



**TÜRKİYE CUMHURİYETİ**  
**ADANA ALPARSLAN TÜRKER SCIENCE AND TECHNOLOGY**  
**UNIVERSITY**

**GRADUATE SCHOOL**  
**BIOENGINEERING DEPARTMENT**

**DRUG REPOSITIONING FOR NEURODEGENERATIVE DISEASES**  
**BASED ON BIOINFORMATICS AND TEXT MINING ANALYSIS**

**KÜBRA TEMİZ**

**M.Sc.**

**ADANA 2024**



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**ADANA, 2024**

## DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

24/07/2024

[Signature]

Kübra TEMİZ

# ÖZET

## BİYOİNFORMATİK VE METİN MADENCİLİĞİ ANALİZİNE DAYALI NÖRODEJENERATİF HASTALIKLAR İÇİN İLAÇ YENİDEN KONUMLANDIRMA

Kübra TEMİZ

Yüksek Lisans, Biyomühendislik Anabilim Dalı

Danışman: Doç. Dr. Esra GÖV

Temmuz 2024, 117 sayfa

Nörodejeneratif hastalıklar (NDH), nörodejenerasyonun olduğu, ilerleyici ve tedavi seçeneği kısıtlı olan hastalıklardır. Bu tezde, makine öğrenmesi ile entegre kapsamlı bir biyoinformatik yaklaşımı kullanarak seçtiğimiz NDH olan Alzheimer Hastalığı (AD), Parkinson Hastalığı (PD), Huntington Hastalığı (HD) ve Amyotrofik Lateral Skleroz (ALS) için ilaç repozisyon çalışmalarına odaklandık.

Hastalık ve sağlıklı örnekleri içeren transkriptomik veriler seçildi ve gruplandırıldı. Farklı şekilde ifade edilen genler (DEG'ler) belirlendi ve ortak DEG'lere yönelik ko-ekspresyon ağı analizi yapıldı. Önemli gen kümelerini belirlemek için Rastgele Orman, Destek Vektör Makinesi (SVM) ve K-En Yakın Komşu (KNN) algoritmaları kullanıldı. Bu gen kümeleri, ilaç yeniden konumlandırma analizi için kullanıldı. Potansiyel ilaç adaylarını belirlemek amacıyla beş makine öğrenimi algoritması (Doğrusal Regresyon, SVR, Rastgele Orman, Gradient Boosting ve Sinir Ağı) uygulandı ve en yüksek öngörülen kat değişimine sahip ilaçlar belirlendi. Bu ilaçların yeniliği, metin madenciliği ile doğrulandı.

AD için 5 aday ilaç: Dmnq, İnterferon beta-1b, Siflutrin, Torcetrapib ve Vx. PD için 9 aday ilaç: İnterferon beta-1b, Aplidin, Androstanolon, Ribavirin, Dmnq, Doğal alfa interferon, İnterferon beta-1a, Klinafloksasin ve Bikalutamid. ALS için 5 aday ilaç: Nilotinib, Trovafloksasin, Apratoxin A, Karboplatin ve Klinafloksasin. Dmnq ve İnterferon beta-1b AD ve PD, Klinafloksasin ise ALS ve PD için ortak olarak belirlendi. Bulgularımızın wet lab çalışmalarıyla doğrulanması, NDH için yeni tedavi seçenekleri sunabilir.

**Anahtar Kelimeler:** ilaç yeniden konumlandırma, makine öğrenmesi, nörodejeneratif hastalıklar

# ABSTRACT

## DRUG REPOSITIONING FOR NEURODEGENERATIVE DISEASES BASED ON BIOINFORMATICS AND TEXT MINING ANALYSIS

Kübra TEMİZ

M.Sc., Department of Bioengineering

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Neurodegenerative diseases (NDDs) are a group of complex diseases with limited treatment options. This thesis presents a comprehensive bioinformatics approach to identify repurposed drug candidates for NDDs, focusing on Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), and Amyotrophic Lateral Sclerosis (ALS). Transcriptomic data including disease and healthy samples were selected and grouped. Differentially expressed genes were identified and co-expression network analysis was performed. Machine learning (ML) algorithms were applied to identify prominent gene clusters. These significant gene clusters were used for drug repositioning analysis. Five ML algorithms (Linear Regression, SVR, Random Forest, Gradient Boosting and Neural Network) were performed to predict the fold change of potential drug candidates. The novelty of these drugs was verified by text mining analysis. For AD, we identified 5 candidate drugs: Dmnq (2,3-dimethoxy-1,4-naphthoquinone), Interferon beta-1b, Cyfluthrin, Torcetrapib, and Vx. For PD, 9 candidate drugs: Interferon beta-1b, Aplidin, Androstanolone, Ribavirin, Dmnq, Natural alpha interferon, Interferon beta-1a, Clinafloxacin, and Bicalutamide. Lastly, for ALS, 5 candidate drugs: Nilotinib, Trovafloxacin, Apratoxin A, Carboplatin, and Clinafloxacin. Among these novel drugs, Dmnq and Interferon beta-1b drugs were common for AD and PD, while Clinafloxacin is common for ALS and PD. Validation of these findings with wet lab studies may provide new treatment options for NDDs.

**Keywords:** drug repurposing, machine learning, neurodegenerative diseases



*To future of myself*

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## LIST OF ABBREVIATIONS

|           |                                    |
|-----------|------------------------------------|
| NDD       | : Neurodegenerative Disease        |
| AD        | : Alzheimer's Disease              |
| PD        | : Parkinson's Disease              |
| HD        | : Huntington's disease             |
| HTT       | : Huntingtin                       |
| ROS       | : Reactive Oxygen Species          |
| RNS       | : Reactive Nitrogen Species        |
| A $\beta$ | : Beta-amyloid                     |
| TDP       | : TAR DNA-binding protein          |
| APP       | : Amyloid Precursor Protein        |
| PSEN1     | : Presenilin 1                     |
| SCNA      | : Alpha-Synuclein Protein          |
| PRKN      | : Parkin                           |
| DEG       | : Differentially Expressed Gene    |
| PINK1     | : PTEN-inducing kinase 1           |
| FC        | : Fold Change                      |
| SCC       | : Spearman Correlation Coefficient |
| PCC       | : Pearson Correlation Coefficient  |
| ML        | : Machine Learning                 |
| RF        | : Random Forest                    |
| SVM       | : Support Vector Machine           |
| SVR       | : Support Vector Regressor         |
| RF        | : Random Forest                    |
| RFR       | : Random Forest Regressor          |

# 1. INTRODUCTION

Neurodegeneration is a process in which the function or structure of neurons is lost. This process usually proceeds slowly and gradually and is unfortunately irreversible (Katsnelson et al., 2016). Neurodegeneration can cause several different conditions. Genetic factors and aging, damage sustained during birth contribute to neurodegeneration. These conditions disrupt the normal functioning of the nervous system by preventing neurons from functioning properly and can lead to various neurodegenerative diseases (NDDs) (Van Horssen et al., 2019).

NDDs have various clinical symptoms that represent the loss of particular neurons and synapses in different parts of the brain; however, current studies have revealed that the pathogenesis of NDDs can overlap at various molecular and cell levels (Azam et al., 2021). In general, inflammatory processes, mitochondrial dysfunctions, damage to reactive oxygen species (Dai et al., 2016), and pathogenic proteins are common problems in NDDs (Katsnelson et al., 2016). In NDDs, common issues include inflammatory processes, mitochondrial dysfunctions, damage caused by reactive oxygen species, and pathogenic proteins. Furthermore, environmental factors and diet increase the risk of age-related neurodegeneration (Słowikowski et al., 2024). Aggregation of cytosolic or nuclear proteins is a common feature in NDDs (Gan et al., 2018; Kujawska et al., 2021). This features the accumulation of beta-amyloid ( $A\beta$ ) plaques in Alzheimer's disease (AD) (Knopman et al., 2021), accumulation of  $\alpha$ -synuclein aggregates and other synucleinopathies in Parkinson's disease (PD) (Poewe et al., 2017), and TAR DNA-binding protein (TDP)-43 residues in Amyotrophic Lateral Sclerosis (ALS) (Masrori & Van Damme, 2020) and frontotemporal dementia. The migration of these aggregates to different parts of the brain is a common phenomenon in NDDs (Gan et al., 2018).

Biomarkers play an essential role in the early diagnosis of diseases, prognosis determination, and treatment monitoring, allowing to personalize the treatment processes of patients (Califf, 2018). However, finding and clinically validating biomarkers involves challenges such as specificity, sensitivity, and biological variability (Bodaghi et al., 2023). Wet lab studies allow direct interaction with biological samples to obtain tangible results but require expensive equipment and time-consuming processes (van der Wijst et al., 2018). Dry lab studies can be fast and cost-effective thanks to data modeling and computer-based analysis. However the accuracy of the results depends on the quality of the models used (Hasin et al., 2017). Both

methods play complementary roles in biomarker research, providing more comprehensive and reliable results.

Network medicine is an approach that refers to understanding and treating complex diseases (Conte et al., 2020). The approach analyzes diseases' genetic, molecular, and phenotypic factors as interacting networks, drawing on disciplines such as network theory and systems biology to comprehend the complexity of biological systems (Turanli et al., 2024). Network medicine approaches can elicit cell signaling, regulatory pathways, and all their associated physical and functional architecture. Network medicine techniques can identify the biological etiology of a pathological disorder (A. J. Singh et al., 2018). The differential network approach, a sub-branch of network medicine, focuses on the dynamic response of systems affected by any disruption. Gene correlation analyses provide an integrated view of the complex interactions between molecular determinants of disease phenotypes (do Valle & Nakaya, 2020). This approach simplifies the unbiased equating of new disease genes and modules with procurement support for practical approaches to therapeutics involving rational polypharmacy (Gov & Arga, 2017; UNAL & Göv, 2022).

The overlap of phenotypic features of NDDs supports the idea that different NDDs have a common pathogenic mechanism and they may be treated with same drugs. Additionally, nearly all NDDs present as proteinopathies distinguished by misfolding, aggregation, and accumulation of a particular disease-causing protein. Arneson et al. conducted a GWAS study and reported that there was no common differentially expressed gene for ALS, PD, and AD in genomic data, but there were common pathways affected in HD, AD, ALS, and PD in transcriptomic data (Arneson et al., 2018).

Discovery, approval, and commercialization of a new drug or small molecule is economical and time-consuming. To solve this problem, it is more rational and necessary to test already discovered drugs against complex diseases. Logarithmic incremental computational methods and the discovery of drug targets have made drug repurposing the most effective method for complex diseases such as NDDs. Recently Bourdakou et al, applied meta-analysis on AD microarray data set and used differentially expressed genes for the drug target (Bourdakou et al., 2023). Another study showed in vivo that Fasudil could be repositioned as a promising drug for AD and PD (Collu et al., 2024). There is also a meta-analysis study on drug repurposing for ALS.

Despite all the studies, the etiology of NDDs is unclear; there is no exact treatment for NDDs yet. Therefore, computational and experimental studies should be conducted to find biomarkers, drug targets and drug candidates for the NDDs. In the present thesis, we investigate the NDDs AD, PD, ALS, and HD with the combination of novel network medicine and machine learning approaches and present repurposed drug candidates that could be used in treatment. We anticipate that the network medicine methods we use can provide promising results for prognosis, diagnosis, and treatment of all diseases and can be helpful worldwide.



## 2. LITERATURE REVIEW

### 2.1. Neurodegeneration

Neurodegeneration is a process that involves the loss of function and death of nerve cells. This process causes a variety of damage to the central and peripheral nervous system and is typically progressive in nature. Besides once neurodegeneration has started, its progression can be slowed down, but not completely reversed that led to initiation of NDDs. NDDs include a variety of clinical syndromes such as AD, PD, HD, and ALS. These disorders are characterized by the degeneration and loss of specific groups of neurons in the brain and spinal cord.

Recent Center Disease for Control and Prevention (CDC) statistics indicate that AD is the most prevalent and vital NDD encountered by mankind (*FastStats*, 2024). Sixty to seventy percent of dementia consists of AD (Ferreira et al., 2014; Silva et al., 2019). AD affects the pathophysiology of the brain like temporal lobe, entorhinal cortex, parietal cortex, and hippocampus (Kumar et al., 2024). Patients who are diagnosed with AD mostly die 9 years after diagnosis. In 2023 6.8 million people in America lived with dementia age 65 or older. AD is a complex disorder and pathologically characterized by the accumulation of amyloid plaques and neurofibrillary tangle in the brain. Also, it can be diagnosed by the presence of a mutation in the amyloid precursor protein (APP), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*). The aetiology of AD is not well understood (Breijyeh & Karaman, 2020). In this case, extended examinations and studies are required to elucidate the underlying mechanism of AD and improve prognostic and drug candidates that will diminish the prevalence of AD.

PD is the most prevalent NDD after AD, approximately 90,000 people diagnosed with PD in the US every year (*Parkinson's Statistics*, 2023). PD is generally characterized by the mutation and misfolding of the alpha-synuclein protein (SCNA) and its accumulation in structures called Lewy bodies. Lewy bodies disrupt the function of neurons and nerve cells, causing neuronal damage and death. This process leads to the motor and cognitive symptoms of PD. Additionally, mutations in several other genes, such as Parkin (PRKN), PTEN-inducing kinase 1 (PINK1), and DJ-1, are also associated with a diagnosis of PD (Poewe et al., 2017). However, despite all this knowledge about PD, there is no exact treatment for PD. Further research into the underlying causes of PD and the identification of new drugs are needed for a complete treatment.

ALS is a mortal NDD defined by progressive degeneration of motor neurons. Both upper motor neurons (in the brain) and lower motor neurons (in the spinal cord and peripheral nerves) are affected, leading to severe symptoms such as muscle weakness, atrophy and ultimately respiratory failure (Petri et al., 2023). While the etiology of ALS is largely unknown, approximately 5-10% of cases are inherited and linked to mutations in genes such as *C9orf72*, *TARDBP*, *FUS* and *SOD1* (Prasad et al., 2019). Mechanisms such as glutamate toxicity (Upadhyaya et al., 2020), oxidative stress, mitochondrial dysfunction and protein misfolding are thought to play a role in the pathophysiology of ALS (Kodavati et al., 2020). Treatment options are limited in this disease and current therapies are generally directed at symptom management. Although drugs such as Riluzole and Edaravone can slow disease progression to some extent, there is still no definitive cure for ALS (Van Es et al., 2017). Tofersen, newly approved by the FDA, reduced concentrations of *SOD1* in cerebrospinal fluid and neurofilament light chain in plasma in people with *SOD1* ALS, but did not improve clinical endpoints and was associated with adverse events (Miller et al., 2022). Future research needs to focus on better understanding the molecular mechanisms of the disease and developing new treatment strategies.

HD is an inherited NDD characterized by progressive motor, cognitive and psychiatric impairments, usually beginning in middle age. HD is caused by a single gene mutation: An abnormal lengthening of CAG trinucleotide repeats in the huntingtin gene (*HTT*). This mutation leads to toxic changes in the huntingtin protein, resulting in neuronal damage and death in the brain, particularly in areas such as the striatum and cortex (H. Yang et al., 2017). Patients initially show involuntary movements (chorea) and impaired motor coordination, and over time experience severe impairments in cognitive function and personality. A cure for HD has not yet been found and focuses on symptom management. Treatment strategies usually consist of pharmacological and psychosocial interventions to alleviate motor and psychiatric symptoms. In recent years, studies have been conducted on gene therapy and other biotechnological approaches targeting the genetic basis of HD, but these methods are still in the clinical trial phase (Ament et al., 2018).

## 2.2. Shared Mechanisms of Neurodegenerative Diseases

### 2.2.1. Aggregation of misfolding proteins

Although NDDs have diverse clinical manifestations, they share common pathological mechanisms. Protein abnormalities, misfolding and aggregation of certain proteins are among the common problems of these diseases (Azam et al., 2021). In AD, excessive phosphorylation of amyloid beta and tau proteins increases diffusion, especially of amyloid beta plaques. As phosphorylation progresses, the density of amyloid beta plaques increases, resulting in the formation of neurofibrillary tangles (Breijyeh & Karaman, 2020).

PD is characterized by protein misfolding and aggregation in consequence of a mutation in the alpha-synuclein gene. When these proteins accumulate in dopaminergic neurons in the substantia nigra, 'Lewy Bodies' are formed, and the formation of inclusions continues irreversible (Poewe et al., 2017).

In ALS, the transcript of the GGGGCC repeat sequences in the *C9orf72* gene produces various disease protein residues (DPRs). These DPRs include poly-GA, poly-GR, poly-PA and poly-PR. DPRs contribute to disease progression by forming toxic aggregates inside the cell and disrupting cellular functions (Gao et al., 2022). These toxic DPRs play an important role in the pathogenesis of ALS and constitute an important research topic in understanding the molecular basis of the disease, as well as developing potential therapeutic targets.

ALS is characterized by degeneration of motor neurons as a result of abnormal aggregation of FUS (fused in sarcoma) and TDP43 (TAR DNA binding protein 43) proteins, (DNA-binding amino acids are specialized regions of proteins that interact directly with DNA, and these interactions are critical for gene expression and regulation of cellular functions.) in the blood and cerebrospinal fluid and the formation of intracellular inclusions (Masrori & Van Damme, 2020).

HD is distinguished by the abnormal length of the HTT protein as a result of a CAG repeat sequence-dependent mutation of the *HTT* gene, known as the Huntington's gene. Abnormal HTT leads to the formation of intracellular inclusions and the accumulation of the mutant protein in the striatum.(Azam et al., 2021)

### 2.2.2. Neuroinflammation and Oxidative Stress in Neurodegenerative Diseases

Neuroinflammation is a type of inflammation triggered with the release of pro-inflammatory cytokines in the central nervous system, particularly the brain and spinal cord, by various immune cells such as astrocytes and microglia. Neuroinflammation has an essential role in the pathogenesis of these diseases. Increasing evidence suggests that neuroinflammation can be triggered by both genetic and environmental factors and accelerates disease progression. Therefore, targeting inflammation could be an important strategy in the treatment of NDDs.(Upadhyaya et al., 2020; W. Zhang et al., 2023)

In AD, A $\beta$  plaques begin to accumulate a decade before clinical signs appear, and Tau pathology usually develops after these plaques. A $\beta$  is recognized to act as a molecular model associated with damage. It activates microglia by binding to receptors such as TLRs (toll-like receptors) (Guijarro-Muñoz et al., 2014), RAGE (receptor for advanced glycation end products) (H. Singh & Agrawal, 2022) and NLRs (nucleotide-binding oligomerization domain-like receptors) (Venegas & Heneka, 2017). Activated microglia and astrocytes attempt to phagocytize A $\beta$ , but in case of failure, chronic inflammation begins and pro-inflammatory cytokines, chemokines, reactive oxygen species (ROS) and nitric oxide (NO) are released. This process enhances immune responses and leads to neurotoxicity. Microglia promote A $\beta$  plaque formation by increasing the expression of *IFITM3* or releasing substances such as iron. Activation of glial cells links A $\beta$  aggregation to tau aggregation and tau hyperphosphorylation leads to the formation of NFTs. Accumulation of tau tangles leads to loss of neuronal function and apoptosis. Furthermore, hyperphosphorylated tau can activate human macrophages via *TLR4*. New research shows that amyloid-laden neurons exhibit a unique inflammatory profile and that intraneuronal accumulation of A $\beta$  is important in the early stages of AD (W. Zhang et al., 2023).

In PD, misfolded  $\alpha$ -synuclein proteins begin to accumulate early in the disease.  $\alpha$ -synuclein activates microglia by binding to receptors such as TLRs and NLRP3 (Y. Li et al., 2021). Activated microglia and astrocytes attempt to phagocytize  $\alpha$ -synuclein, but when they fail, chronic inflammation begins, and they release pro-inflammatory cytokines, chemokines, ROS, and NO. This process enhances both oxidative stress and immune responses and leads to neurotoxicity(Q. Zhang et al., 2017).

In the brain of PD's patients, microglia are activated before the death of dopamine neurons. When misfolded  $\alpha$ -synuclein is released from neurons, it is sensed by microglia and the

inflammatory response begins (Y. Li et al., 2021). This process contributes to neuron death and disease progression. In Lewy bodies, 90% of  $\alpha$ -synuclein is phosphorylated at Ser129 and it has been suggested that this phosphorylation may play a protective role (Ghanem et al., 2022). Chronic activation of microglia exacerbates the disease and promotes the spread of  $\alpha$ -synuclein aggregates. Finally, the adaptive immune system also has a significant role in PD (Badanjak et al., 2021). High numbers of T cell responses to  $\alpha$ -synuclein have been reported of PD patients' brain (Qian et al., 2010). This evidence indicate that inflammation is pivotal in the progression of PD. Early detection and targeting of the immune response are crucial for the treatment of PD.

ALS is a known mortal NDD with a lifespan of approximately 2-4 years after diagnosis (Petri et al., 2023). There are two types of ALS, about 90-95% is sporadic, 5-10% is familial. The familial ALS mostly caused the mutations in *C9orf72* (40%) and *SOD1* (20%) (Damme et al., 2017). In 2006, 97% of ALS patients were found to have hyperphosphorylated TDP-43 inclusions (Prasad et al., 2019). Several factors cause to the onset and progression of ALS. Protein aggregates such as TDP-43 and SOD1 cause neuronal dysfunction and cellular damage. TDP-43 aggregates invade mitochondria, release mtDNA and trigger inflammation by activating the cGAS/STING pathway. SOD1 aggregates lead to oxidative stress and mitochondrial dysfunction. Damage-associated molecular pattern molecules (DAMPs) and protein aggregates released from damaged neurons stimulate microglia activation and the release of proinflammatory cytokines (Masrori et al., 2022). This chronic inflammation damages neurons, exacerbating ALS. Furthermore, people with autoimmune disease have an increased risk of developing ALS, indicating the close association of inflammation with ALS.(W. Zhang et al., 2023)

HD is characterized by a mutation in the *HTT* gene and accumulation of HTT protein aggregates. These aggregates activate microglia and astrocytes, leading to the release of pro-inflammatory cytokines. Chronic inflammation causes damage to striatal and cortical neurons. These inflammatory processes contribute to disease progression (Hsiao et al., 2013; H. Yang et al., 2017).

Finally, endogenous protein aggregations induce neuroinflammation, which accelerates neurodegeneration. Increasing evidence suggests that neuroinflammation plays a central role in the development and progression of NDD. Both genetic and environmental factors accelerate the progression of these diseases by triggering the inflammatory response.

### **2.2.3. Mitochondrial Dysfunction in Neurodegenerative Diseases**

Mitochondria play crucial role in energy production and function of the cells. Brain cells require the highest energy among the other cells. Any functional change in the mitochondria triggers dysfunction of neurons that causes NDD (Azam et al., 2021). Wang et al reported that AD is associated to mitochondrial dysfunction (Sharma et al., 2021). *PINK1*, *Parkin* and *LRRK2* genes which are involved the pathogenesis of the PD regulates the mitochondrial function and morphological integrity of mitochondria (Narendra et al., 2009). Additionally, recent studies demonstrates that mitochondrial dysfunction directly contributes to dysfunction of the motor neuron in ALS (Kodavati et al., 2020). Furthermore, in HD p53 and PGC-1 $\alpha$  dysregulation trigger and exacerbate mitochondrial dysfunction, cell death and neurodegeneration processes (Johri et al., 2013).

### **2.2.4. Oxidative Stress and Redox Balance in Neurodegenerative Disease**

Oxidative stress is a condition in which cellular components are exposed to oxidative damage through the role of ROS and reactive nitrogen species (RNS). Oxidative stress, combined with the inadequacy of antioxidant defense mechanisms in cells over time, leads to impaired cellular function and cell death (Van Horssen et al., 2019). In AD, the accumulation of amyloid beta plaques and tau tangles increases ROS production, leading to lipid peroxidation, protein oxidation and DNA damage (Butterfield & Halliwell, 2019; Van Horssen et al., 2019). Auto-oxidation of dopamine and mitochondrial dysfunction contributes to increase ROS production in dopaminergic neurons in the substantia nigra in PD. This induces to neuronal cell death and disease progression (Dias et al., 2013; Qian et al., 2010). In ALS, *SOD1* mutations increase oxidative stress, leading to mitochondrial dysfunction and cell death in motor neurons (Damme et al., 2017). In HD, mutant HTT protein disrupts mitochondrial function, increasing ROS production and disrupting cellular redox balance. This process accelerates neuronal degeneration and contributes to disease progression (Azam et al., 2021).

### **2.2.5. Cellular Autophagy and Lysosomal Dysfunction in Neurodegenerative Diseases**

Autophagy is a self-renewal, repair and intracellular waste removal mechanism. Autophagy removes accumulated waste products, damaged organelles and unused proteins with the help of the lysosome. Autophagy protects the cells from various diseases by enabling the formation

of new cells or repair of damaged cells. In NDDs, autophagy and lysosomal lead to the accumulation of protein aggregates and cellular toxicity. In AD, disruption of the autophagy-lysosomal system increases the accumulation of amyloid beta and tau proteins and disrupts cellular homeostasis (Uddin et al., 2019). Furthermore, in PD, *PINK1* and *PRK* genes has a crucial role in the clearance of damaged mitochondria through autophagy, called mitophagy (Pickrell & Youle, 2015). Similarly, accumulation of *C9orf72*, *TDP-43* and *SOD1* protein aggregates in ALS (Sasaki, 2011; Webster et al., 2016), and mutant HTT protein in HD disrupt the cellular autophagy process, increasing cellular toxicity and accelerating neuronal degeneration (Martinez-Vicente et al., 2010).

### **2.3. Genetic and Epigenetic Factors in Neurodegenerative Diseases**

Genetic and epigenetic factors play a critical role in the pathogenesis of NDDs. Genetic mutations are a determining factor in many of these diseases, while epigenetic alterations modulate gene expression and cellular functions, affecting disease risk and progression. In AD, genetic risk factors such as the *APOE*  $\epsilon 4$  allele contribute to earlier onset and faster progression of the disease. Epigenetic mechanisms may influence amyloid beta and tau pathologies through DNA methylation and histone modifications (Lozupone et al., 2023; Yuan et al., 2022). In PD, mutations in genes such as *LRRK2*, *SNCA* and *PINK1* play an important role in the pathogenesis of the disease. Epigenetic changes can affect the survival and function of dopaminergic neurons (W. Li et al., 2021; Pickrell & Youle, 2015). In ALS, genetic mutations such as *C9orf72* and *SOD1* play a critical role in the occurrence of the disease. Epigenetic alterations can influence disease progression by modulating motor neuron survival and cellular stress responses (Azam et al., 2021; Masrori & Van Damme, 2020). In HD, CAG repeat expansion in the *HTT* gene is the primary genetic cause of the disease. Epigenetic alterations may contribute to disease pathogenesis by affecting the toxicity of mutant huntingtin protein and cellular responses (Ament et al., 2018).

### **2.4. Bioinformatics and bioinformatics studies in Neurodegeneration**

Bioinformatics, systems biology, and network medicine are interdisciplinary fields that combine biological knowledge with computational technology, mathematics and engineering to understand complex biological systems and diseases (Nguyen et al., 2024).

**Bioinformatics** is the scientific field contributes to develop new methods, tools for analyzing huge complex biological data. Bioinformatics is associated with the collection, storage and analysis of genomic and proteomic data generated by high-throughput technologies. (Chen & Wu, 2013)

**Systems biology** aims to understand the complexity of biological systems and focuses on analyzing the interactions between parts of biological systems. (Chen & Wu, 2013)

**Network medicine** uses network science to explore the molecular complexity of diseases and aims to understand the mechanisms underlying disease by analyzing molecular and phenotypic interactions. (Conte et al., 2020)

All three fields are interconnected and contribute to the development of a more precise approach to medicine that aims to tailor healthcare to individual patient characteristics based on genetic, biomarker and phenotypic data. The goal of these three fields is to identify critical nodes that can be targeted for therapeutic intervention, leading to the development of new drugs and personalized medicine (Chen & Wu, 2013).

Omics data refers to comprehensive datasets covering molecular level information such as genome, transcriptome, proteome metabolome and microbiome. Logarithmic progress in omics technologies provides the opportunity to reveal the molecular mechanisms of diseases and evaluate them from a systems biology perspective (Kori et al., 2023; Unal et al., 2022). Progressions in this field are focused on the use of biomarkers that can predict evolving prognosis, diagnosis, course, and treatment, and the identification of biomarkers that can guide us to comprehend whether there is a therapeutic target to indicate the disease (Bodaghi et al., 2023).

Biological networks, especially gene and protein networks, are valuable data sources that enable us to understand cellular interactions and discover the molecular basis of organisms (Chen & Wu, 2013). Co-expression networks represent groups of co-regulated genes and can help us understand the mechanisms of diseases by analyzing gene correlations between different phenotypes (Kakati et al., 2019). Differences in the correlation between genes in various phenotypes may indicate specific phenotypes and contribute to the discovery of system biomarkers (Kori et al., 2023; Savino et al., 2020). In particular, network analysis methods play an influential role in studies aspired at the discovery of potential biomarkers for early diagnosis and treatment of diseases.

Biomarkers are measurable indicators of specific biological processes, pathophysiological states, or treatment responses (Califf, 2018). In NDDs, as in other diseases, biomarkers play a critical role in early diagnosis of disease, monitoring disease course and assessing treatment responses. Bioinformatics analyses, genetic variations, gene expression changes and proteomic profiles associated with NDDs help us understand the molecular mechanisms of the disease (Ehrenberg et al., 2020). The biomarkers and the nodes can be used as drug target and many studies (Bourdakou et al., 2023; Raza & Muddassar, 2022; Sunildutt et al., 2024) suggest that the biomarkers can be used for drug target for NDDs.

To combat the growing global burden of NDDs, researchers are exploring computational methods for repositioning drugs using bioinformatics, systems biology, and network medicine approaches. Chowdhury et al., studied gene expression microarray and eQTL datasets to explore biomarkers. Firstly they identified differentially expressed genes (DEGs), then analyzed the gene set enrichment, thereafter, they constructed protein-protein interaction (PPI), after that transcription factor (TF) and target gene interaction identified and lastly, they targeted common genes to identify drug candidates for AD, they identified 10 candidate drugs (Chowdhury et al., 2020).

In another study, RNA sequencing was used to determine thyroarytenoid muscle gene expression in rats with PD compared to controls. Differentially expressed genes (DEGs) were identified using EdgeR and statistical methods and enrichment analysis was performed on these genes. To establish the interaction network between the DEGs themselves, STRING analysis was used to identify the most interacting genes and Weighted Gene Co-expression Network Analysis (WGCNA) was performed on these genes. Finally, drug repositioning studies were performed for up- and down-regulated genes. The sequencing dataset was analyzed using a bioinformatics approach and the ENRICH gene analysis tool to compare with biological pathways and processes, disease associations and drug repurposing. The data obtained were compared with a previously published dataset in male rats (Barnett et al., 2023).

Duan et al studied to discover new drug candidates for ALS (Duan et al., 2024), they performed Mendelian randomization (MR) and colocalization analyses using cis-eQTL of druggable gene and ALS GWAS data collections. MR analysis identified *TBK1*, *TNFSF12*, *GPX3* and *CD68* genes as associated with ALS. Drugs such as fostamatinib (R788) and amlexanox (AMX) with repurposing opportunities targeting *TBK1*, *TNFSF12*, and *GPX3* genes were identified. Pharmacological experiments showed that R788 and AMX reduced neuroinflammation in ALS

cell models. The results support the potential of R788 and AMX targeting *TBK1*, *TNFSF12*, and *GPX3* in the treatment of ALS (Duan et al., 2024).

In another study, a transcriptome-wide association study was performed with genome-wide data on HD, assessing genetically determined expression differences in various tissues. Functional validation of priority genes was performed in isogenic HD stem cells and patient brains. Core pathways were identified by enrichment analyses and HD-associated gene co-expression modules were evaluated for associations with neurological phenotypes and to guide drug repurposing analyses. Analyses identified genes associated with age of HD onset and colocalizing with gene expression signals in brain tissue (*FAN1*, *GPR161*, *PMS2*, *SUMF2*). Furthermore, cortical co-expression modules were associated with cognitive decline and HD-related features (Wright et al., 2020).

**Table 2. 1.** Literature review of drug repurposing associated studies for selected neurodegenerative diseases: Alzheimer's Disease, Parkinson's Disease, ALS, and Huntington's Disease

| Disease             | Method                                                                                                                                                                                                                                             | Drug Name                                                                                                                                                                                                                                                                                                                                              | Reference               |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|
| Alzheimer's Disease | <b>Methods for Identification of Differential Expression Genes (DEGs):</b><br>-Limma<br>-Limma-ChDir<br>-mAP-kl<br><b>Drug Repurposing Tools:</b><br>-cMap<br>-sscMap<br>-LINCS-1000<br>SPIEDw: New correlation coefficient-based similarity score | Rottlerin, Dapsone, Biperiden, Skimmianine, Bupropion, Milrinone, Pioglitazone, Rucaparib, Nifedipine, BRD-K05331696, SN-38, Isoliquiritigenin, Forskolin, Vorinostat, Trichostatin-A, HC-toxin, THM-1-94, Panobinostat, Dacinostat, KM-00927, Bisindolylmaleimide, THZ-2-98-01, Enzastaurin, PKCbeta-inhibitor, Inhibitor BEC, SB-216763, Varenicline | (Siavelis et al., 2016) |
|                     | <b>Data Set Selection:</b> AD and control entorhinal cortex samples were selected from the NCBI GEO database (GSE118553, GSE48350).<br>Batch effect was corrected with ComBat, homogeneity was checked with Cochran's Q test.                      | Piperacillin, Riluzole, Thioridazin, Phenylbuta, Temsirolim, Chlorphen, Cisapride, Trimipram,                                                                                                                                                                                                                                                          | (Fagone et al., 2022)   |

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|  | <p><b>In Silico Pharmacology:</b> 749 FDA-approved drugs were analyzed by meta-analysis of LINCS L1000 data, blood-brain barrier permeability was assessed with the B3DB dataset.</p> <p><b>Statistical Analysis:</b> Random effects model was used. Genes with FDR &lt; 0.05 were identified as DEGs and analyzed with NetworkAnalyst.</p> <p><b>Enrichment analysis:</b> Metascape</p> | <p>Biperiden, Ivermectin,<br/>Metronida, Ribavirin,<br/>Dextromet, Fluoxetine,<br/>Fluvoxami, Lamotrigin,<br/>Paroxetine, Tretinoin,<br/>Chlorproth, Clemastine,<br/>Methocarb, Sunitinib,<br/>Tamoxifen, Thiethylpe,<br/>Granisetro, Loperamid,<br/>Praziquant, Diphenylpy,<br/>Droperidol, Pemoline,<br/>Saquinavir, Sertraline,<br/>Atomoxeti, Cytarabine,<br/>Gefitinib, Sulfameth,<br/>Pramipexo, Naloxone,<br/>Nortriptyli, Pentobarb,<br/>Metaxalon, Nalbuphin,<br/>Rivastigmi, Terbinafin,<br/>Cyprohept, Metoclopr,</p> |  |
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|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Naltrexone, Prednicarb,<br>Desoximet, Ethotoin,                                                                                                                                                                                                       |                          |
| Literature Review of published Trials up to December 31, 2020 on PubMed, MEDLINE, and EMBASE                                                                                                                                                                                                                                                                                                                                                                                                                                     | Diuretics, Beta Blockers (BB),<br>Angiotensin-Converting<br>Enzyme Inhibitors (ACE-I),<br>Angiotensin II Receptor<br>Blockers (ARB), Calcium<br>Channel Blockers (CCB), Alpha<br>Blockers, Central Agonists,<br>Vasodilators, Aldosterone<br>Blockers | (Y.-L. Liu et al., 2023) |
| Literature Review of published Trials up to December 31, 2019 in the database of Cochrane, Clinical trials registry, Scopus, and PubMed using the keywords “Pioglitazone” or Thiazolidinediones/therapeutic use” or “hypoglycemic agents/therapeutic use” or “Alzheimer's disease/drug therapy” or “cognitive dysfunction/drug therapy” or “memory loss/drug therapy” or “Diabetes Mellitus, Type 2/drug therapy” or “dementia/drug therapy” or “hypoglycemic agents/therapeutic use” or “randomized controlled clinical trial.” | Pioglitazone                                                                                                                                                                                                                                          | (Basutkar et al., 2023)  |

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|  | <p><b>NRF2-Interaction Partners:</b></p> <ul style="list-style-type: none"> <li>- NRF2 interaction partners were identified based on protein-protein interaction data from the human interactome.</li> </ul> <p><b>Differential Expression of NRF2 and Partners in AD:</b></p> <ul style="list-style-type: none"> <li>- Expression Atlas database.</li> <li>- Differentially expressed genes (DEGs) in the E-GEOD-5281 experiment were identified.</li> </ul> <p><b>Drug Repurposing:</b></p> <ul style="list-style-type: none"> <li>- Drug Gene Budger (DGB) tool</li> <li>- Collection of NRF2 Activating Clinical Drugs: NRF2-activating drugs used in AD clinical trials were collected.</li> </ul> <p><b>Structural Similarity:</b> Candidate drugs and NRF2-related drugs were downloaded in SMILES format and converted into a single SDF file with OpenBabel and clustered with ChemBioServer 2.0.</p> <p><b>ADMET Prediction:</b></p> <ul style="list-style-type: none"> <li>- AdmetSAR web application.</li> </ul> | <p>Curcumin, Trichostatin-A, Panobinostat, Parthenolide, Entinostat</p> | <p>(Bourdakou et al., 2023)</p>  |
|  | <p><b>Extraction of HSA Drugs from DrugBank:</b> Drugs that interact with human serum albumin (HSA) are extracted from the DrugBank database.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <p>Prednisone, Warfarin, Mefenamic acid, Levothyroxine, Propranolol</p> | <p>(Deryusheva et al., 2024)</p> |

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|  | <p><b>Filtering of the Candidates According to a Set of Parameters- Filtering Parameters:</b> Molecular Mass, Water Solubility, Predicted blood-brain barrier (BBB) Penetration, Plasma Concentration. Selection of the Candidates Related to AD- DrugBank and Alzforum.</p> <p><b>Clustering of the Candidate Drugs According to Their Binding Sites on HSA Surface-</b> AutoDock Vina, and candidates are clustered according to these predicted binding sites.</p> <p><b>Ranking of the Candidates Within Each Cluster- Ranking:</b> Candidates are ranked within each cluster based on their binding site characteristics.</p> |                                              |                              |
|  | <p>The in vitro and in silico cholinesterase inhibitor effects of 11 FDA-approved drugs (metformin, <math>\alpha</math>-lipoic acid, trimetazidine, trimebutine, gemfibrozil, theophylline, levamisole, pantoprazole, indomethacin, levetiracetam, and piracetam) were examined to determine their potential for repurposing against AD.</p>                                                                                                                                                                                                                                                                                       | <p>Trimebutine, Theophylline, Levamisole</p> | <p>(Akkaya et al., 2024)</p> |

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|  | <p>This study examined the behavioral and psychological symptoms of dementia (BPSD) in community-dwelling older adults who later developed AD. Researchers used clinical interviews and principal component analysis to categorize BPSD into domains. Post-mortem brain tissues from 60 subjects with BPSD data collected within two years of death were analyzed using RNA-seq. Differential gene expression analysis and weighted gene co-expression network analysis (WGCNA) were performed to identify gene modules associated with BPSD domains. Functional enrichment and cellular decomposition analyses were conducted to understand transcriptional changes related to BPSD in AD.</p> | <p>Ruxolitinib, Vorinostat, Regorafenib, Tofacitinib, Nilotinib, Lapatinib, Palbociclib, Ruxolitinib, Cabozantinib, Baricitinib, Nintedanib, Tofacitinib, Gefitinib, Resveratrol, Dasatinib NVP-TAE684, A443654, Brivanib, Dovitinib, Fedratinib, Torin1, Pelitinib, Fedratinib, H89, Staurosporine Aglycone, GSK1059615, JWE-035, KIN001-111, KW2449, GSK1070916</p> | <p>(Rodriguez et al., 2021)</p> |
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| Parkinson's Disease | <p><b>Data Collection and Network Construction</b> GWAS Data: Extracted Parkinson's disease data from GWAS Catalog on January 7, 2022.</p> <p><b>PPI Data:</b> STRING database</p> <p><b>Network Building:</b> Cytoscape software.</p> <p>Module Building and Gene Set Enrichment Analysis (GSEA) Module Extraction: Girvan-Newman Algorithm GSEA: Conducted with Reactome FIViz, using a module cutoff of 10 and FDR value of 0.1.</p> <p><b>Centrality and Robustness Analysis</b> Centrality Metrics: Calculated Degree, Betweenness, Closeness, and Stress centrality.</p> <p><b>Robustness Metrics:</b> Assessed network robustness using Characteristic path length, Average Neighbors, Network density, and Network Centralization. Data Engineering and Feature Extraction Inhibitors Extraction: From ChEMBL database.</p> <p><b>Dataset Division:</b> Split into strong, weak IC50 groups, and decoys from DUD-E.</p> <p><b>Descriptor Generation:</b> Used Mordred software for 2D/3D descriptors.</p> <p><b>Data Processing:</b> Cleaned and processed with Python, split using SKlearn's Train Test Split, and standardized with Standard Scaler. Model Training, Testing, and Deployment ML Algorithms: Implemented Multilayer Perceptron, Support Vector Machine (SVM), Decision Tree, Random Forest.</p> <p><b>Hyperparameter Optimization:</b> Used GridSearchCV.</p> | <p>tolcapone, lamotrigine, citalopram, diflunisal, diazepam, temazepam, triazolam, felbamate, estazolam, trifluoperazine, zolpidem, escitalopram, flurazepam, haloperidol, alprazolam, salsalate, chlordiazepoxide, clobazam, clomipramine</p> | <p>(Raza &amp; Muddassar, 2022)</p> |
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**Evaluation:** Assessed with Precision, Recall, F1, and Support metrics.

