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DETERMINING THE INFLUENCE OF ANTERO-POSTERIOR AXIAL IDENTITY OF MOTOR NEURONS ON THEIR VULNERABILITY TO AMYOTROPHIC LATERAL SCLEROSIS PREDISPOSING MUTATION BY TRANSCRIPTOME ANALYSIS.

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Abstract

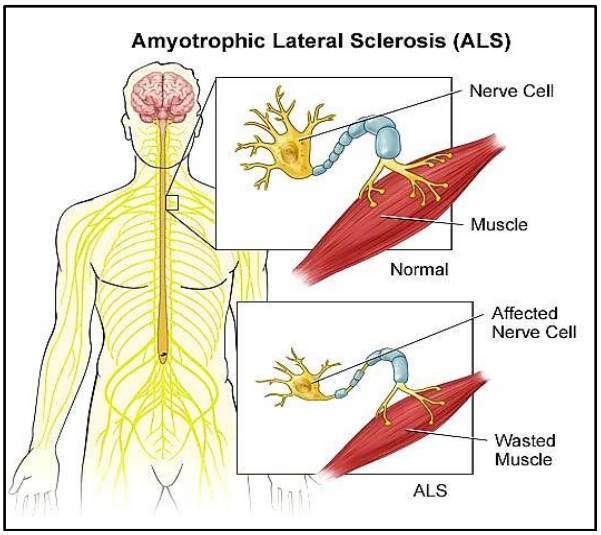
Amyotrophic lateral sclerosis (ALS) is characterized by progressive death of motor-neurons (MNs) by a complex interplay of multiple predisposing-factors. The discovery of miscellaneous ALS-associated mutations signifies the genotypic and phenotypic heterogeneity of ALS. Mutations in the gene for intracellular scavenging enzyme, Cu/Zn-superoxide dismutase (SOD1) are linked to MN-dysfunction in 10-20% of familial-ALS cases and 1-5% of sporadic-ALS cases. However, not every MN-type displays vulnerability to ALS-causing mutation to the same extent and this selective-susceptibility of MN-subpopulations unfolds a striking opportunity to investigate the determinants of ALS-neurodegeneration. The segmental-distribution of MNs along the antero-posterior (A-P) axis is orchestrated by ranked-expression of Hox genes, while the exact contribution of this A-P positional identity to the timing and extent of MN-vulnerability to ALS is not understood clearly. This project was centred on the objective to delineate any link between the A-P axial-identity of MN-columns and their selective-vulnerability to specific ALS-linked mutation by examining transcriptomic changes in spinal-cord samples with two distinct axial-identities (cervical and lumbar) using a publicly-accessible mutant-SOD1 mice (rodent model of SOD1-linked ALS) dataset. Additionally, Gene Ontology-(GO) enrichment analysis was performed on the differentially-expressed (DE) genes in cervical and lumbar spinal-cords to portray a comparison between the vulnerability of these axially-definite MN-subgroups, which revealed an augmented protective inflammatory-response in the mutant-SOD1 cervical spinal-cords. An improved understanding of the influence of A-P axial-identity of MNs on the intricate ALS-linked pathological processes by further experiments may be harnessed for unlocking the way to discover potentially curative therapeutic applications for this deadly neuromuscular disease.

Index Terms: Amyotrophic lateral sclerosis, Axial-Identity, SOD1-mutation, Transcriptome-analysis

1.Introduction:

Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative disorder in the western hemisphere after Alzheimer's disease and Parkinson's disease (Erkkinen et al. 2018; Hirtz et al. 2007). The Greek word 'Amyotrophic' means the atrophy of muscle-fibres due to loss of functionality of corresponding motor-neurons (MNs) (Chiò et al. 2013) ***(Figure–1A)*** and 'Lateral Sclerosis' refers to the hardened nature of lateral and anterior cortico-spinal tracts in ALS disease progression. This clinical term, ALS is used in modern clinical practice to cover a spectrum of neurodegenerative manifestations which initiates with either limb-muscle weakness in spinal-onset ALS (representing about two-thirds of all classical cases) or weakness in the muscles of speaking and swallowing in bulbar-onset ALS (representing about one-third of all classical cases) (Mitchell and Borasio 2007; Rowland and Shneider 2001; Rowland 1998) **(*Figure-1B)***.

A



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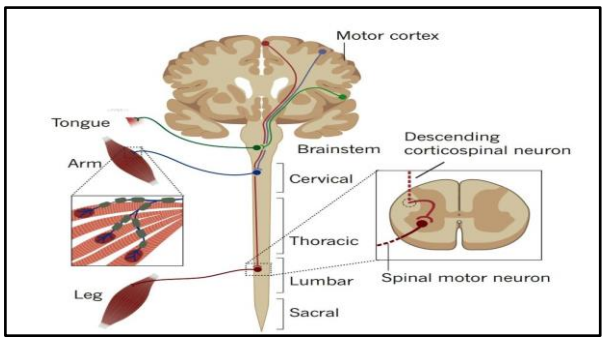
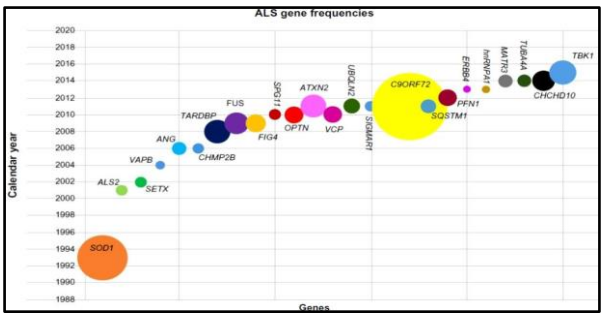


Figure-1: ***(A) Representation of motor neuron (MN) dysfunction in ALS****. The largest nerve cells of body, MNs pass signals from the central nervous system to the effector muscles for controlling voluntary muscle contractions by a chemical synapse formation at the neuromuscular junction in healthy individual. When these innervating MNs are affected in ALS, this deterioration of MN-function results in progressive wasting of peripheral muscle fibres characterised by muscle weakness, tightness (spasticity), cramping and twitching* (Rowland 1998; Swinnen and Robberecht 2014). ***(B) Schematic depicting the affected components of the nervous system in ALS.*** *The progressive motor neuron disease, ALS predominantly attacks the descending axons of corticospinal MNs (upper MNs) projecting from the motor cortex to synapse with MNs in the brainstem and spinal cord, and the bulbar or spinal cord MNs (lower MNs) projecting into neuromuscular junctions of skeletal muscles for controlling voluntary movements. Major ALS-symptoms are muscle atrophy and weakness when MNs along specific regions of the spinal cord are affected in spinal-onset ALS and speech disturbance, swallowing impairment, dysarthria and dysphagia in bulbar-onset ALS due to the demise of specialized nerve cells in corticobulbar area of the brainstem during early stages of ALS* (Taylor et al. 2016).

Most incidences (about 90%) of this genetically heterogenous disease are not associated with any obvious genetic aetiology (sporadic-ALS), whereas in about 10% of ALS-cases are inherited as an autosomal-dominant pattern with age-dependent penetrance (familial-ALS) (Rowland and Shneider 2001) ***(Figure-2B)***. After the demonstration of genetic locus-heterogeneity and linkage of familial-ALS to long (q) arm of chromosome-21 in 1991 (Siddique et al. 1991), mutations in a gene (located on a small region of 21q22.1) coding for metallo-enzyme [Cu/Zn]-superoxide dismutase (SOD1) were identified two years later as the first genetic-link of ALS (Rosen et al. 1993) ***(Figure-2A)*** *which accounts for 20% of familial-ALS-cases (Brown 1995; Renton et al. 2014)* ***(Figure-2B).***

A



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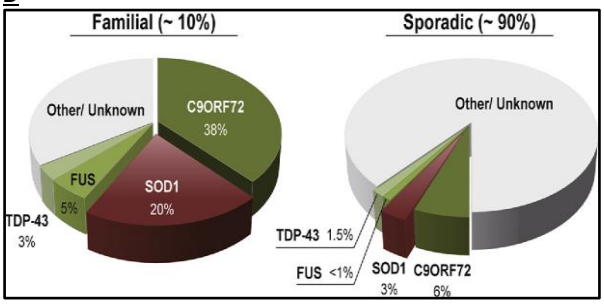
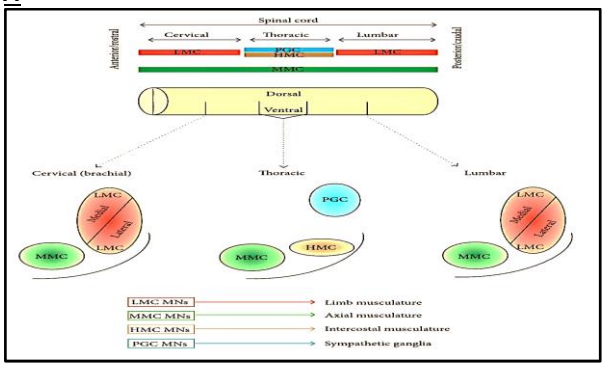


