Enrichr carries out analysis using adjusted P-value(p) of 0.05 and ranking score. The top enriched pathways were represented using a bar diagram.

Analysis of familial experimental samples

Experimental datasets of mice brain sections pertaining to SOD1, FUS, TDP-43 were analyzed. The details of the dataset used and their accession ID is provided in Supplementary figure-1(GSE101391⁶⁹, GSE40652⁷⁰, GSE111775⁷¹). GSEA was performed using Network Analyst. The counts table for the appropriate datasets was downloaded from the GREIN server. Controls and samples were identified using the meta-data available. The table obtained was uploaded in Network analyst and analysis was carried out. Differential gene expression was carried out using the Deseq2 bio-conductor package. GSEA was further carried out using the same portal.

Commonality Analysis

Common pathways enriched between different familial forms of ALS datasets were obtained by plotting a Venn Diagram using an online tool Venny and Draw custom Venn Diagram.

Transformation and Induction of *Saccharomyces cerevisiae* with FUS and TDP-43 plasmids

Wild type and mutant plasmids of FUS (426 Gal-FUS-YFP, 426 Gal-FUS-1-373aa-YFP, 426 Gal-FUS-1-413aa-YFP, 426 Gal-FUS-RRM Mutant-YFP) and TDP-43 (pRS416Gal TDP43 WT YFP and pRS416 Gal Q331K YFP) were obtained from Adgene. *Saccharomyces cerevisiae* (strain BY4741) was transformed with the above plasmids using standard electroporation protocols (1.5KV/25uF/200Ω) with the help of a Bio-Rad electroporator. Transformed cells were grown in Himedia URA-YNB-Dextrose-Agar media at 30°C for 3–4 days. After, the growth of the transformed colonies, a single colony from each plate was inoculated in Himedia URA-YNB-Dextrose liquid broth. The yeast cells were allowed to grow for 12 hours. After 12 hours, 200μl of the culture was inoculated in a raffinose medium containing Himedia URA-YNB-raffinose for 12 hours. After 12 hours, the cells were pelleted and washed with Phosphate Buffer Saline

(pH 7) and were transferred to Himedia URA-YNB-Galactose media for induction. Cells were allowed to remain in galactose media for 7 hours and were harvested at an OD₆₀₀ of 0.8. The induced cells were used for further studies.

Fluorescence Imaging and quantification

Induced Cells were observed under Laben fluorescence microscope at 100X magnification. FUS and TDP-43 protein were tagged with E-YFP which has an excitation wavelength of 510nm and emission at 535nm. The yeast cells were illuminated with a fluorescence LASER and the images were captured. Fluorescence quantification was carried out using Icy software (icy.bioimageanalysis.org) and Microsoft Excel 2019.

RNA Sequencing and measurement of Super Oxide Dismutase Levels

45 million transformed cells were counted using a haemocytometer and the cell pellets were treated with 750µl of Trizol. The mixture was vortexed vigorously and stored in -80°C. RNA isolation was carried out using standard protocols. Library preparation was carried out using the NEB library preparation kit (E7490) for Illumina. Briefly, mRNA was purified using oligo-dT beads from the total RNA. Purified mRNA was subjected to fragmentation at high temperatures and then converted to cDNA. The cDNA fragments are ligated with the adapters and then purified to obtain final libraries. Paired-end sequencing was performed using an Illumina HiSeq 2500. The samples had more than 10 million reads. After the quality check using the Fastqc tool, reads were aligned to the reference *Saccharomyces cerevisiae* S288C strain (Downloaded from The University of California, Santa Cruz (UCSC) genome browser) using bowtie2⁷² with default parameters. The output from bowtie was converted to a binary file using Samtools⁷³. This binary alignment map file (BAM) was used to generate read counts using bedtools⁷⁴. The annotation file used was specific to the *Saccharomyces cerevisiae* S288C strain downloaded from UCSC. DESeq package from Bioconductor software, R⁷⁵ was used to obtain differentially expressed genes. Significant genes were identified with special reference to the expression levels of SOD1 (Super Oxide Dismutase1) and SOD2 (Super Oxide Dismutase2).