**Deployment:** Deployed using JobLib library.

**Drug RepositioningCompound Extraction:** Bio-active compounds for the Nervous system from ChEMBL.

**Feature Extraction and Processing:** Conducted with Python.Automation:  
Automated with Bash for reproducibility.

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| <p><b>Data Sources</b></p> <p><b>Gene Expression Data:</b>NCBI GEO dataset GSE68719, including transcriptomic profiles from Parkinson's disease (PD) patients and healthy controls.</p> <p><b>Network Data:</b>Combined Pathway Commons for protein-protein interactions and KEGG for signaling pathways.</p> <p><b>Autophagy-Related Genes (ARN):</b>Core genes involved in autophagy, significant in PD pathogenesis, obtained from the ARN database.</p> <p><b>Drug-Gene and Drug-Indication Data:</b>Retrieved from DrugBank to understand drug targets and therapeutic uses.</p> <p><b>PDOD Score Calculation</b><b>Conflict Resolution: Algorithm</b> to handle positive and negative interactions between nodes.</p> <p><b>Bell-Shaped Function:</b> Weighs shorter paths more heavily, indicating stronger associations.</p> <p><b>Normalization:</b> Adjusts for varying numbers of drug targets and disease genes, ensuring fair comparisons.</p> <p><b>Gene Expression Analysis</b></p> <p>Log Fold Change (LFC), False Discovery Rate (FDR) Correction</p> <p><b>Random Walk with Restart (RWR) Algorithm:</b> Prioritizes additional disease-associated genes by simulating a random walk from ARN core genes.</p> <p><b>Drug Filtering and Evaluation</b></p> | <p>Apomorphine, Pergolide, Xylometazoline, Clonidine, Isoprenaline, Lutetium Lu 177 dotatate, Somatostatin, Mephentermine, DL-Methylephedrine, Ergotamine, Ergocalciferol, Tizanidine, Racepinephrine, Zolmitriptan, Fenoterol, Formoterol, Arbutamine, Foreskin fibroblast (neonatal), Foreskin keratinocyte (neonatal)</p> | <p>(Kankariya et al., 2024)</p> |
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**PDOD Score and FDR Filtering:** Identifies potential repurposing candidates with significant PDOD scores.

**Random Drug Set Generation:** Creates a baseline for comparison by generating random sets of target genes.

**Proximity Score Calculation:** Prioritizes drugs by measuring deviation from the mean PDOD score of random drug sets, adjusting for biases.

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| <p><b>Data Collection</b></p> <p><b>Disease Marker Genes:</b></p> <ul style="list-style-type: none"> <li>• Sources: Genotator (meta-database), PolySearch, and Pescador (text mining systems).</li> <li>• Converted gene names to HGNC symbols.</li> <li>• Final set of PD-related genes (Gfinal) from the union of the three sources.</li> </ul> <p><b>Drug Information:</b></p> <ul style="list-style-type: none"> <li>• Sources: GeneCards and DrugBank.</li> <li>• Included only "approved" drugs in the ATC Classification.</li> <li>• Excluded "experimental," "withdrawn," and PD-associated drugs.</li> </ul> <p><b>Network Construction</b></p> <p><b>Protein-Protein Interaction Network (PPIN):</b></p> <ul style="list-style-type: none"> <li>• Built using POINeT for genes in Gfinal.</li> </ul> <p><b>Interaction Drug-Target Network (IDTN):</b></p> <ul style="list-style-type: none"> <li>• Based on binary interactions of drugs (Dfinal) with their target proteins in Gfinal.</li> <li>• Visualized using yEd and Cytoscape.</li> </ul> <p><b>Topological Analysis</b></p> <p><b>IDTN and PPIN Analysis:</b></p> <ul style="list-style-type: none"> <li>• Conducted using Cytoscape plug-in CentiScaPe.</li> </ul> | <p>Diethylstilbestrol, Erlotinib, Lidocaine, Dasatinib, Nifedipine, Testosterone, Nicardipine, Melatonin, Sorafenib</p> | <p>(Rakshit et al., 2015)</p> |
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- |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|
| <ul style="list-style-type: none"><li>• Analyzed nodes in IDTN for degree (connectivity).</li><li>• Examined seven topological parameters in PPIN: degree, betweenness, centroid, closeness, eccentricity, radiality, and stress.</li><li>• Identified significant nodes for bidirectional drug repositioning.</li></ul> <p><b>Drug Repositioning</b></p> <p><b>On-Target Ratio (OTR):</b></p> <ul style="list-style-type: none"><li>• Calculated for PD-associated drugs and repositioning candidates.</li><li>• OTR is the ratio of PD-specific targets (TPD) to total drug interactions.</li></ul> |  |  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                    |                                |
|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
|  | <p><b>Adverse Drug Reactions (ADR) Analysis</b></p> <p><b>Entity Extraction:</b></p> <ul style="list-style-type: none"> <li>Extract ADRs from user posts using CHV Wiki to map everyday terms to medical jargon.</li> </ul> <p><b>Association Calculation:</b></p> <ul style="list-style-type: none"> <li>Compute association strength between ADR-drug, ADR-disease, and disease-drug pairs via co-occurrence analysis and association rule mining.</li> </ul> <p><b>Network Construction:</b></p> <ul style="list-style-type: none"> <li>Build networks with nodes (drug, PD, ADR) and links (drug-ADR, PD-ADR, drug-PD) weighted by co-occurrence lifts.</li> </ul> <p><b>Path Mining:</b></p> <ul style="list-style-type: none"> <li>Identify significant ADR-disease paths for drug repositioning.</li> </ul> <p><b>Drug Repositioning:</b></p> | <p>Thalidomide, Glatiramer acetate, Salbutamol, Bupropion, Donepezil, Carbamazepine, Methylphenidate, Citalopram, Clomipramine, Paroxetine, Sertraline, Fluoxetine, Nefazodone, Fluvoxamine, Modafinil, Rivastigmine, Gabapentin, Lamotrigine, Levetiracetam, Topiramate, Valproic acid, Oxcarbazepine, Phenytoin, Mirtazapine</p> | <p>(Zhao &amp; Yang, 2018)</p> |
|--|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|

|  |                                                                                                                                                                                                                                                                                                          |                                      |                                   |
|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------|
|  | <ul style="list-style-type: none"> <li>• Use SIDER for ADR-disease associations.</li> <li>• Create new drug-disease associations via ADR-disease paths, excluding known ones from PharmGKB.</li> </ul>                                                                                                   |                                      |                                   |
|  | <p>The methodology involves extracting relations related to PD from medical literature, fusing the literature knowledge graph with a local medical knowledge base, using knowledge graph completion methods to predict drug candidates, and employing machine learning methods for drug repurposing.</p> | <p>Terazosin, Ambroxol,Nilotinib</p> | <p>(X. Zhang &amp; Che, 2021)</p> |

|     |                                                                                                                                                                                    |                                                                                                                         |                                |
|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| ALS | Protein-Protein Interaction SAveRUNNER algorithm, Random Walk with Restart (RWR), Gene Set Enrichment Analysis (GSEA), Pathways Enrichment Analysis                                | PF-00562271, Cenisertib, Vandetanib                                                                                     | (Fiscon et al., 2021)          |
|     | Protein-Protein and Disease-Gene Interaction Study, Gene Ontology and Pathway Enrichment Study, miRNA-Gene Interaction Analysis, Drug-Gene Interaction Analysis, Survival Analysis | Fostamatinib                                                                                                            | (Duan et al., 2024)            |
|     | Transcriptomic data analysis, Protein-Protein Interaction (PPI) Network, Pathway Enrichment Analysis, Analysis Using Connectivity Map (CMap), Molecular Docking Studies            | Tolterodine, Temozolomide, Phenamil, Nadolol, Lestaurtinib, Fluticasone, Flubendazole, Diphenidol, Cefaclor, Acadesine  | (Sunildutt et al., 2024)       |
|     | Statistical Modeling, Patient Categorization, Identification of Biomarkers, Statistical Modeling, Validation Using Drosophila Model, Exploration of Therapeutic Interventions      | Curcumin, Yohimbine, Ginkgolide-b, Forskolin, Ipriflavone, Ginsenoside, Luteolin, Simvastatin, Atorvastatin, Fenoldopam | (R. G. Huber et al., 2023)     |
|     | Data Set Components and Search Algorithm, Statistical Modeling Methods, ALS Models and Validation, Virtual Screening, Molecular Docking                                            | 9-Methylguanine, Arimoclomol, Belaperidone, Dutasteride, EGCG, Levoleucovorin, Neflumozide,                             | (Galvez-Llompert et al., 2022) |

|                      |                                                                                                                                                                               |                                                                                                                                                                                                                                     |                       |
|----------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Huntington's Disease |                                                                                                                                                                               | Olinciguat, Oxidized coenzyme A                                                                                                                                                                                                     |                       |
|                      | GWAS Summary Statistics of ALS, Transcriptome-Wide Association Study (TWAS), Functional Enrichment Analysis, Drug Repositioning Analysis Using CMap                           | MK-886, STOCK1N-35696, PF-00539758-00, 16, 16-Dimethylprostaglandin E2, Bufexamac, Androsterone, Picrotoxinin, Sulfinpyrazone, Deptropine, Folic acid, Mebendazole, Adenosine phosphate, Tetracycline, Phentolamine, Trichostatin A | (Park et al., 2021)   |
|                      | Microarray Data: Reconstruction of the Transcriptional Regulatory Network, Master Regulator Analysis (MRA), Two-Sided Gene Set Enrichment Analysis, Connectivity Map Analysis | cycloheximide, helveticoside, lanatoside C, loxapine, SR-95639A, strophanthidin, trimethadione, withaferin A                                                                                                                        | (Vargas et al., 2021) |
|                      | Heterogeneous Network, Random Walk with Restart (RWR), Random Walk Model                                                                                                      | Carbamazepine, Dantrolene, Vigabatrin, Lorazepam, Tizanidine                                                                                                                                                                        | (Luo et al., 2019)    |

|                                                   |               |                                         |
|---------------------------------------------------|---------------|-----------------------------------------|
| Drug-Derived Behavioral Signature Analysis (DBSA) | Not specified | (Ambesi-<br>Impiombato<br>et al., 2023) |
|---------------------------------------------------|---------------|-----------------------------------------|

### 3. MATERIALS AND METHODS

The overall design of the analyses conducted in this thesis is provided in **Figure 3.1**.

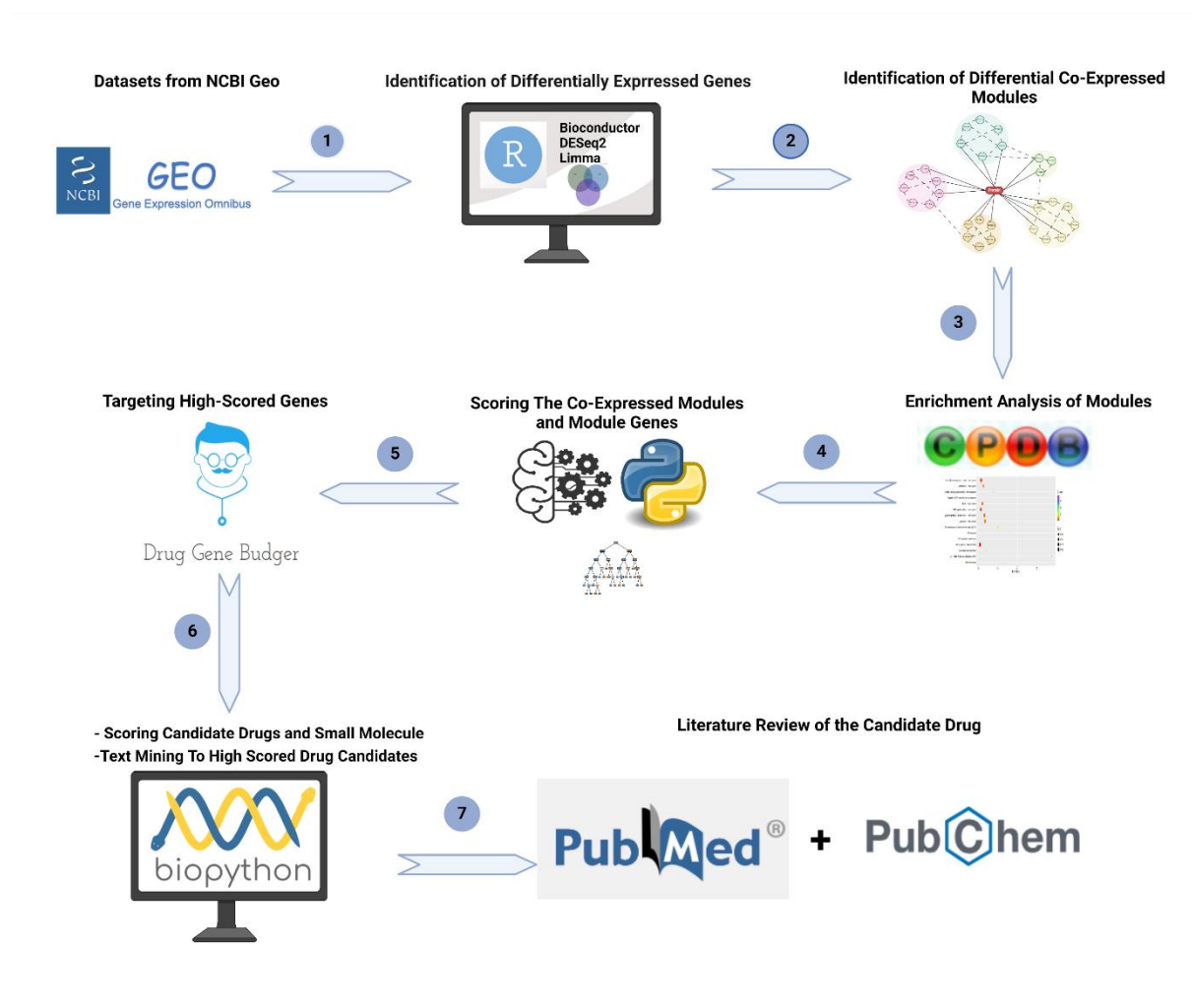


**Figure 3. 1.** Bioinformatics pipeline of the thesis

#### 3.1. Selection of gene expression datasets

Transcriptome raw data sets for AD, PD, ALS and HD were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO)(Barrett et al., 2013) For the selection of datasets, we applied the criteria that the datasets were Homo sapiens microarray or RNA-Seq data and included both diseased and healthy groups (Table 3.1).

**Table 3. 1.** Transcriptome datasets used in the thesis

| Disease name | Data type  | Dataset ID | Cell/ Tissue type | Number of Diseased samples | Number of Control samples |
|--------------|------------|------------|-------------------|----------------------------|---------------------------|
| AD           | Microarray | GSE110226  | Brain             | 39                         | 38                        |
|              |            | GSE36980   |                   |                            |                           |
|              |            | GSE39420   |                   |                            |                           |
|              | Microarray | GSE36980   | Frontal           | 59                         | 77                        |
|              |            | GSE5281    |                   |                            |                           |
|              |            | GSE48350   |                   |                            |                           |
|              | RNASeq     | GSE193438  | Hippocampus       | 8                          | 13                        |
|              | RNASeq     | GSE184942  |                   |                            |                           |
| PD           | Microarray | GSE16759   | Parietal lobe     | 8                          | 8                         |
|              | RNASeq     | GSE193438  |                   |                            |                           |
|              | RNASeq     | GSE68719   | Brain             | 25                         | 27                        |
|              |            | GSE169755  |                   |                            |                           |
|              |            | GSE106608  |                   |                            |                           |
|              | RNASeq     | GSE216281  | Frontal           | 30                         | 35                        |
|              |            | GSE68719   |                   |                            |                           |
|              | Microarray | GSE7621    | Substantia Nigra  | 26                         | 17                        |
| ALS          | RNASeq     | GSE20141   |                   |                            |                           |
|              |            | GSE20186   | Substantia nigra  | 40                         | 31                        |
|              |            | GSE7621    |                   |                            |                           |
|              | Microarray | GSE20141   |                   |                            |                           |
| ALS          | RNASeq     | GSE26276   | Muscle            | 10                         | 10                        |
|              |            | GSE41414   |                   |                            |                           |
|              | RNASeq     | GSE76220   | Motor neuron      | 16                         | 14                        |
|              |            | GSE19332   |                   |                            |                           |
| HD           | RNASeq     | GSE129473  | Frontal           | 38                         | 61                        |
|              |            | GSE64810   |                   |                            |                           |
|              |            | GSE79666   |                   |                            |                           |

### 3.2. Identification of Differentially Expressed Genes

A well-established statistical analysis procedure was used to identify differentially expressed genes (DEGs) for microarray data. Raw data were normalized with the Robust Multi-Array Average (RMA) (Bolstad et al., 2003) expression measure implemented in the Affy package (Gautier et al., 2004) of the R/Bioconductor (W. Huber et al., 2015) platform for each data set. DEGs were identified using the Linear Models for Microarray Data package

(LIMMA)(Ritchie et al., 2015). The raw RNA-seq data was analyzed with DESeq2(Love et al., 2014). The Benjamini-Hochberg method was used to control the false discovery rate (FDR), and an adjusted p value of <0.05 was considered as the cut-off value to determine the statistical significance of DEGs. DEGs will be identified using p-values less than 0.05, and fold changes will be used to determine the regulatory patterns of the DEGs (fold change >1.5 and <0.67). To identify common signatures, the results were comparatively analyzed and common DEGs were used in downstream analyses.

### 3.3. Co-Expression Network Analysis

Co-expression analysis, a specific and established method (Gov & Arga, 2017) was conducted for common DEGs to determine the correlation of gene pairs in the disease and healthy state. This method refers to calculating the mean expression level of common DEGs, and then the values were normalized with z-score. Pearson Correlation Coefficient (PCC) was calculated for each common DEG expression data that conformed to the normal distribution in diseased and healthy conditions, while Spearman Correlation Coefficient (SCC) was calculated for gene expression data that did not fit with normal distribution. These two coefficients assess statistically evaluate changes in gene expression analysis. The threshold p-value < 0.05 was used to obtain statistically significant pairwise correlations of reciprocal DEGs. As a result, co-expression networks containing significantly co-expressed DEG pairs were obtained for disease and healthy samples.

### 3.4. Identification of Differential Co-expression Network Analysis and Clusters

Significantly co-expressed genes were assessed p-critical values for common DEGs were computed by incorporating the acquired pairwise gene expression correlation values with SCCs. The p-critical values ( $P_{critic}$ ), which is the cut-off value of the correlation coefficient, can be found in Equation 1:

$$p_{critic} = \text{mean}SCC + 1.96 * \text{stdof}SCC \quad \text{Equation 1}$$

In order to identify gene pairs with remarkable associations in disease and healthy states, a threshold called  $\varepsilon$  parameter was applied. Equation 2 was used for defining the threshold condition:

$$\varepsilon = |\text{SCCd} - \text{SCCh}| > 0.5 \quad \text{Equation 2}$$

\* $P_{\text{critic}}$  : -p-critical values

\*meanSCC : mean value of Spearman' s Correlation Coefficient

\*stdofSCC : standard deviation of Spearman' s Correlation Coefficient

\*SCCd: Spearman' s Correlation Coefficient of disease

\*SCCch: Spearman' s Correlation Coefficient of healthy

Significant gene clusters require  $\geq 10$  nodes and a network density  $> 40\%$ .

### 3.5. Enrichment Analysis of Co-Expressed Genes

Pathway-based overrepresentation analyses were performed on ConsensusPathDB (Kamburov et al., 2009). Kyoto Encyclopedia of Genes and Genomes (KEGG)(Kanehisa & Goto, 2000) and Reactome (Fabregat et al., 2016, 2016) were preferentially used as pathway databases. In the analyses, KEGG and Reactome pathways with a p-value  $< 0.05$  were considered statistically significant.

### 3.6. Machine learning applied to differentially co-expressed module

For validation analysis, independent gene expression datasets were obtained from NCBI GEO for each disease including GSE28894 for PD (59 healthy and 55 disease samples), GSE13214 and GSE109887 for AD (31 healthy and 47 disease samples) and GSE23297 for ALS (48 healthy and 96 disease samples). The genes of the datasets were designated as nodes of the co-expression network clusters of healthy and diseased samples. The target variable was labeled to indicate healthy (0) and diseased (1) states, and the features were chosen as gene expression values. The data were divided into training (80%) and testing (20%) clusters for each gene independently and normalized. Data were processed, models were created and evaluated using Python programming language for machine learning (ML) analysis. Accuracy scores were calculated using Random Forest (Mahesh Pal, 2005), SVM and KNN models. The Random Forest model was implemented with a 100-tree classifier; SVM was implemented with a linear kernel and parameter  $C=0.1$ ; and KNN was implemented using 5 neighbors. The accuracy scores of each model were calculated for each gene and the results were compared graphically.

### 3.7. Identification of Candidate Drugs through Machine Learning and Text Mining

The drug repurposing analysis was performed using Drug Gene Budger (DGB): a drug repurposing tool that targets genes and aims to change the expression of genes to desired levels using small molecules or drugs. The tool contains experimental data compiled from NCBI GEO, L1000, and the original Connectivity Map (CMap) (Wang et al., 2019). In this study, CREEDS (CRowd Extracted Expression of Differential Signatures) located under the DGB site was used. The analysis process evaluated whether drugs had a reverse on the entered gene expression profile entered. The analysis results include data such as "Drug name", "CREEDS ID", "GEO ID", "p-value", "q-value", "Drug FC" and "Specificity". Threshold values were set as p-value  $<0.05$  and q-value  $<0.05$ . A specific criterion was used to identify drug targets for downregulated and up-regulated genes. This criterion is that the fold change (FC) value of drug is greater than 1.5 or less than 0.67. In this study, significant gene clusters were used for drug repurposing analysis.

For ML analysis, firstly, the input data was constructed from matrices containing gene names and gene FC drug names and drug FC for each gene cluster. Since drug names are categorical data, they were converted into binary variables in numeric format using the One-Hot Encoding method using the `get_dummies` function (Okada et al., 2019). This allowed the model to utilize this information by creating a separate column for each drug. The independent variables (X) consist of the drug names encoded in this way and the target variable (y) consists of the drug fold change (Drug FC). Normalization of the dataset was performed using "StandardScaler" to remove different scales from the data.

Five different machine learning models were applied to the normalized data. Linear Regression (LR) was used to establish a baseline performance and understand the linear relationship between features and the target variable. Support Vector Regression (SVR) was applied to capture complex relationships in the data that might not be evident with linear models (Cortes & Vapnik, 1995). Random Forest Regressor (RFR) was utilized to handle noisy data and intricate relationships between features, providing stability and accuracy (Mahesh Pal, 2005). Gradient Boosting Regressor (GBR) was applied to enhance prediction accuracy and refine model performance by optimizing error correction iteratively (Friedman, 2001). Neural Network (MLPRegressor) was used to model complex non-linear relationships and interactions that other algorithms might miss, given sufficient data and computational resources (Rumelhart

et al., 1986). Each model was trained on the normalized data, and predicted FC were made for each drug. The predicted FC were saved in a DataFrame and then exported to an Excel file to assess the effects of specific drugs on gene expression. The results were visualized using the seaborn library (Waskom, 2021). The performance of each model was evaluated by calculating the mean squared error (MSE) of the predicted FC, which provides a measure of the average squared difference between the predicted FC and actual values.

The novelty of the candidate drugs which the candidate drugs previously used for the treatment of target disease was examined with text mining method through Python. For this purpose, article abstracts were searched separately using two different keyword sets: "drug candidate" and "drug candidate + target disease". Then, the outcomes were explored using the BioPython package (Cock et al., 2009). During the analysis process, term frequency (TF) and inverse document frequency (IDF) were calculated. The TF-IDF score was calculated by multiplying the TF and IDF scores. In the study, drugs with a TF-IDF score of '0' were evaluated as new drug candidates. In this way, drug candidates were associated with the findings of relevant studies in the literature. Finally, whether new drugs have physical interactions with the target gene was examined on PubMed and PubChem.

## 4. RESULTS AND DISCUSSION

### 4.1. Differential gene expression in NDD

For AD, eight gene expression raw data, the data including postmortem parietal tissue, postmortem hippocampus tissue, and frontal cortex tissue and postmortem brain tissue were analyzed individually and statistically to identify DEGs. The datasets showed a wide range in the number of DEGs, ranging from 688 to 4390 genes. The highest number of DEGs (4390) and upregulated genes (3145) were identified in GSE5281. The highest number of downregulated genes was in GSE193438(2113), and the lowest number of DEGs was in GSE36980 (663). Comparative analysis of the resulting DEGs showed that 24 DEGs were common in the frontal cortex, 57 DEGs in the brain, 217 DEGs in the hippocampus, and 139 DEGs in the parietal cortex (**Figure 4.1.**). Downstream analyses were performed using these common DEGs to provide the concurrency and robustness of the analysis.

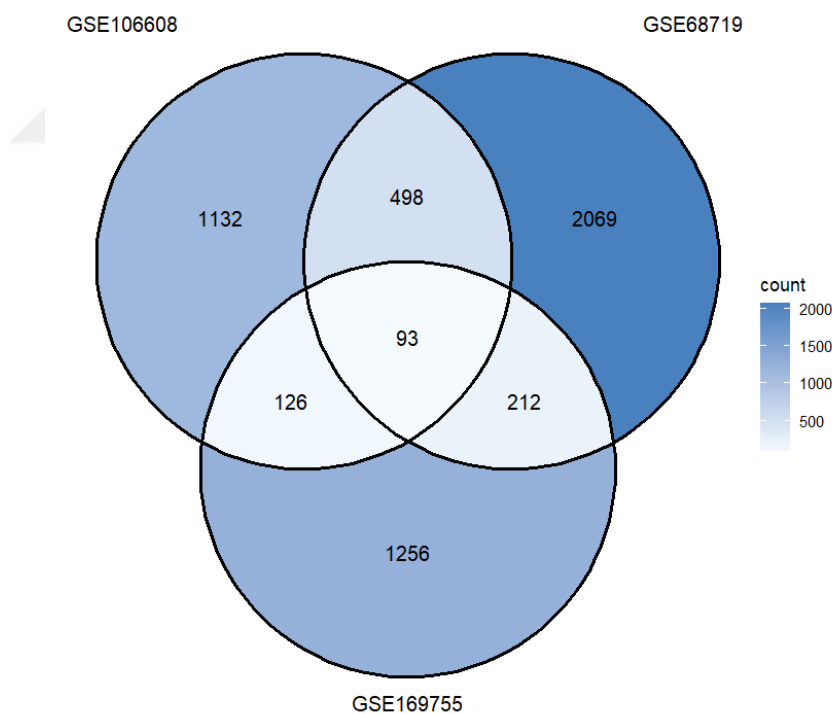


**Figure 4. 1.** Venn diagram showing the common DEGs between all transcriptome data. Brain tissues of AD

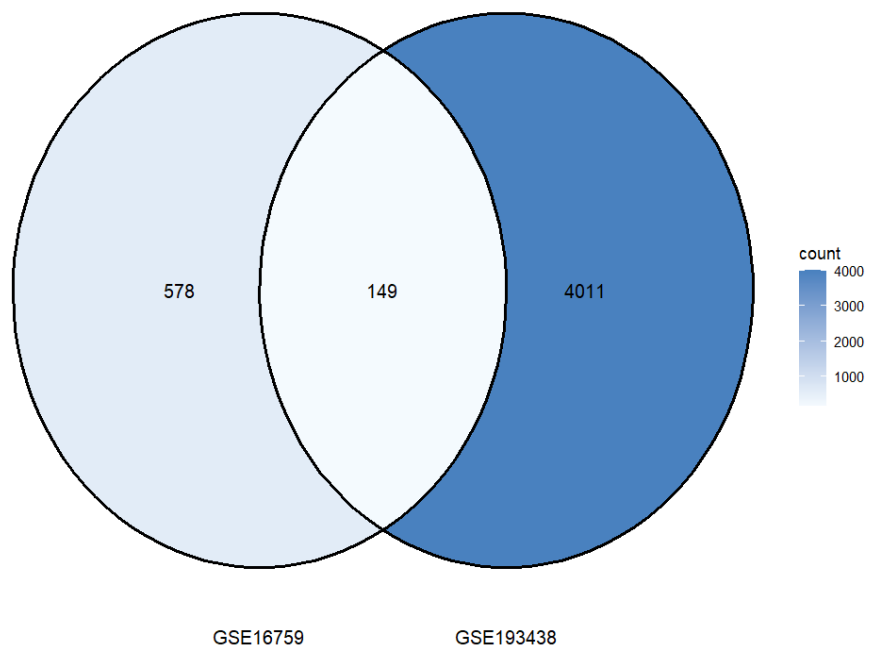


**Figure 4. 2.** Common DEGs parietal tissues of AD

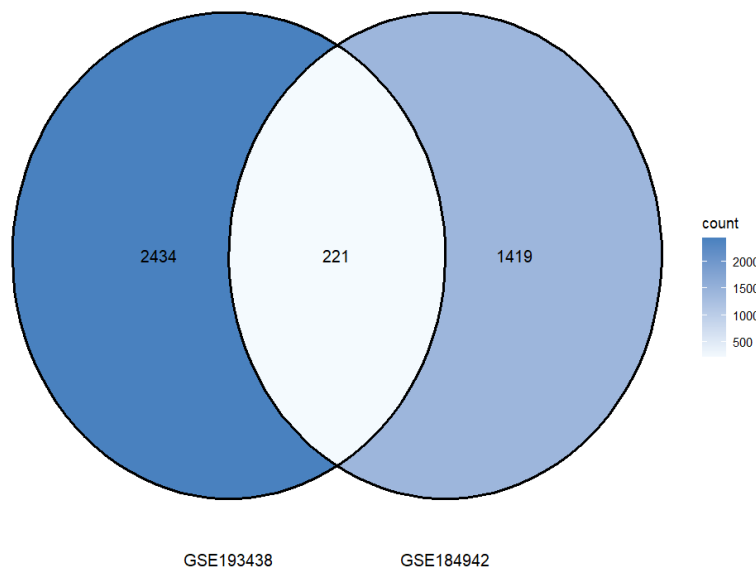


**Figure 4. 3.** Common DEGs hippocampus tissues of AD

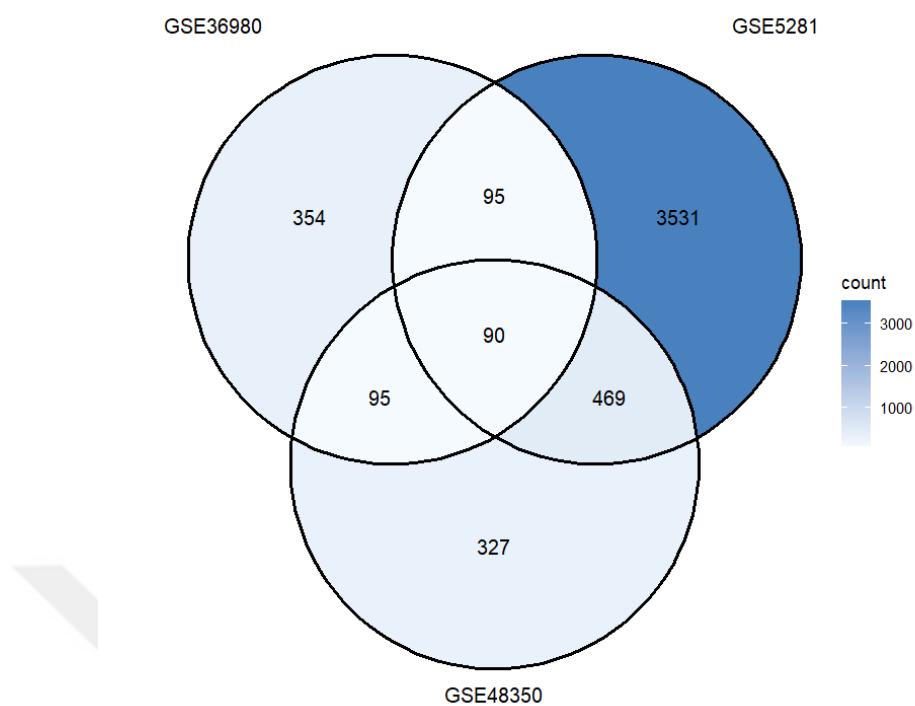


**Figure 4. 4.** Common DEGs frontal tissues of AD

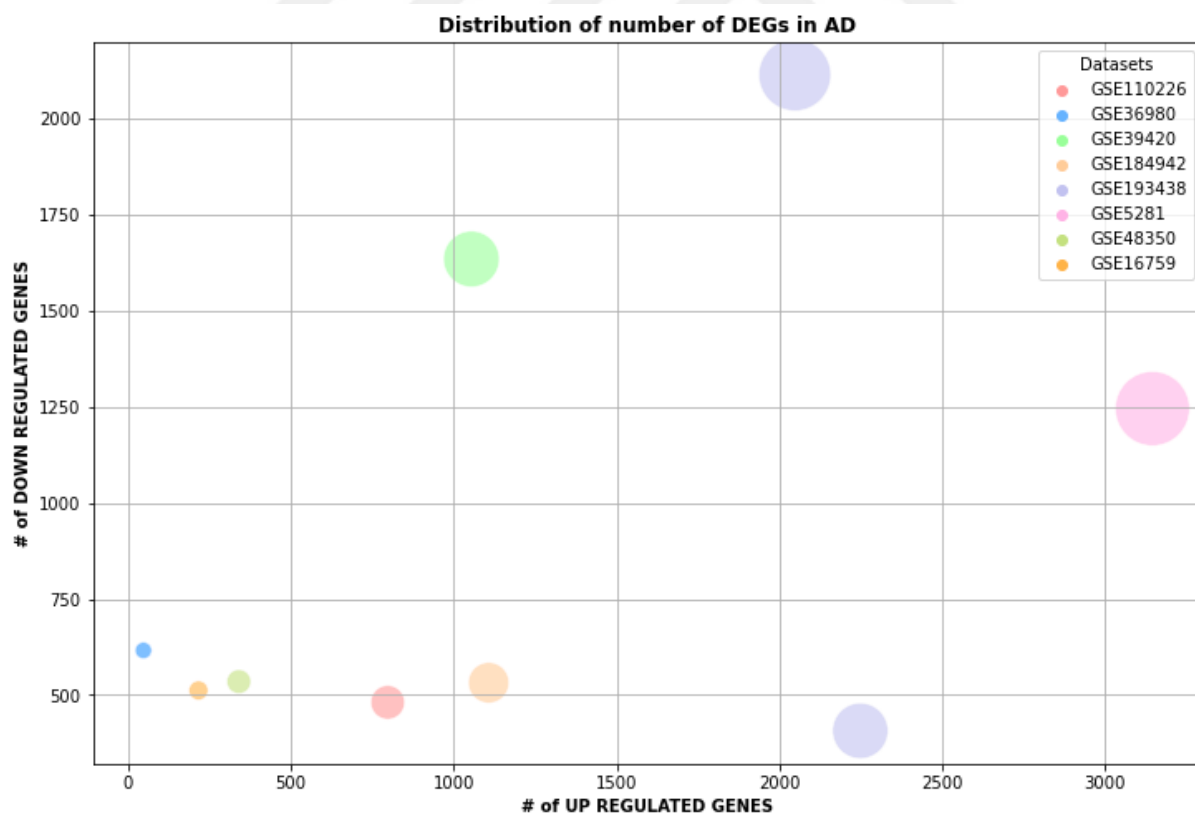


**Figure 4. 5.** Distribution of DEGs in accordance with datasets for AD

PD gene expression raw data were grouped into postmortem brain tissue, frontal cortex and substantia nigra and analyzed. There were 93 common DEGs in brain tissue, 48 DEGs in frontal cortex and 35 DEGs in substantia nigra. However, the removal of the GSE20186 dataset resulted in the identification of 765 common differentially expressed genes (DEGs), which were subjected to further downstream analyses. (**Figure 4.6 – Figure 4.10**). The distribution of DEGs, based on their regulatory signatures, with upregulated genes 334 to 3892 and downregulated genes 37 to 1233. The majority (69%) of DEGs were found to be downregulated.