Figure-2: ***(A) Discovery of genes with ALS-predisposing mutation since 1990.*** *Here, every single gene is plotted against the year of its discovery and the circle size of corresponding gene is signifying the frequency of mutations in the incidences of familial-ALS. SOD1 is the first genetic mutation to be identified in 1993* (Kirby et al. 2016). ***(B) The percentage of ALS incidences in Europe and the proportion of genetic mutations in case of sporadic and familial cases*** *(modified from (Renton et al. 2014)).* ***Until now, mutations in more than 25 genes (namely- C9ORF72, SOD1, TARDBP, FUS, OPTN, VCP, UBQLN2, PFN, SQSTM1 etc.) have been discovered as a causative for hereditary-ALS (Nguyen et al. 2018; Taylor et al. 2016) where SOD1 mutation is responsible for 20% of ALS-cases having an affected family member (Brown 1995; Renton et al. 2014).***

SOD1 is one of the three superoxide-dismutase isoenzymes and a ubiquitously-expressed protein, which is responsible for the conversion of free superoxide-radicals (produced by the cell's metabolism) to hydrogen-peroxide and oxygen (Weisiger and Fridovich 1973). While homozygous-deletion of SOD1 in rodents has not resulted in an ALS-mimicking phenotype (Elchuri et al. 2004; Reaume et al. 1996), conversely transgenic-mice expressing human mutant-form of SOD1 gene have manifested clinical symptoms and underlying ALS-causing pathological mechanisms similar to those identified in ALS patients and thereupon mutant-SOD1 mice are widely used as an excellent animal-model of ALS (Bruijn et al. 1997; Gurney et al. 1994; Dal Canto and Gurney 1995; Nagai et al. 2001; Tu et al. 1996; Wong et al. 1995).

The phenotypical expressions of classical motor neuron disease (ALS) encompass different neurological-regions along the vertebrate antero-posterior (A-P) axis: bulbar, cervical and lumbar, evidencing the gradual degeneration of specifically targeted muscle-controlling nerve cells at all levels of motor system-from primary motor cortex to the anterior horn cells of the spinal cord (Mitchell and Borasio 2007). The MNs along this A-P axis comprise both somatic-MNs and visceral-MNs, where somatic-MNs of cervical and lumbar regions are responsible for transmitting motor impulses from central nervous system to peripheral effector muscles for voluntary movements, whereas visceral-MNs of thoracic and sacral regions control autonomic functions (Stifani 2014). Since embryonic developmental stages of neural tube, MNs get allocated to discrete longitudinally-exhibited columns following a logical anatomical arrangement corresponding to their innervating muscle target(s) (Alaynick et al. 2011; Francius and Clotman 2014; Jessell 2000; Prasad and Hollyday 1991; Tsuchida et al. 1994) ***(Figure-3A)***.

A



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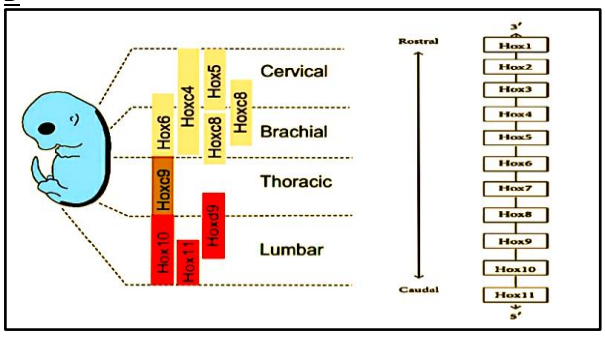


Figure-3*: (A) Summary of segmental distribution of MN columns in relation to the rostro-caudal neuraxis of spinal cord.** *The existence of MMC (median motor column, marked with green colour) MNs is visualized throughout the antero-posterior extent of the spinal cord which accounts for innervation of axial musculature. The location of LMC (lateral motor column, marked with red colour) MNs is noticed at cervical (brachial) and lumbar regions to supply the upper limb and lower limb musculature accordingly. The MNs of HMC (hypaxial motor column, indicated by yellow) and PGC (preganglionic motor column, indicated by blue) are present only in the thoracic portions of spinal cord and supply the intercostal muscles and sympathetic ganglia respectively* (Patani 2016). ***(B) Schematic representation of the role of HOX genes in defining the distinct neuronal fates of MNs along the antero-posterior longitudinal axis of developing spinal cord. *The 39 Hox genes of human genome are arranged in 13 paralogous groups among four clusters advancing in a 3' to 5' expression. Figure* ***(B)*** *is depicting the patterns of rostro-caudal expression of chromosomally linked HOX genes where the HOX genes at the 3' end of the clusters are expressed more anteriorly and HOX genes at the 5' end of the clusters are expressed more posteriorly. While cervical MNs are under the influence of Hox5, Hoxc4, Hox6 genes, more posterior MNs (thoracic and lumbar) are influenced by Hox10, Hox11, HOXd9* (Philippidou and Dasen 2013; Sagner and Briscoe 2019).

Such three-dimensional patterning of MNs as separate anatomical-columns along the spinal rostro-caudal axis is determined by the regulatory activity of 39 highly conserved Homeobox (Hox) genes during embryogenesis (Lewis 1978; Liu et al. 2001; Lumsden and Krumlauf 1996; Trainor and Krumlauf 2001)***;*** Hox4-7 genes decide cervical (anterior) identity and Hox9 defines thoracic identity whereas lumbar (posterior) identity of MNs is conferred by Hox10-11 genes (Philippidou and Dasen 2013; Sagner and Briscoe 2019) ***(Figure-3B)***. These distinct neuronal subpopulations with specific A-P positional identity exhibit vulnerability to ALS-causing mutation to different extents (Carr 2015; Nijssen et al. 2017; Rochat et al. 2016; Ragagnin et al. 2019). While symptoms related to degeneration of spinal-MNs and cholinergic hypoglossal-MNs (located in dorsomedial medulla oblongata) are manifested at early stage of ALS, oculomotor neurons of third cranial nerve (located within the midbrain of brainstem) and Onuf's nuclei-MNs *(*located in the ventral horns of sacral spinal-cord) escape from degeneration even until end stage of this lethal disease (I 1998) **(*Figure-4)***. Although the mechanism of selective-loss of MNs in ALS (Brockington et al. 2013; Kaplan et al. 2014) is not thoroughly explained even 150 years after the first narration of this highly heterogenous disease, this selective-susceptibility of MNs to ALS notably contributes to discover the determining factors for preferential neurodegeneration along the A-P neuraxis.

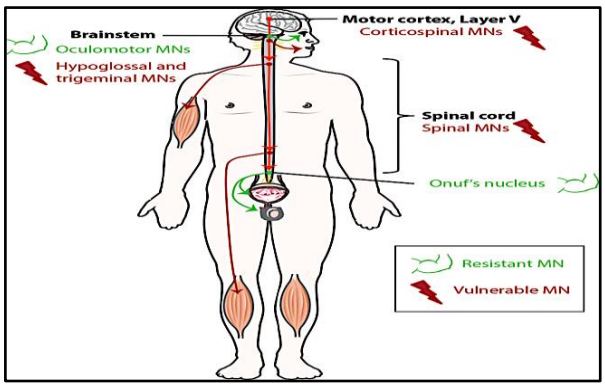


Figure-4: ***Representation of the region-specific selective vulnerability or resistance of MN sub-populations to the classical motor neuron disease ALS.*** *The vulnerable subpopulations of MNs are indicated by red colour and resistant subpopulations of MNs are indicated by green colour. Corticospinal, hypoglossal, and spinal MNs together with the trigeminal nucleus nerve cells progressively fall victim to ALS during the early stage of disease course. On the contrary, MNs located in Onuf's nucleus (accounts for sexual and bladder function) in the sacral position of spinal cord and the oculomotor neurons of third cranial nerves in brainstem (responsible for eye movements) remain unaffected even up to end stage of ALS disease progression* (Rochat et al. 2016).

To date, the most established scientific perspective about neuronal-death pathways in ALS due to the gain of toxic effects by SOD1-mutants is associated with the proclivity of a subfraction of mutant-SOD1 to compose noxious misfolded-protein complexes and aggregates (Bruijn et al. 1998; Durham et al. 1997; Johnston et al. 2000; Subramaniam et al. 2002; Wang et al. 2002) ***(Figure-5)***. Mice hemizygous for G37R-SOD1 transgene (G37R mutant-form of human-SOD1 carrying Glycine-37>Arginine change) is characterised as an enzymatically active, "gain of adverse function" mutation-model expressing a moderate (7-fold) increase in SOD1 activity with the pathology restricted to spinal-MNs and has been tested to resemble human SOD1-linked ALS for understanding disease dynamics (Borchelt et al. 1994; Coussee et al. 2011; Flanagan et al. 2002; Haenggeli et al. 2007; Inoue et al. 2012; McAllum et al. 2013; Milardi et al. 2010; Hong Truong et al. n.d.; Zwiegers et al. 2014). Therefore, to decipher the determining-factors and intrinsic molecular mechanisms behind selective-death of different MN-subpopulations, these SOD1(G37R)-transgenic rodent models can recapitulate accurately the key aspects of human-ALS disease.

