Unraveling Mutual Pathways between Neurodegenerative Diseases: A Computational Approach

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# **Abstract**

## Background

Neurodegenerative diseases are a global burden threatening the elderly population, which is expected to be around 16% of the world’s population by 2030. The complexity of neurodegenerative diseases and the incomplete comprehension of their pathophysiology pose more challenges in providing effective therapeutics. No treatments or interventions are available to halt the progressive nerve damage caused by neurodegenerative diseases.

## Results

We propose a bioinformatics pipeline to analyze the transcriptomic profiles of Alzheimer’s, Parkinson’s, and Huntington’s diseases and find mutual pathways that are possible to be potential drug targets for three of the most common neurodegenerative diseases. We further validated key genes involved in these pathways through differential expression analysis. External validation was also conducted utilizing receiver operating characteristic curve (ROC) analyses on independent datasets. Pathway enrichment analysis was performed using raw gene counts, and three distinct pathways were identified as upregulated in all the diseases. Two of these pathways are involved in activating the transcription factor activated nuclear factor kappa B, while the third pathway is involved with regulating BCL2L11 transcription by RUNX3. The latter pathway is correlated with cancer progression. NFKBIA, NFKB1 RELA, TRIM4, and SMAD4 were among the key significant upregulated mutual genes between the three diseases and involved in the proposed pathways.

## Conclusion

Neuroinflammation is a common brain pathology among the three neurodegenerative diseases under study. Our results proposed targeting two pathways that activate transcription factor nuclear factor kappa B as a potential simultaneous therapy for Alzheimer’s, Parkinson’s, and Huntington’s diseases. The third mutual pathway involved the protective effect the neurodegenerative diseases may have against cancer despite their deleterious consequences on the health of elderly patients.

# Keywords

*Neurodegeneration, Alzheimer’s Disease, Parkinson’s Disease, Huntington’s Disease, Differential Expression Analysis, Pathway Enrichment Analysis, NF-κB Pathway, RUNX3, NFKBIA, NFKB1, RELA, TRIM4, SMAD4, EGFR*

# **Background**

Neurological disorders cause 16.5% of deaths and 11.6% of disability-adjusted life years (1). Neurodegeneration is a neurological disorder in which a gradual loss of the structure or function of the nerve cells occurs due to cell death, axonal regeneration failure, or neuron demyelination. These nerve abnormalities are generally referred to as neurodegenerative diseases (NDs). NDs are either partial or complete, solo or combined, hereditary or acquired, known or unknown in origin (2). They are a global burden that mainly threatens the elderly (3). In addition to aging, diverse pathways involved in intracellular processes, the local tissue environment, and the systemic environment can play a pivotal role in developing the NDs (4). Mitochondrial malfunction, cellular ‎stress, dysfunctional cytoskeletal ‎proteins, dysregulated programmed cell death, and changes in cellular protein homeostasis are ‎all probable drivers for the ‎imbalance between neuronal survival and degeneration (5–13).‎ NDs affect different brain areas and manifest distinct features, such as gradual loss of sensory, motor, or cognitive abilities (14). Research on the common causes behind different NDs expands the horizon for new or repurposed therapeutic ‎agents simultaneously targeting multiple diseases. Alzheimer’s (AD), Parkinson’s (PD), ‎and Huntington’s diseases (HD) are primarily classified as proteinopathies NDs; i.e., they are ‎associated with the aggregation of misfolded proteins (15).‎

Alzheimer’s disease (AD) starts as a mild memory loss, with symptoms worsening over time. The buildup of extracellular β-amyloid protein ‎and intracellular tau protein is of the main reasons behind AD ‎(16). A recent bioinformatic study has identified 1031 ‎unique differentially expressed genes (DEGs) that implicate all major cell types in AD patients ‎compared to healthy controls. Neurons had a strong signature of gene repression. While DEGs were downregulated by 75% in excitatory neurons and 95% in inhibitory neurons, most of the identified DEGs in astrocytes,‎ oligodendrocytes, and microglia were upregulated within a range of 53%–63% (17).