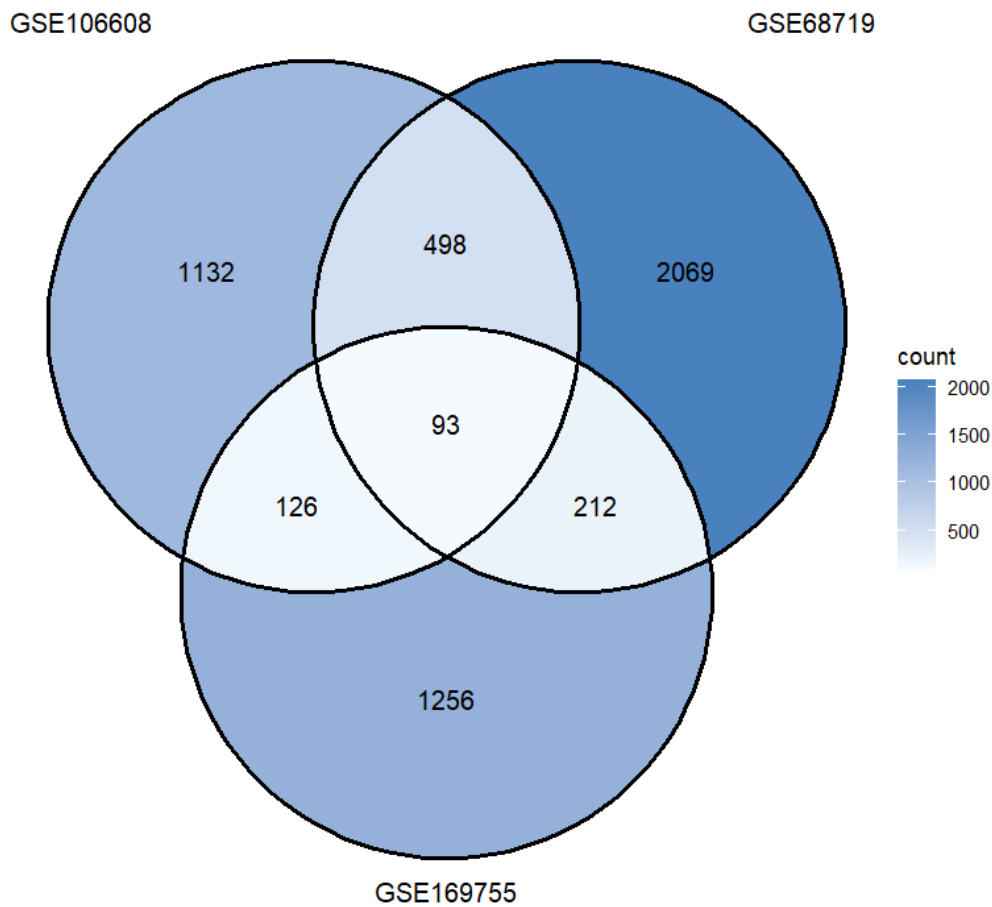


**Figure 4. 6.** Common DEGs brain tissues of PD

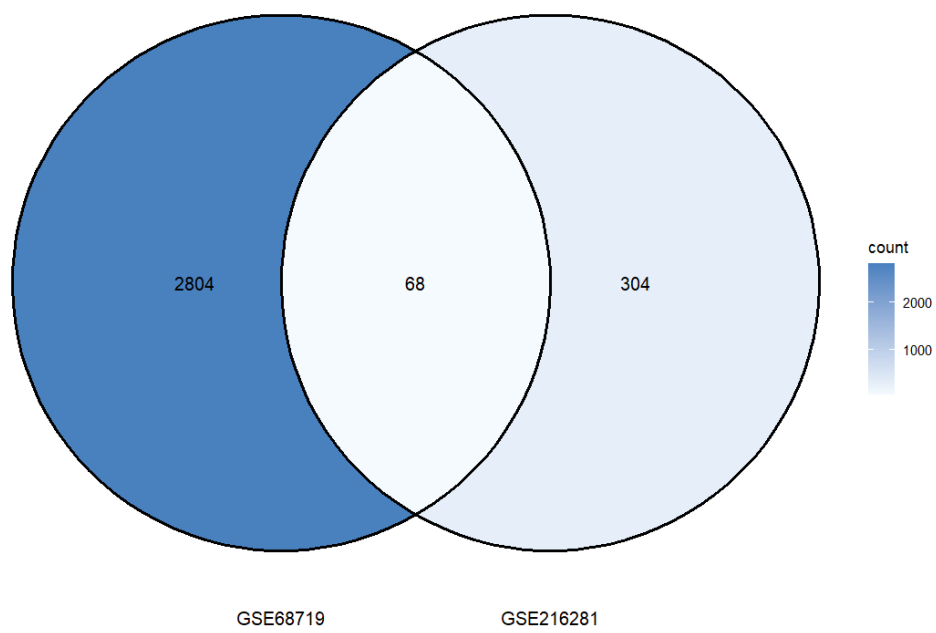


**Figure 4. 7.** Common DEGs frontal tissues of PD

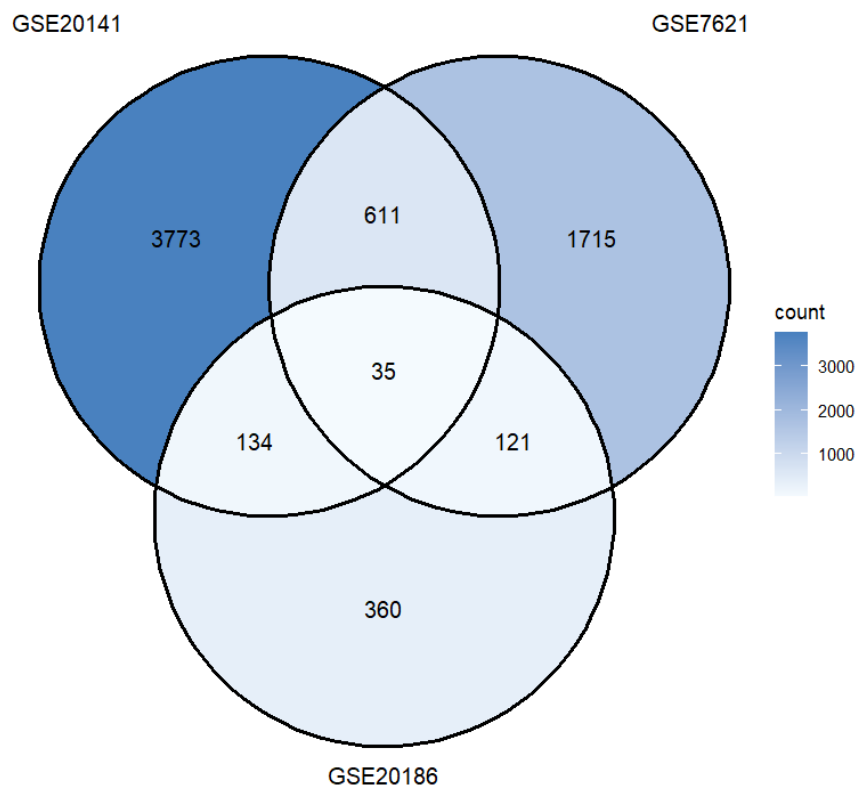


**Figure 4. 8.** Common DEGs substantia nigra tissues of PD

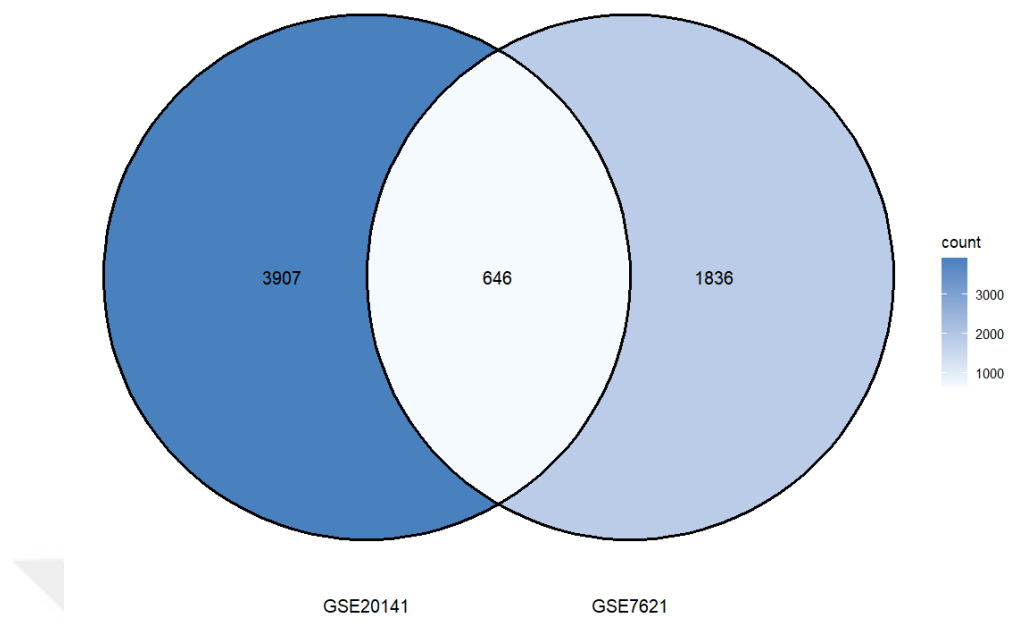


**Figure 4. 9.** Common DEGs substantia nigra tissues of PD

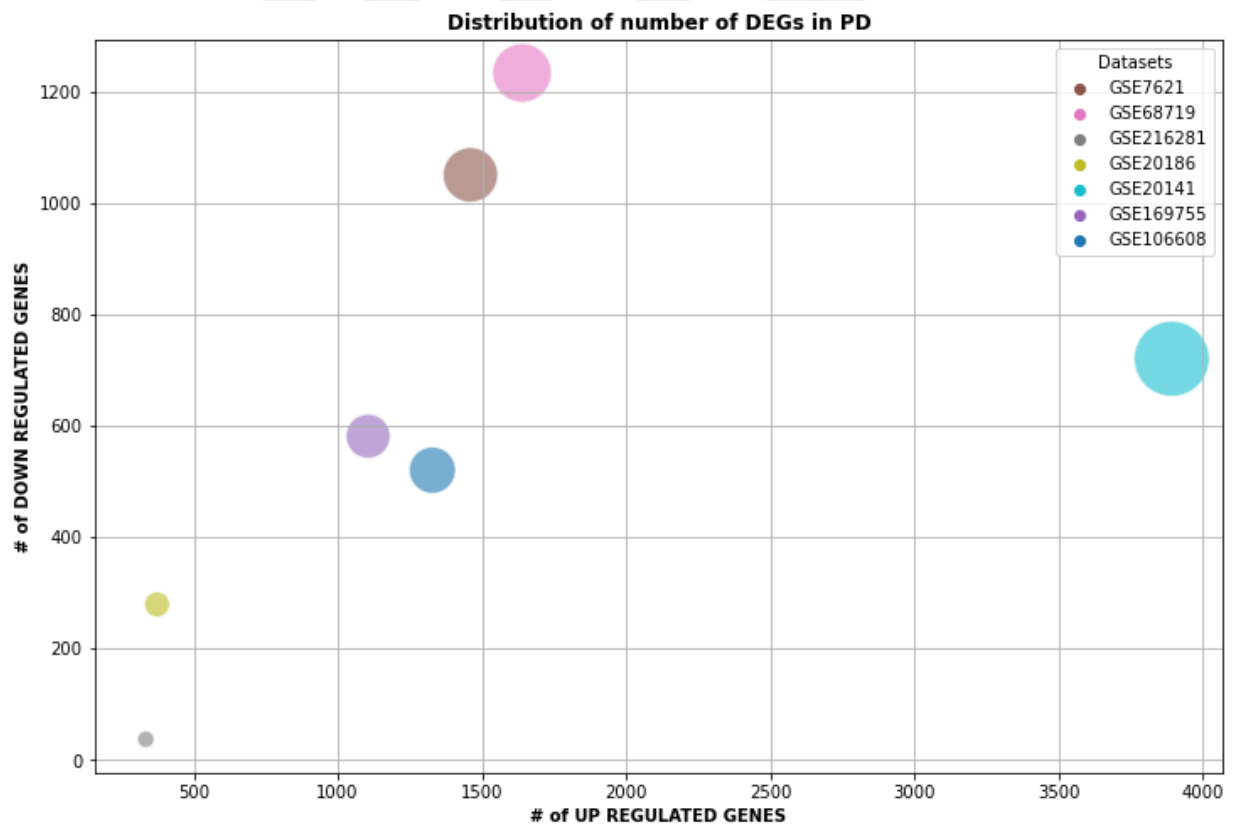


**Figure 4. 10.** Distribution of DEGs in accordance with datasets for PD

ALS gene expression raw data was divided into two tissue groups: muscle and motor neuron. A total of 121 common DEGs were identified in muscle tissues, while 274 common DEGs were identified in motor neuron cells. (**Figure 4.12-Figure 4.13**) In muscle tissues 63.93% of DEGs were upregulated, 84 % of DEGs were upregulated in motor neuron tissues (**Figure 4.14**).

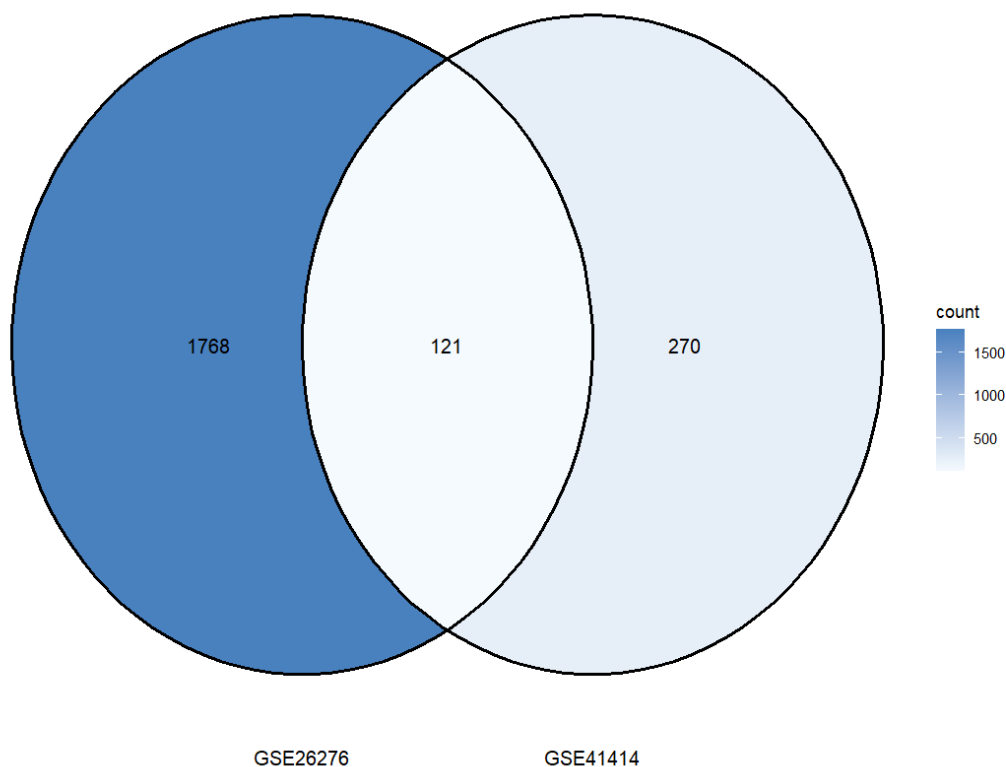


**Figure 4. 11.** Common DEGs muscle tissues of ALS

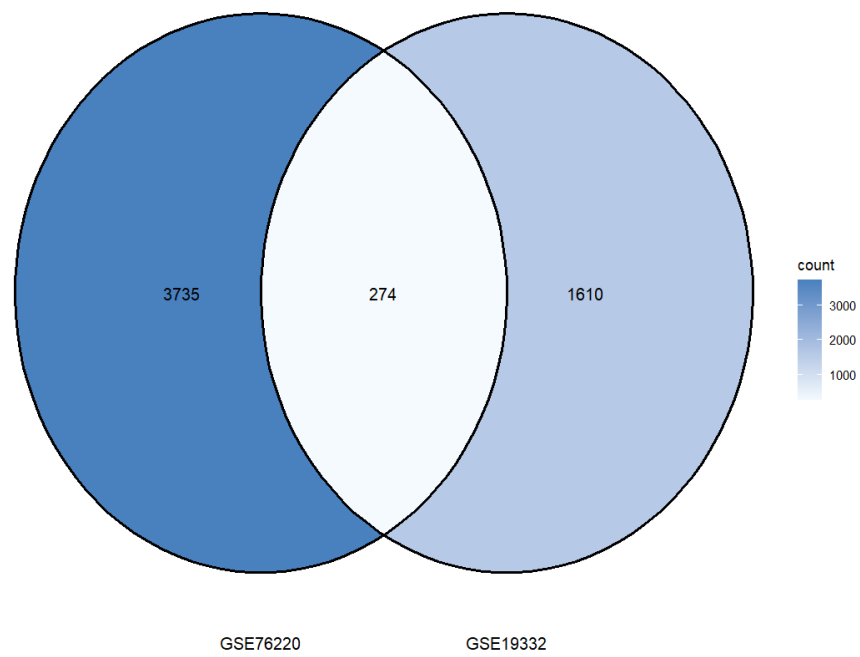


**Figure 4. 12.** Common DEGs motor neuron cells of ALS

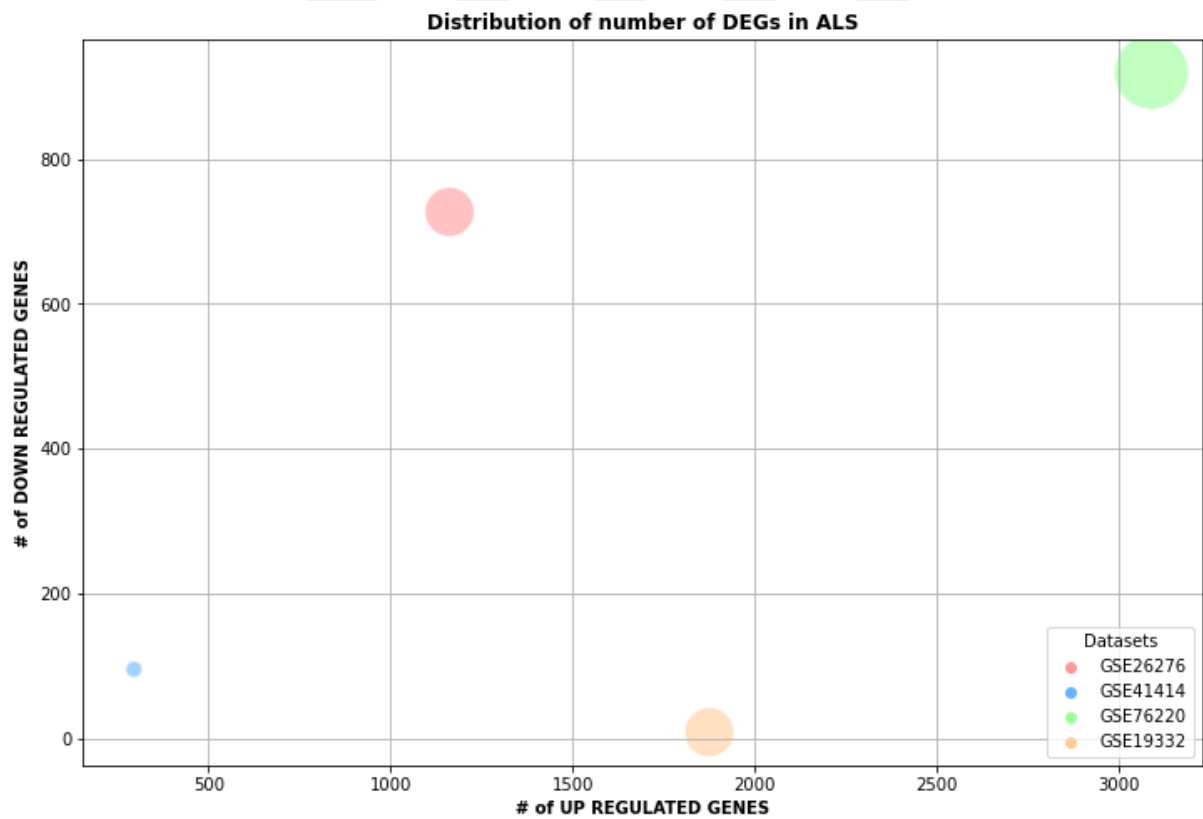


**Figure 4. 13.** Distribution of DEGs in accordance with datasets for ALS

Three datasets were analyzed for Huntington's Disease (HD). A total of 43 common differentially expressed genes (DEGs) were identified (**Figure 4.14**). Of these DEGs, 52.09% were upregulated, while 47.91% were downregulated, indicating a nearly equal distribution (**Figure 4.15**).

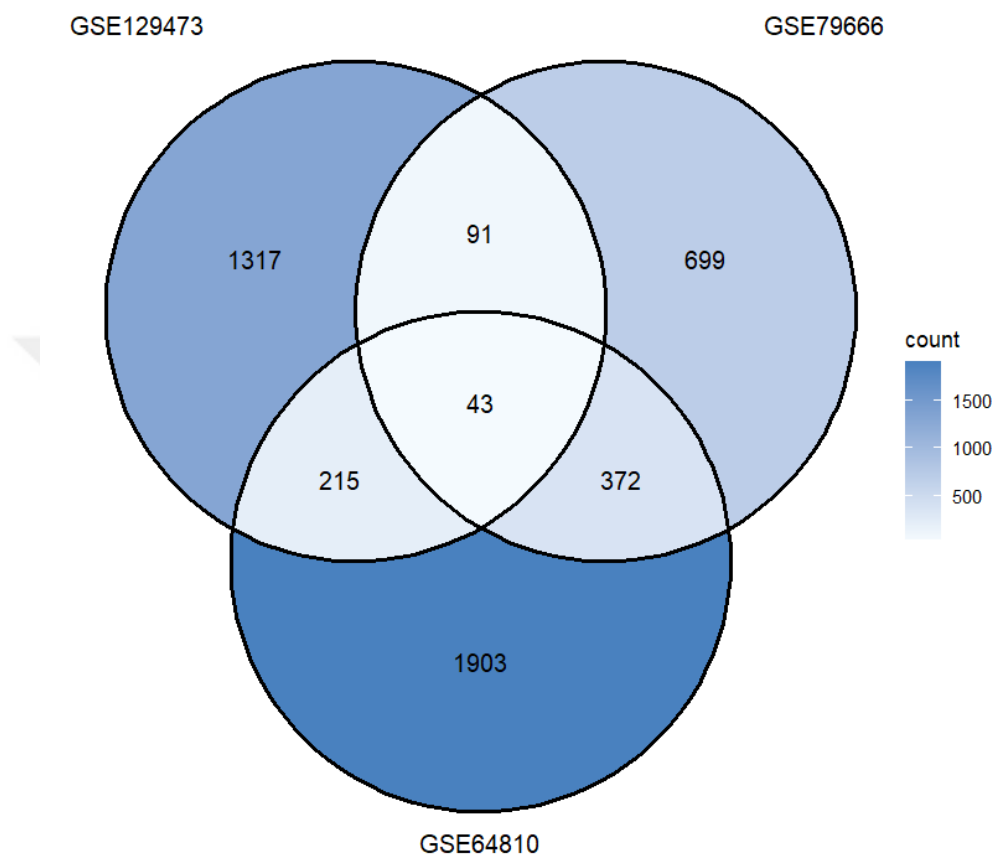


**Figure 4. 14.** Common DEGs in HD

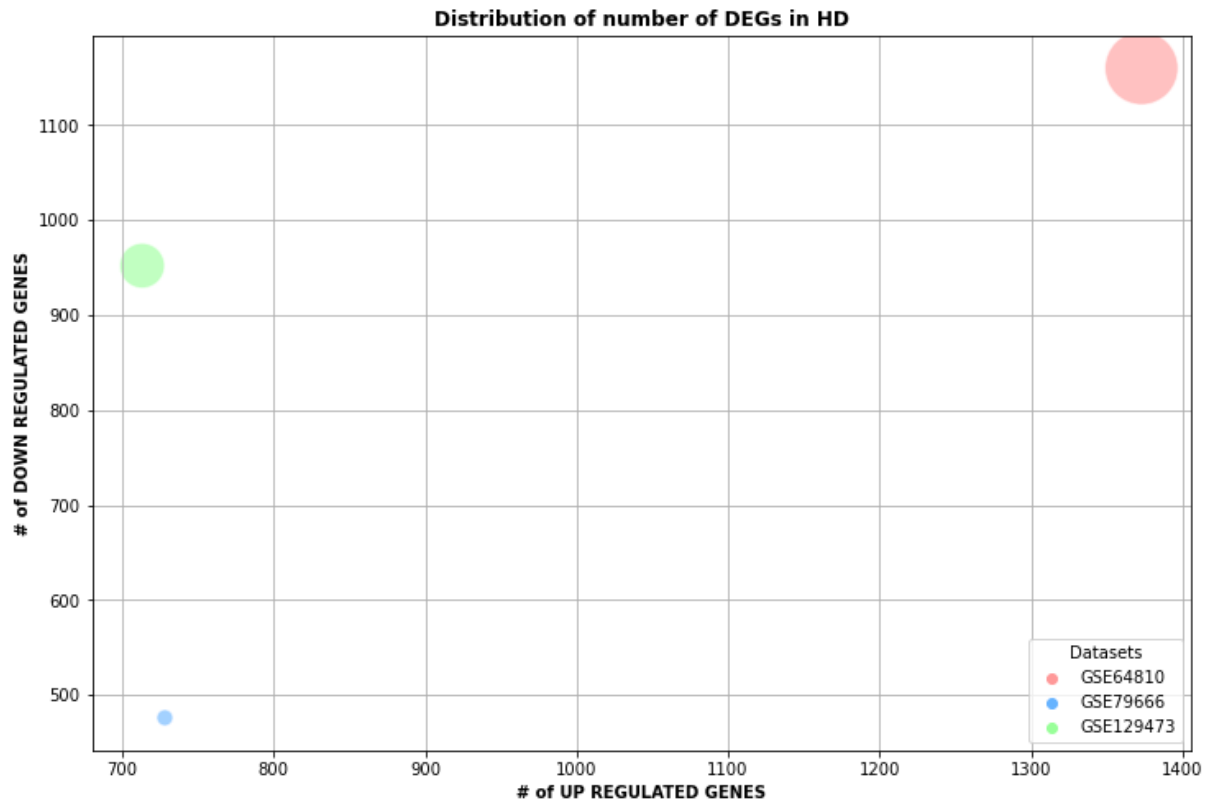


**Figure 4. 15.** Distribution of DEGs in accordance with datasets for HD

## 4.2. Differential Co-expression Analysis and Clusters and Enrichment Analysis and Machine Learning Applied to Cluster's Results

### 4.2.1. AD

Following the identification of common differentially expressed genes (DEGs), we calculated SCCs of binary common DEGs to identify crucial gene pairs exhibiting co-expression. Within the constructed a co-expressed network for AD state, a total of 15 co-expressed clusters were initially recognized. However, our criteria for detecting gene clusters, co-expressed clusters (requiring nodes  $\geq 10$  and network density  $> 40\%$ ) led to the identification of only three modules meeting our specified parameters for AD state.

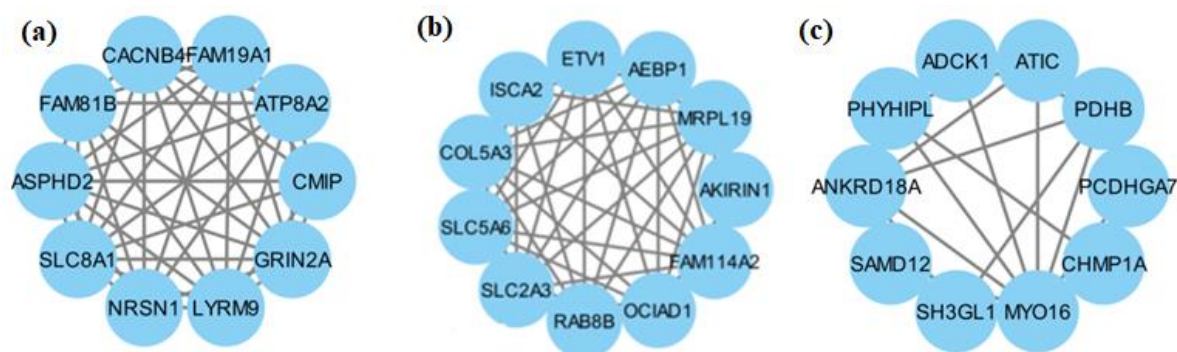


**Figure 4. 16.** Co-expressed clusters in AD. (a) Gene cluster in brain tissues of AD (b) Gene cluster in parietal tissues of AD. (c) Gene cluster in hippocampus tissues of AD.

In the differentially co-expression analysis of the common DEGs of brain tissues in AD, two clusters were identified only one of was significant, consisting of 10 genes. This cluster exhibited an edge count of 42 and a network density of 93%, resulting in an mCode score of 9.33. Similarly, analysis of the common DEGS of hippocampus tissue in AD revealed seven clusters, among which one statistically significant cluster (Cluster 5) comprised 10 genes, with an edge count of 20 and a network density of 44%, yielding an mCode score of 4.44. Lastly, examination of the common DEGs of parietal lobe uncovered five clusters, with one statistically significant cluster (Cluster 2) consists of 11 genes. This cluster exhibited an edge count of 44, a network density of 80%, and an mCode score of 8.80. However, analysis of the common DEGs of frontal cortex tissues, no clusters were observed. **(Figure 4.16)**

The gene enrichment analysis targeting common DEGs in Alzheimer's Disease (AD) identified several pathways that are significantly enriched, indicating their potential involvement in the disease's pathogenesis. Key pathways such as "Transmission across Chemical Synapses (Pathak et al., 2021)," "Synaptic Vesicle Cycle (Morfini et al., 2009)," "Serotonin Neurotransmitter Release Cycle", "Neurotransmitter Release Cycle," (Kandimalla & Reddy, 2017)," "Nicotine Addiction (López-Arrieta & Sanz, 2001)," "Muscle Contraction (Burtscher et al., 2021)," "Morphine Addiction," and "Calcium Signaling Pathway (Pchitskaya et al., 2018)" were found to be consistently enriched across different region of brain in AD. These pathways are critical for synaptic function and neuronal communication, which are known to be impaired in most NDD and AD. **(Figure 4.17-4.20)**

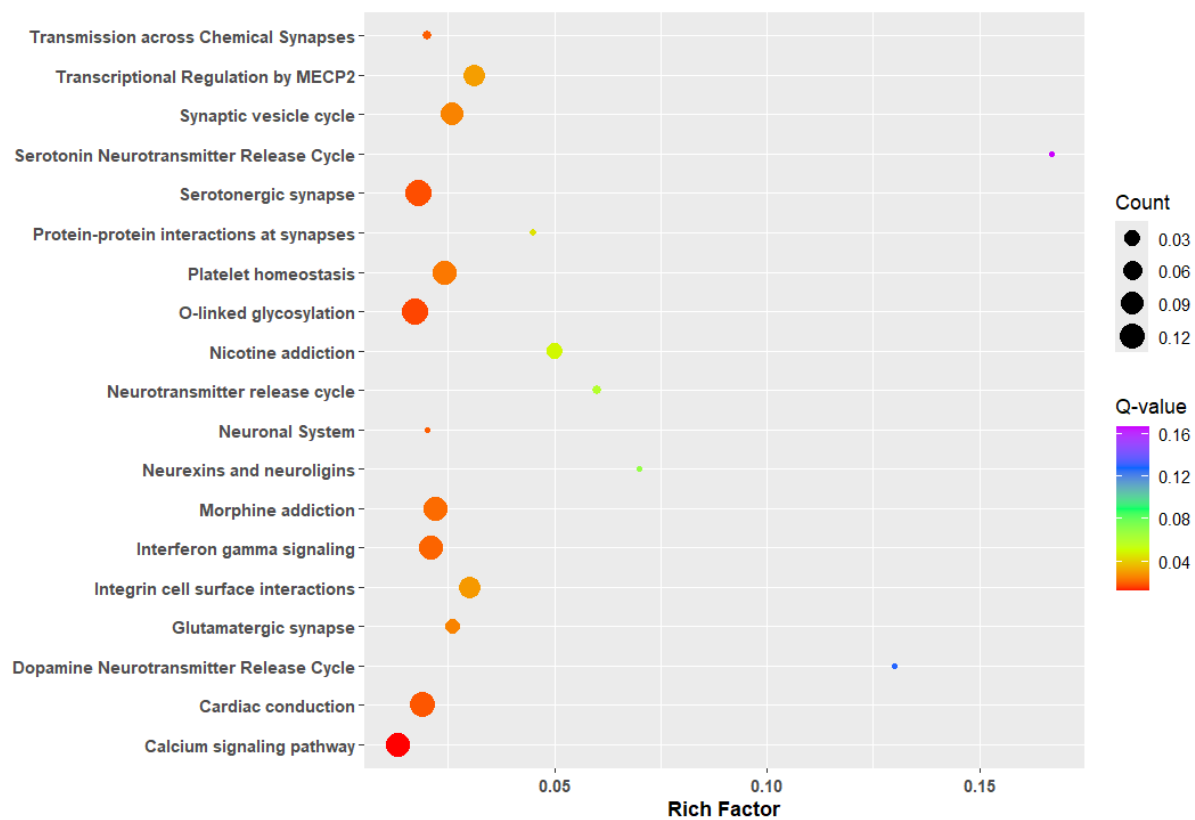


**Figure 4. 17.** Gene enrichment analysis of common DEGs in brain tissue of AD

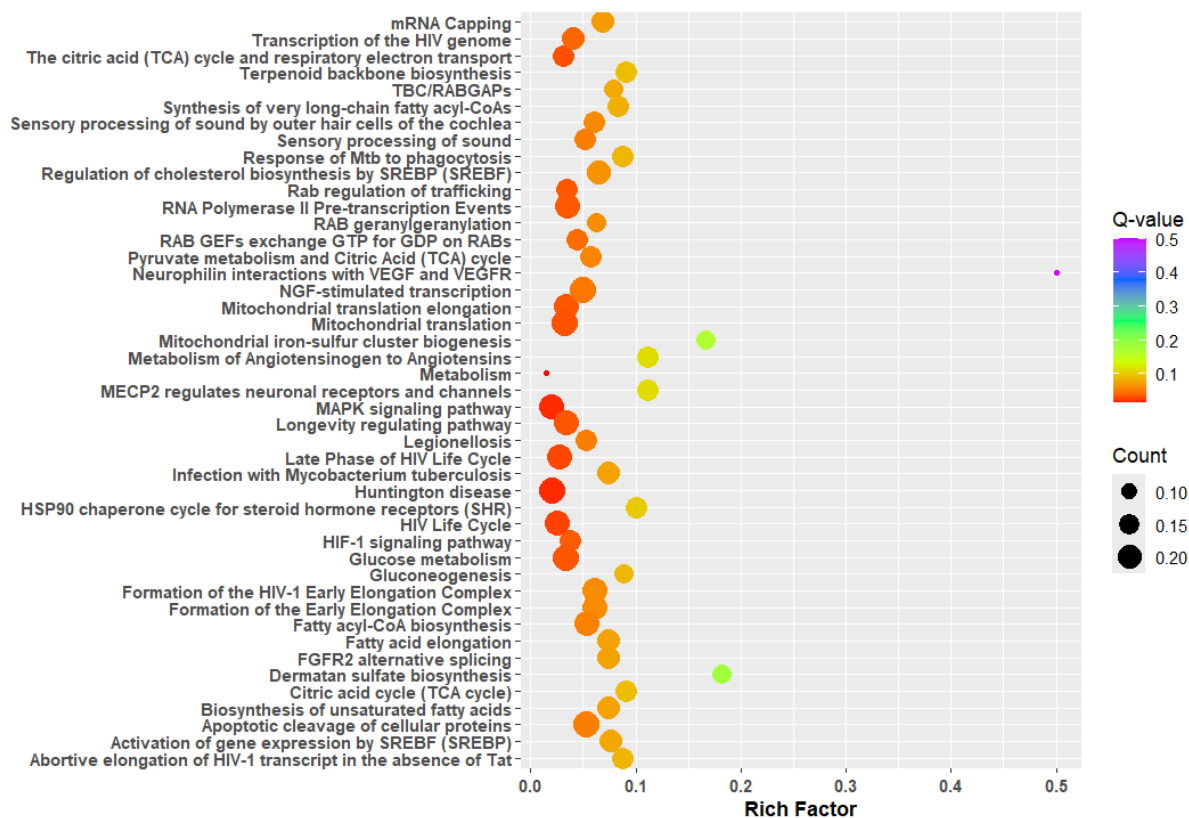


**Figure 4. 18.** Gene enrichment analysis of common DEGs in parietal tissue of AD

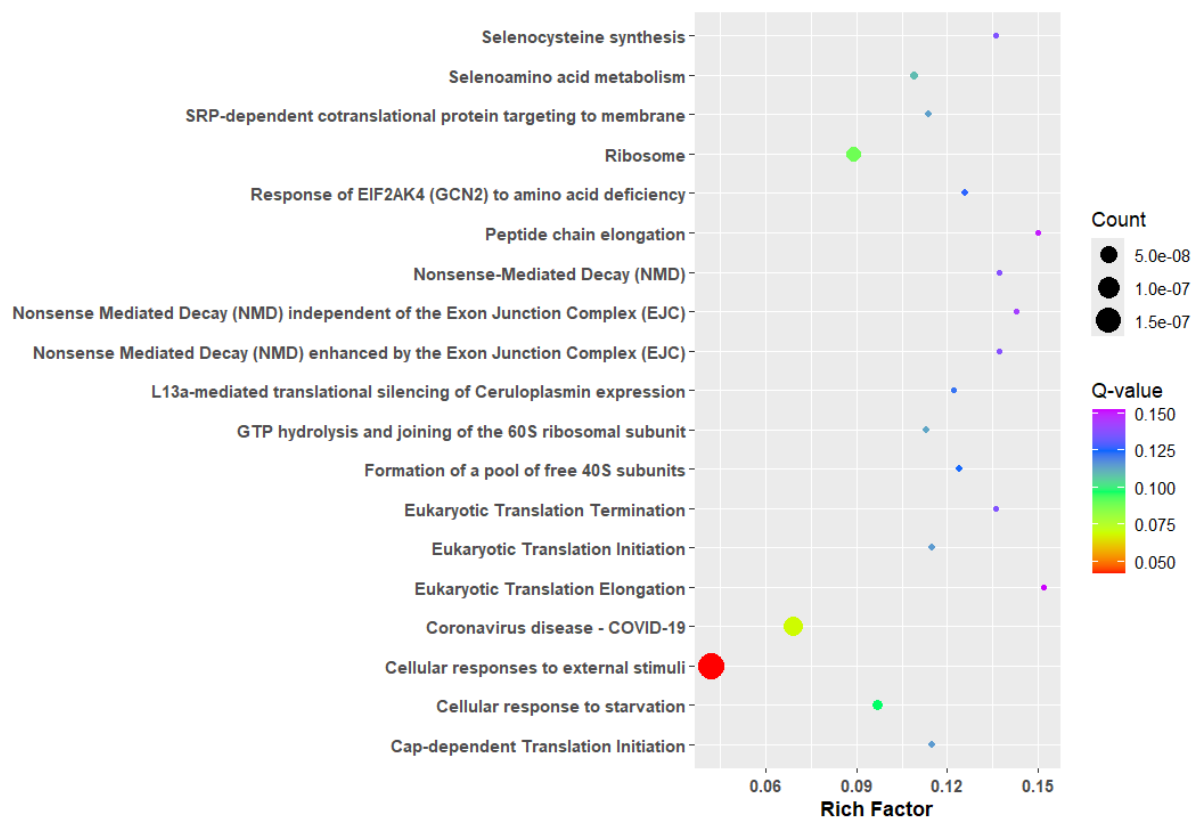


**Figure 4. 19.** Gene enrichment analysis of common DEGs in hippocampus tissue of AD

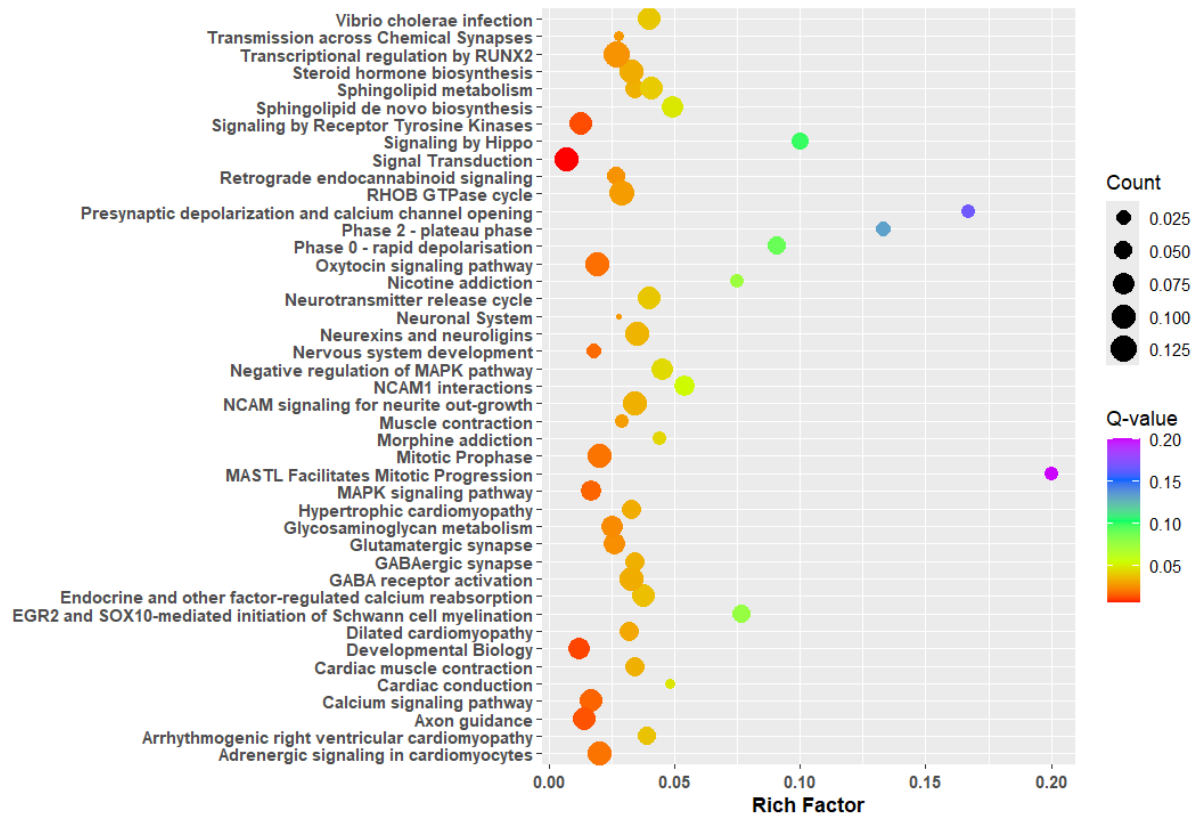


**Figure 4. 20.** Gene enrichment analysis of common DEGs in frontal tissue of AD

The repeated appearance of these pathways in our analysis underscores their relevance and suggests that disruptions in synaptic transmission and neurotransmitter cycling are central to the disease mechanism. Moreover, the involvement of addiction-related pathways such as "Nicotine Addiction(López-Arrieta & Sanz, 2001)" and "Morphine Addiction(L.-W. Liu et al., 2013)" points to potential overlaps in the molecular mechanisms underlying addiction and AD, possibly through common pathways of neuroinflammation and neurodegeneration. The identification of the "Calcium Signaling Pathway" also aligns with previous studies that have highlighted dysregulated calcium homeostasis as a key factor in AD's pathology and also involved to HD and PD (Pchitskaya et al., 2018). Overall, these findings provide a comprehensive view of the molecular alterations in AD and highlight potential targets for therapeutic intervention.