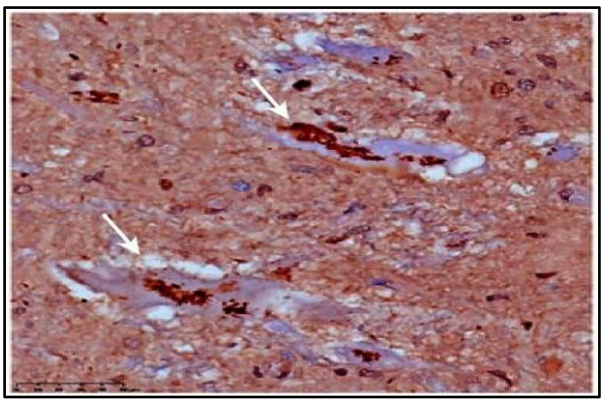


Figure- 5: ***Intracellular accumulation of SOD1 misfolded protein aggregates (white arrows) in spinal MNs is a typical pathological feature of SOD1-related familial ALS*** (Taylor et al. 2016)**.**

If any impact of A-P axial-identity of MNs on ALS can be evidenced from the perspective of selective neuronal susceptibility, this will unveil the prospect of pre-clinical drug testing and development of novel therapeutic targets specific for each neurological region along the spinal A-P axis. Considering the findings of previous scientific papers and existing gaps in this research field, my project was based upon the aim to examine if the same ALS-predisposing mutation affects MNs of distinct axial-identity in the same or different manner. For detecting any link between the differential-vulnerability of MNs and their A-P regional identity, this study has aspired to draw a contrast between differentially-expressed (DE) gene profiles (ALS-affected versus healthy) of two distinct spinal-MN samples varying in A-P positional identity (cervical and lumbar). This was done by comparative transcriptome analysis using a publicly available mutant-SOD1(G37R) mice dataset (from GEO database of NCBI). Afterwards, Gene Ontology (GO)-based functional enrichment analysis was conducted on both common and exclusive DE-gene sets (upregulated and downregulated) in cervical and lumbar spinal-cords of SOD1-mice (obtained from comparing spinal-cord samples of SOD1-mice with wild-type controls) to identify the significantly enriched candidate biological pathways and signalling cascades related to ALS-pathogenesis at cervical and lumbar spinal-cord levels.

2.*Materials and methods:*

2.1. Dataset selection for RNA-seq analysis:

A dataset consisted of 44 samples displaying high throughput mRNA-sequencing data of G37R-SOD1 mice (mimicking rodent-model of ALS) was selected from *GEO*-database (Gene Expression Omnibus, managed by *NCBI*, National Centre for Biotechnology Information) (Barrett et al. 2013; Barrett et al. 2011; Barrett et al. 2009) with *GEO* accession number: *GSE135539*, submitted on August 07,2019 by *Salk Institute for Biological Studies, USA*. Transcriptome

data of cervical and lumbar spinal-cords from ALS model mice (stable-overexpression of mutant-SOD1(G37R) transgene in mice, not wild-variety of SOD1) and wild-type healthy mice as their control counterparts were reported, where time points include end-stage disease animals, as well as mid-stage disease animals ***(Figure-6)***.

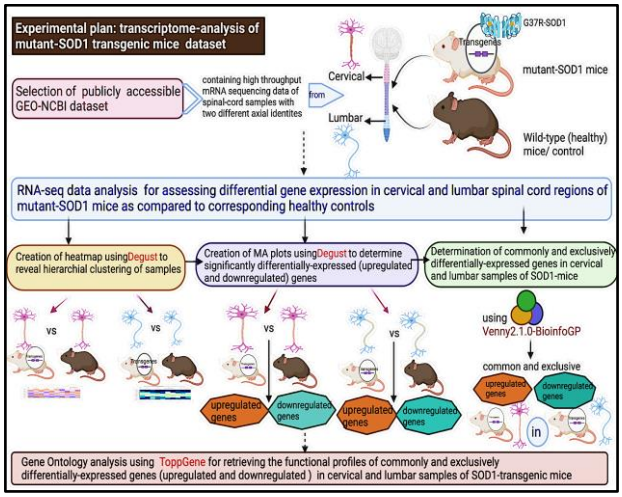


Figure-6: ***Sequential methods in transcriptome analysis of selected SOD1-mice dataset.*** *(Created with Biorender.com)*

2.2. Creation of MA plots and Heatmaps using Degust:

Degust is an integrative web-portal for comprehensive analysis of gene expression data and assessing differential gene expression using edgeR (Dai et al. 2014) or limma-voom method (Law et al. 2014) which accepts the input file in CSV-format (comma-separated file) of RNA-seq counts where each row representing a measured gene and each column representing a different biological sample (Anon 2016). The *GSE135539_gene_counts.csv file of* selected publicly available dataset was uploaded in *Degust* web-tool that was provided in the *GEO*-database. Next, configuration settings were conducted by categorizing samples as four discrete conditions: wild-type control cervical, mutant-SOD1 cervical, wild-type control lumbar and mutant-SOD1 lumbar. While creating MA plots and heatmaps by *edgeR*-method (empirical analysis of digital gene expression in R programming) for analysing RNA-seq gene expression data using *Degust*; FDR (False Discovery Rate) cut-off was set at 0.05 and absolute log fold change (abs logFC) was set at 1.5.

2.3. Determination of the overlap of significant DE-genes in cervical and lumbar spinal cords of mutant-SOD1 mice using Venny 2.1.0- BioinfoGP:

After attaining DE-gene lists (comparing mutant-SOD1 spinal-cord samples of both cervical and lumbar regions with their corresponding wild-type counterparts) via *Degust, Microsoft Excel (version 2019)* from *Microsoft Office 365* was used to sort out the lists of genes with enhanced and depleted expression at transcript level, by filtering the DE-gene lists of separate comparisons (*mutant-SOD1 cervical vs wild-type control cervical*; *mutant-SOD1 lumbar vs wild-type control lumbar* and *wild-type control lumbar vs wild-type control cervical*). To identify overlap among significantly upregulated and downregulated gene-lists obtained from different comparisons, *Venny 2.1.0-BioinfoGP* (Oliveros 2015) was used for creating Venn-diagrams (Bennett 2015) which exhibited the commonly and exclusively upregulated or downregulated genes in cervical and lumbar replicates.

2.4. Gene Ontology (GO) analysis by ToppGene:

For retrieving the functional profile of common and exclusive DE-gene lists (upregulated or downregulated) and for extensive understanding of underlying biological processes related to the DE-gene sets in case of both cervical and lumbar specimens, *ToppFun* portal from *ToppGene Suite* was used (Chen et al. 2009). Finally, the biological pathways with a P-value ≤ 0.05 and abs logFC ≥ 1.5 were screened and analysed.

2.5. Statistical analysis of data:

For generating clustered bar charts of GO-term plots and pie chart (to illustrate numerical proportion of upregulated and downregulated genes in cervical and lumbar samples), *Prism-GraphPad* (version 9.2.0) was used.

3. Results:

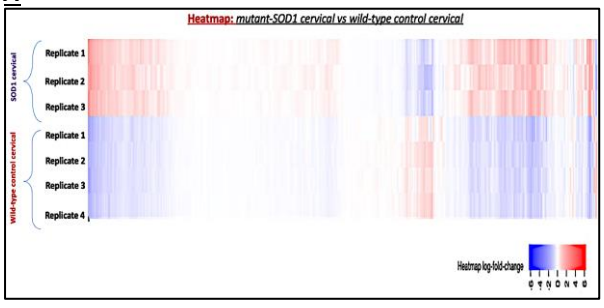
For investigating the association of A-P axial-identity of ALS-affected spinal-MNs with ensuing genotypic and phenotypic alterations , transcriptome profiling by RNA-seq (RNA sequencing) analysis can be used as a revolutionary methodology to reveal and quantify DE-genes in diseased tissue as RNA-transcript count (Nagalakshmi et al. 2010). In this study, analysis of transcriptomic data of a publicly-accessible mutant-SOD1(G37R) mice dataset from GEO-database (a public functional genomics data-repository maintained by *NCBI*) was performed. The aim of this study was to evaluate transcriptomic alterations detected in the two groups of mutant-SOD1 spinal-MNs carrying distinct A-P positional identity (cervical and lumbar) when spinal-cord samples of transgenic mice (mutant-SOD1:ALS) were compared to those of age-matched wild-type mice. Then GO enrichment analysis was performed on DE-gene sets (with enhanced or depleted expression at transcript level) in mutant-SOD1 transgenic mice as compared to their healthy counterparts for identifying the biological processes that are impacted in ALS at different spinal cord levels aa well as drawing any comparison between the vulnerability of cervical or lumbar MNs to ALS owing to their axial-identity.