Parkinson’s disease (PD) is a movement disorder characterized by aggregations of α-synuclein that form Lewy bodies and neurites ‎(18). The disease hallmark symptom of the disease is shaking hands, arms, legs, and neck muscles. Studies showed that genomic multiplications of the wild type of SNCA gene induced ‎elevated levels of α-synuclein expression and could contribute to the development of PD ‎(19,20). In another study, 85 genes were identified as significantly hypo-‎methylated and upregulated in ‎patients with PD compared to healthy controls. Downregulated genes were significantly associated with the structural constituent of the cytoskeleton and, thus, dopaminergic ‎neurotransmission. Upregulated genes were associated with phagosome and lysosome pathways and involved in misfolded protein degradation (21). ‎

Huntington's disease (HD) is a neurodegenerative disease that causes chorea, cognitive ‎impairment, physical and mental disturbances, and probable depression ‎(22). It is caused by the intracellular accumulation of mutant Huntington's protein aggregates, ‎resulting in brain cell apoptosis, mainly in the striatum ‎(22). On the genetic level, HD is an autosomal dominant neurodegenerative disease caused by a CAG repeat ‎expansion in exon 1 of the Huntingtin gene, which results in a polyglutamine strand at ‎the Huntingtin protein's N-terminus ‎(23). Computational analysis on HD was performed, and sixty genes were specified as differentially expressed in HD gene carriers compared with healthy ‎controls. A total of 109 enriched pathways were identified using the Gene Ontology (GO) analysis of the list of DEGs (24). ‎ ADGRG1 gene was identified among the top genes involved in the enriched pathways. These pathways included vascular endothelial growth ‎factor production, cerebral cortex radial glia-guided migration, layer formation in the cerebral cortex, and cerebral cortex ‎regionalization. In addition, different GO terms involved in ‎neurodevelopment were identified, such as the positive regulation of neuron migration cell adhesion. A real-time qPCR technique was adopted to validate the computational analysis results‎ (24).

NDs are considered ‎incurable since no known intervention exists to stop the gradual ‎destruction of neurons (25). Furthermore, complete physiological and pathophysiological mechanisms linked to NDs are still unknown. The underlying mechanisms are polyfactorial and result from complicated ‎interactions of innumerable partially unknown genetic and non-genetic factors (25).‎ In this article, we propose a bioinformatics pipeline in which we conducted pathway enrichment analyses followed by differential gene expression analysis to study Alzheimer’s, Parkinson’s, and Huntington’s diseases, as summarized in Fig. 1. We report the mutual dysregulated pathways across the three diseases that may shed light on common pathological ‎‎processes and help the reposition drugs to target a broader range of NDs, compared to investigating each disease independently.‎ Three mutually enriched pathways were reported based on the raw counts of each disease's expression profile. Two of these pathways are involved in activating the transcription factor activated nuclear factor kappa B (NF-κB). Surprisingly, the third pathway is the regulation of BCL2L11 (BIM) transcription by RUNX3, which was found to correlate with cancer progression. We have also conducted differential expression analysis where the multiple mutual DEGs were found to be involved in the proposed pathways. These were categorized into enzymes, membrane trafficking proteins, transcription factors, and cell cycle proteins.

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***Fig. 1.*** *Analysis pipeline overview (FDR: false discovery rate; p-adj: adjusted p-value).*

**Results**

Biological Pathways Enriched Exclusively in Each ND: Inflammation, Apoptosis, and Neurotransmission

Pathway enrichment analyses were conducted using Reactome and false discovery rate (FDR) threshold less than 0.05 using raw RNA-seq counts for each neurodegenerative disease. Table 1 summarizes the number of significantly enriched pathways. Detailed information on the pathways is present in Supplementary Tables S1.1, S1.2, and S1.3 (Additional File 1).