### 4.2.2. PD

In PD, no clusters were observed in brain and frontal cortex tissues, but 6 clusters were observed in substantia nigra, two of which were statistically significant: Cluster 1 and Cluster 2. Cluster 1 is a highly clustered genes consisting of 159 nodes with 5380 edges and 43% network density, with an mCode score of 68.10. Cluster 2 is clustered with 53 nodes and 1215 edges with 88% network density and an mCode score of 46.73. In the healthy state, 10 clusters are observed and 3 of them are significantly clustered. (**Figure 4.21-Figure 4.22**)

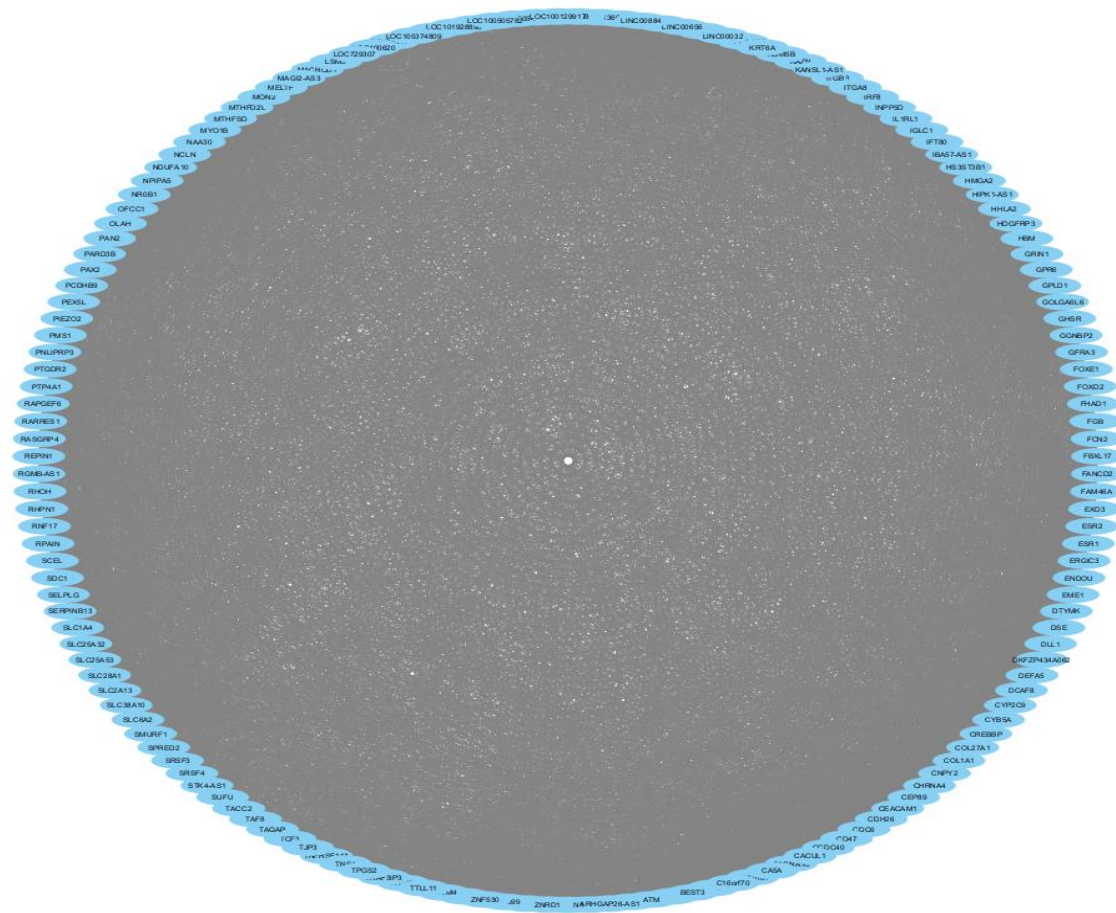


**Figure 4. 21.** Cluster 1 in the substantia nigra of PD identified by co-expression network analysis

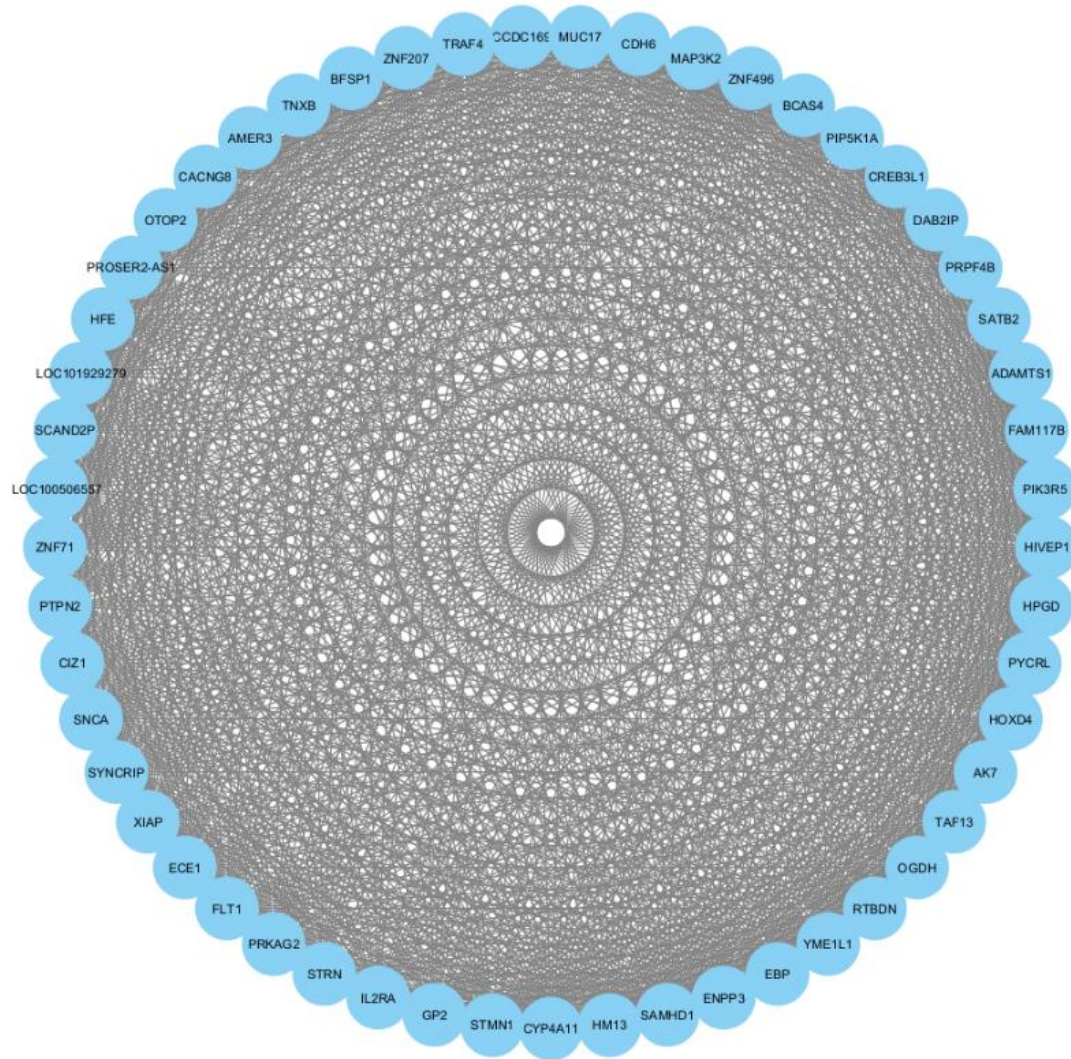


**Figure 4. 22.** Cluster 2 in the substantia nigra of PD identified by co-expression network analysis.

Our enrichment analysis for PD clusters revealed significant enrichment in several pathways, with "MAPK signaling pathway," "ECM-receptor interaction," and "Metabolism of lipids" being prominently featured. The "MAPK signaling pathway" is critical for cell communication and apoptosis, which are disrupted in PD. The consistent involvement of "ECM-receptor interaction" suggests alterations in extracellular matrix components, which can impact neuronal stability and survival. Moreover, the lipid metabolism pathway was also found to be important among the results of our overrepresentation analysis, pointing to potential perturbations in lipid metabolism that may contribute to the neuronal degeneration seen in NDD and PD (L. G. Yang et al., 2023). These findings highlight crucial molecular mechanisms potentially driving PD pathology, providing insights into potential therapeutic targets. (**Figure 4.23-Figure 4.24**)

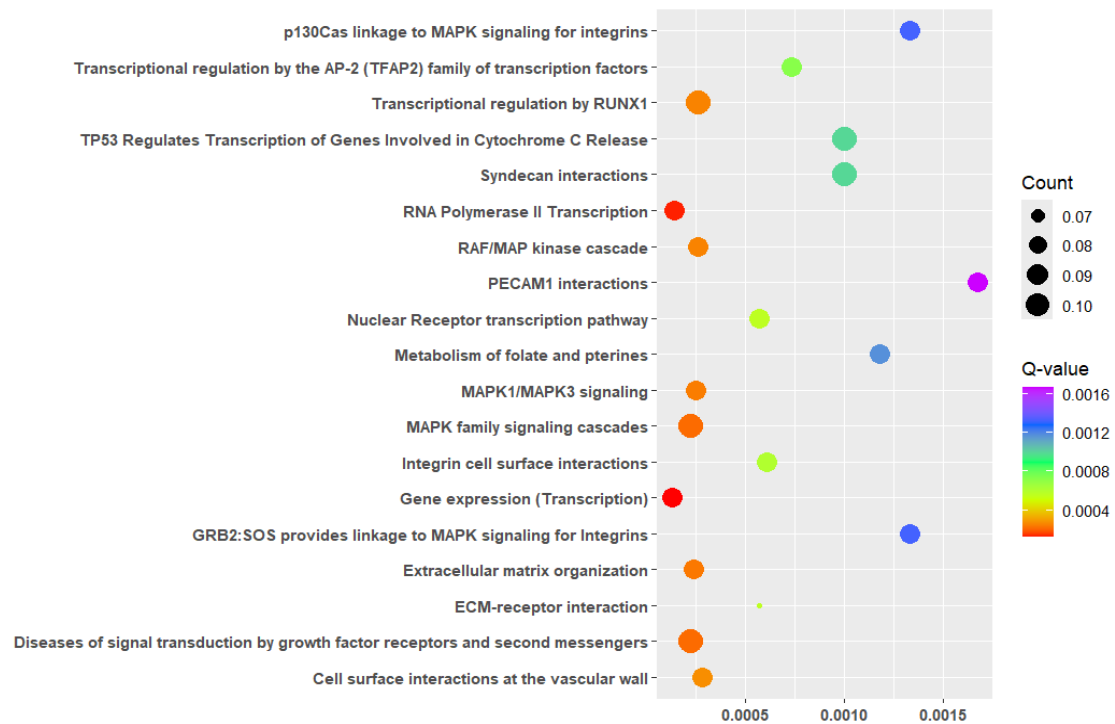


**Figure 4. 23.** Gene enrichment analysis of cluster 1 in Parkinson's Disease.

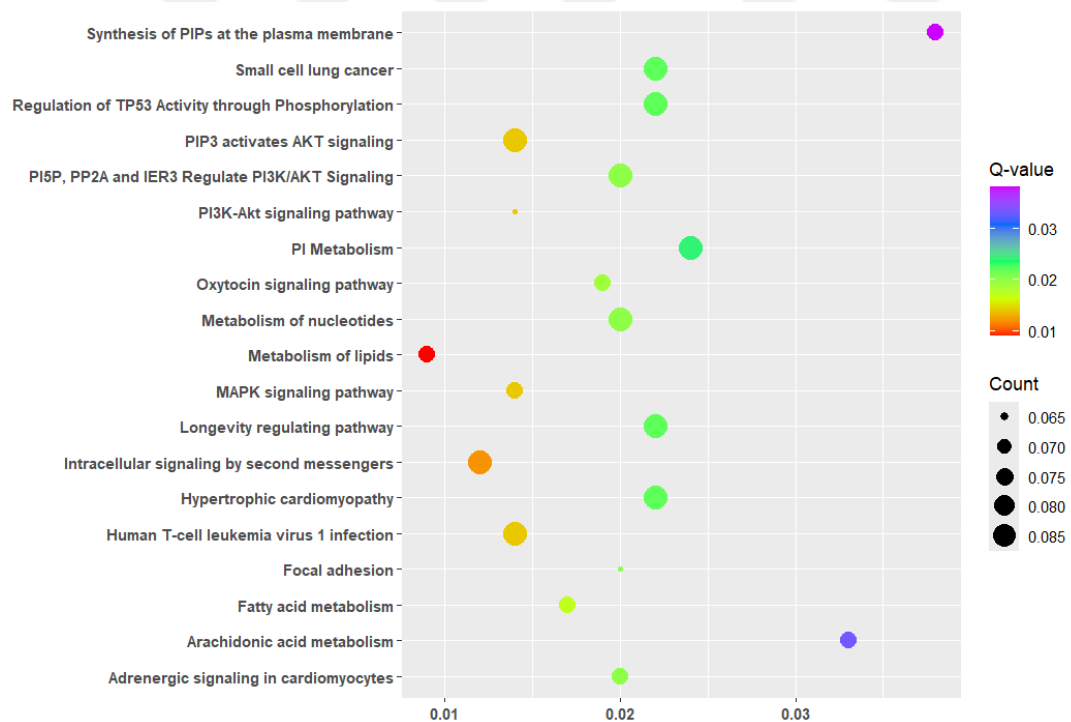


**Figure 4. 24.** Gene enrichment analysis of cluster 2 in Parkinson's Disease.

### 4.2.3. ALS

For motor neuron cells in ALS, we identified four clusters, but only one met our threshold criteria. Cluster 1 consists of 20 nodes, 130 edges, and has a network density of 68%, with an mCode score of 13.68 (**Figure 4.25**). For muscle cells, we identified five clusters, but only Cluster 1 met our significant cluster criteria. Cluster 1 consists of 28 nodes, 174 edges, and has a network density of 46%, with an mCode score of 12.89 (**Figure 4.26**). No clusters were observed in HD.

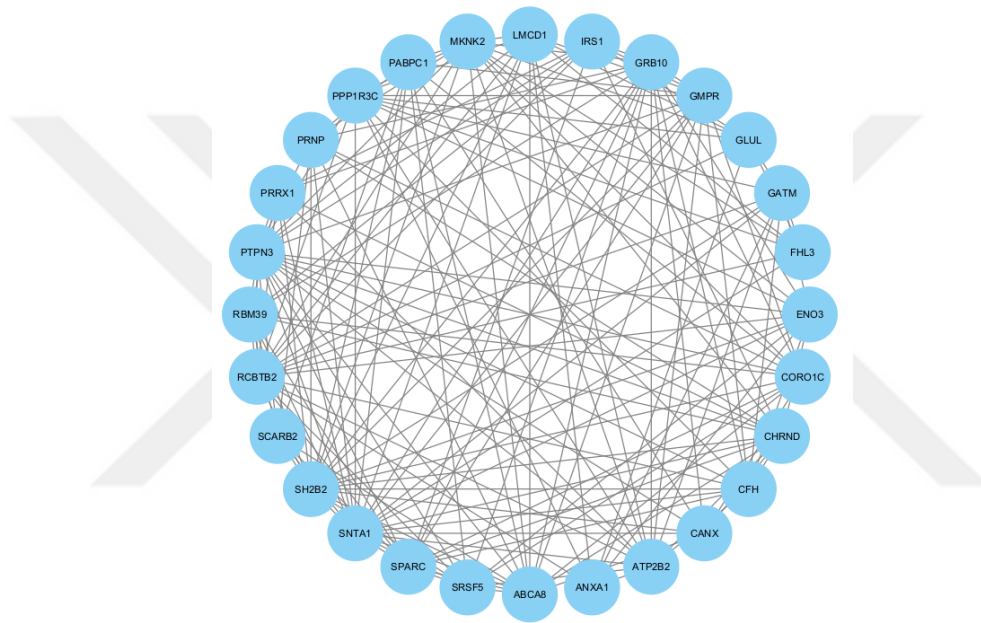


**Figure 4. 25.** Cluster 1 in muscle tissue of ALS identified by co-expression network analysis

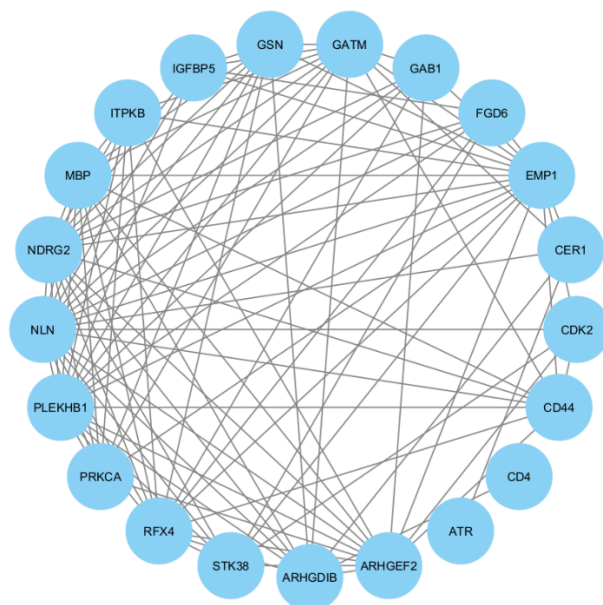


**Figure 4. 26.** Cluster 1 in motor neuron cell for ALS identified by co-expression network analysis

In the comprehensive overrepresentation analysis of cluster genes in ALS, several pathways including "mTOR signaling pathway," "cGMP-PKG signaling pathway," "Signaling by SCF-KIT," "Signaling by Receptor Tyrosine Kinases," "Neurotrophin signaling pathway," "Muscle contraction," and "HIF-1 signaling pathway" were identified statistically significant. Impaired mTOR signaling is associated with NDD and ALS also play crucial role in autophagy and protein homeostasis(Querfurth & Lee, 2021). cGMP PKG signaling pathway and Signaling by SCF-KIT, which are taken a role in cellular signaling and neuroprotection pathways, were also found to be among the important pathways(Peixoto et al., 2015). Furthermore, the "HIF-1 signaling pathway" points to hypoxia-related mechanisms that may exacerbate the progression of NDD and ALS diseases (Merelli et al., 2018). (**Figure 4.27 - Figure 4.28**)

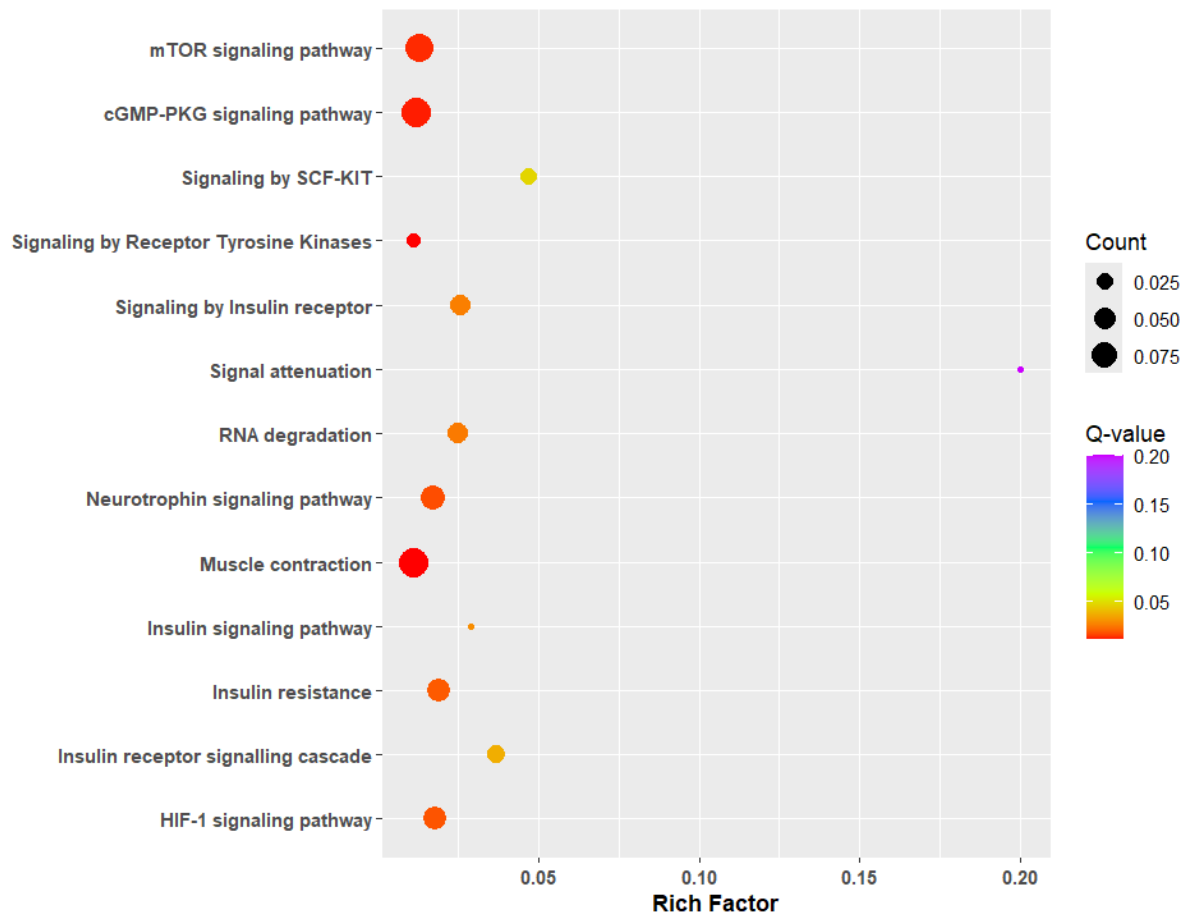


**Figure 4. 27.** Cluster 2 in muscle tissues of ALS identified by co-expression network analysis



**Figure 4. 28.** Gene enrichment analysis of cluster 2 in muscle tissues of ALS

### 4.3. Machine Learning applied to resultant gene clusters

#### 4.3.1. AD

Overall, in the gene cluster analysis of brain tissue in of AD, the SVM model accuracy with hyperparameter tuning was 0.3077, while the Random Forest model achieved an accuracy of 0.5385 for all genes, and the SVM model achieved an accuracy of 0.3846 for all genes. In the cluster genes, only *FAM19A1*, *ATP8A2*, and *FAM81B* had scores higher than 0.6. *FAM19A1* had the highest score (0.62) in KNN compared to other machine learning algorithms (RF: 0.31, SVM: 0.31). *ATP8A2* achieved the highest score in the Random Forest model (0.62) among other algorithms (SVM: 0.31, KNN: 0.38). Similarly, *FAM81B* had the highest score in the Random Forest model (0.62) compared to other algorithms (SVM: 0.46, KNN: 0.38). (Figure 4. 29)

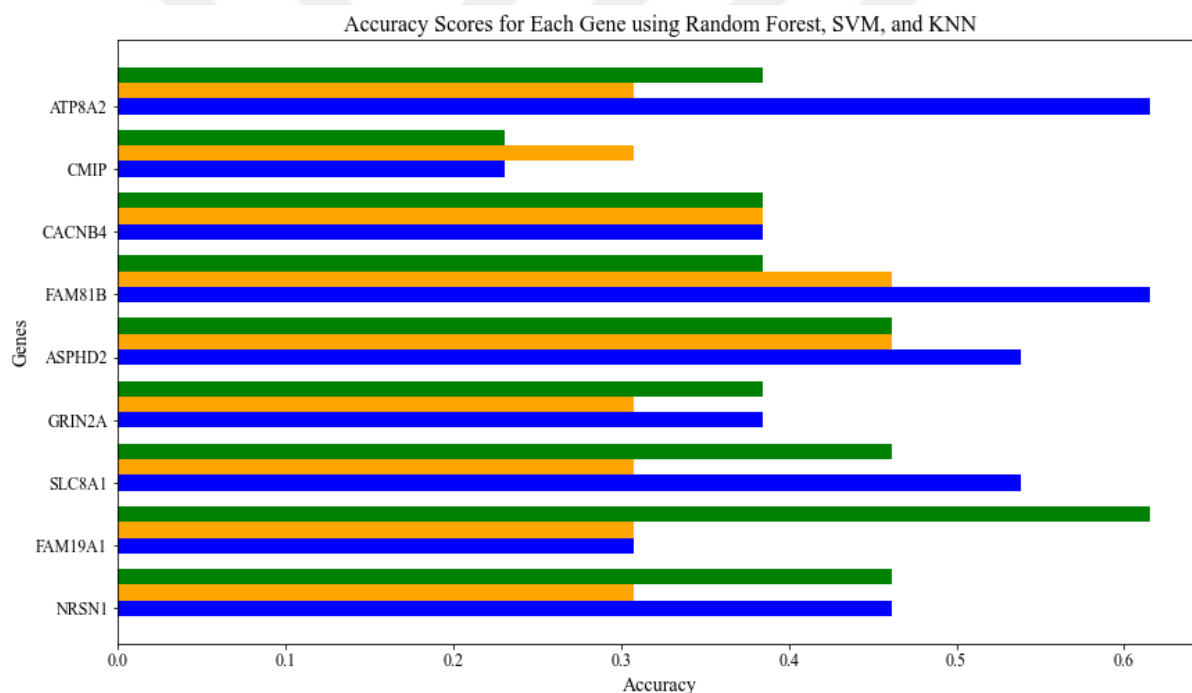


**Figure 4. 29.** Machine learning analysis of gene cluster in brain tissues of AD

The gene cluster in parietal tissue of AD demonstrated superior classification performance, with accuracy scores of 0.75 for both the RF and KNN models, and 0.625 for the SVM model. Notably, all genes within the parietal cluster, exception for *AKIRIN1*, achieved accuracy scores exceeding 62%. (Figure 4. 30)

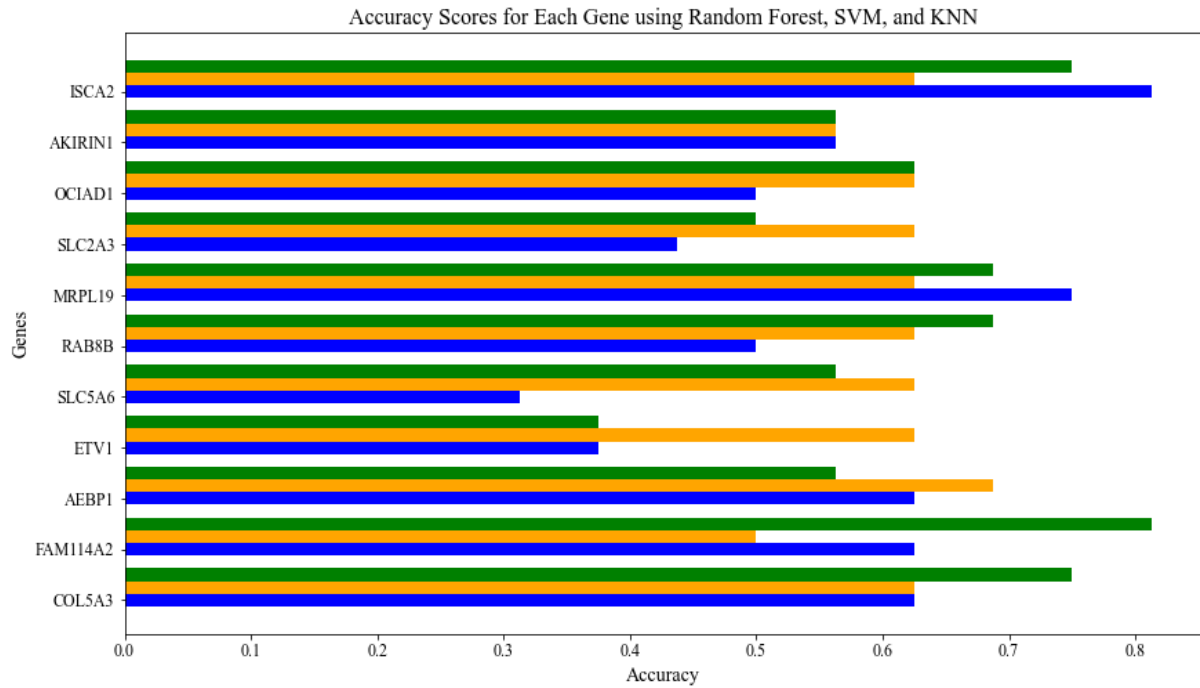


**Figure 4. 30.** Machine learning analysis of gene cluster in parietal tissues of AD

Overall, the hippocampus tissue gene expression cluster exhibited high accuracy scores across different models: 0.75 for Random Forest, 0.8125 for SVM, and 0.75 for KNN. Among the cluster genes, *ANKRD18A* had the highest scores across all algorithms, with 0.875 for Random Forest, 0.625 for SVM, and 0.625 for KNN. In the hippocampus cluster, the genes with scores higher than 70% were *ANKRD18A* and *SH3GL1*. (**Figure 4. 31**)

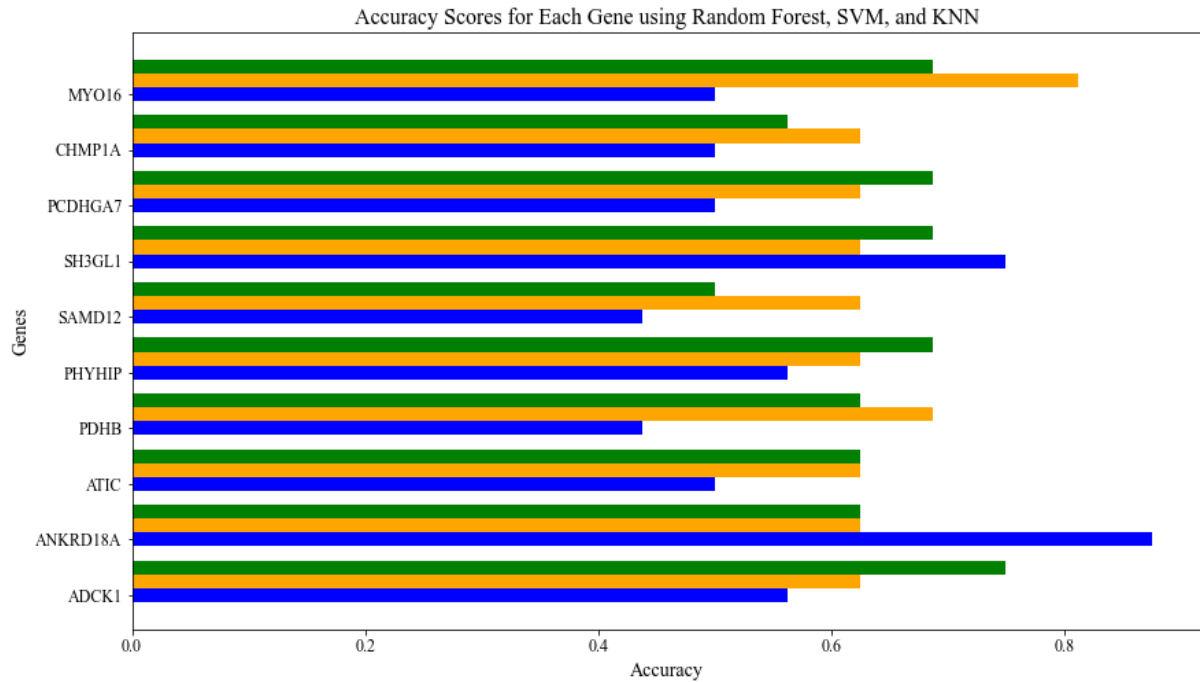


**Figure 4. 31.** Machine learning analysis of gene cluster in hippocampus tissues of AD

#### 4.3.2. PD

The gene cluster 2 in substantia nigra tissues in PD, various genes exhibited different levels of classification accuracy across the RF, SVM, KNN algorithms. Notably, *MAP3K2* achieved the highest accuracy with RF 0.7391, followed by SVM 0.6087 and KNN 0.6522. *PRPF4B* and *FLT1* both showed higher accuracy with RF (0.6522) compared to SVM and KNN, with *PRPF4B* demonstrating particularly low performance in KNN (0.3913). Conversely, *AK7* had its highest accuracy with KNN (0.6957), though it performed relatively lower with RF (0.5652) and SVM (0.4348). *CREB3L1* also stood out with its performance in RF (0.5217) and SVM (0.6522), compared to KNN (0.6087). Interestingly, genes such as *HM13*, *ZNF71*, and *IL2RA* consistently scored lower across all algorithms, with *HM13* achieving its highest accuracy with RF (0.5217). Furthermore, genes like *OTOP2* and *CDH6* showed uniform accuracy across all three models (0.4783), whereas others like *STRN* had relatively balanced performance with RF and SVM (both 0.5217) and KNN (0.6087). It is worth noting that the gene *AKIRIN1* was the only one to score below 62% accuracy, demonstrating varying performance across the different classification models. (Figure 4.32 – Figure 4.33)

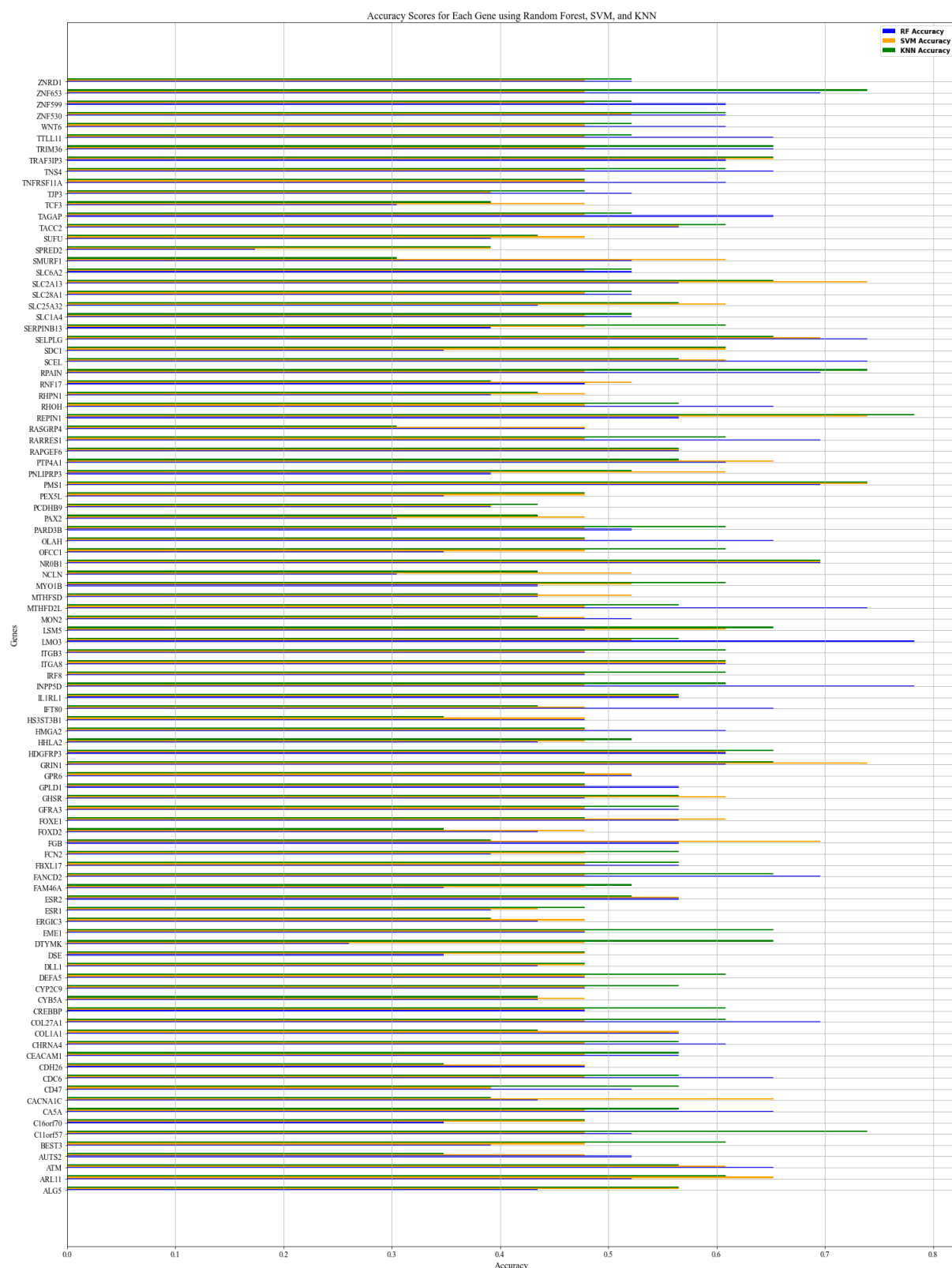


**Figure 4. 32.** Machine learning analysis of gene cluster 1 in substantia nigra tissues of PD

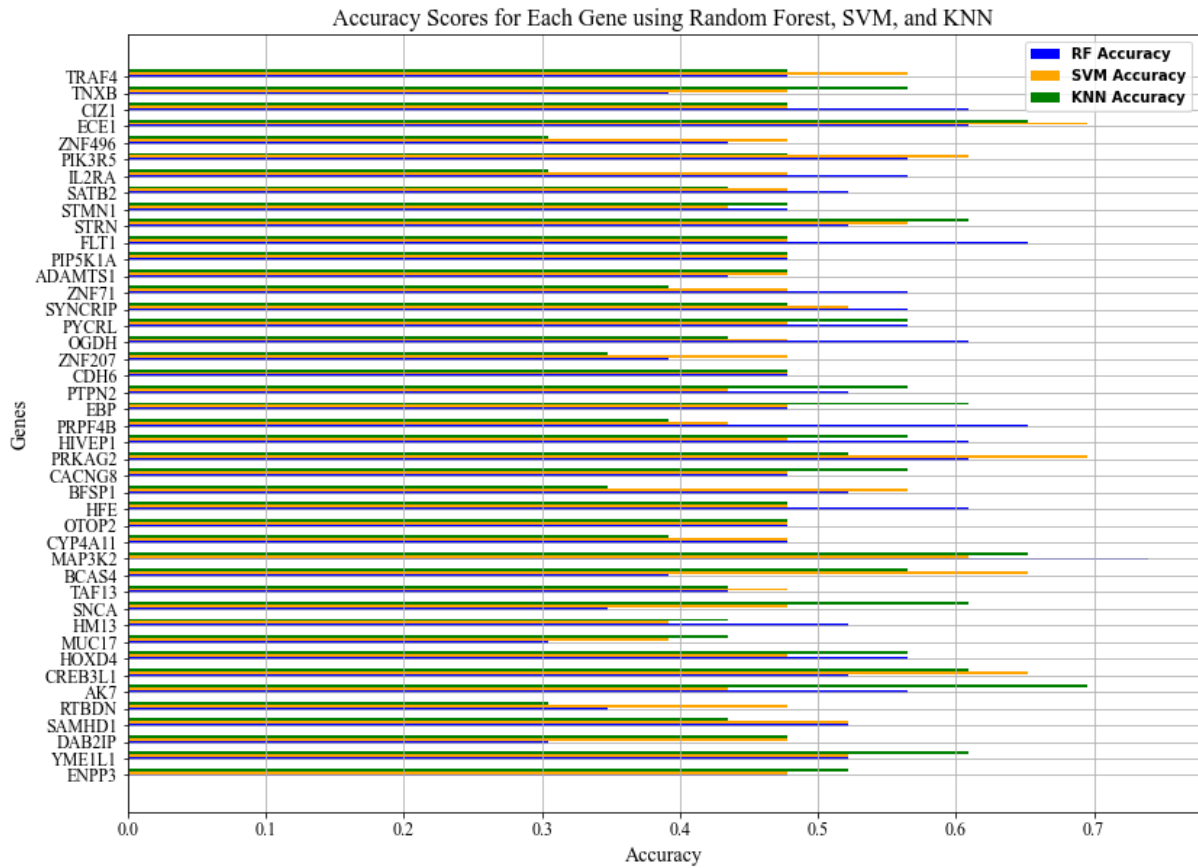


**Figure 4. 33.** Machine learning analysis of cluster 2 in substantia nigra tissues of PD

#### 4.3.3. ALS

The classification performance of motor neuron and muscle clusters in ALS was evaluated using SVM and Random Forest (RF) algorithms. The SVM model performance metrics for the motor neuron cluster are as follows: Accuracy: 0.6207, Precision: 0.6667, Recall: 0.9000, F1 Score: 0.7660. The metrics obtained for the best Random Forest model (n\_estimators=10) are Accuracy: 0.7586, Precision: 0.7826, Recall: 0.9000, F1 Score: 0.8372. For the muscle cluster, the SVM model performance metrics are as follows: Accuracy: 0.6897, Precision: 0.7037, Recall: 0.9500, F1 Score: 0.8085. The metrics obtained for the best Random Forest model (n\_estimators=10) are Accuracy: 0.6897, Precision: 0.7391, Recall: 0.8500, F1 Score: 0.7907. SVM and RF models performed similarly in the muscle cluster. *SRSF5*, *PABPC1*, *CFH*, *CORO1C*, *CANX* and *PRNP* were the genes with high classification scores for muscle clusters, while *CD44*, *NDRG2* and *FGD6* genes also had high scores.