3.1. Examining transcriptomic alterations in cervical and lumbar spinal-cord samples of SOD1-mice by RNA-seq analysis:

At first, Degust (an interactive web-tool for analysing RNA-seq data) was used to perform differential expression analysis (DEA) of transcriptome data (RNA-seq read-counts) pertaining to mutant-SOD1(G37R) cervical and lumbar spinal-cord samples and their wild-type controls. The results of RNA-seq analysis of selected SOD1-mice dataset revealed discrete transcriptomic profiles (quantification of gene expression at transcription-RNA level) of mutant-SOD1 cervical and mutant-SOD1 spinal-cord lumbar samples contrasted to their wild-type healthy counterparts.

In an attempt to visualize the expression-pattern of genes across four different spinal-cord samples, two separate heatmaps (Wilkinson and Friendly 2012) of RNA-seq results were generated (using *edgeR* method in *Degust*) for cervical and lumbar identities (*mutant-SOD1 cervical vs wild-type control cervical and mutant-SOD1 lumbar vs wild-type control lumbar*). The replicates of each specified spinal-cord condition (mutant-SOD1 cervical, wild-type control cervical, mutant-SOD1 lumbar, wild-type control lumbar) exhibited similar gene expression-pattern as indicated by the colour and intensity matching of the data-matrices in the heatmaps ***(Figure-7A,B)***. Accordingly, the comparable pattern of gene expression changes noticed among all the representatives of each mutant-SOD1 spinal-cord sample (in comparison to corresponding wild-type control) established the hierarchical clustering in data-matrices (Johnson 1967; Navarro et al. 1997) exhibited among the replicates of four separate spinal-cord conditions.

A



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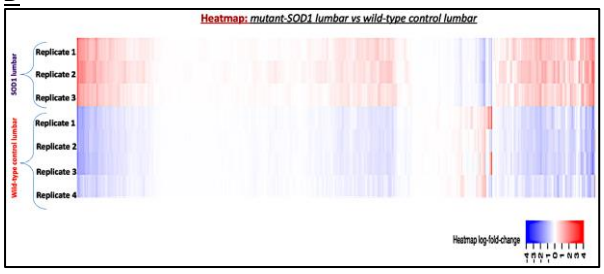
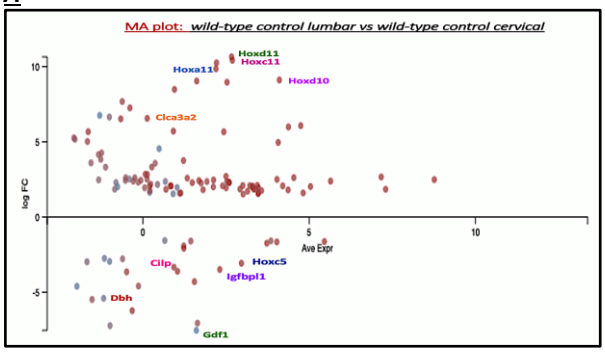


Figure-7: ***Heatmaps revealing hierarchical cluster analysis of tissue gene expression data in cervical and lumbar spinal cord samples. (A)*** *Heatmap comparing mutant-SOD1 cervical with wild-type control cervical samples displays data in a grid where each row is representing a replicate of mutant-SOD1 or wild-type (healthy) control cervical samples and each column is representing a differentially expressed gene. Red represents upregulated genes and blue represents downregulated genes.* ***(B)*** *Heatmap comparing mutant-SOD1 lumbar with wild-type control lumbar samples displays data in a grid where each row is representing a replicate of mutant-SOD1 or wild-type (healthy) control lumbar samples and each column is representing a differentially expressed gene. Red represents upregulated genes and blue represents downregulated genes.*

The next step was to substantiate the A-P axial identity (cervical or lumbar) of mutant-SOD1 and wild-type (control) spinal-cord samples. For that purpose, two MA plots (log ratio versus average expression signal) were created using *Degust* comparing the gene-expression data of cervical spinal-cords with lumbar variety of samples for both SOD1-mice and wild-type mice. MA plot is a variety of scatter plot for visual representation of differential gene-expression data between two group of samples, where each dot represents one DE-gene; when log2 fold change (M) is plotted against Y-axis and the average gene-expression (at transcript level) of the samples is plotted against X-axis (Robinson et al. 2010). With FDR (false discovery rate) cut-off ≤ 0.05 (Benjamini et al. 2001; Reiner et al. 2003) and abs logFC (absolute log fold change) ≥ 1.5 (Dembélé and Kastner 2014), the generated MA plot showing gene-expression changes between wild-type control lumbar and wild-type control cervical representatives revealed 91 DE-genes except 33 ribosomal protein (RP) pseudogenes (75 gene-transcripts with enhanced expression and 16 gene-transcripts with depleted expression) ***(Figure-8A)***. Since the expression of Hoxd11, Hoxc11, Hoxa11, Hoxd10, Hoxa13, Hoxc10, Hoxa10 genes were upregulated and the expression of Hoxc5, Hoxc6, Hoxa5 were downregulated in lumbar spinal-cords of wild-type mice in comparison to wild-type cervical samples ***(Figure-8A, Supplementary table-1,2)***, the A-P positional identity of lumbar and cervical samples was verified; as anterior (cervical/brachial) MNs are Hox5, Hoxc4, Hox6-positive and posterior (lumbosacral) segments were under the influence of Hox10 to Hox13 markers (Philippidou and Dasen 2013). The upregulation of Hoxc11, Hox d11, Hoxa11, Hoxd10, Hoxa13, Hoxc10, Hoxa10 gene-expression in mutant-SOD1 lumbar samples when compared to mutant-SOD1 cervical samples also confirmed the A-P axial-identity of spinal-cord samples in selected mice dataset ***(Figure-8B)***.

A



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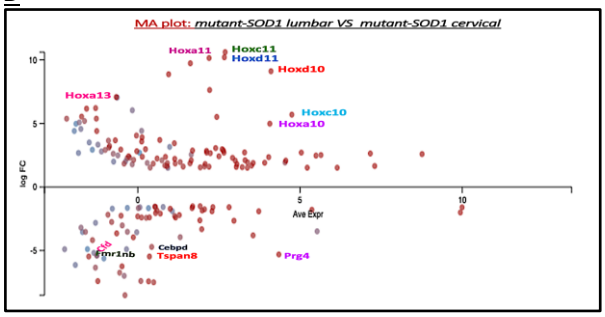


Figure- 8: *(**A**) MA plot: **wild-type control lumbar vs wild-type control cervical**; created with Degust web-tool (FDR cut-off ≤ 0.05, abs logFC ≥ 1.5) showing top-ranked DE genes in wild-type (healthy) lumbar samples when compared with wild-type spinal cord samples of cervical region. **(B)** MA plot: **mutant-SOD1 lumbar vs mutant-SOD1 cervical**; created with Degust web-tool (FDR cut-off ≤ 0.05, abs logFC ≥ 1.5) displaying top DE genes in mutant-SOD1 lumbar spinal-cords in comparison to mutant-SOD1 cervical spinal-cord samples. If expression is significantly different between lumbar and cervical replicates, the dots are red. If not, they are blue. (In Degust, significant means FDR <0.05.) Here, ribosomal protein (RP) pseudogenes are not highlighted in the MA plots as they lack protein-coding exons.*

Following the verification of A-P axial identity of the mice spinal-cord samples, two MA plots were generated using *Degust* comparing gene-expression data between mutant-SOD1 samples and wild-type controls separately for cervical and lumbar regions to examine if transcriptomic changes (genes expressed at higher or lower level) in mutant-SOD1 mice are similar or different at cervical and lumbar spinal-cord levels as compared to corresponding wild-type counterparts.