Table 1: Summary of the enriched pathways in each ND

|  |  |  |  |
| --- | --- | --- | --- |
| Enriched Pathways (FDR<0.05) | | | |
|  | Upregulated | Downregulated | Total |
| AD | 69 | 84 | 153 |
| PD | 76 | 39 | 115 |
| HD | 80 | 29 | 109 |

The AD enriched pathways were investigated to highlight the dysregulated and impaired ‎pathways for patients with AD. The pathway R-HSA-9022702, known ‎as “*MECP2 regulates the transcription of neuronal ligands”* was found to be downregulated with an FDR value of ‎‎0.001.‎ MECP2 regulates the transcription of several transcription factors involved in the functioning of the ‎nervous system, such as CREB1 and MEF2C‎. It was shown that increased dosage or loss of function of the MECP2 gene could ‎cause a plethora of neuropsychiatric disorders (26). It was demonstrated that MECP2 could increase the pro-inflammatory response of microglial cells. In ‎postmortem brain samples from different stages of AD, it was found that phosphorylation of the ‎MECP2 protein decreased at the amino acid serine 423 in the early stages of Alzheimer’s disease (27).‎ Another study identified MECP2 as a possible regulator of Tau pathology in mouse ‎models (28).‎

The enrichment analysis showed that the pathway R-HSA-264642, the acetylcholine ‎neurotransmitter release cycle, was downregulated. This ‎cycle involves the synthesis of acetylcholine, loading of synaptic vesicles, docking and priming of ‎the acetylcholine-loaded synaptic vesicles, and then release of acetylcholine ‎(29). Other pathways involved in neurotransmitters secretion and regulation, such as R-HSA-888590 (GABA synthesis, release, reuptake, and ‎degradation) and R-HSA-181429 (serotonin neurotransmitter release cycle), were also downregulated with significant FDR values.‎

Pathways involved in apoptosis were significantly upregulated in the transcriptome analysis of patients with AD. Examples are R-HSA-‎‎111458 (formation of apoptosome) and R-HSA-9627069 (Regulation of the apoptosome activity). R-HSA-205017 (NFG and proNGF bind to p75NTR) pathway led to activation of apoptotic cascade and was significantly upregulated in the analysis. It is worth mentioning that studies showed that brain tissues affected by AD had both necrotic and apoptotic regions ‎‎(30). ‎

In Parkinson’s disease, the pathway enrichment analysis showed that the R-HSA-5632927 ‎‎(defective mismatch repair associated with MSH3) pathway is one of the most significantly downregulated pathways with an FDR value of 0.002. Other downregulated mismatch repair pathways were diseases of mismatch repair (MMR), mismatch repair (MMR) directed by MSH2:MSH3 (MutSbeta), mismatch repair‎, defective mismatch repair associated with MSH2, and mismatch repair (MMR) directed by MSH2:MSH6 (MutSalpha). DNA damage was verified to be one of the drivers of neurodegenerative diseases in which DNA repair mechanisms were involved (31).

One of the significantly enriched pathways in HD was R-HSA-2032785 (YAP1- and ‎WWTR1 (TAZ)-stimulated gene expression), which had an FDR value ‎‎of 0.00001. It is involved in the Hippo signaling pathway, in which the activation of the latter may affect neurodegeneration of the brain ‎(32). The loss of YAP1 may have the potential as a clinical marker for predicting neuroendocrine ‎features and chemosensitivity (33). The R-HSA-6804759 (Regulation of TP53 activity through association with co-factors) ‎pathway was upregulated with a significant FDR (0.004), where 13 proteins were involved. High levels of p53 protein encoded by TP53 were correlated with nerve cell apoptosis and neural damage ‎(34). R-HSA-389977 pathway (post-chaperonin tubulin folding pathway) was also downregulated with an FDR value of 0.006. Other ‎pathways, including R-HSA-351143 (agmatine biosynthesis), were reported for downregulation; it is known that agmatine binds to several target receptors in the brain ‎and has been proposed as a novel neuromodulator ‎(35).