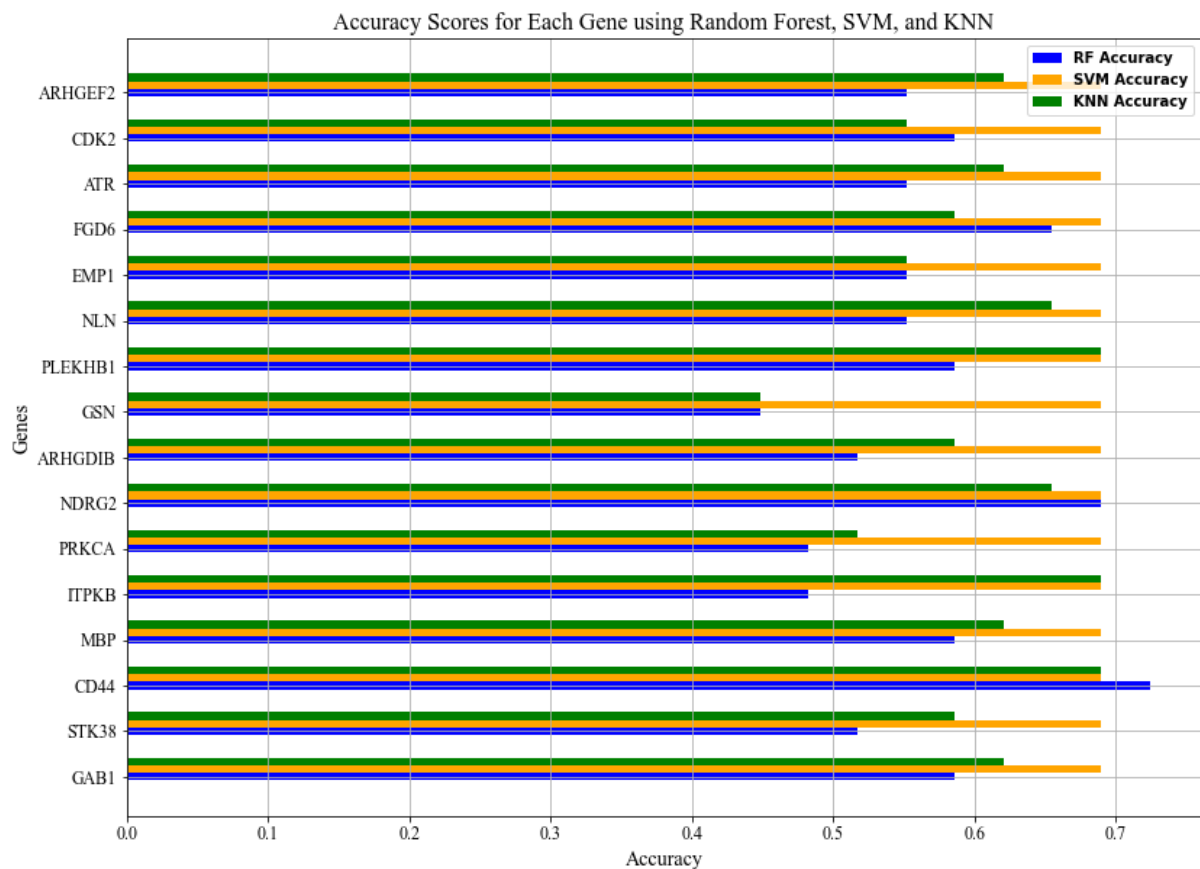


**Figure 4. 34.** Machine learning analysis of cluster 2 in motor neuron cells of ALS

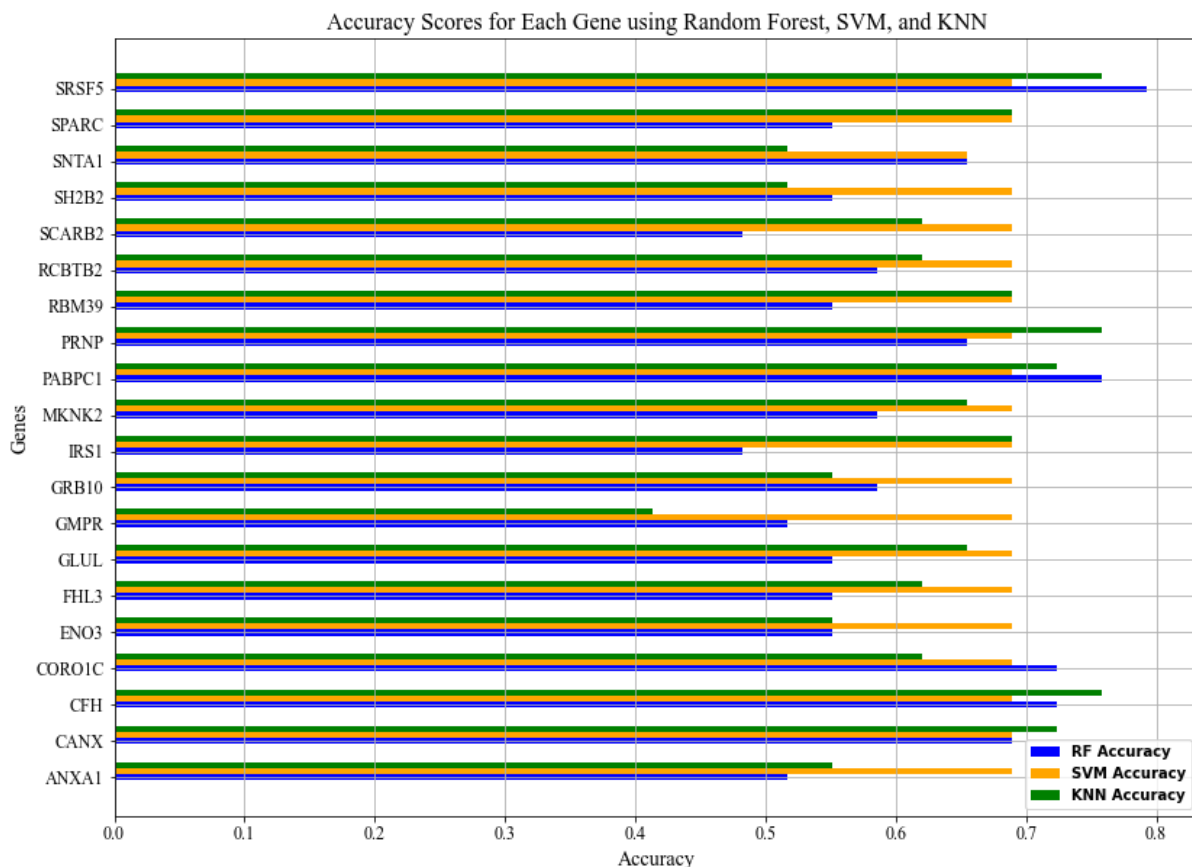


**Figure 4. 35.** Machine learning analysis of cluster 2 in muscle tissue of ALS

#### 4.4. Drug Repurposing

We implemented a drug repurposing approach using the DGB search engine based on significant gene clusters (**Result 4.2**). Drug repurposing was conducted separately for each cluster. Following the subsequent filtering step (**Method 3.7**), common drugs were identified, which are as follows: Natural alpha interferon, Interferon beta-1b, Triiodothyronine-[13c] hydrochloride (T3 thyronine), Doxorubicin, Cisplatin, Vanadium pentoxide, Vemurafenib, Estradiol, Imatinib, and Tretinoin (**Figure 4.36**).

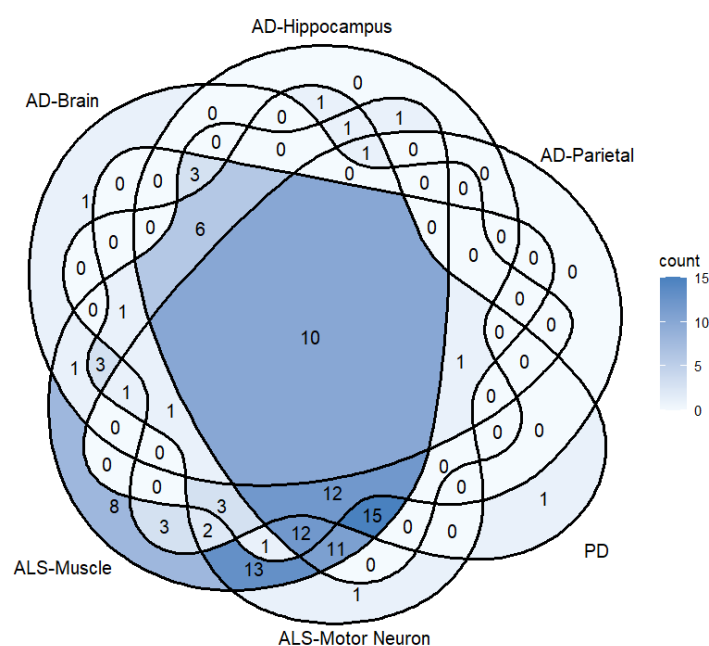


**Figure 4. 36.** Common repurposed drugs across all gene clusters

#### 4.4.1. Machine Learning to Repurposed Drugs

All candidate repurposed drugs identified with DGB were re-analyzed with ML algorithms.

##### 4.4.1.1. AD

In the drug repositioning analysis for AD, drug candidates derived from gene clusters including brain, hippocampus and parietal tissues were evaluated by five different machine learning algorithms such as Linear Regression, Support Vector Regression, Random Forest Regressor, Gradient Boosting Regressor and Neural Network Regressor. In AD's drug repositioning analysis, drugs such as Doxorubicin, Lipopolysaccharide and Sulforaphane stood out, with the lowest error values generally achieved using the SVR model. Especially in the overall analysis, the SVR model provided the lowest Mean Square Error (MSE) values compared to the other models. In the AD Parietal Cluster analysis, low MSE values of 0.389 MSE for Doxorubicin, 0.391 MSE for Lipopolysaccharide and 0.392 MSE for Sulforaphane were obtained and these values were provided by the SVR model. For AD Hippocampus Cluster, the lowest MSE values of 0.384 MSE for Doxorubicin and 0.386 MSE for Sulforaphane were provided by the SVR model.

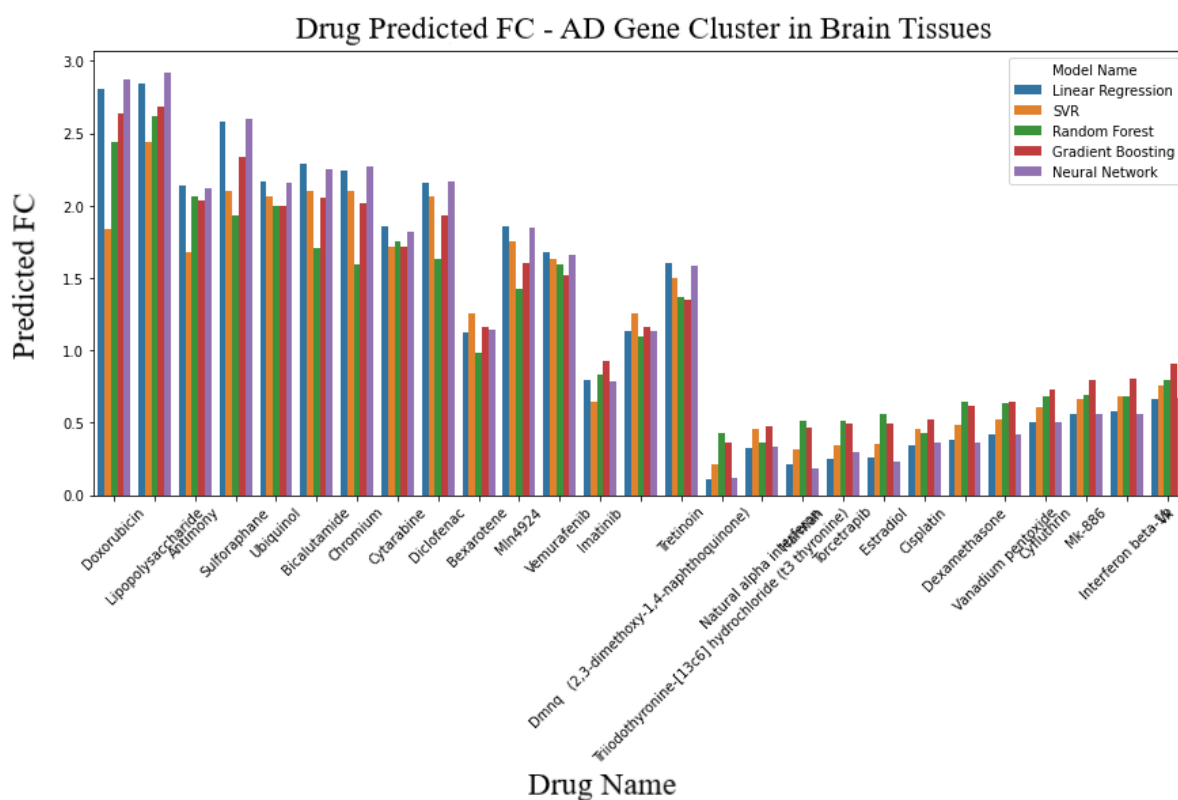


**Figure 4. 37.** Machine learning analysis for candidate drugs targeting Cluster 1 in brain tissues of AD

As a result of ML algorithms to candidate repurposed drugs for AD, Doxorubicin, Lipopolysaccharide, Sulforaphane, Natural Alpha Interferon, Interferon Beta-1b, Triiodothyronine-[13c6] Hydrochloride (T3 Thyronine), Cisplatin, Vanadium Pentoxide, Vemurafenib, Estradiol, Imatinib, Tretinoin, Donepezil, Rivastigmine, Galantamine, Memantine, Vitamin E, Gingko Biloba, Resveratrol, Curcumin were found to the top 10 drugs in accordance with ML results.

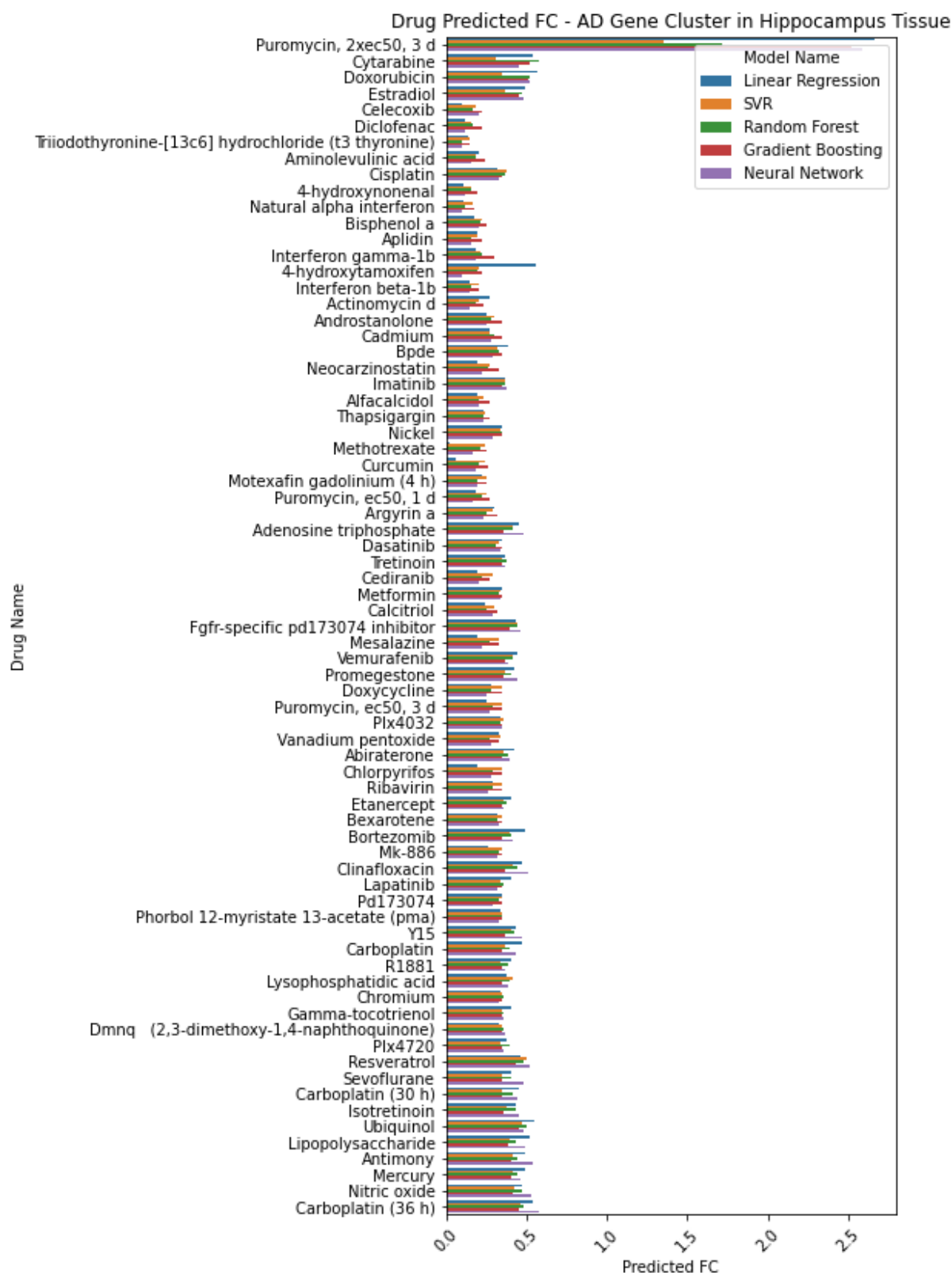


**Figure 4. 38.** Machine learning analysis for candidate drugs targeting Cluster 5 in hippocampus tissue of AD

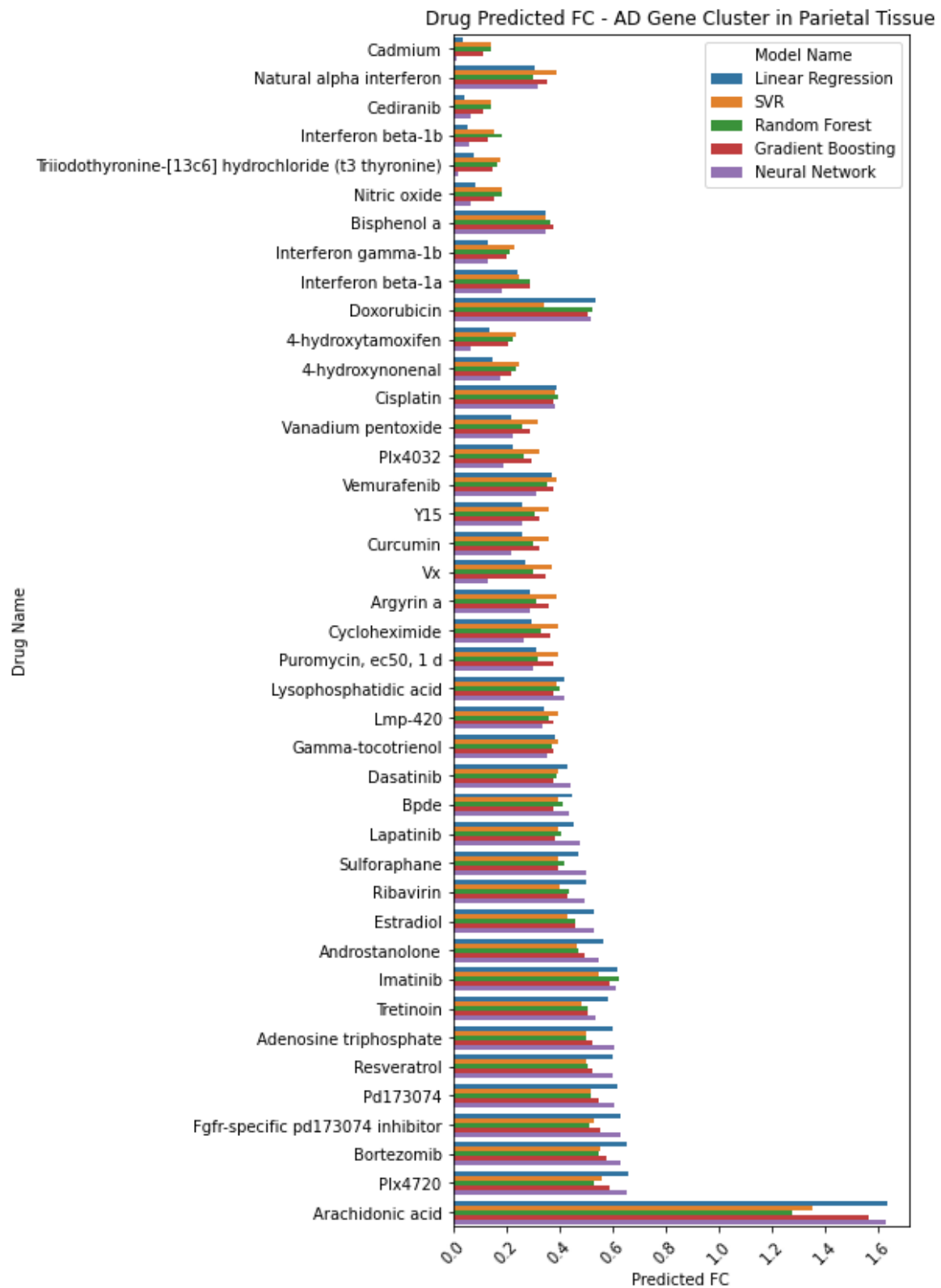


**Figure 4. 39.** Machine learning analysis for candidate drugs targeting Cluster 5 in parietal tissue of AD

#### **4.4.1.2. PD**

Five different machine learning models were used in the analysis for the evaluation of drug candidates in PD. Linear regression, SVR, Random Forest, Gradient Boosting, Gradient Boosting and Neural Network models were used to identify several potentially effective drug candidates for the treatment of PD. For example, the SVR model for Bisphenol A showed the lowest error value of 0.276, while the Neural Network model for Nickel achieved the highest error value of 1.159. In the process of evaluating the potential effects of various drug candidates for the progression of PD, the SVR model generally provided the lowest error rates. **(Supplementary Table 4.2)**

The ML results indicate that Bisphenol A, Nickel, Levodopa, Pramipexole, Selegiline, Entacapone, Benztropine, Amantadine, Sulforaphane, Natural Alpha Interferon, Ropinirole, Rasagiline, Apomorphine, Rotigotine, Tolcapone, Trihexyphenidyl, Bromocriptine, Pergolide, Safinamide, Istradefylline are identified candidate repurposed drugs for PD.

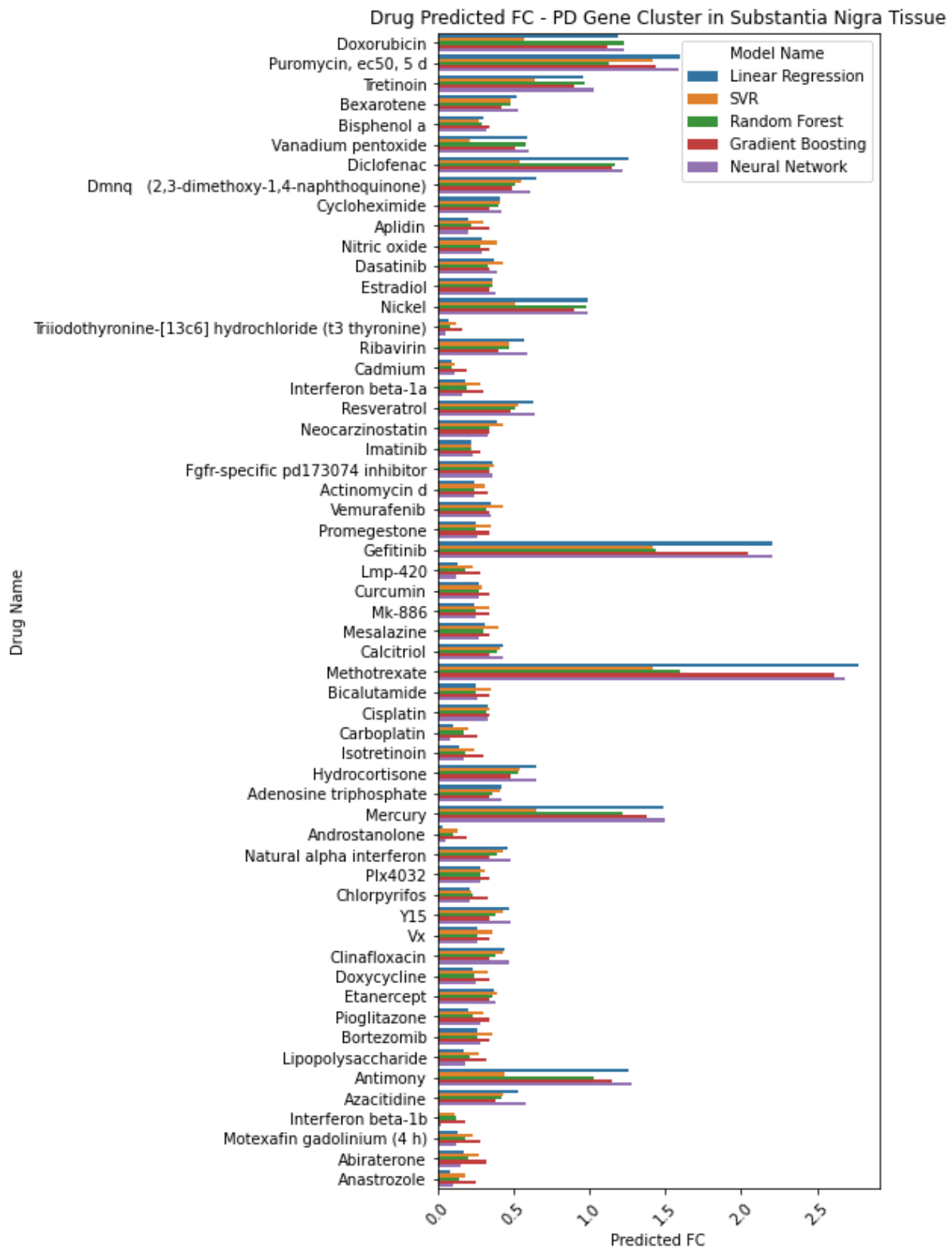


**Figure 4. 40.** Machine learning analysis for candidate drugs targeting cluster genes in substantia nigra tissue of PD

#### **4.4.1.3. ALS**

The mean error rates obtained as a result of the evaluation of ALS motor neuron cluster drug candidates with machine learning models allow us to compare the performance of the models. Gradient Boosting has the lowest average error rate with 0.574, followed by the SVR model with 0.581. The Random Forest model ranked third with an error rate of 0.605, while the Neural Network model ranked fourth with 0.658. The model with the highest average error rate was Linear Regression with 0.712. These results show that Gradient Boosting and SVR models perform better than other models in predicting FC of ALS motor neuron cluster drug candidates.



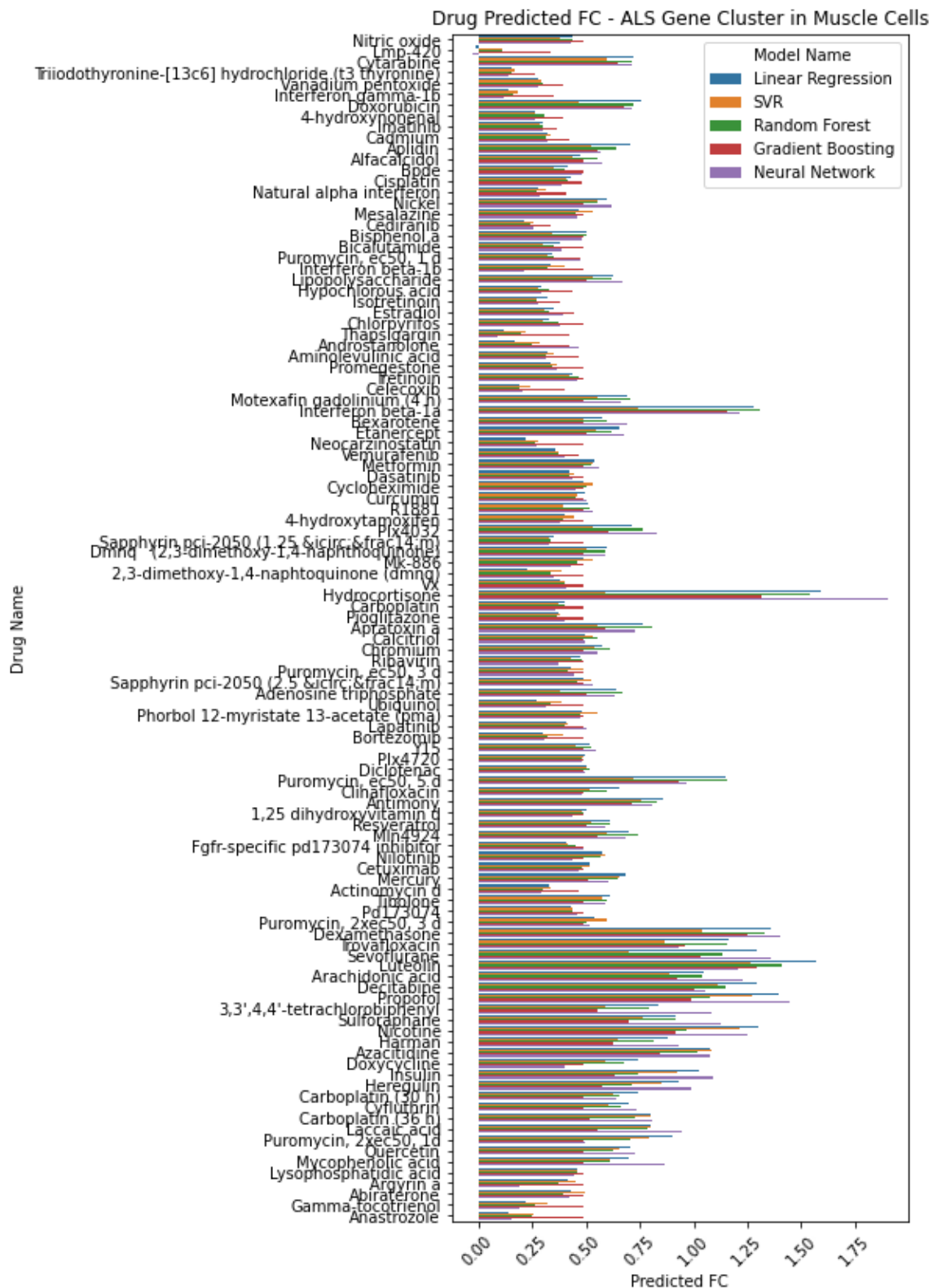


**Figure 4. 41.** Machine learning analysis of repurposed drugs for the gene cluster in muscle with ALS

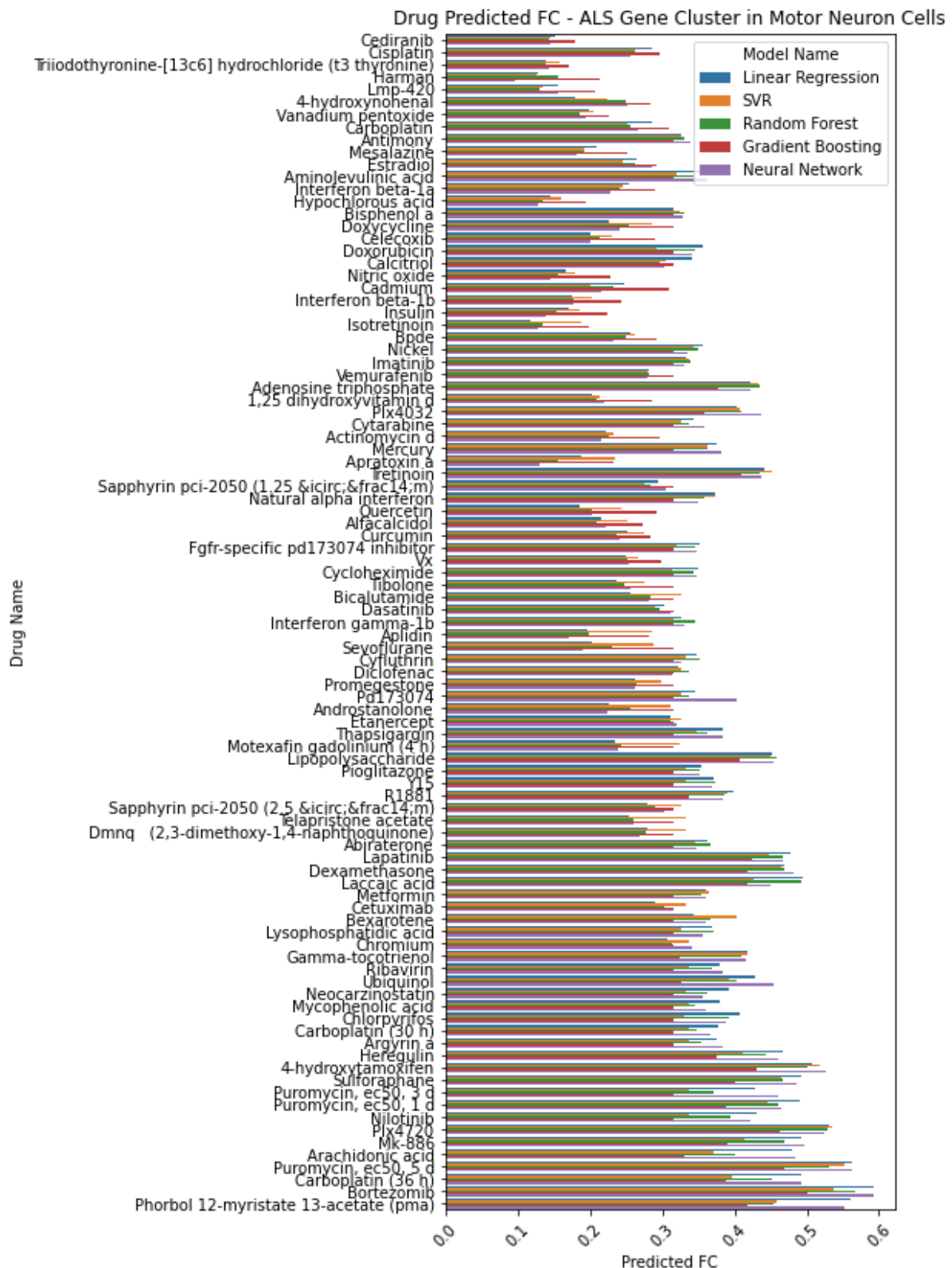


**Figure 4. 42.** Machine learning analysis of repurposed drugs for the gene cluster in motor neuron cells with ALS

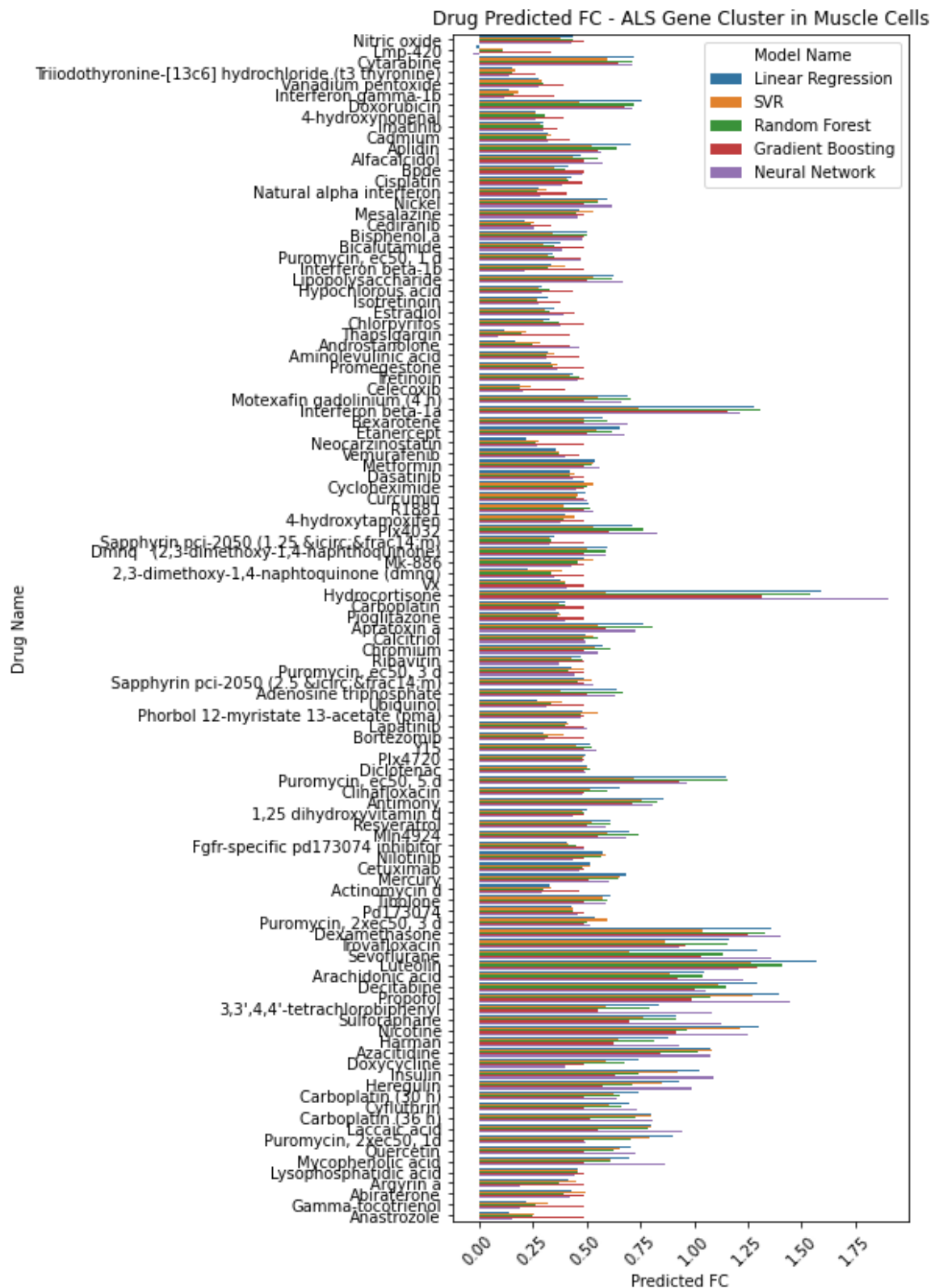


**Figure 4. 43.** Machine learning analysis of repurposed drugs for the gene cluster in muscle tissue with ALS

In the analysis of cluster's drug candidates for muscle tissues with ALS, the effects of various drugs on nitric oxide production were evaluated with different machine learning models. The results vary significantly depending on the combinations of drugs and models. For example, the best performing model on nitric oxide was the Random Forest model (0.443). Similarly for other drugs, the performance of each model varied, and the best results were generally obtained from Gradient Boosting and Neural Network models. These findings can be an important resource for evaluating the effectiveness of machine learning models in drug development and research on nitric oxide production.