When *mutant-SOD1 cervical vs wild-type control cervical*- MA plot was created using *Degust* with FDR cut-off ≤ 0.05 and abs logFC ≥ 1.5, 1187 DE-genes were identified in cervical spinal-cord samples of SOD1-mice as compared to wild-type healthy controls (except 323 RP genes); where the expression of 979 genes was upregulated, and the expression of 208 genes was downregulated at transcript level. Here, the top significantly upregulated-genes were: Cd5l (CD5 Antigen-like), Atp6v0d2 (ATPase, H+ transporting. Lysosomal V0 subunit D2), Mcoln3 (Mucolipin TRP Cation Channel 3), Gpnmb (Glycoprotein Non-metastatic Melanoma Protein B), Htr2b (5-Hydroxytryptamine Receptor-2B), Apoc2 (Apolipoprotein-C2), Tnfsf11 (TNF Superfamily Member-11), Asb11 (Ankyrin Repeat and SOCS Box Containing 11), Cd300lb (CD300 Molecule Like Family Member-B) and Cd200r4 (Cell Surface Glycoprotein CD200 Receptor 4) etc. ***(Figure-9)***. Among these genes: Cd5l (induces autophagy in macrophages) (Mohanty et al. 2020; Xu et al. 2017), Atp6v0d2 (facilitates autophagosome-lysosome fusion) (Xia et al. 2019), Mcoln3 (restores autophagy in ALS) (Santoni et al. 2020) are involved in modulating autophagy (a cellular recycling-process). Tnfsf11 (involved in T-cell dependent immune response) (Odgren et al. 2009) and Cd300lb (related with immunoregulatory interactions between lymphoid and non-lymphoid cells) (Borrego 2013) code for proteins responsible for immune response, while Cd200r4 coded protein is linked with regulation of neuroinflammatory response (Walker et al. 2009). Besides, Gpnmb (inhibits MN death in ALS) and Htr2b (inhibits mononuclear phagocytes degeneration in spinal cord) proteins have been found to have neuroprotective role in ALS (Tanaka et al. 2012; El Oussini et al. 2016)**.** On the other hand, the top significantly downregulated genes were: Ddx3y (DEAD-Box Helicase 3 Y-linked), Eif2s3y (Eukaryotic Translation Initiation Factor 2 Subunit-3) , Npcd (Neuronal Pentraxin Chromo Domain), Uty (Ubiquitously Transcribed Tetratricopeptide Repeat Containing, Y-linked), Kdm5d (Lysine-specific demethylase 5D), F2rl3 (F2R Like Thrombin or Trypsin Receptor 3), Nlrp6 (NLR Family Pyrin Domain Containing 6), Edn2 (Endothelin-2), Dbh (Dopamine Beta-Hydroxylase) and Fam122a (Family With Sequence Similarity-122A) etc. **(*Figure-9*).** Among these downregulated gene-transcripts, Npcd coded protein plays role in regulation of axon growth (Chen and Bixby 2005); Dbh coded enzyme catalyses the conversion of dopamine to norepinephrine (Weinshilboum and Axelrod 1971); NLRP6 protein has been discovered to act as a negative regulator of inflammatory signalling and innate immunity in one study (Anand et al. 2012) whereas protein coded by Ddx3y probably plays multifunctional role in neuronal differentiation (Vakilian et al. 2015).

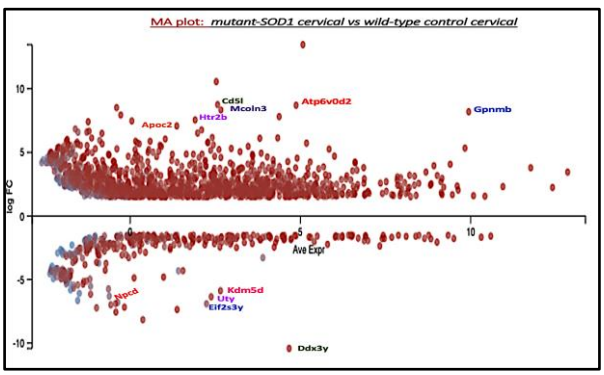


Figure-9: *MA plot:* ***mutant-SOD1 cervical vs wild-type control cervical*****;** *created with Degust web-tool (FDR cut-off ≤ 0.05, abs logFC ≥ 1.5) displaying top DE genes in mutant-SOD1 cervical spinal cord tissues in comparison to their wild-type healthy controls. If expression is significantly different between mutant-SOD1 samples and wild-type controls, the dots are red. If not, they are blue. (In Degust, significant means FDR <0.05). Here, ribosomal protein (RP) pseudogenes are not highlighted in the MA plots as they lack protein-coding exons.*

Additionally, when *mutant-SOD1 lumbar vs wild-type control lumbar* MA plot was created, it displayed 815 DE-genes (except 189 RP genes) in lumbar spinal-cord samples from mutant-SOD1 mice as compared to their wild-type controls with FDR cut-off ≤ 0.05 and abs logFC ≥ 1.5; where transcripts of 730 genes showed enriched expression, while the expression of 85 gene-transcripts was depleted. Mcoln3, Atp6v0d2, Htr2b, Gpnmb, Cd5l these five genes were commonly upregulated and Ddx3y, Eif2s3y, Uty, Kdm5d these four genes were commonly downregulated in both mutant-SOD1 lumbar and mutant-SOD1 cervical samples. The top exclusively upregulated-genes in mutant-SOD1 lumbar spinal-cords were Gdf1 (Growth Differentiation Factor1), Tgm1 (Transglutaminase 1), Mmp3 (Matrix Metallopeptidase 3), Serpinf2 (Serine Proteinase Inhibitor, Clade F, Member 2), Gdf3 (Growth Differentiation Factor3) etc. ***(Figure-10).*** Protein coded by Gdf1 is involved in BMP (Bone morphogenic protein)-signalling pathway and SMAD-protein signal transduction (signal transducers for receptors of transforming growth factor-beta) during embryonic development (Lee 1990; Söderström and Ebendal 1999; Tanaka et al. 2007). Gdf3 coded protein plays a role in the establishment of antero-posterior identity and controls the formation of anterior visceral endoderm during the pre-gastrulation stage of embryogenesis (Andersson et al. 2007; Levine and Brivanlou 2006). On the contrary, the top exclusively downregulated-genes in lumbar samples of SOD1-mice were: Myh4 (Myosin Heavy Chain-4), Tmem233 (Transmembrane Protein-233), Slc17a7 (Vesicular Glutamate Transporter-1), Tlx2 (T cell Leukaemia Homeobox-2), Scn11a (Sodium Voltage Gated Channel Alpha Subunit-11) and Tspan8 (Tetraspanin-8) etc. ***(Figure-10).*** Myosin-4 protein by Myh4 gene is involved in ATP hydrolysis for kinetic energy transduction (Weiss et al. 1999), while Tlx2 gene is essential for physiological cell death of enteric neurons in the gastrointestinal tract and normal development of the enteric nervous system (Borghini et al. 2007; Ohara et al. 2021)

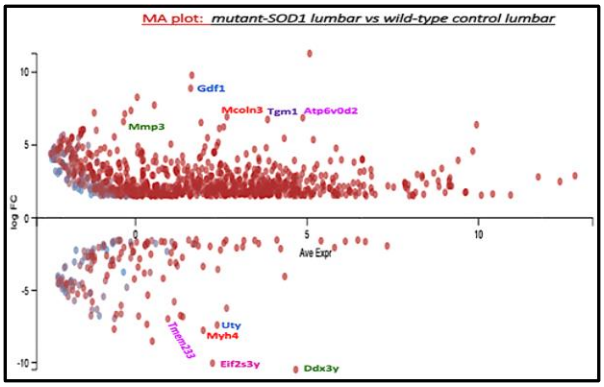
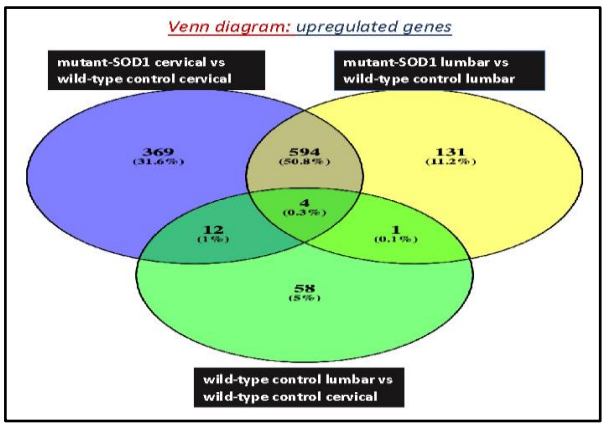


Figure- 10: *MA plot:* ***mutant-SOD1 lumbar vs wild-type control lumbar****; created with Degust web-tool (FDR cut-off ≤ 0.05, abs logFC ≥ 1.5) showing top DE genes in mutant-SOD1 lumbar spinal cord samples when contrasted to their wild-type healthy counterparts. If expression is significantly different between mutant-SOD1 samples and wild-type controls, the dots are red. If not, they are blue. (In Degust, significant means FDR <0.05). Here, ribosomal protein (RP) pseudogenes are not highlighted in the MA plots as they lack protein-coding exons.*

3.2. Ascertaining commonly and exclusively upregulated and downregulated genes in mutant-SOD1 cervical and lumbar samples:

When Venn-diagram was created to determine the overlapping of differential gene-expression among three comparisons (*mutant-SOD1 cervical vs wild-type control cervical, mutant-SOD1 lumbar vs wild-type control lumbar and wild-type control lumbar vs wild-type control cervical*), we achieved the lists of both common and exclusive transcripts (enriched and depleted) for cervical and lumbar spinal-cord regions. From the Venn-diagram showing overlapping elements among upregulated gene-sets, we found that 369 genes were exclusively upregulated in mutant-SOD1 cervical samples, and 131 genes were exclusively upregulated in mutant-SOD1 lumbar samples when compared to corresponding healthy control counterparts. Again, 594 genes were commonly upregulated in cervical and lumbar regions of SOD1-mice (when FDR cut off was set at 0.05 and abs logFC was set at 1.5) ***(Figure-11A).*** The Venn-diagram showing overlapping elements among downregulated gene-sets displayed that 29 genes were commonly downregulated in mutant-SOD1 cervical and mutant-SOD1 lumbar samples, whereas 178 genes were exclusively downregulated in mutant-SOD1 cervical samples, and 56 genes were exclusively downregulated in lumbar spinal-cords transcriptome profiling of SOD1-mice as compared to corresponding healthy controls ***(Figure- 11B).***