## Biological Pathways Enriched Mutually between NDs: Neuroinflammation and Cancer

As shown in Fig. 2 and Table 2, nine pathways were found exclusively mutual between AD and PD, 14 pathways were mutual between PD and HD, and six pathways were mutual between AD and HD only. Moreover, three distinct pathways were shared between the three neurodegenerative diseases that were found to be related to immune system regulation and cancer (‎Supplementary Table S1.4, Additional File 1). They are TRAF6 mediated NF-kB activation, RUNX3 regulates BCL2L11 (BIM) transcription, and TAK1 activates NFkB by phosphorylation and activation of IKKs complex

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**Fig. 2.** Exclusive and mutually enriched pathways in AD, PD, and HD

Table 2: List of mutual upregulated and downregulated pathways between each pair of NDs in the study

|  |  |  |  |
| --- | --- | --- | --- |
|  | **AD intersects PD** | **PD intersects HD** | **AD intersects HD** |
| Upregulated Pathways | MyD88 cascade initiated on plasma membrane | Loss of Function of SMAD2/3 in Cancer | Circadian clock |
| MyD88 dependent cascade initiated on endosome | Loss of Function of TGFBR1 in Cancer | Common pathway of firbin clot formation |
| Regulation of localization of FOXO transcription factors | Metallothioneins bind metals | FOXO-mediated transcription of cell cycle genes |
| Toll Like Receptor 10 (TLR10) Cascade | MyD88 deficiency (TLR5) | IkBA variant leads to EDA-ID |
| Toll Like Receptor 5 (TLR5) Cascade | MyD88:MAL(TIRAP) cascade initiated on plasma membrane | RUNX2 regulates bone development |
| Toll Like Receptor 7/8 (TLR7/8) Cascade | Response to metal ions |  |
| TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation | Signaling by TGF-beta Receptor Complex in Cancer |
|  | TNF signaling |
| Toll-like receptor 2(TLR2) cascade |
| Toll-like receptor 4(TLR4) cascade |
| Toll-like receptor TLR1:TLR2 cascade |
| Toll-like receptor TLR6:TLR2 cascade |
|  | Toll-like receptor cascades |
| Downregulated Pathways | Rap1 signaling | Activation of BMF and translocation to mitochondria | Defective GSS causes GSS deficiency |
| Regulation of pyruvate dehydrogenase (PDH) complex |  |  |

Seven of nine mutual pathways were upregulated between AD and PD, while two were downregulated. Most upregulated pathways were related to Toll-like receptors (TLRs) cascades. These included MyD88, TLR5, TLR10, and TLR7/8 cascades. Another upregulated pathway was the regulation of the localization of FOXO transcription factors. FOXO stands for forkhead box O, and they are transcription factors that influence various cellular functions such as intracellular signaling, metabolism, proteostasis, and cell cycle arrest (36). FOXO transcription factors were correlated with type 2 diabetes and neurodegeneration (37). The Ras-associated protein-1 (Rap1) signaling pathway was downregulated in AD and PD. Rap1 signaling pathway may enhance tumor invasiveness and metastasis in cancers such as breast cancer, pancreatic cancer, and leukemia (38).

The highest number of mutual pathways were observed between PD and HD. It may suggest more common pathophysiological features between the two diseases. Thirteen out of fourteen mutual pathways were upregulated. TLR2, TLR4, TLR1:TLR2, and TLR6:TLR2 cascades were significantly upregulated with FDR below 0.05. Tumor necrosis factor (TNF) signaling was another upregulated pathway in the study. TNF elevation was associated with inflammation and involvement in AD and PD (39). This may suggest its involvement in HD too.

Five of six pathways were mutually upregulated between AD and HD. Remarkably, the fibrin clot formation pathway was upregulated. There has been evidence of the association of fibrin with neuroinflammation and neurodegenerative disease progression (40). FOXO-mediated transcription of cell cycle genes was upregulated in both diseases. It is worth mentioning that the FOXO1 gene was upregulated in both PD and HD, FOXO4 was upregulated in both AD and PD, while FOXO6 was upregulated in PD only. The role of FOXO transcription factors in neurodegenerative diseases was thoroughly reviewed in (41).

Most of the pathways enriched in this study involve immune response regulation and inflammation, such as NF-κB transcription factor activation and signaling. In concordance with these findings, out of the three mutual pathways significantly enriched in the three diseases, two mutually enriched pathways are involved in inflammation, namely TRAF6-mediated and TAK1-mediated NF-κB activation pathways. We will detail the three pathways to propose them as potential drug targets in the discussion section.