The result of ML demonstrates that Riluzole, Edaravone, N-acetylcysteine, Lithium, Gabapentin, Ceftriaxone, Thalidomide, Tamoxifen, Interferon Beta-1b, Tretinoin, Arimoclomol, Mexiletine, Prednisone, Sodium Phenylbutyrate, Trihexyphenidyl, Cyclophosphamide, Quinidine, Dextromethorphan, Erythropoietin, Valproate were identified the repurposed drugs for ALS.

#### 4.4.2. Text mining analysis for common repurposed drugs

We targeted the significant clusters we identified by downstream analysis of transcriptomic data with bioinformatics and ML. In order to uncover the novelty of the significant ML-scored drugs, we calculated TF-IDF values by data mining through Biopython (Table 4.1 – Table 4.3). Drug candidates that have not yet been studied in the target disease (i.e. those with TF-IDF values of '0') were considered novel drug candidates for the target disease.

**Table 4. 1.** Proteins expressed by cluster genes that interact with repurposed novel drugs according to literature searches

| Disease names | Candidate Repurposed Drug Names | Protein names                                     | References                                                       |
|---------------|---------------------------------|---------------------------------------------------|------------------------------------------------------------------|
| AD            | Torcetrapib                     | SLC2A3, AKIRIN1                                   | (Fan et al., 2012)                                               |
| PD            | Aplidin                         | CYP4A11                                           | (Brandon et al., 2007)                                           |
| PD            | Androstanolone                  | COL1A1, CREBBP, CYB5A, ALG5, DCAF8, ESR1, TRIM36, | (Lin et al., 2007),<br>(Shah et al., 2012),<br>(Dumontet et al., |

|    |              |                                           |                                                                                                                               |
|----|--------------|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
|    |              | MYO1B, PNLIPRP3, ESR2, HM13, EBP, ADAMTS1 | 2018), (Haggard et al., 2018), (Kojima et al., 2003), (Sehrawat et al., 2018), (Hess-Wilson et al., 2007), (Wen et al., 2006) |
| PD | Bicalutamide | CREBBP, ESR1, CYP2C9, CDC6, ESR2, EBP     | (Comuzzi et al., 2004), (Sipes et al., 2013), (Cockshott, 2004), (Bai et al., 2005)                                           |

#### **4.4.2.1. Text mining analysis of repurposed drugs for AD**

We identified five novel drug candidates (Dmnq (2,3-dimethoxy-1,4-naphthoquinone), Interferon beta-1b, Cyfluthrin, Torcetrapib, and Vx) for the treatment of AD. Dmnq is a potent inducer of oxidative stress (Sathish et al., 2011). It is often used in biochemical studies to promote the production of ROS. DMNQ is widely used as a model compound to study cellular antioxidant defense mechanisms and cellular responses to oxidative damage (Ishihara et al., 2011). Interferon beta-1b is a type of recombinant protein and has significant regulatory effects on the immune system. It is used in the treatment of Multiple sclerosis (MS), a neurodegenerative disease, and reduces the frequency and severity of attacks by slowing the course of the disease (“Interferon Beta,” 2006). VX, a highly toxic organophosphate nerve agent, is also among the candidate drugs (Misik et al., 2015). Torcetrapib is a drug developed as a cholesterol ester transfer protein (CETP) inhibitor (Su et al., 2020). Interaction information with AD cluster genes SLC2A3 and AKIRIN1 is also available (Fan et al., 2012).

**Table 4. 2.** Text Mining Results for Drug Candidates Identified by ML for brain tissue of AD

| <b>Candidate Drugs<br/>in AD</b>                               | <b># of Drugs<br/>Related to<br/>Articles</b> | <b># of<br/>Drugs-AD<br/>Related to<br/>Articles</b> | <b>TF</b> | <b>IDF</b> | <b>TF-IDF</b> |
|----------------------------------------------------------------|-----------------------------------------------|------------------------------------------------------|-----------|------------|---------------|
| Doxorubicin                                                    | 81653                                         | 39                                                   | 0.0210    | 0.8236     | 0.0173        |
| Lipopolysaccharide                                             | 100033                                        | 1016                                                 | 0.5480    | 0.7355     | 0.4030        |
| Antimony                                                       | 7035                                          | 4                                                    | 0.0022    | 1.8883     | 0.0041        |
| Sulforaphane                                                   | 2522                                          | 30                                                   | 0.0162    | 2.3339     | 0.0378        |
| Ubiquinol                                                      | 1961                                          | 14                                                   | 0.0076    | 2.4431     | 0.0184        |
| Bicalutamide                                                   | 1463                                          | 1                                                    | 0.0005    | 2.5704     | 0.0014        |
| Chromium                                                       | 31277                                         | 16                                                   | 0.0086    | 1.2404     | 0.0107        |
| Cytarabine                                                     | 6948                                          | 1                                                    | 0.0005    | 1.8937     | 0.0010        |
| Diclofenac                                                     | 13300                                         | 20                                                   | 0.0108    | 1.6118     | 0.0174        |
| Bexarotene                                                     | 661                                           | 59                                                   | 0.0318    | 2.9154     | 0.0928        |
| Mln4924                                                        | 395                                           | 1                                                    | 0.0005    | 3.1390     | 0.0017        |
| Vemurafenib                                                    | 2115                                          | 2                                                    | 0.0011    | 2.4103     | 0.0026        |
| Imatinib                                                       | 13119                                         | 20                                                   | 0.0108    | 1.6177     | 0.0175        |
| Dmnq (2,3-<br>dimethoxy-1,4-<br>naphthoquinone)                | 112                                           | 0                                                    | 0         | 3.6864     | 0             |
| Tretinoin                                                      | 1279                                          | 1                                                    | 0.0005    | 2.6287     | 0.0014        |
| Triiodothyronine-<br>[13c6]<br>hydrochloride (t3<br>thyronine) | 20398                                         | 37                                                   | 0.0200    | 1.4260     | 0.0285        |
| Natural alpha<br>interferon                                    | 38980                                         | 49                                                   | 0.0264    | 1.1448     | 0.0303        |
| Harman                                                         | 490                                           | 1                                                    | 0.0005    | 3.0454     | 0.0016        |

|                    |       |     |        |        |        |
|--------------------|-------|-----|--------|--------|--------|
| Torcetrapib        | 177   | 0   | 0      | 3.4876 | 0      |
| Estradiol          | 86398 | 388 | 0.2093 | 0.7991 | 0.1672 |
| Cisplatin          | 68114 | 31  | 0.0167 | 0.9024 | 0.0151 |
| Dexamethasone      | 63332 | 120 | 0.0647 | 0.9340 | 0.0605 |
| Vanadium pentoxide | 493   | 1   | 0.0005 | 3.0428 | 0.0016 |
| Cyfluthrin         | 721   | 0   | 0.0000 | 2.8777 | 0      |
| Mk-886             | 385   | 3   | 0.0016 | 3.1501 | 0.0051 |
| Interferon beta-1b | 629   | 0   | 0      | 2.9370 | 0      |
| Vx                 | 16    | 0   | 0      | 4.5315 | 0      |

#### 4.4.2.2. Text mining analysis of repurposed drugs for PD

We identified nine novel drug candidates (i.e.: Interferon beta-1b, Aplidin, Androstanolone, Ribavirin, Dmnq (2,3-dimethoxy-1,4-naphthoquinone), Natural alpha interferon, Interferon beta-1a, Clinafloxacin, and Bicalutamide). Interferon beta-1b and Dmnq is also novel drug candidates for AD. Dmnq is used for oxidative damage and cellular antioxidant defense system studies. Interferon beta-1b is also used for the immune system regulatory. Aplidin, also known as plitidepsin, is an antineoplastic agent and a cyclic depsipeptide. It stops the growth of cancer cells by inhibiting intracellular protein translation and induces apoptosis (Leisch et al., 2018). The drug also has interaction with CYP4A11 gene which is also the PD Clusters gene (Brandon et al., 2007). Androstanolone, also known as dihydrotestosterone (DHT), is a powerful androgen hormone. It can also be effective in increasing muscle mass and strength, as well as in conditions such as hair loss and prostate enlargement. The drug also interacts with 13 cluster genes encoding proteins including *COL1A1*, *CREBBP*, *CYB5A*, *ALG5*, *DCAF8*, *ESR1*, *TRIM36*, *MYO1B*, *PNLIPRP3*, *ESR2*, *HM13*, *EBP*, *ADAMTS1* in PD. Ribavirin is a nucleoside analog with broad-spectrum antiviral properties. Ribavirin inhibits viral RNA and DNA replication, preventing viruses from replicating (Nyström et al., 2019). Natural alpha interferon is a protein and a type of cytokine produced by the immune system, and it has antiviral, antiproliferative and immunomodulatory effects (Yanai et al., 2001). Bicalutamide is a nonsteroidal antiandrogen drug, known for stopping the growth of cancer cells by blocking the

effects of testosterone and other androgens. A recent study showed that in a mouse model of spinal and bulbar muscular atrophy characterized by motor neuron degeneration, Bicalutamide improved motor behavior (Galbiati et al., 2023). Moreover, Bicalutamide interact with 6 PD cluster genes (*CREBBP* (Comuzzi et al., 2004), *ESR1* (Sipes et al., 2013), *CYP2C9* (Cockshott, 2004), *CDC6* (Bai et al., 2005), *ESR2* (Bilancio et al., 2017), and *EBP* (Bouchal et al., 2005))

**Table 4. 3.** Text Mining Results for Drug Candidates Identified by ML for PD

| <b>Candidate Drugs in PD</b>                         | <b># of Drugs Related to Articles</b> | <b># of Drugs-AD Related to Articles</b> | <b>TF</b> | <b>IDF</b> | <b>TF-IDF</b> |
|------------------------------------------------------|---------------------------------------|------------------------------------------|-----------|------------|---------------|
| Triiodothyronine-[13c6] hydrochloride (t3 thyronine) | 20398                                 | 4                                        | 0.0169    | 1.1005     | 0.0186        |
| Cadmium                                              | 50411                                 | 33                                       | 0.1392    | 0.7075     | 0.0985        |
| Interferon beta-1b                                   | 629                                   | 0                                        | 0         | 2.6114     | 0             |
| Aplidin                                              | 49                                    | 0                                        | 0         | 3.7199     | 0             |
| Pioglitazone                                         | 5335                                  | 43                                       | 0.1814    | 1.6829     | 0.3053        |
| Androstanolone                                       | 68                                    | 0                                        | 0.0000    | 3.5776     | 0.0000        |
| Abiraterone                                          | 2314                                  | 1                                        | 0.0042    | 2.0457     | 0.0086        |
| Ribavirin                                            | 12431                                 | 0                                        | 0         | 1.3156     | 0             |
| Dmnq (2,3-dimethoxy-1,4-naphthoquinone)              | 112                                   | 0                                        | 0         | 3.3609     | 0             |
| Adenosine triphosphate                               | 21591                                 | 99                                       | 0.4177    | 1.0758     | 0.4494        |
| Cycloheximide                                        | 20712                                 | 26                                       | 0.1097    | 1.0939     | 0.1200        |
| Dasatinib                                            | 3765                                  | 2                                        | 0.0084    | 1.8343     | 0.0155        |
| Natural alpha interferon                             | 38980                                 | 0                                        | 0         | 0.8192     | 0             |

|                    |       |    |        |        |        |
|--------------------|-------|----|--------|--------|--------|
| Nickel             | 51544 | 17 | 0.0717 | 0.6979 | 0.0501 |
| Actinomycin d      | 14122 | 8  | 0.0338 | 1.2602 | 0.0425 |
| Interferon beta-1a | 1078  | 0  | 0      | 2.3775 | 0      |
| Isotretinoin       | 3318  | 1  | 0.0042 | 1.8892 | 0.0080 |
| Clinafloxacin      | 230   | 0  | 0.0000 | 3.0483 | 0      |
| Bortezomib         | 8534  | 3  | 0.0127 | 1.4789 | 0.0187 |
| Bicalutamide       | 1463  | 0  | 0      | 2.2448 | 0      |

#### 4.4.2.1. Text mining analysis of repurposed drugs for ALS

The result of text mining analysis of ALS candidate genes identified 5 novel drug candidates including Nilotinib, Trovafloxacin, Apratoxin A, Carboplatin, and Clinafloxacin (Table4.3). Among these drugs, Nilotinib, Apratoxin A, and Carboplatin are chemotherapeutic agents, whereas Clinafloxacin and Trovafloxacin are antibacterial agents. Nilotinib is a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukemia (CML) and is preferred in patients resistant to imatinib therapy or in case of imatinib intolerance(Sachdeva et al., 2024). Apratoxin A is a natural product isolated from moorea producens, a marine organism. It shows antitumor activity and stops the growth of cancer cells by inhibiting the cell cycle (Nagle et al., 2004). Carboplatin causes the death of cancer cells by binding to DNA and inhibiting its repair (Kramer et al., 2017).

**Table 4. 4.** Text Mining Results for Drug Candidates Identified by ML for ALS

| <b>Candidate<br/>Drugs in ALS</b> | <b># of Drug<br/>Related to<br/>Articles</b> | <b># of Drug-AD<br/>Related to<br/>Articles</b> | <b>TF</b> | <b>IDF</b> | <b>TF-IDF</b> |
|-----------------------------------|----------------------------------------------|-------------------------------------------------|-----------|------------|---------------|
| Insulin                           | 361574                                       | 191                                             | 0.5701    | 0.2625     | 0.1496        |
| Luteolin                          | 6265                                         | 2                                               | 0.0060    | 2.0237     | 0.0121        |
| Sevoflurane                       | 10326                                        | 10                                              | 0.0299    | 1.8067     | 0.0539        |
| Decitabine                        | 2074                                         | 1                                               | 0.0030    | 2.5038     | 0.0075        |
| Propofol                          | 23143                                        | 19                                              | 0.0567    | 1.4562     | 0.0826        |
| Dexamethasone                     | 63333                                        | 11                                              | 0.0328    | 1.0190     | 0.0335        |

|                  |       |    |        |        |        |
|------------------|-------|----|--------|--------|--------|
| Nicotine         | 43943 | 11 | 0.0328 | 1.1778 | 0.0387 |
| Arachidonic acid | 36896 | 15 | 0.0448 | 1.2537 | 0.0561 |
| Sulforaphane     | 2522  | 3  | 0.0090 | 2.4189 | 0.0217 |
| Nilotinib        | 2686  | 0  | 0.0000 | 2.3915 | 0      |
| Trovafloxacin    | 677   | 0  | 0.0000 | 2.9901 | 0      |
| Pioglitazone     | 5335  | 10 | 0.0299 | 2.0935 | 0.0625 |
| Apratoxin a      | 39    | 0  | 0.0000 | 4.2296 | 0      |
| Actinomycin d    | 14122 | 3  | 0.0090 | 1.6708 | 0.0150 |
| Hydrocortisone   | 19255 | 5  | 0.0149 | 1.5361 | 0.0229 |
| Antimony         | 7003  | 2  | 0.0060 | 1.9754 | 0.0118 |
| Carboplatin      | 15922 | 0  | 0.0000 | 1.6187 | 0      |
| Lapatinib        | 2502  | 2  | 0.0060 | 2.4224 | 0.0145 |
| Mercury          | 43833 | 50 | 0.1493 | 1.1788 | 0.1759 |
| Clinafloxacin    | 230   | 0  | 0.0000 | 3.4589 | 0      |

## 5. CONCLUSION

Holistic and integrated approaches are crucial to uncover the mechanisms underlying complex and heterogeneous diseases. Finding of molecular signatures, pathways and repurposed drugs for such diseases is only possible through integrated network medicine and systems biology approaches. Transcriptomic data provides us with information about the disease and its understanding is only possible through statistical calculations and bioinformatics approaches. In the last decades, the logarithmic development in x-ome data analysis methods and machine learnings are contributing to the treatment of diseases, spurring the discovery of promising drugs for many diseases or innovative methods for drug repurposing.

This thesis conducted a comprehensive meta-analysis of a total number of 22 transcriptomic datasets (RNASeq and microarray) separated 11 grouped across AD, PD, ALS, and HD. Each grouped were analyzed and determined the common DEGs. Then to reveal if there any network between these common DEGs, the common DEGs analyzed using a co-expression network analysis method.

- For AD,
  - Brain tissue: 2 clusters were obtained but only one of them was selected according to our cluster selection criteria: Brain Cluster 1 (10 nodes with 42 edges, and 93% of network density and the mCode Score is 9.33).
  - Frontal group: No cluster were obtained according to our cluster selection criteria
  - Hippocampus: A total 7 clusters were obtained but only one of the was met with our criteria: Hippocampus Cluster 5 (10 nodes with 20 edges, and 44% of network density and the mCode Score is 4.44).
  - Parietal lobe: 5 clusters were obtained but only one of them was selected: Parietal Cluster 2 (11 nodes with 44 edges, and 80% of network density and the mCode Score is 8.80).
- In PD, only in the dataset of substantia nigra has a total number 6 clusters, only two of them selected: Cluster 1 and Cluster 2
  - Cluster 1 consists of 159 nodes- 5380 edges with 43% network density and 68.10 mCode score.
  - Cluster 2 consists of 53 nodes- 1215 edges with 88% network density and 46.73 mCode score.

- In ALS, both groups motor neuron and muscle has selected clusters according to our cluster selection criteria.
  - Motor neuron group had 4 clusters and one of them was selected: Cluster 1 consists of 20 nodes- 130 edges with 68% network density and 13.68 mCode score.
  - Muscle group had 5 clusters and one of them was selected: Cluster 1 consists of 28 nodes- 174 edges with 46% network density and 12.89 mCode score.
- In HD no clusters were observed.

The overrepresentation analysis of significant clusters was performed using Consensus PathDB. However, the number of AD clusters was insufficient, and we couldn't obtain any pathways. Therefore, we performed the overrepresentation analysis on common DEGs for each group. Almost all the significant pathways were related to neuronal systems and its progression.

We tested the gene clusters using classification ML methods, including SVM, KNN, and RF. All clusters had an accuracy score greater than 60%, except for the gene cluster in the brain tissue of AD, affirming the correctness of our methodology. Furthermore, the genes in the clusters were scored using these three ML algorithms.

The downstream analysis continued with drug repurposing for the genes of the clusters. We obtained target drugs that could reversibly change the expression of these genes. We prepared the repurposed drug candidates and tested them with ML algorithms, including Linear Regression, SVR, RF, Gradient Boosting, and Neural Networks. The top candidate repurposed drugs were obtained, and their novelty was confirmed using text mining analysis. For AD, we identified 5 candidate drugs: Dmnq (2,3-dimethoxy-1,4-naphthoquinone), Interferon beta-1b, Cyfluthrin, Torcetrapib, and Vx. For PD, 9 candidate drugs: Interferon beta-1b, Aplidin, Androstanolone, Ribavirin, Dmnq (2,3-dimethoxy-1,4-naphthoquinone), Natural alpha interferon, Interferon beta-1a, Clinafloxacin, and Bicalutamide. Lastly, for ALS, 5 candidate drugs: Nilotinib, Trovafloxacin, Apratoxin A, Carboplatin, and Clinafloxacin. Among these novel drugs, Dmnq and Interferon beta-1b drugs were common for AD and PD, while Clinafloxacin is common for ALS and PD.

## REFERENCES

- Akkaya, D., Seyhan, G., Sari, S., & Barut, B. (2024). In vitro and in silico investigation of FDA-approved drugs to be repurposed against Alzheimer's disease. *Drug Development Research*, 85(3), e22184. <https://doi.org/10.1002/ddr.22184>
- Ambesi-Impiombato, A., Cox, K., Ramboz, S., Brunner, D., Bansal, M., & Leahy, E. (2023). Enrichment analysis of phenotypic data for drug repurposing in rare diseases. *Frontiers in Pharmacology*, 14, 1128562. <https://doi.org/10.3389/fphar.2023.1128562>
- Ament, S. A., Pearl, J. R., Cantle, J. P., Bragg, R. M., Skene, P. J., Coffey, S. R., Bergey, D. E., Wheeler, V. C., MacDonald, M. E., Baliga, N. S., Rosinski, J., Hood, L. E., Carroll, J. B., & Price, N. D. (2018). Transcriptional regulatory networks underlying gene expression changes in Huntington's disease. *Molecular Systems Biology*, 14(3), e7435. <https://doi.org/10.15252/msb.20167435>
- Arneson, D., Zhang, Y., Yang, X., & Narayanan, M. (2018). Shared mechanisms among neurodegenerative diseases: From genetic factors to gene networks. *Journal of Genetics*, 97(3), 795–806. <https://doi.org/10.1007/s12041-018-0963-3>
- Azam, S., Haque, Md. E., Balakrishnan, R., Kim, I.-S., & Choi, D.-K. (2021). The Ageing Brain: Molecular and Cellular Basis of Neurodegeneration. *Frontiers in Cell and Developmental Biology*, 9, 683459. <https://doi.org/10.3389/fcell.2021.683459>
- Badanjak, K., Fixemer, S., Smajić, S., Skupin, A., & Grünwald, A. (2021). The Contribution of Microglia to Neuroinflammation in Parkinson's Disease. *International Journal of Molecular Sciences*, 22(9), 4676. <https://doi.org/10.3390/ijms22094676>
- Bai, V. U., Cifuentes, E., Menon, M., Barrack, E. R., & Reddy, G. P.-V. (2005). Androgen receptor regulates Cdc6 in synchronized LNCaP cells progressing from G1 to S phase. *Journal of Cellular Physiology*, 204(2), 381–387. <https://doi.org/10.1002/jcp.20422>

Barnett, D. G. S., Lechner, S. A., Gammie, S. C., & Kelm-Nelson, C. A. (2023).

Thyroarytenoid Oxidative Metabolism and Synaptic Signaling Dysregulation in the Female *Pink1*  $-/-$  Rat. *The Laryngoscope*, 133(12), 3412–3421.

<https://doi.org/10.1002/lary.30768>

Barrett, T., Wilhite, S. E., Ledoux, P., Evangelista, C., Kim, I. F., Tomashevsky, M.,

Marshall, K. A., Phillippy, K. H., Sherman, P. M., Holko, M., Yefanov, A., Lee, H.,

Zhang, N., Robertson, C. L., Serova, N., Davis, S., & Soboleva, A. (2013). NCBI

GEO: Archive for functional genomics data sets--update. *Nucleic Acids Research*,

41(Database issue), D991-995. <https://doi.org/10.1093/nar/gks1193>

Basutkar, R. S., Sudarsan, P., Robin, S. M., Bhaskar, V., Viswanathan, B., & Sivasankaran, P.

(2023). Drug Repositioning of Pioglitazone in Management and Improving the

Cognitive Function among the Patients with Mild to Moderate Alzheimer's Disease: A

Systematic Review and Meta-Analysis. *Neurology India*, 71(6), 1132.

<https://doi.org/10.4103/0028-3886.391397>

Bilancio, A., Bontempo, P., Donato, M. D., Conte, M., Giovannelli, P., Altucci, L.,

Migliaccio, A., & Castoria, G. (2017). Bisphenol A induces cell cycle arrest in

primary and prostate cancer cells through EGFR/ERK/p53 signaling pathway

activation. *Oncotarget*, 8(70), 115620–115631.

<https://doi.org/10.18632/oncotarget.23360>

Bodaghi, A., Fattahi, N., & Ramazani, A. (2023). Biomarkers: Promising and valuable tools

towards diagnosis, prognosis and treatment of Covid-19 and other diseases. *Heliyon*,

9(2), e13323. <https://doi.org/10.1016/j.heliyon.2023.e13323>

Bolstad, B. M., Irizarry, R. A., Astrand, M., & Speed, T. P. (2003). A comparison of

normalization methods for high density oligonucleotide array data based on variance

- and bias. *Bioinformatics (Oxford, England)*, 19(2), 185–193.  
<https://doi.org/10.1093/bioinformatics/19.2.185>
- Bouchal, J., Baumforth, K. R. N., Šváchová, M., Murray, P. G., von Angerer, E., & Kolář, Z. (2005). Microarray analysis of bicalutamide action on telomerase activity, p53 pathway and viability of prostate carcinoma cell lines. *Journal of Pharmacy and Pharmacology*, 57(1), 83–92. <https://doi.org/10.1211/0022357055164>
- Bourdakou, M. M., Fernández-Ginés, R., Cuadrado, A., & Spyrou, G. M. (2023). Drug repurposing on Alzheimer’s disease through modulation of NRF2 neighborhood. *Redox Biology*, 67, 102881. <https://doi.org/10.1016/j.redox.2023.102881>
- Brandon, E. F. A., Sparidans, R. W., Van Ooijen, R. D., Meijerman, I., Lazaro, L. L., Manzanares, I., Beijnen, J. H., & Schellens, J. H. M. (2007). In vitro characterization of the human biotransformation pathways of aplidine, a novel marine anti-cancer drug. *Investigational New Drugs*, 25(1), 9–19. <https://doi.org/10.1007/s10637-006-7589-7>
- Breijyeh, Z., & Karaman, R. (2020). Comprehensive Review on Alzheimer’s Disease: Causes and Treatment. *Molecules*, 25(24), 5789. <https://doi.org/10.3390/molecules25245789>
- Burtscher, J., Millet, G. P., Place, N., Kayser, B., & Zanou, N. (2021). The Muscle-Brain Axis and Neurodegenerative Diseases: The Key Role of Mitochondria in Exercise-Induced Neuroprotection. *International Journal of Molecular Sciences*, 22(12), 6479. <https://doi.org/10.3390/ijms22126479>
- Butterfield, D. A., & Halliwell, B. (2019). Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nature Reviews Neuroscience*, 20(3), 148–160.  
<https://doi.org/10.1038/s41583-019-0132-6>
- Califf, R. M. (2018). Biomarker definitions and their applications. *Experimental Biology and Medicine*, 243(3), 213–221. <https://doi.org/10.1177/1535370217750088>

- Chen, B.-S., & Wu, C.-C. (2013). Systems Biology as an Integrated Platform for Bioinformatics, Systems Synthetic Biology, and Systems Metabolic Engineering. *Cells*, 2(4), 635–688. <https://doi.org/10.3390/cells2040635>
- Chowdhury, U. N., Islam, M. B., Ahmad, S., & Moni, M. A. (2020). Systems biology and bioinformatics approach to identify gene signatures, pathways and therapeutic targets of Alzheimer's disease. *Informatics in Medicine Unlocked*, 21, 100439. <https://doi.org/10.1016/j.imu.2020.100439>
- Cock, P. J. A., Antao, T., Chang, J. T., Chapman, B. A., Cox, C. J., Dalke, A., Friedberg, I., Hamelryck, T., Kauff, F., Wilczynski, B., & de Hoon, M. J. L. (2009). Biopython: Freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics*, 25(11), 1422–1423. <https://doi.org/10.1093/bioinformatics/btp163>
- Cockshott, I. D. (2004). Bicalutamide. *Clinical Pharmacokinetics*, 43(13), 855–878. <https://doi.org/10.2165/00003088-200443130-00003>
- Collu, R., Yin, Z., Giunti, E., Daley, S., Chen, M., Morin, P., Killick, R., Wong, S. T. C., & Xia, W. (2024). Effect of the ROCK inhibitor fasudil on the brain proteomic profile in the tau transgenic mouse model of Alzheimer's disease. *Frontiers in Aging Neuroscience*, 16, 1323563. <https://doi.org/10.3389/fnagi.2024.1323563>
- Comuzzi, B., Nemes, C., Schmidt, S., Jasarevic, Z., Lodde, M., Pycha, A., Bartsch, G., Offner, F., Culig, Z., & Hobisch, A. (2004). The androgen receptor co-activator CBP is up-regulated following androgen withdrawal and is highly expressed in advanced prostate cancer. *The Journal of Pathology*, 204(2), 159–166. <https://doi.org/10.1002/path.1609>
- Conte, F., Fiscon, G., Licursi, V., Bizzarri, D., D'Antò, T., Farina, L., & Paci, P. (2020). A paradigm shift in medicine: A comprehensive review of network-based approaches.

- Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1863(6), 194416. <https://doi.org/10.1016/j.bbagr.2019.194416>
- Cortes, C., & Vapnik, V. (1995). Support-vector networks. *Machine Learning*, 20(3), 273–297. <https://doi.org/10.1007/BF00994018>
- Dai, C.-Q., Luo, T.-T., Luo, S.-C., Wang, J.-Q., Wang, S.-M., Bai, Y.-H., Yang, Y.-L., & Wang, Y.-Y. (2016). p53 and mitochondrial dysfunction: Novel insight of neurodegenerative diseases. *Journal of Bioenergetics and Biomembranes*, 48(4), 337–347. <https://doi.org/10.1007/s10863-016-9669-5>
- Damme, P. van, Robberecht, W., & Bosch, L. V. D. (2017). Modelling amyotrophic lateral sclerosis: Progress and possibilities. *Disease Models & Mechanisms*. <https://www.semanticscholar.org/paper/Modelling-amyotrophic-lateral-sclerosis%3A-progress-Damme-Robberecht/149fb7eab92275887f61c9629869663ea24e2525>
- Deryusheva, E. I., Shevelyova, M. P., Rastrygina, V. A., Nemashkalova, E. L., Vologzhannikova, A. A., Machulin, A. V., Nazipova, A. A., Permyakova, M. E., Permyakov, S. E., & Litus, E. A. (2024). In Search for Low-Molecular-Weight Ligands of Human Serum Albumin That Affect Its Affinity for Monomeric Amyloid  $\beta$  Peptide. *International Journal of Molecular Sciences*, 25(9), Article 9. <https://doi.org/10.3390/ijms25094975>
- Dias, V., Junn, E., & Mouradian, M. M. (2013). The Role of Oxidative Stress in Parkinson's Disease. *Journal of Parkinson's Disease*, 3(4), 461–491. <https://doi.org/10.3233/JPD-130230>
- do Valle, I. F., & Nakaya, H. I. (2020). Network Medicine: Methods and Applications. In F. A. B. da Silva, N. Carels, M. Trindade dos Santos, & F. J. P. Lopes (Eds.), *Networks*

- in Systems Biology: Applications for Disease Modeling* (pp. 3–18). Springer International Publishing. [https://doi.org/10.1007/978-3-030-51862-2\\_1](https://doi.org/10.1007/978-3-030-51862-2_1)
- Duan, Q.-Q., Wang, H., Su, W.-M., Gu, X.-J., Shen, X.-F., Jiang, Z., Ren, Y.-L., Cao, B., Li, G.-B., Wang, Y., & Chen, Y.-P. (2024). TBK1, a prioritized drug repurposing target for amyotrophic lateral sclerosis: Evidence from druggable genome Mendelian randomization and pharmacological verification in vitro. *BMC Medicine*, 22(1), 96. <https://doi.org/10.1186/s12916-024-03314-1>
- Dumontet, T., Sahut-Barnola, I., Septier, A., Montanier, N., Plotton, I., Roucher-Boulez, F., Ducros, V., Lefrançois-Martinez, A.-M., Pointud, J.-C., Zubair, M., Morohashi, K.-I., Breault, D. T., Val, P., & Martinez, A. (2018). PKA signaling drives reticularis differentiation and sexually dimorphic adrenal cortex renewal. *JCI Insight*, 3(2). <https://doi.org/10.1172/jci.insight.98394>
- Ehrenberg, A. J., Khatun, A., Coomans, E., Betts, M. J., Capraro, F., Thijssen, E. H., Senkevich, K., Bharucha, T., Jafarpour, M., Young, P. N. E., Jagust, W., Carter, S. F., Lashley, T., Grinberg, L. T., Pereira, J. B., Mattsson-Carlsson, N., Ashton, N. J., Hanrieder, J., Zetterberg, H., ... Paterson, R. W. (2020). Relevance of biomarkers across different neurodegenerative diseases. *Alzheimer's Research & Therapy*, 12(1), 56. <https://doi.org/10.1186/s13195-020-00601-w>
- Fabregat, A., Sidiropoulos, K., Garapati, P., Gillespie, M., Hausmann, K., Haw, R., Jassal, B., Jupe, S., Korninger, F., McKay, S., Matthews, L., May, B., Milacic, M., Rothfels, K., Shamovsky, V., Webber, M., Weiser, J., Williams, M., Wu, G., ... D'Eustachio, P. (2016). The Reactome pathway Knowledgebase. *Nucleic Acids Research*, 44(D1), D481–D487. <https://doi.org/10.1093/nar/gkv1351>

- Fagone, P., Mangano, K., Martino, G., Quattropiani, M. C., Pennisi, M., Bella, R., Fisicaro, F., Nicoletti, F., & Petralia, M. C. (2022). Characterization of Altered Molecular Pathways in the Entorhinal Cortex of Alzheimer's Disease Patients and In Silico Prediction of Potential Repurposable Drugs. *Genes*, 13(4), 703. <https://doi.org/10.3390/genes13040703>
- Fan, S., Geng, Q., Pan, Z., Li, X., Tie, L., Pan, Y., & Li, X. (2012). Clarifying off-target effects for torcetrapib using network pharmacology and reverse docking approach. *BMC Systems Biology*, 6(1), 152. <https://doi.org/10.1186/1752-0509-6-152>
- FastStats. (2024, January 17). <https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm>
- Ferreira, D., Perestelo-Perez, L., Westman, E., Wahlund, L., Sarria, A., & Serrano-Aguilar, P. (2014). Meta-Review of CSF Core Biomarkers in Alzheimer's Disease: The State-of-the-Art after the New Revised Diagnostic Criteria. *Frontiers in Aging Neuroscience*, 6. <https://www.frontiersin.org/articles/10.3389/fnagi.2014.00047>
- Fiscon, G., Conte, F., Amadio, S., Volonté, C., & Paci, P. (2021). Drug Repurposing: A Network-based Approach to Amyotrophic Lateral Sclerosis. *Neurotherapeutics*, 18(3), 1678–1691. <https://doi.org/10.1007/s13311-021-01064-z>
- Friedman, J. H. (2001). Greedy function approximation: A gradient boosting machine. *The Annals of Statistics*, 29(5), 1189–1232. <https://doi.org/10.1214/aos/1013203451>
- Galbiati, M., Meroni, M., Boido, M., Cescon, M., Rusmini, P., Crippa, V., Cristofani, R., Piccolella, M., Ferrari, V., Tedesco, B., Casarotto, E., Chierichetti, M., Cozzi, M., Mina, F., Cicardi, M. E., Pedretti, S., Mitro, N., Caretto, A., Risè, P., ... Poletti, A. (2023). Bicalutamide and Trehalose Ameliorate Spinal and Bulbar Muscular Atrophy Pathology in Mice. *Neurotherapeutics*, 20(2), 524–545. <https://doi.org/10.1007/s13311-023-01343-x>

- Galvez-Llompart, M., Zanni, R., Garcia-Domenech, R., & Galvez, J. (2022). How Molecular Topology Can Help in Amyotrophic Lateral Sclerosis (ALS) Drug Development: A Revolutionary Paradigm for a Merciless Disease. *Pharmaceuticals*, 15(1), Article 1. <https://doi.org/10.3390/ph15010094>
- Gan, L., Cookson, M. R., Petrucelli, L., & La Spada, A. R. (2018). Converging pathways in neurodegeneration, from genetics to mechanisms. *Nature Neuroscience*, 21(10), 1300–1309. <https://doi.org/10.1038/s41593-018-0237-7>
- Gao, J., Mewborne, Q. T., Girdhar, A., Sheth, U., Coyne, A. N., Punathil, R., Kang, B. G., Dasovich, M., Veire, A., DeJesus Hernandez, M., Liu, S., Shi, Z., Dafinca, R., Fouquerel, E., Talbot, K., Kam, T.-I., Zhang, Y.-J., Dickson, D., Petrucelli, L., ... Zhang, K. (2022). Poly(ADP-ribose) promotes toxicity of C9ORF72 arginine-rich dipeptide repeat proteins. *Science Translational Medicine*, 14(662), eabq3215. <https://doi.org/10.1126/scitranslmed.abq3215>
- Gautier, L., Cope, L., Bolstad, B. M., & Irizarry, R. A. (2004). affy—Analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics (Oxford, England)*, 20(3), 307–315. <https://doi.org/10.1093/bioinformatics/btg405>
- Ghanem, S. S., Majbour, N. K., Vaikath, N. N., Ardah, M. T., Erskine, D., Jensen, N. M., Fayyad, M., Sudhakaran, I. P., Vasili, E., Melachroinou, K., Abdi, I. Y., Poggiolini, I., Santos, P., Dorn, A., Carloni, P., Vekrellis, K., Attems, J., McKeith, I., Outeiro, T. F., ... El-Agnaf, O. M. A. (2022).  $\alpha$ -Synuclein phosphorylation at serine 129 occurs after initial protein deposition and inhibits seeded fibril formation and toxicity. *Proceedings of the National Academy of Sciences of the United States of America*, 119(15), e2109617119. <https://doi.org/10.1073/pnas.2109617119>