A



B

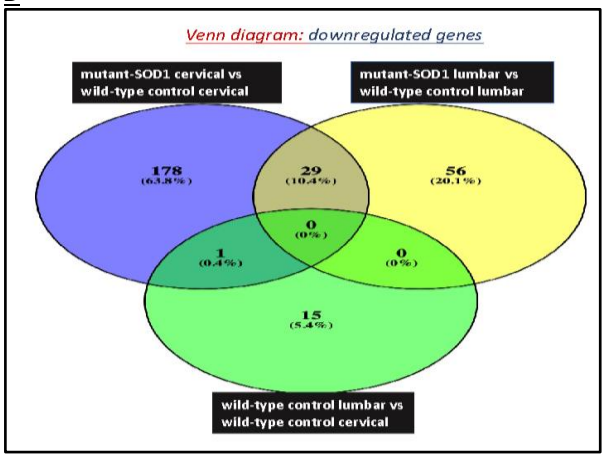


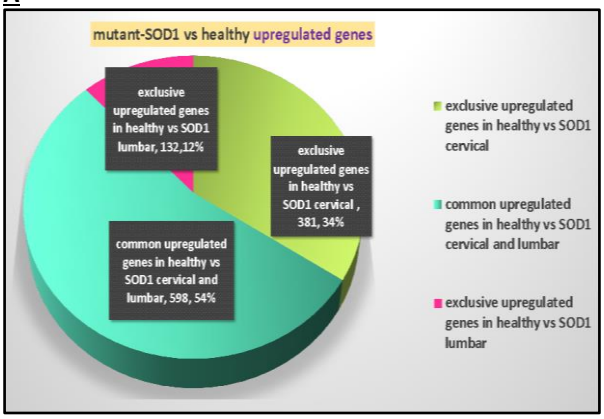
Figure- 11: ***(A) Venn diagram exhibiting overlapping of transcripts with enriched expression.*** *While 594 transcripts were commonly enriched in mutant-SOD1 spinal cord samples of both cervical and lumbar regions, 369 transcripts showed enriched expression exclusively in mutant-SOD1 cervical samples and 131 transcripts showed enriched expression exclusively in mutant-SOD1 lumbar samples as compared to their corresponding wild-type control counterparts.* (Here, FDR cut-off was at 0.05 and abs logFC was at 1.5 for each comparison.)

(B) Venn diagram displaying overlapping of transcripts with depleted expression.

While only 29 transcripts were commonly depleted in mutant-SOD1 spinal cord samples of both cervical and lumbar regions, 178 transcripts showed depleted expression exclusively in mutant-SOD1 cervical samples and 56 transcripts showed depleted expression exclusively in mutant-SOD1 lumbar samples as compared to their corresponding wild-type control counterparts. (Here, FDR cut-off was at 0.05 and abs logFC was at 1.5 for each comparison.)

Two pie charts (separately for upregulated and downregulated genes) were created afterwards with the number of common and exclusive DE-genes at cervical and lumbar levels of spinal-cord of SOD1-mice. The pie chart arraying upregulated-genes between mutant-SOD1 vs healthy control showed that 54% of upregulated-genes were common in both cervical and lumbar regions of mutant-SOD1 mice. On the contrary, 34% of upregulated-genes exhibited enhanced expression exclusively in mutant-SOD1 cervical samples and rest 12% of upregulated-genes showed enhanced expression in mutant-SOD1 lumbar spinal-cord tissues (**Figure-12A)**. From the pie chart presenting mutant-SOD1 vs healthy downregulated genes, it was detected that higher percentage (68%) of downregulated-genes in mutant-SOD1 mice showed depleted expression at cervical level than in lumbar spinal-cords (21%), whereas only 11% of downregulated-genes were common in cervical and lumbar samples of mutant-SOD1 mice ***(Figure-12B).*** Therefore, I conclude that a higher number of gene expression changes were observed in mutant-SOD1 cervical samples than in mutant-SOD1 lumbar representatives.

A



B

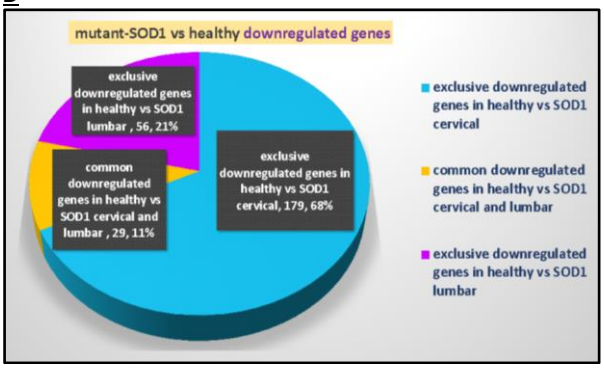


Figure-12: ***(A)*** Pie chart showing upregulated genes statistics ***(B)*** Pie chart showing downregulated genes statistics

3.3. Gene ontology (GO) analysis in biological process aspect:

Lastly, to gain further insight into the biological processes implicated in SOD1-ALS pathology at cervical and lumbar spinal-cord levels, GO enrichment analysis was performed using ToppFun web-tool on the DE-gene sets (common and exclusive) with increased or decreased expression in cervical and lumbar spinal-cord samples of SOD1-mice.

GO-term plot exhibiting the biological processes associated with the commonly upregulated-genes in mutant-SOD1 cervical and mutant-SOD1 lumbar samples has demonstrated that these genes are remarkably involved in defence response, leukocyte activation, regulation of immune system process, immune effector process, inflammatory response, response to biotic stimulus etc. (considering the number of genes involved in specific biological processes and the P-value colour scale) (***Figure -13).*** Similarly, the most enriched pathways concerned with the exclusively upregulated-genes in both cervical and lumbar regions of mutant-SOD1 mice are defence response, immune effector process, leukocyte activation, cytokine production, regulation of immune system process, inflammatory response etc. ***(Figure-14-A,B).***Therefore, both commonly and exclusively upregulated-genes in SOD1 cervical or lumbar MNs are associated mostly with the same GO terms.

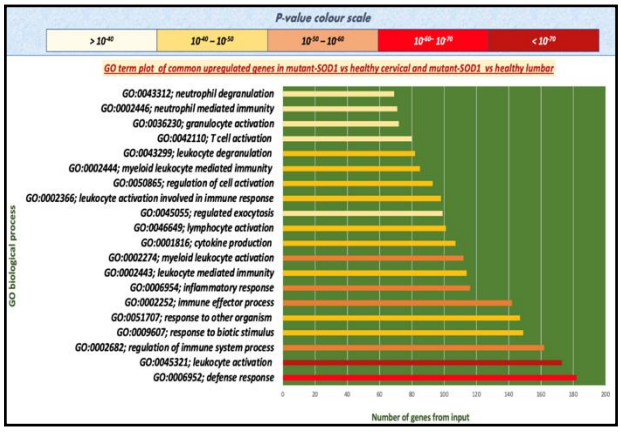
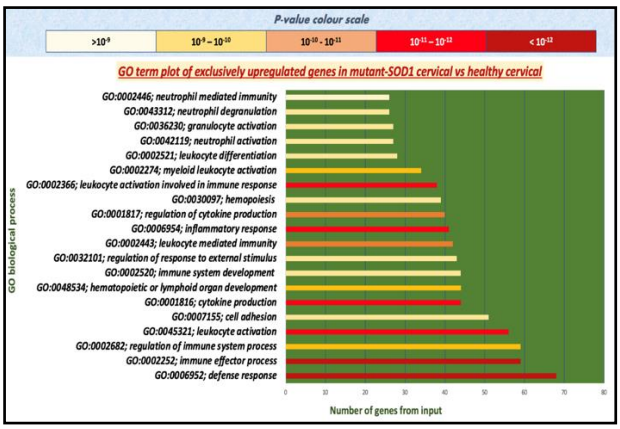


Figure-13: ***Gene Ontology (GO) analysis of commonly upregulated genes at cervical and lumbar spinal-cord levels in mutant-SOD1 vs healthy control comparison.*** *GO term plot showing significantly enriched biological processes associated with the commonly upregulated genes in mutant-SOD1 samples vs healthy controls of both cervical and lumbar spinal cords.*

A



B

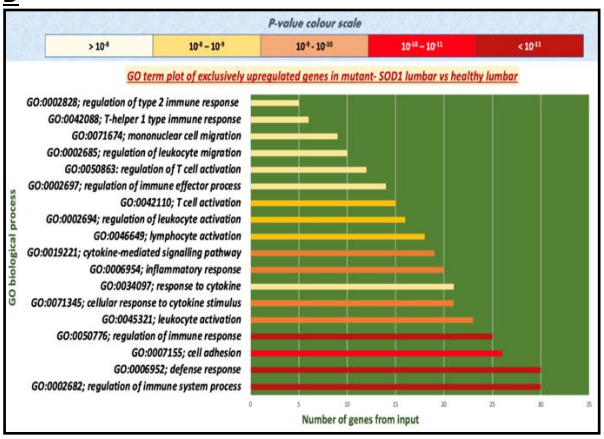


Figure-14: ***Gene Ontology (GO) analysis of exclusively upregulated genes in mutant-SOD1 vs healthy control.*** *(Diagram of GO (Biological Process) terms that are significantly enriched in differentially expressed genes.)* ***(A)*** *GO term plots showing the biological processes associated with the exclusively upregulated genes in mutant-SOD1 cervical vs healthy control cervical samples.* ***(B)*** *GO term plots showing the biological processes associated with the exclusively upregulated genes in mutant-SOD1 lumbar vs healthy control lumbar samples.*