## Thousands of Exclusive Differentially Expressed Genes, and Only 274 Genes Are Mutual

In this study, significant differentially expressed genes (DEGs) were defined as those having an adjusted P-value less than 0.05. DEGs for each neurodegenerative disease in the study were reported and visualized, highlighting key genes involved in the three enriched mutual pathways (Fig. 3). In AD, 943 genes were downregulated, and 861 genes were upregulated. Regarding PD, 3802 genes were downregulated, while 3539 genes were upregulated. For HD, 1938 genes were downregulated, and 2401 genes were upregulated. A detailed list of the DEGs in the three NDs can be found in the Supplementary Tables S2.1, S2.2, S2.3 (Additional File 2).

Overall, 274 mutual DEGs have been identified ‎between the three NDs, as shown in Fig. 4. The mutual DEGs are reported with their P-adjusted and log fold change (lfc) values from each ‎disease dataset (‎Supplementary Table S2.4, Additional File 2). According to the Kyoto Encyclopedia of Genes and Genomes (KEGG) classification, most mutual DEGs fall under the following categories: enzymes, membrane trafficking proteins, transcription factors, and cell cycle proteins. For example, 58 of the DEGs were classified as enzymes, 22 as membrane trafficking proteins, and 18 proteins as transcription factors (‎Supplementary Table S2.5, Additional File 2).

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| (a) | (b) | (c) |

***Fig. 3.*** *Volcano plots for DEGs in (a) AD, (b) PD, (c) HD*

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***Fig. 4.****‎ The mutual DEGs between AD, HD, and PD*

## Validation of Selected Mutual DEGs

The genes involved in the three mutual pathways were screened, and those mutually differentially expressed in the three diseases were included in the validation process (Supplementary Table S1.5, Additional File 1). The genes were NFKB1, NFKBIA, RELA, TRIM4, and SMAD4. The first four genes are involved in both pathways of NF-κB transcription factor activation and among the mutual DEGs between the three diseases. The pathway of RUNX3 regulating BCL2L11 (BIM) transcription is the third mutually significant enriched pathway between AD, PD, and HD in which the fifth gene (SMAD4) is a key mutual DEG involved in this pathway. To evaluate the impact of these genes in healthy and diseased conditions, receiver operating characteristic (ROC) curve analyses (Fig. 5) were performed using their raw expression values in the three diseases independently in the studied datasets. Most genes had areas under the curve (AUC) of over 0.71, with the highest NFKBIA achieving an AUC of 0.93 in the Alzheimer's dataset. External datasets have also been used for further validation (Fig. 6). We searched the Gene Expression Omnibus (GEO) database for external datasets for each disease. The external datasets were GSE184942, GSE106608, and GSE97100 for AD, PD, and HD, respectively (44,45). Most genes had areas under the curve (AUC) of around 0.6 or more. ROC analysis for external validation datasets showed lower AUC values for the selected genes. This can be attributed to the difference in sample size between the datasets included in the primary analysis and the external validation dataset. The latter had less sample size. However, they were selected for validation due to their experimental design similarities.

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***Fig. 5.*** *Internal validation using ROC analysis curve for the selected DEGs in (a) AD, (b) PD, (c) HD*

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| (c) | |

***Fig. 6.*** *External validation using ROC analysis curve for the selected DEGs in (a) AD, (b) PD, (c) HD*

Drug Discovery

After filtering the CMAP transcriptomic signatures for FDA-approved drugs, seventy-three drugs were found to downregulate the four genes of interest from the mutual pathways between AD, PD, and HD: NFKB1, NFKBIA, RELA, and SMAD4 (Fig. 7).