Inhibitor and knock-out studies using Fluorescence Imaging

Inhibitor treatment studies were carried out on *Saccharomyces cerevisiae* transformed with FUS, TDP-43, and its mutants. Transformed cells were treated with a non-toxic concentration of inhibitors during galactose induction. Fluorescence imaging was carried out after 7 hours of induction along with non-treated controls. Fluorescence quantification was performed using Icy software (icy.bioimageanalysis.org) and Microsoft Excel 2019. Yeast knock out library was procured from Dharmacon (Product No. YSC1054). Yeast knock-outs pertaining to mitochondrial complex-III (Δ QCR8) and complex-IV (Δ COX8) were used for the study. Knock-outs were transformed and fluorescence study was carried out as stated above.

Declarations

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Author’s contribution: The study was equally contributed by S.S.R and A.P.S. S.S.P and B.P helped in standardizing and processing several bio-informatic pipelines used for the study. B.P also contributed by providing “Agilent Genespring software “used for the study. R.R.K and M.M performed the transcriptomic analysis and assisted in analysis of the results. B.C helped in analysis and interpretation of transcriptomic yeast datasets. S.V conceptualized the entire idea, interpreted the results and played a major role in the preparation of manuscript.

Addition Information:

Competing interest’s’ statement: The authors declare no competing Interests

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Figures

Figure 1

Results obtained from gene set enrichment analysis of cortex section in human brain (GSE124439).
 FIGURE-1B: Results obtained from gene set enrichment analysis of cerebellum section in human brain (GSE67196). (Figures were made using www.networkanalyst.ca and Microsoft Excel 2019).

Figure 2

Results obtained from pathway enrichment analysis of proteomics data pertaining to cortex section of brain. FIGURE-2B: Results obtained from pathway enrichment analysis of proteomics data pertaining to cerebro-spinal fluid. (Figures were made using maayanlab.cloud/Enrichr and Microsoft Excel 2019)

FIGURE-2C: Figure and table representing common pathways enriched between different brain datasets

(Figures were made using bioinfogp.cnb.csic.es/tools/venny and bioinformatics.psb.ugent.be/webtools/Venn).

Figure 3

Results obtained from gene set enrichment analysis of SOD1 mice brain (GSE101391). Results obtained from gene set enrichment analysis of FUS mice brain (GSE40652). Results obtained from gene set

enrichment analysis of TDP-43 mice brain (GSE111775). ((Figures were made using www.networkanalyst.ca and Microsoft Excel 2019).

Figure 4

Venn diagram representing common pathways enriched between different mice brain sections pertaining to familial forms of ALS. Table representing common pathways enriched between different mice brain

sections pertaining to familial forms of ALS. ((Figures were made using bioinfogp.cnb.csic.es/tools/venny and bioinformatics.psb.ugent.be/webtools/Venn))

Figure 5

List of oxidative phosphorylation genes enriched in post-mortem human brain datasets. Results of cell type analysis of human cortex sections. (Figures were made using maayanlab.cloud/Enrichr and

Microsoft Excel 2019). Results of cell type analysis of human cerebellum sections. (Figures were made using maayanlab.cloud/Enrichr and Microsoft Excel 2019).

Figure 6

Results obtained from gene set enrichment analysis of muscle biopsies of ALS patients (GSE41414). Graph representing results obtained from cell type analysis of ALS muscle biopsies (GSE41414). (Figures were made using maayanlab.cloud/Enrichr and Microsoft Excel 2019).

Figure 7

Saccharomyces cerevisiae transformed with FUS was used to validate the results. Imaging was carried out in dark field (blue light) and white light. Inhibition of complex-3 and 4 lead to decreased amyloidogenesis. Further, knock-out of COX8 (cytochrome c oxidase sub-unit) and QCR8 showed absence of amyloids. Saccharomyces cerevisiae transformed with TDP-43 was used to validate the results. inhibition of complex-4 leads to decreased amyloidogenesis. further, knock-out of COX8

(cytochrome c oxidase sub-unit) showed absence of amyloids. Florescence quantification of the figures are provided in the supplementary section (SUPPLEMENTARY -2). Table representing levels of SOD1 and SOD2 in FUS, TDP-43 and their mutants.

Figure 8

Figure summarizing the results obtained from the study.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SUPPLEMENTARYFIGURE1.pdf](#)
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