Clustered bar charts showing GO-term plot exhibiting the most enriched biological processes associated with commonly downregulated-genes in mutant-SOD1 cervical and mutant-SOD1 lumbar spinal-cord samples, has indicated that these genes are notably involved in organic hydroxy-compound metabolic process, steroid metabolic process, cholesterol metabolic process, secondary alcohol metabolic process, sterol metabolic process etc. (considering P-value colour scale and the number of genes related to these processes) ***(Figure-15).*** On the other hand, the exclusively downregulated-genes in cervical spinal-cords of SOD1-mice are implicated in transmembrane transport, cation transport, ion-transmembrane transport, cell-cell signalling, inorganic ion transmembrane transport, synaptic signalling etc***. (Figure-16A)*** whereas in mutant-SOD1 lumbar samples exclusively downregulated genes are related with cell fate commitment, cell fate specification, neuron fate specification, autonomic nervous system development and negative regulation of dendrite morphogenesis ***(Figure-16B).***

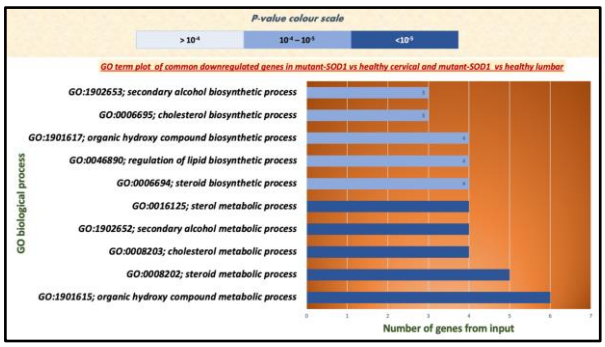
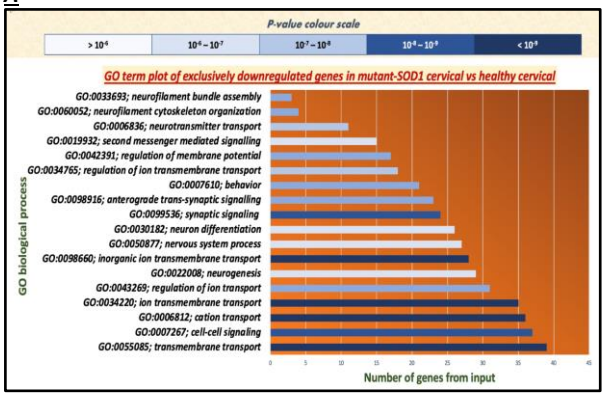


Figure-15: ***Gene Ontology (GO) analysis of commonly downregulated genes at cervical and lumbar spinal-cord levels in mutant-SOD1 vs healthy control comparison.*** *GO term plot showing biological processes associated with the commonly downregulated genes in mutant-SOD1 samples vs healthy controls of both cervical and lumbar spinal cords.*

A



B

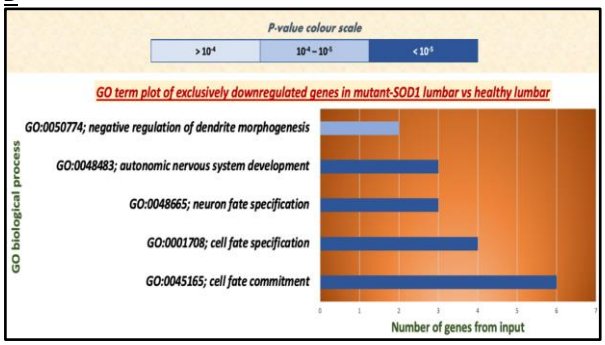


Figure-16: ***Gene Ontology (GO) analysis of exclusively downregulated genes in mutant-SOD1 vs healthy control.*** ***(****Diagram of GO (Biological Process) terms that are significantly enriched in differentially expressed genes.)* ***(B)*** *GO term plots showing the biological processes associated with the exclusively downregulated genes in mutant-SOD1 cervical vs healthy control cervical samples.* ***(C)*** *GO term plots showing the biological processes associated with the exclusively downregulated genes in mutant-SOD1 lumbar vs healthy control lumbar samples.*

4. Discussion:

Since the first transcriptomics study of ALS in 2001 (Malaspina et al. 2001), gene expression profiles have been generated by transcriptome analysis to understand ALS pathogenesis using autopsy specimens of spinal-cords (Dangond et al. 2004; Ishigaki et al. 2002; Jiang et al. 2005) or post-mortem motor cortex collected from ALS patients (Lederer et al. 2007; Wang et al. 2009; Ferraiuolo et al. 2007; Chen et al. 2010), transgenic rodent-models of ALS (Olsen et al. 2001; D'Arrigo et al. 2010; Yoshihara et al. 2002; Ferraiuolo et al. 2007; Chen et al. 2010) and in vitro-models of ALS (Kim et al. 2017; Onodera et al. 2020). However, very few scientific literatures have been found expounding the regulation of selective-susceptibility of MNs to ALS by A-P axial-identity. For investigating the differential-vulnerability of MNs in relation to their A-P positional identities, one SOD1-ALS transgenic-mice dataset (consisting of RNA sequencing profiles of spinal-cord samples from mutant-SOD1(G37R) mice and their wild-type counterparts) has been analysed in this study to collate the transcriptomic changes descried in cervical and lumbar spinal-cords of SOD1-mice as compared to healthy controls.

The unbiased transcriptome analysis in this study discovered the enhanced expression of autophagy-associated genes (Cd5l, Atp6v0d2, Mcoln3 etc.) in mutant-SOD1 mice. Cd5l, the most upregulated gene (8.75-fold higher) at cervical spinal-cord level (in mutant-SOD1 versus healthy mice comparison) supports the upregulation of complement-pathway in ALS patients validated by raised potential plasma biomarker Cd5l level in some previous studies (Mohanty et al. 2020; Xu et al. 2017; Xu et al. 2018). The second top-ranked upregulated gene (Atp6v0d2) for macrophage-specific subunit for vacuolar ATPase promotes autophagy by facilitating autophagosome-lysosome fusion (Xia et al. 2019). The Mcoln3 gene coding for a pH-regulated Ca^{2+}-channel contributes to lysosomal calcium release, induces autophagic influx and leads to MN-survival (Martina et al. 2009; Santoni et al. 2020). In postmitotic cells like neurons, where defective organelles and proteins are more likely to accumulate, autophagy functions as an essential lysosomal degradation pathway and so neural-specific defects in autophagy result in neurodegeneration (Hara et al. 2006; Komatsu et al. 2006; Mariño et al. 2011; Rubinsztein et al. 2004; Ventruti and Cuervo 2007). The neurodegenerative disease, ALS is characterised by aberrant intracellular accumulation of ubiquitinated protein-aggregates ***(Figure-5)***, cytoplasmic inclusions or damaged mitochondria in affected MNs (Al-Chalabi et al. 2012; Blokhuis et al. 2013; Lee et al. 2015) and thus it provides the evidence of insufficient clearance of proteins and organelles by degradation in degenerating MNs of ALS patients. Furthermore, defective retrograde axonal-transport has been noticed in SOD1-mouse model of ALS (Kieran et al. 2005; Nixon 2007) which most likely interrupts fusion of autophagosome and lysosome leading to massive aggregation of autophagy-intermediates. As

misfolded SOD1-proteins is found as a component of ALS-associated cytoplasmic inclusions in MNs, aggregated SOD1-proteins have been detected to inhibit autophagosome/lysosome fusion by interacting with dynein for altering its position (Zhang et al. 2007). However, delayed MN death in ALS can be achieved by autophagic clearance of mutant SOD1 (Crippa et al. 2010; Hetz et al. 2009). Therefore, the upregulation of genes involved in restoring autophagy and lysosome function in ALS (Cd5l, Atp6v0d2, Mcoln3) evinced the augmented neuroinflammatory cascades and striving cell death machinery triggering body defence mechanism against ALS-pathology.