- Gov, E., & Arga, K. Y. (2017). Differential co-expression analysis reveals a novel prognostic gene module in ovarian cancer. *Scientific Reports*, 7(1), 4996.
- Guijarro-Muñoz, I., Compte, M., Álvarez-Cienfuegos, A., Álvarez-Vallina, L., & Sanz, L. (2014). Lipopolysaccharide activates Toll-like receptor 4 (TLR4)-mediated NF- $\kappa$ B signaling pathway and proinflammatory response in human pericytes. *The Journal of Biological Chemistry*, 289(4), 2457–2468. <https://doi.org/10.1074/jbc.M113.521161>
- Haggard, D. E., Noyes, P. D., Waters, K. M., & Tanguay, R. L. (2018). Transcriptomic and phenotypic profiling in developing zebrafish exposed to thyroid hormone receptor agonists. *Reproductive Toxicology*, 77, 80–93. <https://doi.org/10.1016/j.reprotox.2018.02.006>
- Hasin, Y., Seldin, M., & Lusi, A. (2017). Multi-omics approaches to disease. *Genome Biology*, 18(1), 83. <https://doi.org/10.1186/s13059-017-1215-1>
- Hess-Wilson, J. K., Webb, S. L., Daly, H. K., Leung, Y.-K., Boldison, J., Comstock, C. E. S., Sartor, M. A., Ho, S.-M., & Knudsen, K. E. (2007). Unique Bisphenol A Transcriptome in Prostate Cancer: Novel Effects on ER $\beta$  Expression That Correspond to Androgen Receptor Mutation Status. *Environmental Health Perspectives*, 115(11), 1646–1653. <https://doi.org/10.1289/ehp.10283>
- Hsiao, H.-Y., Chen, Y.-C., Chen, H.-M., Tu, P.-H., & Chern, Y. (2013). A critical role of astrocyte-mediated nuclear factor- $\kappa$ B-dependent inflammation in Huntington's disease. *Human Molecular Genetics*, 22(9), 1826–1842. <https://doi.org/10.1093/hmg/ddt036>
- Huber, R. G., Pandey, S., Chhangani, D., Rincon-Limas, D. E., Staff, N. P., & Yeo, C. J. J. (2023). Identification of potential pathways and biomarkers linked to progression in

- ALS. *Annals of Clinical and Translational Neurology*, 10(2), 150–165.  
<https://doi.org/10.1002/acn3.51697>
- Huber, W., Carey, V. J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B. S., Bravo, H. C., Davis, S., Gatto, L., Girke, T., Gottardo, R., Hahne, F., Hansen, K. D., Irizarry, R. A., Lawrence, M., Love, M. I., MacDonald, J., Obenchain, V., Oleś, A. K., ... Morgan, M. (2015). Orchestrating high-throughput genomic analysis with Bioconductor. *Nature Methods*, 12(2), 115–121. <https://doi.org/10.1038/nmeth.3252>
- Interferon Beta. (2006). In *Drugs and Lactation Database (LactMed®)*. National Institute of Child Health and Human Development.  
<http://www.ncbi.nlm.nih.gov/books/NBK501168/>
- Ishihara, Y., Ishii, S., Sakai, Y., Yamamura, N., Onishi, Y., & Shimamoto, N. (2011). Crucial role of cytochrome P450 in hepatotoxicity induced by 2,3-dimethoxy-1,4-naphthoquinone in rats. *Journal of Applied Toxicology*, 31(2), 173–178.  
<https://doi.org/10.1002/jat.1578>
- Johri, A., Chandra, A., & Beal, M. F. (2013). PGC-1 $\alpha$ , Mitochondrial Dysfunction and Huntington's Disease. *Free Radical Biology & Medicine*, 62, 37.  
<https://doi.org/10.1016/j.freeradbiomed.2013.04.016>
- Kakati, T., Bhattacharyya, D. K., Barah, P., & Kalita, J. K. (2019). Comparison of Methods for Differential Co-expression Analysis for Disease Biomarker Prediction. *Computers in Biology and Medicine*, 113, 103380.  
<https://doi.org/10.1016/j.combiomed.2019.103380>
- Kamburov, A., Wierling, C., Lehrach, H., & Herwig, R. (2009). ConsensusPathDB--a database for integrating human functional interaction networks. *Nucleic Acids Research*, 37(Database issue), D623-628. <https://doi.org/10.1093/nar/gkn698>

- Kandimalla, R., & Reddy, P. H. (2017). Therapeutics of Neurotransmitters in Alzheimer's Disease. *Journal of Alzheimer's Disease : JAD*, 57(4), 1049–1069.  
<https://doi.org/10.3233/JAD-161118>
- Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, 28(1), 27–30. <https://doi.org/10.1093/nar/28.1.27>
- Kankariya, P., Rode, R., Mudaliar, K., & Hatode, P. P. (2024). *Drug Repurposing for Parkinson's Disease Using Random Walk With Restart Algorithm and the Parkinson's Disease Ontology Database* (arXiv:2404.08711). arXiv.  
<https://doi.org/10.48550/arXiv.2404.08711>
- Katsnelson, A., De Strooper, B., & Zoghbi, H. Y. (2016). Neurodegeneration: From cellular concepts to clinical applications. *Science Translational Medicine*, 8(364).  
<https://doi.org/10.1126/scitranslmed.aal2074>
- Knopman, D. S., Amieva, H., Petersen, R. C., Chételat, G., Holtzman, D. M., Hyman, B. T., Nixon, R. A., & Jones, D. T. (2021). Alzheimer disease. *Nature Reviews Disease Primers*, 7(1), 33. <https://doi.org/10.1038/s41572-021-00269-y>
- Kodavati, M., Wang, H., & Hegde, M. L. (2020). Altered Mitochondrial Dynamics in Motor Neuron Disease: An Emerging Perspective. *Cells*, 9(4), 1065.  
<https://doi.org/10.3390/cells9041065>
- Kojima, H., Iida, M., Katsura, E., Kanetoshi, A., Hori, Y., & Kobayashi, K. (2003). Effects of a diphenyl ether-type herbicide, chlornitrofen, and its amino derivative on androgen and estrogen receptor activities. *Environmental Health Perspectives*, 111(4), 497–502.  
<https://doi.org/10.1289/ehp.5724>
- Kori, M., Temiz, K., & Gov, E. (2023). Network medicine approaches for identification of novel prognostic systems biomarkers and drug candidates for papillary thyroid

- carcinoma. *Journal of Cellular and Molecular Medicine*, 27(24), 4171–4180.  
<https://doi.org/10.1111/jcmm.18002>
- Kramer, D., Stark, N., Schulz-Heddergott, R., Erytch, N., Edmunds, S., Roßmann, L., Bastians, H., Concin, N., Moll, U. M., & Dobbelsstein, M. (2017). Strong antitumor synergy between DNA crosslinking and HSP90 inhibition causes massive premitotic DNA fragmentation in ovarian cancer cells. *Cell Death & Differentiation*, 24(2), 300–316. <https://doi.org/10.1038/cdd.2016.124>
- Kujawska, M., Domanskyi, A., & Kreiner, G. (2021). Editorial: Common Pathways Linking Neurodegenerative Diseases—The Role of Inflammation. *Frontiers in Cellular Neuroscience*, 15, 754051. <https://doi.org/10.3389/fncel.2021.754051>
- Kumar, A., Sidhu, J., Goyal, A., & Tsao, J. W. (2024). Alzheimer Disease. In *StatPearls*. StatPearls Publishing. <http://www.ncbi.nlm.nih.gov/books/NBK499922/>
- Leisch, M., Egle, A., & Greil, R. (2018). Plitidepsin: A Potential New Treatment For Relapsed/Refractory Multiple Myeloma. *Future Oncology*, 15(2), 109–120.  
<https://doi.org/10.2217/fon-2018-0492>
- Li, W., Fu, Y., Halliday, G. M., & Sue, C. M. (2021). PARK Genes Link Mitochondrial Dysfunction and Alpha-Synuclein Pathology in Sporadic Parkinson’s Disease. *Frontiers in Cell and Developmental Biology*, 9.  
<https://doi.org/10.3389/fcell.2021.612476>
- Li, Y., Xia, Y., Yin, S., Wan, F., Hu, J., Kou, L., Sun, Y., Wu, J., Zhou, Q., Huang, J., Xiong, N., & Wang, T. (2021). Targeting Microglial  $\alpha$ -Synuclein/TLRs/NF-kappaB/NLRP3 Inflammasome Axis in Parkinson’s Disease. *Frontiers in Immunology*, 12.  
<https://doi.org/10.3389/fimmu.2021.719807>

- Lin, I. C., Slemper, A. E., Hwang, C., Sena-Esteves, M., Nah, H.-D., & Kirschner, R. E. (2007). Dihydrotestosterone Stimulates Proliferation and Differentiation of Fetal Calvarial Osteoblasts and Dural Cells and Induces Cranial Suture Fusion. *Plastic and Reconstructive Surgery*, 120(5), 1137.  
<https://doi.org/10.1097/01.prs.0000279527.99734.bf>
- Liu, L.-W., Lu, J., Wang, X.-H., Fu, S.-K., Li, Q., & Lin, F.-Q. (2013). Neuronal apoptosis in morphine addiction and its molecular mechanism. *International Journal of Clinical and Experimental Medicine*, 6(7), 540–545.
- Liu, Y.-L., Kong, Z., Tao, C., & Chen, Y. (2023, July 16). *Drug repositioning for Alzheimer's disease and related dementias: Network meta-analysis using individual participant data*. Alzheimer's Association International Conference.  
<https://alz.confex.com/alz/2023/meetingapp.cgi/Paper/80731>
- López-Arrieta, J., & Sanz, F. J. F. (2001). Nicotine for Alzheimer's disease. *The Cochrane Database of Systematic Reviews*, 2001(2), CD001749.  
<https://doi.org/10.1002/14651858.CD001749>
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 550.  
<https://doi.org/10.1186/s13059-014-0550-8>
- Lozupone, M., Dibello, V., Sardone, R., Castellana, F., Zupo, R., Lampignano, L., Bortone, I., Daniele, A., Bellomo, A., Solfrizzi, V., & Panza, F. (2023). The Impact of Apolipoprotein E (APOE) Epigenetics on Aging and Sporadic Alzheimer's Disease. *Biology*, 12(12), Article 12. <https://doi.org/10.3390/biology12121529>
- Luo, H., Wang, J., Li, M., Luo, J., Ni, P., Zhao, K., Wu, F.-X., & Pan, Y. (2019). Computational Drug Repositioning with Random Walk on a Heterogeneous Network.

- IEEE/ACM Transactions on Computational Biology and Bioinformatics*, 16(6), 1890–1900. <https://doi.org/10.1109/TCBB.2018.2832078>
- Mahesh Pal. (2005). Random forest classifier for remote sensing classification. *International Journal of Remote Sensing*, 26, 217–222.
- Martinez-Vicente, M., Tallochy, Z., Wong, E., Tang, G., Koga, H., Kaushik, S., de Vries, R., Arias, E., Harris, S., Sulzer, D., & Cuervo, A. M. (2010). Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. *Nature Neuroscience*, 13(5), 567–576. <https://doi.org/10.1038/nn.2528>
- Masrori, P., Beckers, J., Gossye, H., & Van Damme, P. (2022). The role of inflammation in neurodegeneration: Novel insights into the role of the immune system in C9orf72 HRE-mediated ALS/FTD. *Molecular Neurodegeneration*, 17(1), 22. <https://doi.org/10.1186/s13024-022-00525-z>
- Masrori, P., & Van Damme, P. (2020). Amyotrophic lateral sclerosis: A clinical review. *European Journal of Neurology*, 27(10), 1918–1929. <https://doi.org/10.1111/ene.14393>
- Merelli, A., Rodríguez, J. C. G., Folch, J., Regueiro, M. R., Camins, A., & Lazarowski, A. (2018). Understanding the Role of Hypoxia Inducible Factor During Neurodegeneration for New Therapeutics Opportunities. *Current Neuropharmacology*, 16(10), 1484–1498. <https://doi.org/10.2174/1570159X16666180110130253>
- Miller, T. M., Cudkowicz, M. E., Genge, A., Shaw, P. J., Sobue, G., Bucelli, R. C., Chiò, A., Van Damme, P., Ludolph, A. C., Glass, J. D., Andrews, J. A., Babu, S., Benatar, M., McDermott, C. J., Cochrane, T., Chary, S., Chew, S., Zhu, H., Wu, F., ... Fradette, S.

- (2022). Trial of Antisense Oligonucleotide Tofersen for *SOD1* ALS. *New England Journal of Medicine*, 387(12), 1099–1110. <https://doi.org/10.1056/NEJMoa2204705>
- Misik, J., Pavlikova, R., Cabal, J., & Kuca, K. (2015). Acute toxicity of some nerve agents and pesticides in rats. *Drug and Chemical Toxicology*, 38(1), 32–36. <https://doi.org/10.3109/01480545.2014.900070>
- Morfini, G. A., Burns, M., Binder, L. I., Kanaan, N. M., LaPointe, N., Bosco, D. A., Brown, R. H., Brown, H., Tiwari, A., Hayward, L., Edgar, J., Nave, K.-A., Garberrn, J., Atagi, Y., Song, Y., Pigino, G., & Brady, S. T. (2009). Axonal Transport Defects in Neurodegenerative Diseases. *Journal of Neuroscience*, 29(41), 12776–12786. <https://doi.org/10.1523/JNEUROSCI.3463-09.2009>
- Nagle, D. G., Zhou, Y.-D., Mora, F. D., Mohammed, K. A., & Kim, Y.-P. (2004). Mechanism Targeted Discovery of Antitumor Marine Natural Products. *Current Medicinal Chemistry*, 11(13), 1725–1756. <https://doi.org/10.2174/0929867043364991>
- Narendra, D., Tanaka, A., Suen, D.-F., & Youle, R. J. (2009). Parkin-induced mitophagy in the pathogenesis of Parkinson disease. *Autophagy*, 5(5), 706–708. <https://doi.org/10.4161/auto.5.5.8505>
- Nguyen, T.-T., Dao, T.-K., Pham, D.-T., & Duong, T.-H. (2024). Exploring the Molecular Terrain: A Survey of Analytical Methods for Biological Network Analysis. *Symmetry*, 16(4), Article 4. <https://doi.org/10.3390/sym16040462>
- Nyström, K., Waldenström, J., Tang, K.-W., & Lagging, M. (2019). Ribavirin: Pharmacology, Multiple Modes of Action and Possible Future Perspectives. *Future Virology*, 14(3), 153–160. <https://doi.org/10.2217/fvl-2018-0166>

- Okada, S., Ohzeki, M., & Taguchi, S. (2019). Efficient partition of integer optimization problems with one-hot encoding. *Scientific Reports*, 9(1), 13036.  
<https://doi.org/10.1038/s41598-019-49539-6>
- Park, S., Kim, D., Song, J., & Joo, J. W. J. (2021). An Integrative Transcriptome-Wide Analysis of Amyotrophic Lateral Sclerosis for the Identification of Potential Genetic Markers and Drug Candidates. *International Journal of Molecular Sciences*, 22(6), 3216. <https://doi.org/10.3390/ijms22063216>
- Parkinson's statistics: How common is it?* (2023, August 4).  
<https://www.medicalnewstoday.com/articles/how-common-is-parkinsons-disease>
- Pathak, G. A., Wendt, F. R., Levey, D. F., Mecca, A. P., van Dyck, C. H., Gelernter, J., & Polimanti, R. (2021). Pleiotropic effects of telomere length loci with brain morphology and brain tissue expression. *Human Molecular Genetics*, 30(14), 1360–1370. <https://doi.org/10.1093/hmg/ddab102>
- Pchitskaya, E., Popugaeva, E., & Bezprozvanny, I. (2018). Calcium signaling and molecular mechanisms underlying neurodegenerative diseases. *Cell Calcium*, 70, 87–94.  
<https://doi.org/10.1016/j.ceca.2017.06.008>
- Peixoto, C. A., Nunes, A. K. S., & Garcia-Osta, A. (2015). Phosphodiesterase-5 Inhibitors: Action on the Signaling Pathways of Neuroinflammation, Neurodegeneration, and Cognition. *Mediators of Inflammation*, 2015(1), 940207.  
<https://doi.org/10.1155/2015/940207>
- Petri, S., Grehl, T., Grosskreutz, J., Hecht, M., Hermann, A., Jesse, S., Lingor, P., Löscher, W., Maier, A., Schoser, B., Weber, M., & Ludolph, A. C. (2023). Guideline “Motor neuron diseases” of the German Society of Neurology (Deutsche Gesellschaft für

- Neurologie). *Neurological Research and Practice*, 5(1), 25.  
<https://doi.org/10.1186/s42466-023-00251-x>
- PGC-1 $\alpha$* , *Mitochondrial Dysfunction and Huntington's Disease.pdf*. (n.d.).
- Pickrell, A. M., & Youle, R. J. (2015). The Roles of PINK1, Parkin, and Mitochondrial Fidelity in Parkinson's Disease. *Neuron*, 85(2), 257–273.  
<https://doi.org/10.1016/j.neuron.2014.12.007>
- Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A.-E., & Lang, A. E. (2017). Parkinson disease. *Nature Reviews Disease Primers*, 3(1), 17013. <https://doi.org/10.1038/nrdp.2017.13>
- Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., & Patel, B. K. (2019). Molecular Mechanisms of TDP-43 Misfolding and Pathology in Amyotrophic Lateral Sclerosis. *Frontiers in Molecular Neuroscience*, 12. <https://doi.org/10.3389/fnmol.2019.00025>
- Qian, L., Flood, P. M., & Hong, J.-S. (2010). Neuroinflammation is a key player in Parkinson's disease and a prime target for therapy. *Journal of Neural Transmission*, 117(8), 971–979. <https://doi.org/10.1007/s00702-010-0428-1>
- Querfurth, H., & Lee, H.-K. (2021). Mammalian/mechanistic target of rapamycin (mTOR) complexes in neurodegeneration. *Molecular Neurodegeneration*, 16(1), 44.  
<https://doi.org/10.1186/s13024-021-00428-5>
- Raza, A., & Muddassar, M. (2022). *Network Based Identification of Holistic Drug Target for Parkinson Disease and Deep Learning assisted Drug Repurposing*.  
<https://doi.org/10.1101/2022.11.18.515243>
- Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, 43(7), e47–e47. <https://doi.org/10.1093/nar/gkv007>

- Rodriguez, S., Hug, C., Todorov, P., Moret, N., Boswell, S. A., Evans, K., Zhou, G., Johnson, N. T., Hyman, B. T., Sorger, P. K., Albers, M. W., & Sokolov, A. (2021). Machine learning identifies candidates for drug repurposing in Alzheimer's disease. *Nature Communications*, 12, 1033. <https://doi.org/10.1038/s41467-021-21330-0>
- Rumelhart, D. E., Hinton, G. E., & Williams, R. J. (1986). Learning representations by back-propagating errors. *Nature*, 323(6088), 533–536. <https://doi.org/10.1038/323533a0>
- Sachdeva, M., Singh, J., Dayal, S., & Gupta, A. (2024). Imatinib- and Nilotinib-Induced Lichenoid Eruption in Chronic Myeloid Leukemia: A Rare Case Report. *Indian Dermatology Online Journal*, 15(3), 492. [https://doi.org/10.4103/idoj.idoj\\_229\\_23](https://doi.org/10.4103/idoj.idoj_229_23)
- Sasaki, S. (2011). Autophagy in Spinal Cord Motor Neurons in Sporadic Amyotrophic Lateral Sclerosis. *Journal of Neuropathology & Experimental Neurology*, 70(5), 349–359. <https://doi.org/10.1097/NEN.0b013e3182160690>
- Sathish, P., Paramasivan, V., Palani, V., & Sivanesan, K. (2011). N-acetylcysteine attenuates dimethylnitrosamine induced oxidative stress in rats. *European Journal of Pharmacology*, 654(2), 181–186. <https://doi.org/10.1016/j.ejphar.2010.10.080>
- Savino, A., Provero, P., & Poli, V. (2020). Differential Co-Expression Analyses Allow the Identification of Critical Signalling Pathways Altered during Tumour Transformation and Progression. *International Journal of Molecular Sciences*, 21(24), 9461. <https://doi.org/10.3390/ijms21249461>
- Sehrawat, A., Gao, L., Wang, Y., Bankhead, A., McWeeney, S. K., King, C. J., Schwartzman, J., Urrutia, J., Bisson, W. H., Coleman, D. J., Joshi, S. K., Kim, D.-H., Sampson, D. A., Weinmann, S., Kallakury, B. V. S., Berry, D. L., Haque, R., Van Den Eeden, S. K., Sharma, S., ... Alumkal, J. J. (2018). LSD1 activates a lethal prostate cancer gene network independently of its demethylase function. *Proceedings of the National*

- Academy of Sciences*, 115(18), E4179–E4188.  
<https://doi.org/10.1073/pnas.1719168115>
- Shah, S., Prasad, S., & Knudsen, K. E. (2012). Targeting Pioneering Factor and Hormone Receptor Cooperative Pathways to Suppress Tumor Progression. *Cancer Research*, 72(5), 1248–1259. <https://doi.org/10.1158/0008-5472.CAN-11-0943>
- Sharma, C., Kim, S., Nam, Y., Jung, U. J., & Kim, S. R. (2021). Mitochondrial Dysfunction as a Driver of Cognitive Impairment in Alzheimer’s Disease. *International Journal of Molecular Sciences*, 22(9), 4850. <https://doi.org/10.3390/ijms22094850>
- Siavelis, J. C., Bourdakou, M. M., Athanasiadis, E. I., Spyrou, G. M., & Nikita, K. S. (2016). Bioinformatics methods in drug repurposing for Alzheimer’s disease. *Briefings in Bioinformatics*, 17(2), 322–335. <https://doi.org/10.1093/bib/bbv048>
- Silva, M. V. F., Loures, C. de M. G., Alves, L. C. V., de Souza, L. C., Borges, K. B. G., & Carvalho, M. das G. (2019). Alzheimer’s disease: Risk factors and potentially protective measures. *Journal of Biomedical Science*, 26(1), 33.  
<https://doi.org/10.1186/s12929-019-0524-y>
- Singh, A. J., Ramsey, S. A., Filtz, T. M., & Kioussi, C. (2018). Differential gene regulatory networks in development and disease. *Cellular and Molecular Life Sciences*, 75(6), 1013–1025. <https://doi.org/10.1007/s00018-017-2679-6>
- Singh, H., & Agrawal, D. K. (2022). Therapeutic potential of targeting the receptor for advanced glycation end products (RAGE) by small molecule inhibitors. *Drug Development Research*, 83(6), 1257–1269. <https://doi.org/10.1002/ddr.21971>
- Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., & Knudsen, T. B. (2013). Profiling 976 ToxCast

- Chemicals across 331 Enzymatic and Receptor Signaling Assays. *Chemical Research in Toxicology*, 26(6), 878–895. <https://doi.org/10.1021/tx400021f>
- Su, X., Li, G., Deng, Y., & Chang, D. (2020). Cholesteryl ester transfer protein inhibitors in precision medicine. *Clinica Chimica Acta*, 510, 733–740. <https://doi.org/10.1016/j.cca.2020.09.012>
- Sunildutt, N., Ahmed, F., Chethikkattuveli Salih, A. R., Lim, J. H., & Choi, K. H. (2024). Integrating Transcriptomic and Structural Insights: Revealing Drug Repurposing Opportunities for Sporadic ALS. *ACS Omega*, 9(3), 3793–3806. <https://doi.org/10.1021/acsomega.3c07296>
- Turanli, B., Gulfidan, G., Aydogan, O. O., Kula, C., Selvaraj, G., & Arga, K. Y. (2024). Genome-scale metabolic models in translational medicine: The current status and potential of machine learning in improving the effectiveness of the models. *Molecular Omics*, 20(4), 234–247. <https://doi.org/10.1039/D3MO00152K>
- Uddin, Md. S., Mamun, A. A., Labu, Z. K., Hidalgo-Lanussa, O., Barreto, G. E., & Ashraf, G. M. (2019). Autophagic dysfunction in Alzheimer's disease: Cellular and molecular mechanistic approaches to halt Alzheimer's pathogenesis. *Journal of Cellular Physiology*, 234(6), 8094–8112. <https://doi.org/10.1002/jcp.27588>
- Unal, U., Comertpay, B., Demirtas, T. Y., & Gov, E. (2022). Drug repurposing for rheumatoid arthritis: Identification of new drug candidates via bioinformatics and text mining analysis. *Autoimmunity*, 55(3), 147–156.
- UNAL, U., & Göv, E. (2022). *Bioinformatics prediction and analysis of co-altered genes as prognostic targets for papillary thyroid carcinom*. <https://doi.org/10.21203/rs.3.rs-1697373/v1>

- Upadhyaya, R., Zingg, W., Shetty, S., & Shetty, A. K. (2020). Astrocyte-derived extracellular vesicles: Neuroreparative properties and role in the pathogenesis of neurodegenerative disorders. *Journal of Controlled Release*, 323, 225–239.  
<https://doi.org/10.1016/j.jconrel.2020.04.017>
- van der Wijst, M. G. P., Brugge, H., de Vries, D. H., Deelen, P., Swertz, M. A., & Franke, L. (2018). Single-cell RNA sequencing identifies celltype-specific cis-eQTLs and co-expression QTLs. *Nature Genetics*, 50(4), 493–497. <https://doi.org/10.1038/s41588-018-0089-9>
- Van Es, M. A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R. J., Veldink, J. H., & Van Den Berg, L. H. (2017). Amyotrophic lateral sclerosis. *The Lancet*, 390(10107), 2084–2098. [https://doi.org/10.1016/S0140-6736\(17\)31287-4](https://doi.org/10.1016/S0140-6736(17)31287-4)
- Van Horssen, J., Van Schaik, P., & Witte, M. (2019). Inflammation and mitochondrial dysfunction: A vicious circle in neurodegenerative disorders? *Neuroscience Letters*, 710, 132931. <https://doi.org/10.1016/j.neulet.2017.06.050>
- Vargas, D. M., De Bastiani, M. A., Parsons, R. B., & Klamt, F. (2021). Parkinson's Disease Master Regulators on Substantia Nigra and Frontal Cortex and Their Use for Drug Repositioning. *Molecular Neurobiology*, 58(4), 1517–1534.  
<https://doi.org/10.1007/s12035-020-02203-x>
- Venegas, C., & Heneka, M. T. (2017). Danger-associated molecular patterns in Alzheimer's disease. *Journal of Leukocyte Biology*, 101(1), 87–98.  
<https://doi.org/10.1189/jlb.3MR0416-204R>
- Wang, Z., He, E., Sani, K., Jagodnik, K. M., Silverstein, M. C., & Ma'ayan, A. (2019). Drug Gene Budger (DGB): An application for ranking drugs to modulate a specific gene

- based on transcriptomic signatures. *Bioinformatics*, 35(7), 1247–1248.  
<https://doi.org/10.1093/bioinformatics/bty763>
- Waskom, M. (2021). seaborn: Statistical data visualization. *Journal of Open Source Software*, 6(60), 3021. <https://doi.org/10.21105/joss.03021>
- Webster, C. P., Smith, E. F., Bauer, C. S., Moller, A., Hautbergue, G. M., Ferraiuolo, L., Myszczyńska, M. A., Higginbottom, A., Walsh, M. J., Whitworth, A. J., Kaspar, B. K., Meyer, K., Shaw, P. J., Grierson, A. J., & De Vos, K. J. (2016). The C9orf72 protein interacts with Rab1a and the ULK1 complex to regulate initiation of autophagy. *The EMBO Journal*, 35(15), 1656–1676.  
<https://doi.org/10.15252/emj.201694401>
- Wen, J., Zhu, H., Murakami, S., Leung, P. C. K., & MacCalman, C. D. (2006). Regulation of A Disintegrin And Metalloproteinase with Thrombospondin Repeats-1 Expression in Human Endometrial Stromal Cells by Gonadal Steroids Involves Progestins, Androgens, and Estrogens. *The Journal of Clinical Endocrinology & Metabolism*, 91(12), 4825–4835. <https://doi.org/10.1210/jc.2006-1567>
- Wright, G. E. B., Caron, N. S., Ng, B., Casal, L., Casazza, W., Xu, X., Ooi, J., Pouladi, M. A., Mostafavi, S., Ross, C. J. D., & Hayden, M. R. (2020). Gene expression profiles complement the analysis of genomic modifiers of the clinical onset of Huntington disease. *Human Molecular Genetics*, 29(16), 2788–2802.  
<https://doi.org/10.1093/hmg/ddaa184>
- Yanai, Y., Sanou, O., Kayano, T., Ariyasu, H., Yamamoto, K., Yamauchi, H., Ikegami, H., & Kurimoto, M. (2001). Analysis of the Antiviral Activities of Natural IFN- $\alpha$  Preparations and Their Subtype Compositions. *Journal of Interferon & Cytokine Research*, 21(10), 835–841. <https://doi.org/10.1089/107999001753238088>

- Yang, H., Yang, S., Huang, S., Tang, B., & Guo, J. (2017). Microglial Activation in the Pathogenesis of Huntington's Disease. *Frontiers in Aging Neuroscience*.  
<https://www.semanticscholar.org/paper/Microglial-Activation-in-the-Pathogenesis-of-Yang-Yang/2ff3c759075786ce3b095e35f31f17bbe851ff7e>
- Yang, L. G., March, Z. M., Stephenson, R. A., & Narayan, P. S. (2023). Apolipoprotein E in lipid metabolism and neurodegenerative disease. *Trends in Endocrinology & Metabolism*, 34(8), 430–445. <https://doi.org/10.1016/j.tem.2023.05.002>
- Yuan, L., Guo, Y., Wen, S., & Deng, H. (2022). Editorial: Genetic and Epigenetic Basis of Neurodegenerative Diseases. *Frontiers in Aging Neuroscience*, 14.  
<https://doi.org/10.3389/fnagi.2022.958847>
- Zhang, Q., Heng, Y., Yuan, Y., & Chen, N. (2017). Pathological  $\alpha$ -synuclein exacerbates the progression of Parkinson's disease through microglial activation. *Toxicology Letters*.  
<https://www.semanticscholar.org/paper/Pathological-%CE%B1-synuclein-exacerbates-the-of-disease-Zhang-Heng/7c6ee4437ea50f14ddfeeff3a5259bbaf177f2fa>
- Zhang, W., Xiao, D., Mao, Q., & Xia, H. (2023). Role of neuroinflammation in neurodegeneration development. *Signal Transduction and Targeted Therapy*, 8(1), 1–32. <https://doi.org/10.1038/s41392-023-01486-5>
- Zhang, X., & Che, C. (2021). Drug Repurposing for Parkinson's Disease by Integrating Knowledge Graph Completion Model and Knowledge Fusion of Medical Literature. *Future Internet*, 13(1), Article 1. <https://doi.org/10.3390/fi13010014>
- Zhao, M., & Yang, C. C. (2018). Drug Repositioning to Accelerate Drug Development Using Social Media Data: Computational Study on Parkinson Disease. *Journal of Medical Internet Research*, 20(10), e271. <https://doi.org/10.2196/jmir.9646>

## APPENDICES

**Supplementary Table 4. 1.** Machine learning analysis for candidate drugs targeting Cluster 5 genes in hippocampus tissue of AD

| Drug Name                               | GBR    | LR     | MLPRegressor | RFR    | SVR    |
|-----------------------------------------|--------|--------|--------------|--------|--------|
| 4-hydroxynonenal                        | 0.1969 | 0.1117 | 0.1210       | 0.1603 | 0.1607 |
| 4-hydroxytamoxifen                      | 0.2188 | 0.5571 | 0.1013       | 0.1908 | 0.2024 |
| Abiraterone                             | 0.3455 | 0.4242 | 0.3932       | 0.3816 | 0.3555 |
| Actinomycin d                           | 0.2339 | 0.2679 | 0.1466       | 0.1859 | 0.2051 |
| Adenosine triphosphate                  | 0.3596 | 0.4554 | 0.4771       | 0.4187 | 0.4109 |
| Alfacalcidol                            | 0.2706 | 0.1898 | 0.2064       | 0.2052 | 0.2325 |
| Aminolevulinic acid                     | 0.2434 | 0.2054 | 0.1597       | 0.1836 | 0.1881 |
| Androstanolone                          | 0.3455 | 0.2523 | 0.2561       | 0.2802 | 0.2988 |
| Antimony                                | 0.4050 | 0.4946 | 0.5404       | 0.4449 | 0.4151 |
| Aplidin                                 | 0.2206 | 0.1898 | 0.1549       | 0.1581 | 0.1954 |
| Argyria a                               | 0.3205 | 0.2992 | 0.2300       | 0.2529 | 0.2917 |
| Bexarotene                              | 0.3455 | 0.3227 | 0.3283       | 0.3164 | 0.3515 |
| Bisphenol a                             | 0.2485 | 0.1742 | 0.2030       | 0.2159 | 0.2245 |
| Bortezomib                              | 0.3455 | 0.4867 | 0.4161       | 0.4082 | 0.3944 |
| Bpde                                    | 0.3455 | 0.3851 | 0.2944       | 0.3272 | 0.3204 |
| Cadmium                                 | 0.3455 | 0.2679 | 0.2803       | 0.2973 | 0.2753 |
| Calcitriol                              | 0.3149 | 0.2445 | 0.2861       | 0.2485 | 0.3029 |
| Carboplatin                             | 0.3455 | 0.4711 | 0.4351       | 0.4003 | 0.3693 |
| Carboplatin (30 h)                      | 0.3455 | 0.4555 | 0.4398       | 0.4147 | 0.3515 |
| Carboplatin (36 h)                      | 0.4502 | 0.5415 | 0.5791       | 0.4828 | 0.4653 |
| Cediranib                               | 0.2733 | 0.1976 | 0.2080       | 0.2203 | 0.2870 |
| Celecoxib                               | 0.2189 | 0.0961 | 0.1994       | 0.1612 | 0.1818 |
| Chlorpyrifos                            | 0.3455 | 0.1899 | 0.2837       | 0.2904 | 0.3515 |
| Chromium                                | 0.3455 | 0.3383 | 0.3331       | 0.3584 | 0.3515 |
| Cisplatin                               | 0.3455 | 0.3226 | 0.3327       | 0.3643 | 0.3729 |
| Clinafloxacin                           | 0.3666 | 0.4711 | 0.5103       | 0.4470 | 0.4128 |
| Curcumin                                | 0.2636 | 0.0649 | 0.1836       | 0.2008 | 0.2456 |
| Cytarabine                              | 0.5202 | 0.5414 | 0.4538       | 0.5730 | 0.3139 |
| Dasatinib                               | 0.3455 | 0.3461 | 0.3408       | 0.3069 | 0.3315 |
| Diclofenac                              | 0.2240 | 0.1195 | 0.1146       | 0.1643 | 0.1533 |
| Dmnq (2,3-dimethoxy-1,4-naphthoquinone) | 0.3455 | 0.3305 | 0.3660       | 0.3579 | 0.3515 |
| Doxorubicin                             | 0.5095 | 0.5726 | 0.5240       | 0.5161 | 0.3489 |
| Doxycycline                             | 0.3455 | 0.2758 | 0.2527       | 0.2794 | 0.3494 |
| Estradiol                               | 0.4510 | 0.4867 | 0.4823       | 0.4739 | 0.3643 |
| Etanercept                              | 0.3455 | 0.4008 | 0.3601       | 0.3806 | 0.3538 |
| Egfr-specific pd173074 inhibitor        | 0.3957 | 0.4320 | 0.4660       | 0.4420 | 0.4446 |
| Gamma-tocotrienol                       | 0.3455 | 0.4008 | 0.3543       | 0.3581 | 0.3515 |

|                                                      |        |        |        |        |        |
|------------------------------------------------------|--------|--------|--------|--------|--------|
| Imatinib                                             | 0.3455 | 0.3656 | 0.3753 | 0.3641 | 0.3654 |
| Interferon beta-1b                                   | 0.2022 | 0.1508 | 0.1464 | 0.1596 | 0.2042 |
| Interferon gamma-1b                                  | 0.2982 | 0.1859 | 0.1827 | 0.2196 | 0.2103 |
| Isotretinoin                                         | 0.3587 | 0.4321 | 0.4562 | 0.4351 | 0.3743 |
| Lapatinib                                            | 0.3455 | 0.4008 | 0.3175 | 0.3533 | 0.3424 |
| Lipopolysaccharide                                   | 0.3877 | 0.5180 | 0.4956 | 0.4361 | 0.3969 |
| Lysophosphatidic acid                                | 0.3455 | 0.3773 | 0.3896 | 0.3952 | 0.4113 |
| Mercury                                              | 0.4053 | 0.4946 | 0.4625 | 0.4452 | 0.4155 |
| Mesalazine                                           | 0.3332 | 0.1899 | 0.2245 | 0.2730 | 0.3246 |
| Metformin                                            | 0.3455 | 0.3500 | 0.3368 | 0.3314 | 0.3342 |
| Methotrexate                                         | 0.2486 | 0.0258 | 0.1618 | 0.2095 | 0.2392 |
| Mk-886                                               | 0.3455 | 0.2602 | 0.3156 | 0.3266 | 0.3515 |
| Motexafin gadolinium (4 h)                           | 0.2471 | 0.2250 | 0.1938 | 0.1922 | 0.2478 |
| Natural alpha interferon                             | 0.1773 | 0.1078 | 0.1026 | 0.1206 | 0.1694 |
| Neocarzinostatin                                     | 0.3260 | 0.1937 | 0.2231 | 0.2647 | 0.2670 |
| Nickel                                               | 0.3455 | 0.3461 | 0.2876 | 0.3462 | 0.3342 |
| Nitric oxide                                         | 0.4099 | 0.4712 | 0.5306 | 0.4693 | 0.4276 |
| Pd173074                                             | 0.3455 | 0.3462 | 0.2895 | 0.3322 | 0.3515 |
| Phorbol 12-myristate 13-acetate (pma)                | 0.3455 | 0.3383 | 0.3317 | 0.3443 | 0.3515 |
| Plx4032                                              | 0.3455 | 0.3383 | 0.3489 | 0.3342 | 0.3544 |
| Plx4720                                              | 0.3455 | 0.3793 | 0.3593 | 0.3931 | 0.3424 |
| Promegestone                                         | 0.3530 | 0.4261 | 0.4427 | 0.4035 | 0.3634 |
| Puromycin, 2xec50, 3 d                               | 2.5201 | 2.6665 | 2.5912 | 1.7192 | 1.3499 |
| Puromycin, ec50, 1 d                                 | 0.2665 | 0.1821 | 0.1644 | 0.2276 | 0.2527 |
| Puromycin, ec50, 3 d                                 | 0.3455 | 0.2524 | 0.2692 | 0.2874 | 0.3515 |
| R1881                                                | 0.3455 | 0.4086 | 0.3634 | 0.3830 | 0.3342 |
| Resveratrol                                          | 0.4306 | 0.4633 | 0.5170 | 0.4816 | 0.4988 |
| Ribavirin                                            | 0.3455 | 0.2915 | 0.2643 | 0.2949 | 0.3515 |
| Sevoflurane                                          | 0.3455 | 0.4008 | 0.4771 | 0.4035 | 0.3515 |
| Thapsigargin                                         | 0.2749 | 0.2328 | 0.2290 | 0.2293 | 0.2382 |
| Tretinoin                                            | 0.3455 | 0.3627 | 0.3706 | 0.3784 | 0.3500 |
| Triiodothyronine-[13c6] hydrochloride (t3 thyronine) | 0.1423 | 0.1390 | 0.1008 | 0.0987 | 0.1498 |
| Ubiquinol                                            | 0.4544 | 0.5521 | 0.4842 | 0.4976 | 0.4737 |
| Vanadium pentoxide                                   | 0.3306 | 0.3329 | 0.2794 | 0.2708 | 0.3342 |
| Vemurafenib                                          | 0.3642 | 0.4468 | 0.3890 | 0.4148 | 0.4107 |
| Y15                                                  | 0.3629 | 0.4333 | 0.4674 | 0.4260 | 0.4098 |