Additionally, the findings of GO enrichment analysis has demonstrated that for statistically significant upregulated-genes in cervical and lumbar spinal-cords of SOD1-mice most enrichment occurred in defence response to restrict damage to organism or in processes modulating the frequency, rate, extent of the immune response to an immunogenic stimulus. Although genes implicated in inflammatory or immunological pathways were affected in SOD1-mice at both cervical (genes like- Cd5l, Atp6v0d2, Mcoln3, Tnfsf11, Cd300lb, Cd200r4 etc.) and lumbar (genes like- Mcoln3, Atp6v0d2, Cd5l) spinal-levels, an escalated protective inflammatory-response was perceived in cervical region of transgenic mice with detectable mutant-SOD1 pathology. The activation of complement-mediated immune-response (Cd5l, Cd300lb, Cd200r4 etc. upregulation) in cervical spinal-cords with SOD1-mutation insinuates that antibody treatment against the cell-surface receptors of immune cells can improve MN-survival in ALS-patients by reducing neuroinflammation and delaying the activation of adaptive (T-cell mediated) or innate immune-system as demonstrated in another study (Lincecum et al. 2010).

In this study, the expression of Gpnmb gene in the spinal-cord samples of SOD1-mice has been found as significantly increased with the progression of ALS disease. It has been discovered that Gpnmb can prevent MN-death (induced by transfection of mutant-SOD1) and therefore can act as a neuro-protective factor by slowing the onset of ALS symptoms in SOD1-mice model. Thus, contribution of Gpnmb to MN-survival reveals that this transmembrane glycoprotein as a potential therapeutic target for ALS (Tanaka et al. 2012). The upregulation of Htr2b gene for serotonin 2B receptor (expressed in mononuclear phagocytes of central nervous system, microglia) in spinal-cord regions of ALS-mouse models has also been reported by other studies and increased expression of this receptor limits microglial degeneration in spinal cord and delays disease manifestation in ALS-mice (Arnoux and Dupuis 2021; El Oussini et al. 2016; Roumier and Béchade 2021).

Next, if we focus on the transcripts with decreased expression in mutant-SOD1 versus healthy comparison, most of the genes downregulated at cervical spinal-cord level were unique to that region (178 exclusive genes among 208 total downregulated-genes in mutant-SOD1 cervical samples). However, the number of exclusively downregulated-genes was less in mutant-SOD1 lumbar samples (56 unique genes except 29 downregulated-genes common with cervical region) in comparison to those in mutant-SOD1 cervical samples. In this study, the 178 transcripts with depleted expression that were identified exclusively in the cervical spinal-cords of mutant-SOD1 mice are involved in specific biological processes, mainly including- transmembrane transport, cell-cell signalling, neurogenesis, neurofilament cytoskeleton organization etc. Conversely, the 56 downregulated transcripts that were exclusive for lumbar spinal-cords of SOD1-mice are mainly related to commitment of cells to specific cell fates and their capacity to differentiate into specific type of cells.

The aberrant accumulation of neurofilament (the major intermediate filaments in the neuronal cytoskeleton of adult MNs, abundant in myelinated axonal fibres) aggregates in the proximal axons of MNs is a common pathological hallmark of both SOD1-linked familial and sporadic ALS (Carpenter 1968; Hirano et al. 1984; Hirano 1991; Rouleau et al. 1996) giving rise to the hypothesis that defective neurofilament organization may be accountable for ALS pathogenesis. After neurofilament-disorganization was reported in degenerating MNs of mutant-SOD1 transgenic mice (Gurney et al. 1994), a marked reduction in the rates of MN-axonal transport has been documented not only for neurofilaments, but also for other cytoskeletal proteins (tubulin, actin etc.) and organelles (mitochondria) (Julien 2011). This defective axonal transport in MNs by cytoskeletal derangements was also observed in my study associated with the exclusively downregulated-genes in mutant-SOD1 cervical samples ***(Figure-16A),*** providing an explanation for the selective vulnerability of cervical-MNs in ALS (Williamson et al. 1998) as MNs with large, myelinated axons are particularly susceptible to disruption of the neurofilament network owing to their higher amount of neurofilament protein synthesis (Oblinger and Lasek 1984). Accordingly, the potentiality of suppressing neurofilament cytoskeleton disorganization with drugs blazes a trail for the development of advanced therapeutic approaches for this inexorable neurodegenerative disease.

In our study, the significant downregulation of Myh4 gene transcription (coding for fast myosin heavy chain-2b isoform in very fast glycolytic myofibers (Schiaffino and Reggiani 2011)) was observed exclusively in mutant-SOD1 lumbar samples which corresponds to the loss of innervation of fast-twitch glycolytic muscle fibres (type IIb) by fast-fatigable MNs at the early stage of disease progression (Pun et al. 2006). On a different note, about 5-fold decrease in the expression of Dbh gene in cervical spinal-cords of SOD1-mice signifies the damage of central dopaminergic system observed in ALS-patients through immunohistochemical, neurochemical and imaging techniques (Borasio et al. 1998; Kato et al. 1993; Sofic et al. 1991; Takahashi et al. 1993).

As a remarkable finding, the gene-transcripts that were found depleted in both cervical and lumbar spinal-cord samples of SOD1-mice than wild-type healthy mice are involved in lipid biosynthesis and metabolism. This result coincides with the lipidomic profiles of SOD1(G86R) mice by Henriques et al. where reduction in the levels of most lipid species (phospholipids and sphingolipids) (Henrique et al. 2015) was observed in spinal-cord of pre-symptomatic and symptomatic SOD1 murine models. Earlier in 2007, Niebroj-Dobosz et al. published a paper presenting the myelin composition of spinal-cord in SOD1(G93A)-mice based on biochemical and electron microscopic examinations findings which revealed considerable deviations in lipid composition of spinal-cord myelin sheath including most pronounced reduction in cholesterol component (Niebroj-Dobosz et al. 2007). The main architectural constituent of compact myelin is lipid (cholesterol) representing 70% to 80% of total composition (Norton and Cammer 1984) which maintains the fluidity and permeability of the myelin membranes. Therefore, evidence of decreased myelin-cholesterol even in symptom-free rodent models might elucidate the destabilization of the molecular structure of myelin membrane and likely play an essential role in the patho-mechanism of ALS at early stage. Differential expression of genes responsible for lipid metabolism in the CNS was documented by several studies (Kirby et al. 2011; Malaspina et al. 2001; Offen et al. 2008; Wang et al. 2009). These findings suggest an existing link between alterations in lipid metabolism and pathology behind inexorable MN-death in ALS and may proffer novel therapeutic targets for ALS. Diets enriched in fats might ameliorate the time frame between the onset of ALS-manifestations and death significantly as demonstrated in transgenic mouse model of ALS (Dupuis et al. 2004; Mattson et al. 2007).

In summary, aforementioned analysis provided a snapshot of the deregulated-pathways related to SOD1-pathology at cervical and lumbar spinal-cords uncovering that neuroinflammation is common in both regions of SOD1-mice, but transcriptomic fluctuations are more significant at cervical rather than at lumbar spinal-cord level. However, the major limitations of this study entail absence of transcriptome profile analysis of visceral-MNs (thoracic and sacral), limited sample size, lack of RNA-seq data regarding other ALS-associated genetic causes (C9ORF72, TARDBP, FUS), unavailability of spinal-cord transcriptome data from human with SOD1-ALS etc. Further RNA-seq analyses of spinal-cord data-sets containing large sample size (e.g.- collected from *TargetALS* foundation, https://www.targetals.org) encompassing animal-models with different ALS-predisposing mutations and post-mortem ALS-materials or transcriptomic reports on in vitro-derived (induced pluripotent stem cell-based) MNs with different A-P axial identities may unveil the comprehensive influence of A-P regional identity of MNs on the gene signatures and molecular targets associated with selective-vulnerability of MNs in ALS.

5. Conclusion:

In spite of enormous leaps in technological innovation and scientific research in medical science, the most frequent form of motor neuron disease (ALS) still prevails as a life-threatening neurologic condition with few approved drugs (Riluzole and Edaravone) which can only extend lifespan by a few months in ALS-patients. For mitigating disease progression in ALS, various new therapeutic approaches consisting of novel drug therapy, immunotherapy, gene therapy, stem cell-exosomal therapy etc. are being evaluated in preclinical and clinical trial, yet we have not achieved success in discovering efficacious and curative treatment option for this debilitating neurological disorder. As a part of advancement in regenerative medicine field, clinical trials for stem cell transplants in ALS are being conducted to slow the MN degeneration by generating neuroprotective milieu and replacing diseased MNs. Despite decades of research in molecular pathogenesis of SOD1-linked ALS, very few have focused on the variation in the degree of proneness of spinal MNs to ALS-inducing mutations among MN subpopulations based on their A-P axial identity. This study can be appraised as a starting point for future experiments addressing the candidate biological processes involved in the differential neurodegenration in different A-P axial regions of spinal cord caused by ALS-predisposing mutations. If our proposition regarding the association between the intrinsic vulnerability of MNs to ALS and their A-P positional identity can be established and the distinct mutation-linked pathogenic pathways related to neurodegeneration at different spinal cord levels in ALS can be evidenced by further studies, then we can envisage the identification of rostro-caudal position-specific therapeutic targets intending to develop regional therapies for this devastating neurodegenerative disease. In time, neural restoration by replacing electrophysiologically active MNs (with specific A-P axial-identity) identical to the degenerated ones and protecting remaining healthy neurons by different region-specific treatment protocols will be the forthcoming cure for ALS in the near future, not a reverie.

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