**Supplementary Table 4. 2.** Machine learning analysis for candidate drugs targeting cluster1 genes in brain tissue of AD

| Drug Name | GBR | LR | MLPRegressor | RFR | SVR |
|-----------|-----|----|--------------|-----|-----|
|-----------|-----|----|--------------|-----|-----|

|                                                      |        |        |        |        |        |
|------------------------------------------------------|--------|--------|--------|--------|--------|
| Antimony                                             | 2.0393 | 2.1430 | 2.1375 | 2.1131 | 1.6768 |
| Bexarotene                                           | 1.1616 | 1.1202 | 1.1424 | 1.1251 | 1.2571 |
| Bicalutamide                                         | 2.0526 | 2.2915 | 2.2942 | 1.8956 | 2.0998 |
| Chromium                                             | 2.0132 | 2.2384 | 2.2131 | 1.6116 | 2.0998 |
| Cisplatin                                            | 0.5199 | 0.3477 | 0.3542 | 0.4439 | 0.4523 |
| Cyfluthrin                                           | 0.7328 | 0.5032 | 0.5300 | 0.6643 | 0.6033 |
| Cytarabine                                           | 1.7194 | 1.8547 | 1.8287 | 1.7796 | 1.7115 |
| Dexamethasone                                        | 0.6190 | 0.3814 | 0.3949 | 0.5151 | 0.4814 |
| Diclofenac                                           | 1.9292 | 2.1593 | 2.1676 | 1.5303 | 2.0592 |
| Dmnq (2,3-dimethoxy-1,4-naphthoquinone)              | 1.1616 | 1.1301 | 1.1122 | 1.1022 | 1.2571 |
| Doxorubicin                                          | 2.6374 | 2.8106 | 2.8673 | 2.6321 | 1.8365 |
| Estradiol                                            | 0.4979 | 0.2550 | 0.2833 | 0.4291 | 0.3553 |
| Harman                                               | 0.4650 | 0.2125 | 0.2170 | 0.4238 | 0.3123 |
| Imatinib                                             | 0.9229 | 0.7950 | 0.8130 | 0.8193 | 0.6477 |
| Interferon beta-1b                                   | 0.8086 | 0.5808 | 0.5884 | 0.7219 | 0.6807 |
| Lipopolysaccharide                                   | 2.6831 | 2.8425 | 2.8690 | 2.5111 | 2.4355 |
| Mk-886                                               | 0.7968 | 0.5627 | 0.5492 | 0.5708 | 0.6626 |
| Mln4924                                              | 1.6054 | 1.8574 | 1.8360 | 1.4449 | 1.7577 |
| Natural alpha interferon                             | 0.4785 | 0.3237 | 0.3198 | 0.4246 | 0.4523 |
| Sulforaphane                                         | 2.3359 | 2.5784 | 2.6044 | 1.9416 | 2.0998 |
| Torcetrapib                                          | 0.4946 | 0.2474 | 0.2997 | 0.5445 | 0.3474 |
| Tretinoin                                            | 1.3499 | 1.6000 | 1.6207 | 1.4024 | 1.5002 |
| Triiodothyronine-[13c6] hydrochloride (t3 thyronine) | 0.3594 | 0.1114 | 0.0946 | 0.4769 | 0.2117 |
| Ubiquinol                                            | 2.0019 | 2.1671 | 2.2020 | 1.9074 | 2.0618 |
| Vanadium pentoxide                                   | 0.6471 | 0.4172 | 0.3935 | 0.6102 | 0.5175 |
| Vemurafenib                                          | 1.5184 | 1.6814 | 1.6871 | 1.5769 | 1.6300 |
| Vx                                                   | 0.9093 | 0.6613 | 0.6734 | 0.7051 | 0.7610 |

**Supplementary Table 4. 3.** Machine learning analysis for candidate drugs targeting cluster1 genes in parietal tissue of AD

| Drug Name              | GBR    | LR     | MLPRegressor | RFR    | SVR    |
|------------------------|--------|--------|--------------|--------|--------|
| 4-hydroxynonenal       | 0.2215 | 0.1469 | 0.1775       | 0.2378 | 0.2473 |
| 4-hydroxytamoxifen     | 0.2049 | 0.1341 | 0.0657       | 0.2259 | 0.2340 |
| Adenosine triphosphate | 0.5259 | 0.5987 | 0.6062       | 0.5015 | 0.4987 |
| Androstanolone         | 0.4966 | 0.5669 | 0.5484       | 0.4724 | 0.4669 |
| Arachidonic acid       | 1.5636 | 1.6333 | 1.6247       | 1.2768 | 1.3495 |
| Argyria                | 0.3590 | 0.2867 | 0.2883       | 0.3116 | 0.3867 |
| Bisphenol a            | 0.3769 | 0.3506 | 0.3468       | 0.3672 | 0.3453 |
| Bortezomib             | 0.5795 | 0.6552 | 0.6279       | 0.5484 | 0.5552 |
| Bpde                   | 0.3769 | 0.4504 | 0.4387       | 0.4096 | 0.3935 |
| Cadmium                | 0.1104 | 0.0396 | 0.0113       | 0.1452 | 0.1396 |
| Cediranib              | 0.1125 | 0.0419 | 0.0638       | 0.1413 | 0.1417 |
| Cisplatin              | 0.3769 | 0.3892 | 0.3853       | 0.3952 | 0.3845 |

|                                                         |        |        |        |        |        |
|---------------------------------------------------------|--------|--------|--------|--------|--------|
| Curcumin                                                | 0.3261 | 0.2574 | 0.2175 | 0.3008 | 0.3573 |
| Cycloheximide                                           | 0.3681 | 0.2927 | 0.2636 | 0.3289 | 0.3927 |
| Dasatinib                                               | 0.3769 | 0.4310 | 0.4445 | 0.3909 | 0.3935 |
| Doxorubicin                                             | 0.5091 | 0.5328 | 0.5173 | 0.5213 | 0.3394 |
| Estradiol                                               | 0.4596 | 0.5291 | 0.5309 | 0.4617 | 0.4291 |
| Fgfr-specific pd173074 inhibitor                        | 0.5538 | 0.6295 | 0.6302 | 0.5095 | 0.5294 |
| Gamma-tocotrienol                                       | 0.3769 | 0.3804 | 0.3543 | 0.3708 | 0.3935 |
| Imatinib                                                | 0.5861 | 0.6192 | 0.6146 | 0.6210 | 0.5494 |
| Interferon beta-1a                                      | 0.2889 | 0.2408 | 0.1850 | 0.2873 | 0.2509 |
| Interferon beta-1b                                      | 0.1292 | 0.0542 | 0.0579 | 0.1808 | 0.1540 |
| Interferon gamma-1b                                     | 0.2013 | 0.1292 | 0.1315 | 0.2103 | 0.2293 |
| Lapatinib                                               | 0.3850 | 0.4534 | 0.4772 | 0.4039 | 0.3935 |
| Lmp-420                                                 | 0.3769 | 0.3438 | 0.3360 | 0.3611 | 0.3935 |
| Lysophosphatidic acid                                   | 0.3769 | 0.4154 | 0.4164 | 0.4019 | 0.3895 |
| Natural alpha interferon                                | 0.3563 | 0.3052 | 0.3205 | 0.2982 | 0.3895 |
| Nitric oxide                                            | 0.1523 | 0.0839 | 0.0685 | 0.1813 | 0.1839 |
| Pd173074                                                | 0.5450 | 0.6159 | 0.6066 | 0.5155 | 0.5158 |
| Plx4032                                                 | 0.2949 | 0.2250 | 0.1900 | 0.2684 | 0.3253 |
| Plx4720                                                 | 0.5902 | 0.6579 | 0.6522 | 0.5293 | 0.5578 |
| Puromycin, ec50, 1 d                                    | 0.3769 | 0.3104 | 0.3016 | 0.3195 | 0.3935 |
| Resveratrol                                             | 0.5259 | 0.6002 | 0.6018 | 0.5042 | 0.5002 |
| Ribavirin                                               | 0.4313 | 0.5032 | 0.4922 | 0.4354 | 0.4034 |
| Sulforaphane                                            | 0.3956 | 0.4708 | 0.5011 | 0.4199 | 0.3935 |
| Tretinoin                                               | 0.5048 | 0.5800 | 0.5340 | 0.5083 | 0.4805 |
| Triiodothyronine-[13c6]<br>hydrochloride (t3 thyronine) | 0.1480 | 0.0783 | 0.0169 | 0.1685 | 0.1782 |
| Vanadium pentoxide                                      | 0.2918 | 0.2196 | 0.2251 | 0.2593 | 0.3194 |
| Vemurafenib                                             | 0.3769 | 0.3699 | 0.3114 | 0.3534 | 0.3895 |
| Vx                                                      | 0.3474 | 0.2721 | 0.1284 | 0.3005 | 0.3726 |
| Y15                                                     | 0.3261 | 0.2572 | 0.2573 | 0.3050 | 0.3574 |

**Supplementary Table 4. 4.** Machine learning analysis for candidate drugs targeting Cluster 1-2 genes in substantia nigra tissue of PD

| Drug Name              | GBR    | LR     | MLPRegresso<br>r | RFR    | SVR    |
|------------------------|--------|--------|------------------|--------|--------|
| Abiraterone            | 0.3253 | 0.1756 | 0.1522           | 0.2029 | 0.2754 |
| Actinomycin d          | 0.3320 | 0.2456 | 0.2447           | 0.2459 | 0.3165 |
| Adenosine triphosphate | 0.3424 | 0.4195 | 0.4225           | 0.3654 | 0.4073 |
| Anastrozole            | 0.2504 | 0.0864 | 0.1044           | 0.1415 | 0.1864 |
| Androstanolone         | 0.1894 | 0.0282 | 0.0489           | 0.1004 | 0.1283 |
| Antimony               | 1.1517 | 1.2598 | 1.2781           | 1.0305 | 0.4413 |
| Aplidin                | 0.3424 | 0.2007 | 0.2013           | 0.2264 | 0.3004 |
| Azacitidine            | 0.3798 | 0.5351 | 0.5810           | 0.4198 | 0.4350 |
| Bexarotene             | 0.4191 | 0.5249 | 0.5300           | 0.4846 | 0.4856 |

|                                                      |        |        |        |        |        |
|------------------------------------------------------|--------|--------|--------|--------|--------|
| Bicalutamide                                         | 0.3424 | 0.2493 | 0.2579 | 0.2510 | 0.3490 |
| Bisphenol a                                          | 0.3424 | 0.3026 | 0.3231 | 0.2939 | 0.2764 |
| Bortezomib                                           | 0.3424 | 0.2598 | 0.2788 | 0.2600 | 0.3604 |
| Cadmium                                              | 0.1884 | 0.0963 | 0.1164 | 0.0951 | 0.1125 |
| Calcitriol                                           | 0.3424 | 0.4302 | 0.4302 | 0.3928 | 0.4073 |
| Carboplatin                                          | 0.2580 | 0.1058 | 0.0844 | 0.1717 | 0.2057 |
| Chlorpyrifos                                         | 0.3292 | 0.2170 | 0.2159 | 0.2273 | 0.2175 |
| Cisplatin                                            | 0.3424 | 0.3270 | 0.3294 | 0.3264 | 0.3380 |
| Clinafloxacin                                        | 0.3424 | 0.4428 | 0.4754 | 0.3782 | 0.4309 |
| Curcumin                                             | 0.3424 | 0.2716 | 0.2769 | 0.2763 | 0.2918 |
| Cycloheximide                                        | 0.3424 | 0.4089 | 0.4171 | 0.4050 | 0.4073 |
| Dasatinib                                            | 0.3424 | 0.3684 | 0.3885 | 0.3327 | 0.4309 |
| Diclofenac                                           | 1.1527 | 1.2623 | 1.2239 | 1.1737 | 0.5429 |
| Dmnq (2,3-dimethoxy-1,4-naphthoquinone)              | 0.4886 | 0.6540 | 0.6065 | 0.5148 | 0.5541 |
| Doxorubicin                                          | 1.1240 | 1.1880 | 1.2299 | 1.2288 | 0.5675 |
| Doxycycline                                          | 0.3424 | 0.2325 | 0.2544 | 0.2463 | 0.3321 |
| Estradiol                                            | 0.3424 | 0.3652 | 0.3807 | 0.3607 | 0.3619 |
| Etanercept                                           | 0.3424 | 0.3716 | 0.3838 | 0.3596 | 0.3909 |
| Egfr-specific pd173074 inhibitor                     | 0.3424 | 0.3661 | 0.3648 | 0.3438 | 0.3716 |
| Gefitinib                                            | 2.0451 | 2.2070 | 2.2037 | 1.4371 | 1.4160 |
| Hydrocortisone                                       | 0.4856 | 0.6463 | 0.6534 | 0.5273 | 0.5462 |
| Imatinib                                             | 0.2853 | 0.2272 | 0.2303 | 0.2208 | 0.2255 |
| Interferon beta-1a                                   | 0.2976 | 0.1871 | 0.1621 | 0.1947 | 0.2818 |
| Interferon beta-1b                                   | 0.1827 | 0.0175 | 0.0229 | 0.1261 | 0.1176 |
| Isotretinoin                                         | 0.3031 | 0.1445 | 0.1685 | 0.1857 | 0.2446 |
| Lipopolysaccharide                                   | 0.3252 | 0.1743 | 0.1859 | 0.2107 | 0.2744 |
| Lmp-420                                              | 0.2832 | 0.1299 | 0.1186 | 0.1802 | 0.2300 |
| Mercury                                              | 1.3792 | 1.4907 | 1.5028 | 1.2185 | 0.6463 |
| Mesalazine                                           | 0.3424 | 0.3117 | 0.2731 | 0.3023 | 0.4061 |
| Methotrexate                                         | 2.6193 | 2.7753 | 2.6825 | 1.5948 | 1.4160 |
| Mk-886                                               | 0.3424 | 0.2432 | 0.2551 | 0.2479 | 0.3429 |
| Motexafin gadolinium (4 h)                           | 0.2834 | 0.1309 | 0.1176 | 0.1792 | 0.2310 |
| Natural alpha interferon                             | 0.3424 | 0.4635 | 0.4818 | 0.3929 | 0.4309 |
| Neocarzinostatin                                     | 0.3424 | 0.3885 | 0.3337 | 0.3405 | 0.4309 |
| Nickel                                               | 0.8977 | 0.9914 | 0.9909 | 0.9763 | 0.5116 |
| Nitric oxide                                         | 0.3424 | 0.2895 | 0.2905 | 0.2826 | 0.3901 |
| Pioglitazone                                         | 0.3424 | 0.1998 | 0.2846 | 0.2318 | 0.2999 |
| Plx4032                                              | 0.3424 | 0.2774 | 0.2809 | 0.2808 | 0.3148 |
| Promegestone                                         | 0.3424 | 0.2566 | 0.2581 | 0.2565 | 0.3567 |
| Puromycin, ec50, 5 d                                 | 1.4369 | 1.6012 | 1.5891 | 1.1334 | 1.4160 |
| Resveratrol                                          | 0.4782 | 0.6309 | 0.6377 | 0.5106 | 0.5311 |
| Ribavirin                                            | 0.4047 | 0.5692 | 0.5942 | 0.4695 | 0.4693 |
| Tretinoin                                            | 0.9032 | 0.9581 | 1.0321 | 0.9690 | 0.6400 |
| Triiodothyronine-[13c6] hydrochloride (t3 thyronine) | 0.1614 | 0.0707 | 0.0516 | 0.0792 | 0.1193 |

|                    |        |        |        |        |        |
|--------------------|--------|--------|--------|--------|--------|
| Vanadium pentoxide | 0.5077 | 0.5958 | 0.5967 | 0.5831 | 0.2107 |
| Vemurafenib        | 0.3424 | 0.3522 | 0.3479 | 0.3199 | 0.4309 |
| Vx                 | 0.3424 | 0.2592 | 0.2625 | 0.2572 | 0.3594 |
| Y15                | 0.3424 | 0.4743 | 0.4842 | 0.3850 | 0.4309 |

**Supplementary Table 4. 5.** Machine learning analysis for candidate drugs targeting Cluster 1 genes in muscle tissue of ALS

| Drug Name                              | GBR    | LR     | MLPRegressor | RFR    | SVR    |
|----------------------------------------|--------|--------|--------------|--------|--------|
| 1,25 dihydroxyvitamin d                | 0.4878 | 0.5065 | 0.4355       | 0.4874 | 0.4825 |
| 2,3-dimethoxy-1,4-naphtoquinone (dmnq) | 0.4878 | 0.2292 | 0.3536       | 0.3396 | 0.3859 |
| 3,3',4,4'-tetrachlorobiphenyl          | 0.5549 | 0.8346 | 1.0827       | 0.7939 | 0.5909 |
| 4-hydroxynonenal                       | 0.3913 | 0.2604 | 0.2655       | 0.3049 | 0.2669 |
| 4-hydroxytamoxifen                     | 0.4878 | 0.4050 | 0.3826       | 0.3957 | 0.4439 |
| Abiraterone                            | 0.4878 | 0.4327 | 0.4204       | 0.3959 | 0.4950 |
| Actinomycin d                          | 0.4683 | 0.3307 | 0.2915       | 0.2971 | 0.3365 |
| Adenosine triphosphate                 | 0.5022 | 0.6393 | 0.6347       | 0.6718 | 0.3771 |
| Alfacalcidol                           | 0.4878 | 0.4753 | 0.5729       | 0.5542 | 0.4372 |
| Aminolevulinic acid                    | 0.4699 | 0.3229 | 0.3125       | 0.3146 | 0.3487 |
| Anastrozole                            | 0.4878 | 0.1398 | 0.1534       | 0.2488 | 0.2540 |
| Androstanolone                         | 0.4224 | 0.1706 | 0.4643       | 0.2454 | 0.2846 |
| Antimony                               | 0.7121 | 0.8581 | 0.8042       | 0.8290 | 0.7590 |
| Aplidin                                | 0.5504 | 0.7057 | 0.5694       | 0.6437 | 0.5266 |
| Apratoxin a                            | 0.5929 | 0.7643 | 0.7302       | 0.8079 | 0.5544 |
| Arachidonic acid                       | 0.9264 | 1.0495 | 1.2276       | 1.0413 | 0.8895 |
| Argyrin a                              | 0.4878 | 0.4128 | 0.1877       | 0.3700 | 0.4495 |
| Azacitidine                            | 0.8433 | 1.0729 | 1.0733       | 1.0176 | 1.0860 |
| Bexarotene                             | 0.4878 | 0.5729 | 0.6916       | 0.5954 | 0.4875 |
| Bicalutamide                           | 0.4878 | 0.3776 | 0.3903       | 0.3525 | 0.2992 |
| Bisphenol a                            | 0.4878 | 0.5026 | 0.4832       | 0.5018 | 0.3466 |
| Bortezomib                             | 0.4878 | 0.3034 | 0.3040       | 0.3209 | 0.3963 |
| Bpde                                   | 0.4878 | 0.4128 | 0.4790       | 0.4020 | 0.3537 |
| Cadmium                                | 0.4263 | 0.3190 | 0.3207       | 0.3136 | 0.3356 |
| Calcitriol                             | 0.4878 | 0.4948 | 0.4932       | 0.5506 | 0.5345 |
| Carboplatin                            | 0.4878 | 0.4050 | 0.3568       | 0.4002 | 0.3696 |
| Carboplatin (30 h)                     | 0.4878 | 0.7409 | 0.6409       | 0.6570 | 0.6230 |
| Carboplatin (36 h)                     | 0.5195 | 0.7995 | 0.8045       | 0.7255 | 0.7985 |
| Cediranib                              | 0.3377 | 0.2136 | 0.2570       | 0.2403 | 0.2591 |
| Celecoxib                              | 0.4045 | 0.1901 | 0.2059       | 0.1887 | 0.2432 |
| Cetuximab                              | 0.4878 | 0.5143 | 0.4672       | 0.4816 | 0.5164 |
| Chlorpyrifos                           | 0.4878 | 0.3307 | 0.3763       | 0.3697 | 0.2974 |
| Chromium                               | 0.4878 | 0.5768 | 0.5531       | 0.6127 | 0.5374 |
| Cisplatin                              | 0.4803 | 0.4323 | 0.3853       | 0.4131 | 0.4079 |
| Clinafloxacin                          | 0.4878 | 0.6550 | 0.4777       | 0.5968 | 0.5164 |

|                                         |        |         |         |        |        |
|-----------------------------------------|--------|---------|---------|--------|--------|
| Curcumin                                | 0.4878 | 0.4987  | 0.4999  | 0.4523 | 0.4570 |
| Cycloheximide                           | 0.4878 | 0.4909  | 0.4519  | 0.5001 | 0.5345 |
| Cyfluthrin                              | 0.4878 | 0.6979  | 0.7330  | 0.6658 | 0.6036 |
| Cytarabine                              | 0.6511 | 0.7175  | 0.7149  | 0.7102 | 0.5996 |
| Dasatinib                               | 0.4878 | 0.4245  | 0.4371  | 0.4243 | 0.4459 |
| Decitabine                              | 1.0065 | 1.2917  | 1.0556  | 1.1496 | 1.1137 |
| Dexamethasone                           | 1.2475 | 1.3620  | 1.4046  | 1.3305 | 1.0393 |
| Diclofenac                              | 0.4878 | 0.5026  | 0.4940  | 0.5197 | 0.5061 |
| Dmnq (2,3-dimethoxy-1,4-naphthoquinone) | 0.4878 | 0.6003  | 0.5926  | 0.5870 | 0.5039 |
| Doxorubicin                             | 0.6773 | 0.7565  | 0.7101  | 0.7188 | 0.4660 |
| Doxycycline                             | 0.4878 | 0.7409  | 0.3982  | 0.6798 | 0.5909 |
| Estradiol                               | 0.4426 | 0.3503  | 0.3952  | 0.3313 | 0.3080 |
| Etanercept                              | 0.5035 | 0.6550  | 0.6794  | 0.6215 | 0.5445 |
| Egfr-specific pd173074 inhibitor        | 0.4878 | 0.4089  | 0.4886  | 0.4520 | 0.4130 |
| Gamma-tocotrienol                       | 0.4878 | 0.2233  | 0.1874  | 0.2665 | 0.3245 |
| Harman                                  | 0.6283 | 0.8776  | 0.9317  | 0.8115 | 0.6462 |
| Heregulin                               | 0.5766 | 0.9327  | 0.9875  | 0.7099 | 0.8535 |
| Hydrocortisone                          | 1.3123 | 1.5905  | 1.9009  | 1.5393 | 0.5909 |
| Hypochlorous acid                       | 0.4383 | 0.2917  | 0.2939  | 0.3264 | 0.2802 |
| Imatinib                                | 0.3686 | 0.2975  | 0.2967  | 0.3022 | 0.2842 |
| Insulin                                 | 0.6336 | 1.0265  | 1.0877  | 0.7446 | 0.9233 |
| Interferon beta-1a                      | 1.1524 | 1.2780  | 1.2131  | 1.3059 | 0.7396 |
| Interferon beta-1b                      | 0.4878 | 0.3366  | 0.2132  | 0.3240 | 0.4027 |
| Interferon gamma-1b                     | 0.3506 | 0.1432  | 0.1157  | 0.1620 | 0.1849 |
| Isotretinoin                            | 0.3814 | 0.3229  | 0.2791  | 0.2704 | 0.2743 |
| Laccaic acid                            | 0.5518 | 0.8034  | 0.9463  | 0.7894 | 0.8015 |
| Lapatinib                               | 0.4878 | 0.4089  | 0.5030  | 0.4000 | 0.4154 |
| Lipopolysaccharide                      | 0.5015 | 0.6237  | 0.6709  | 0.6196 | 0.5306 |
| Lmp-420                                 | 0.3348 | -0.0130 | -0.0266 | 0.1092 | 0.1113 |
| Luteolin                                | 1.2963 | 1.5671  | 1.2035  | 1.4126 | 1.2649 |
| Lysophosphatidic acid                   | 0.4878 | 0.4596  | 0.4452  | 0.4620 | 0.4613 |
| Mercury                                 | 0.5068 | 0.6862  | 0.6052  | 0.6482 | 0.6579 |
| Mesalazine                              | 0.4878 | 0.4675  | 0.4617  | 0.4493 | 0.5334 |
| Metformin                               | 0.4878 | 0.5417  | 0.5619  | 0.5265 | 0.5345 |
| Mk-886                                  | 0.4878 | 0.4889  | 0.4318  | 0.4566 | 0.5345 |
| Mln4924                                 | 0.5519 | 0.6979  | 0.6869  | 0.7455 | 0.6003 |
| Motexafin gadolinium (4 h)              | 0.4878 | 0.6882  | 0.6638  | 0.7033 | 0.5544 |
| Mycophenolic acid                       | 0.4878 | 0.7023  | 0.8638  | 0.6098 | 0.6134 |
| Natural alpha interferon                | 0.4109 | 0.2761  | 0.2849  | 0.2734 | 0.3142 |
| Neocarzinostatin                        | 0.4878 | 0.2214  | 0.2683  | 0.2672 | 0.2780 |
| Nickel                                  | 0.4878 | 0.5983  | 0.6180  | 0.5549 | 0.5560 |
| Nicotine                                | 0.9187 | 1.3038  | 1.2533  | 0.9706 | 1.2144 |
| Nilotinib                               | 0.4878 | 0.5749  | 0.4353  | 0.5670 | 0.5909 |
| Nitric oxide                            | 0.4878 | 0.4401  | 0.4304  | 0.4402 | 0.3831 |
| Pd173074                                | 0.4878 | 0.4304  | 0.4614  | 0.4393 | 0.4365 |

|                                                         |        |        |        |        |        |
|---------------------------------------------------------|--------|--------|--------|--------|--------|
| Phorbol 12-myristate 13-acetate (pma)                   | 0.4878 | 0.4811 | 0.4758 | 0.4770 | 0.5544 |
| Pioglitazone                                            | 0.4878 | 0.3688 | 0.4008 | 0.3626 | 0.3786 |
| Plx4032                                                 | 0.6054 | 0.7165 | 0.8303 | 0.7624 | 0.5301 |
| Plx4720                                                 | 0.4878 | 0.4958 | 0.4843 | 0.4822 | 0.4867 |
| Promegestone                                            | 0.4878 | 0.3395 | 0.3668 | 0.3466 | 0.3645 |
| Propofol                                                | 0.9913 | 1.3937 | 1.4427 | 1.0751 | 1.2728 |
| Puromycin, 2xec50, 1d                                   | 0.4878 | 0.9015 | 0.4959 | 0.7073 | 0.7951 |
| Puromycin, 2xec50, 3 d                                  | 0.4878 | 0.5421 | 0.5153 | 0.5048 | 0.5972 |
| Puromycin, ec50, 1 d                                    | 0.4739 | 0.3405 | 0.4719 | 0.3506 | 0.3211 |
| Puromycin, ec50, 3 d                                    | 0.4878 | 0.4304 | 0.4482 | 0.4160 | 0.4907 |
| Puromycin, ec50, 5 d                                    | 0.9295 | 1.1501 | 0.9659 | 1.1579 | 0.7209 |
| Quercetin                                               | 0.4878 | 0.7057 | 0.7304 | 0.6297 | 0.6565 |
| R1881                                                   | 0.4878 | 0.5104 | 0.5291 | 0.5155 | 0.3912 |
| Resveratrol                                             | 0.4999 | 0.6081 | 0.5872 | 0.6109 | 0.5224 |
| Ribavirin                                               | 0.4878 | 0.4704 | 0.3709 | 0.4814 | 0.4326 |
| Sapphyrin pci-2050 (1.25<br>&icirc;&frac14;m)           | 0.4878 | 0.3542 | 0.3291 | 0.3354 | 0.3283 |
| Sapphyrin pci-2050 (2.5<br>&icirc;&frac14;m)            | 0.4878 | 0.4870 | 0.5289 | 0.4604 | 0.5246 |
| Sevoflurane                                             | 1.0300 | 1.2917 | 1.3588 | 1.1367 | 0.6958 |
| Sulforaphane                                            | 0.6975 | 0.9196 | 1.1264 | 0.9174 | 0.7620 |
| Thapsigargin                                            | 0.4224 | 0.1198 | 0.0929 | 0.1962 | 0.2227 |
| Tibolone                                                | 0.4878 | 0.6091 | 0.5924 | 0.5991 | 0.5746 |
| Tretinoin                                               | 0.4878 | 0.4382 | 0.4581 | 0.4660 | 0.4227 |
| Triiodothyronine-[13c6] hydrochloride<br>(t3 thyronine) | 0.2651 | 0.1554 | 0.1422 | 0.1581 | 0.1720 |
| Trovaflaxacin                                           | 0.9608 | 1.1628 | 0.9337 | 1.1529 | 0.8668 |
| Ubiquinol                                               | 0.4878 | 0.2726 | 0.3121 | 0.3332 | 0.3857 |
| Vanadium pentoxide                                      | 0.3931 | 0.2790 | 0.2807 | 0.2992 | 0.2937 |
| Vemurafenib                                             | 0.4644 | 0.3613 | 0.3978 | 0.3708 | 0.3705 |
| Vx                                                      | 0.4878 | 0.3789 | 0.4088 | 0.4032 | 0.4009 |
| Y15                                                     | 0.4878 | 0.5148 | 0.5470 | 0.5236 | 0.4513 |

**Supplementary Table 4. 6.** Machine learning analysis for candidate drugs targeting Cluster 1 genes in motor neuron cells of ALS

| Drug Name               | GBR    | LR     | MLPRegressor | RFR    | SVR    |
|-------------------------|--------|--------|--------------|--------|--------|
| 1,25 dihydroxyvitamin d | 0.2866 | 0.2034 | 0.2194       | 0.2088 | 0.2140 |
| 4-hydroxynonenal        | 0.2837 | 0.1800 | 0.2524       | 0.2502 | 0.2250 |
| 4-hydroxytamoxifen      | 0.4298 | 0.5081 | 0.5270       | 0.5010 | 0.5188 |
| Abiraterone             | 0.3158 | 0.3636 | 0.3466       | 0.3673 | 0.3465 |
| Actinomycin d           | 0.2969 | 0.2230 | 0.2162       | 0.2260 | 0.2321 |
| Adenosine triphosphate  | 0.3770 | 0.4222 | 0.4228       | 0.4356 | 0.4319 |
| Alfacalcidol            | 0.2727 | 0.2152 | 0.2219       | 0.2103 | 0.2528 |
| Aminolevulinic acid     | 0.3158 | 0.3753 | 0.3633       | 0.3441 | 0.3195 |

|                                         |        |        |        |        |        |
|-----------------------------------------|--------|--------|--------|--------|--------|
| Androstanolone                          | 0.3158 | 0.2269 | 0.2242 | 0.2553 | 0.3113 |
| Antimony                                | 0.3158 | 0.3265 | 0.3396 | 0.3301 | 0.3272 |
| Aplidin                                 | 0.2824 | 0.1956 | 0.1705 | 0.1986 | 0.2863 |
| Apratoxin a                             | 0.2323 | 0.1878 | 0.1312 | 0.1552 | 0.2349 |
| Arachidonic acid                        | 0.3316 | 0.4789 | 0.4830 | 0.4011 | 0.3702 |
| Argyria                                 | 0.3158 | 0.3754 | 0.3838 | 0.3547 | 0.3372 |
| Bexarotene                              | 0.3158 | 0.3441 | 0.3614 | 0.3671 | 0.4040 |
| Bicalutamide                            | 0.3158 | 0.2562 | 0.2810 | 0.2846 | 0.3272 |
| Bisphenol a                             | 0.3158 | 0.3148 | 0.3290 | 0.3306 | 0.3240 |
| Bortezomib                              | 0.5014 | 0.5921 | 0.5928 | 0.5667 | 0.5364 |
| Bpde                                    | 0.2927 | 0.2562 | 0.2317 | 0.2493 | 0.2616 |
| Cadmium                                 | 0.3089 | 0.2484 | 0.2162 | 0.2325 | 0.2006 |
| Calcitriol                              | 0.3158 | 0.3421 | 0.3031 | 0.2975 | 0.3044 |
| Carboplatin                             | 0.3089 | 0.2855 | 0.2662 | 0.2556 | 0.2528 |
| Carboplatin (30 h)                      | 0.3158 | 0.3773 | 0.3676 | 0.3481 | 0.3372 |
| Carboplatin (36 h)                      | 0.3883 | 0.4925 | 0.4931 | 0.4515 | 0.3960 |
| Cediranib                               | 0.1799 | 0.1527 | 0.1456 | 0.1440 | 0.1456 |
| Celecoxib                               | 0.2906 | 0.2015 | 0.1998 | 0.2125 | 0.2296 |
| Cetuximab                               | 0.3158 | 0.2894 | 0.3149 | 0.3032 | 0.3332 |
| Chlorpyrifos                            | 0.3158 | 0.4066 | 0.3876 | 0.3927 | 0.3304 |
| Chromium                                | 0.3158 | 0.3070 | 0.3421 | 0.3128 | 0.3372 |
| Cisplatin                               | 0.2966 | 0.2855 | 0.2568 | 0.2626 | 0.2630 |
| Curcumin                                | 0.2835 | 0.2523 | 0.2415 | 0.2368 | 0.2759 |
| Cycloheximide                           | 0.3158 | 0.3499 | 0.3481 | 0.3431 | 0.3130 |
| Cyfluthrin                              | 0.3158 | 0.3480 | 0.3270 | 0.3515 | 0.3332 |
| Cytarabine                              | 0.3158 | 0.3441 | 0.3584 | 0.3368 | 0.3272 |
| Dasatinib                               | 0.3158 | 0.3030 | 0.3124 | 0.2959 | 0.2899 |
| Dexamethasone                           | 0.4172 | 0.4691 | 0.4818 | 0.4700 | 0.4641 |
| Diclofenac                              | 0.3158 | 0.3226 | 0.3139 | 0.3377 | 0.3272 |
| Dmnq (2,3-dimethoxy-1,4-naphthoquinone) | 0.3158 | 0.2796 | 0.2680 | 0.2781 | 0.3332 |
| Doxorubicin                             | 0.3158 | 0.3558 | 0.3417 | 0.3445 | 0.2920 |
| Doxycycline                             | 0.3158 | 0.2269 | 0.2416 | 0.2540 | 0.2861 |
| Estradiol                               | 0.2929 | 0.2640 | 0.2860 | 0.2616 | 0.2460 |
| Etanercept                              | 0.3158 | 0.3109 | 0.3203 | 0.3109 | 0.3272 |
| Egfr-specific pd173074 inhibitor        | 0.3158 | 0.3519 | 0.3467 | 0.3454 | 0.3205 |
| Gamma-tocotrienol                       | 0.3250 | 0.4183 | 0.4151 | 0.4101 | 0.4179 |
| Harman                                  | 0.2135 | 0.1292 | 0.0970 | 0.1565 | 0.1262 |
| Heregulin                               | 0.3753 | 0.4671 | 0.4608 | 0.4445 | 0.4124 |
| Hypochlorous acid                       | 0.1944 | 0.1448 | 0.1287 | 0.1344 | 0.1606 |
| Imatinib                                | 0.3158 | 0.3323 | 0.3308 | 0.3385 | 0.3374 |
| Insulin                                 | 0.2244 | 0.1702 | 0.1390 | 0.1545 | 0.1855 |
| Interferon beta-1a                      | 0.2892 | 0.2542 | 0.2294 | 0.2410 | 0.2447 |
| Interferon beta-1b                      | 0.2428 | 0.1761 | 0.1781 | 0.1783 | 0.2030 |
| Interferon gamma-1b                     | 0.3158 | 0.3275 | 0.3304 | 0.3446 | 0.3149 |
| Isotretinoin                            | 0.1977 | 0.1175 | 0.1279 | 0.1348 | 0.1887 |

|                                                         |        |        |        |        |        |
|---------------------------------------------------------|--------|--------|--------|--------|--------|
| Laccaic acid                                            | 0.4185 | 0.4944 | 0.4489 | 0.4924 | 0.4264 |
| Lapatinib                                               | 0.4246 | 0.4778 | 0.4673 | 0.4668 | 0.4472 |
| Lipopolysaccharide                                      | 0.4082 | 0.4515 | 0.4545 | 0.4589 | 0.4492 |
| Lmp-420                                                 | 0.2079 | 0.1566 | 0.1568 | 0.1313 | 0.1357 |
| Lysophosphatidic acid                                   | 0.3158 | 0.3685 | 0.3565 | 0.3706 | 0.3256 |
| Mercury                                                 | 0.3158 | 0.3763 | 0.3827 | 0.3628 | 0.3633 |
| Mesalazine                                              | 0.2528 | 0.2083 | 0.1809 | 0.1933 | 0.1919 |
| Metformin                                               | 0.3158 | 0.3597 | 0.3598 | 0.3537 | 0.3654 |
| Mk-886                                                  | 0.3898 | 0.4925 | 0.4964 | 0.4685 | 0.4140 |
| Motexafin gadolinium (4 h)                              | 0.3158 | 0.2357 | 0.2387 | 0.2425 | 0.3250 |
| Mycophenolic acid                                       | 0.3158 | 0.3793 | 0.3597 | 0.3462 | 0.3372 |
| Natural alpha interferon                                | 0.3158 | 0.3724 | 0.3489 | 0.3581 | 0.3729 |
| Neocarzinostatin                                        | 0.3158 | 0.3929 | 0.3563 | 0.3617 | 0.3332 |
| Nickel                                                  | 0.3158 | 0.3558 | 0.3340 | 0.3506 | 0.3432 |
| Nilotinib                                               | 0.3158 | 0.4301 | 0.4214 | 0.3946 | 0.3372 |
| Nitric oxide                                            | 0.2293 | 0.1673 | 0.1460 | 0.1553 | 0.1786 |
| Pd173074                                                | 0.3158 | 0.3460 | 0.4039 | 0.3367 | 0.3272 |
| Phorbol 12-myristate 13-acetate (pma)                   | 0.4177 | 0.5609 | 0.5512 | 0.4543 | 0.4585 |
| Pioglitazone                                            | 0.3158 | 0.3548 | 0.3519 | 0.3528 | 0.3324 |
| Plx4032                                                 | 0.3577 | 0.4036 | 0.4376 | 0.4102 | 0.4083 |
| Plx4720                                                 | 0.4619 | 0.5306 | 0.5244 | 0.5280 | 0.5351 |
| Promegestone                                            | 0.3158 | 0.2630 | 0.2617 | 0.2643 | 0.2993 |
| Puromycin, ec50, 1 d                                    | 0.3885 | 0.4896 | 0.4652 | 0.4615 | 0.4459 |
| Puromycin, ec50, 3 d                                    | 0.3158 | 0.4281 | 0.4606 | 0.3704 | 0.3372 |
| Puromycin, ec50, 5 d                                    | 0.4694 | 0.5628 | 0.5620 | 0.5315 | 0.5527 |
| Quercetin                                               | 0.2933 | 0.1854 | 0.2023 | 0.2026 | 0.2436 |
| R1881                                                   | 0.3370 | 0.3992 | 0.3836 | 0.3866 | 0.3906 |
| Ribavirin                                               | 0.3158 | 0.3797 | 0.3831 | 0.3681 | 0.3365 |
| Sapphyrin pci-2050 (1.25<br>&icirc;&frac14;m)           | 0.3158 | 0.2943 | 0.3042 | 0.2844 | 0.2744 |
| Sapphyrin pci-2050 (2.5<br>&icirc;&frac14;m)            | 0.3158 | 0.2806 | 0.3038 | 0.2895 | 0.3272 |
| Sevoflurane                                             | 0.3158 | 0.2035 | 0.1904 | 0.2299 | 0.2875 |
| Sulforaphane                                            | 0.4009 | 0.4915 | 0.4859 | 0.4674 | 0.4651 |
| Telapristone acetate                                    | 0.3158 | 0.2542 | 0.2608 | 0.2605 | 0.3332 |
| Thapsigargin                                            | 0.3158 | 0.3846 | 0.3837 | 0.3634 | 0.3483 |
| Tibolone                                                | 0.3158 | 0.2366 | 0.2563 | 0.2467 | 0.2746 |
| Tretinoin                                               | 0.4089 | 0.4422 | 0.4373 | 0.4345 | 0.4519 |
| Triiodothyronine-[13c6] hydrochloride<br>(t3 thyronine) | 0.1699 | 0.1380 | 0.1424 | 0.1385 | 0.1575 |
| Ubiquinol                                               | 0.3271 | 0.4290 | 0.4547 | 0.4033 | 0.3932 |
| Vanadium pentoxide                                      | 0.2274 | 0.1988 | 0.1948 | 0.1856 | 0.2045 |
| Vemurafenib                                             | 0.3158 | 0.2820 | 0.2791 | 0.2821 | 0.2790 |
| Vx                                                      | 0.2985 | 0.2491 | 0.2530 | 0.2522 | 0.2659 |
| Y15                                                     | 0.3158 | 0.3721 | 0.3698 | 0.3727 | 0.3336 |