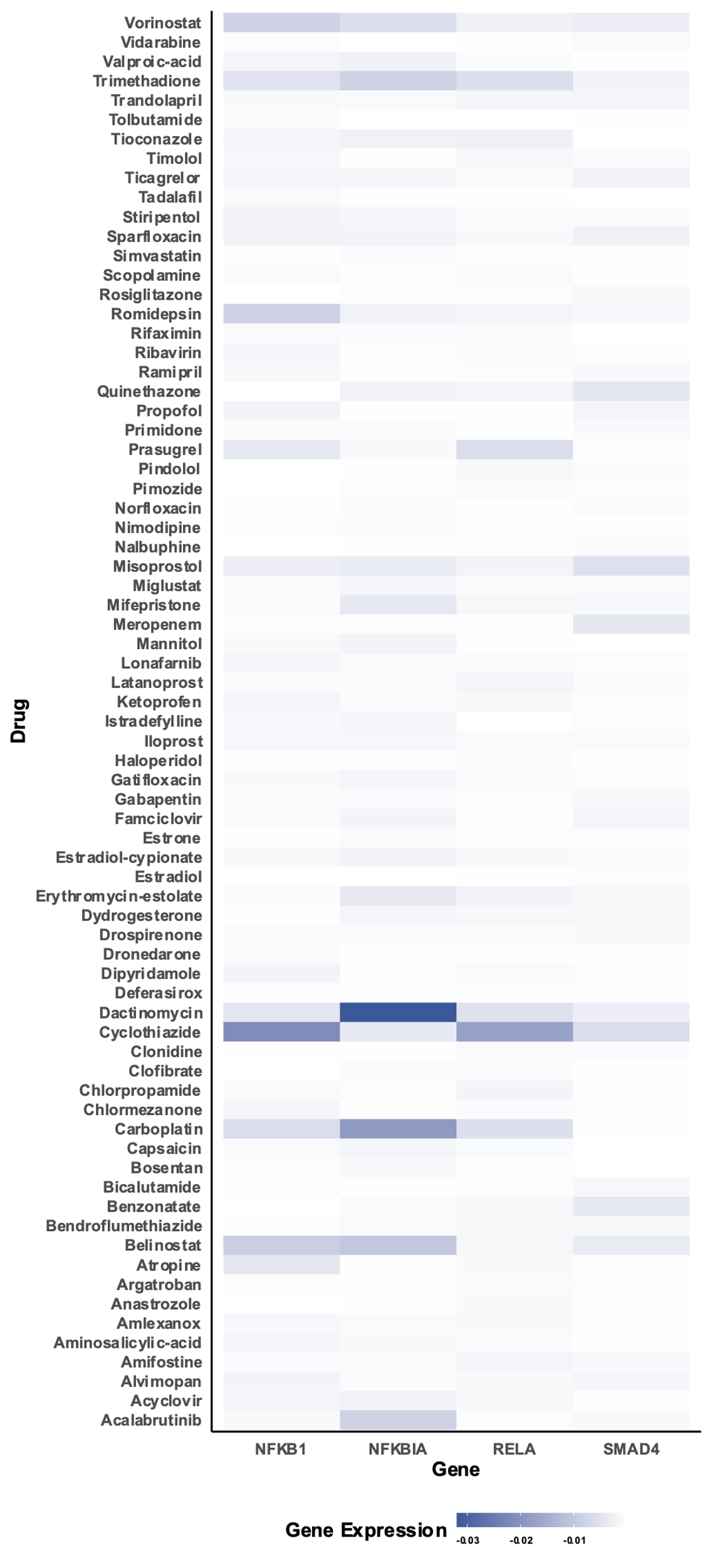


Fig. 7. Connectivity map (CMAP) gene expression profile of the FDA-approved drugs that can downregulate mutual upregulated genes in AD, PD, and HD.

# **Discussion**

## Pathways: “Activated Nuclear Factor Kappa B (NF-κB)”

Due to their complex and progressive nature, research efforts are being expended to conclude the resemblances and discrepancies between different NDs. Early diagnostic tests and therapeutic interventions are sought to help limit the inevitable nerve damage threatening the patients’ quality of life and reduce the global burden of NDs. Most NDs share common pathophysiological pathways. In addition to accumulating irregular peptides or proteins, inflammation is a potential cause of NDs. Neuroinflammation was verified to be involved in the progression of the three diseases under study (44–46). While local inflammation is one hypothesis, constant systematic inflammation with elevated proinflammatory and inflammatory mediators can also cause neuronal damage (47,48).

Nuclear factor kappa B (NF-κB) is a B cell-specific inducible transcriptional factor that regulates the transcription of proinflammatory mediators such as chemokines and cytokines. Under normal conditions, NF-κB finetunes inflammation and maintains cellular protection. It is present in the central nervous system glial cells and cerebral blood vessels (49–51). NF-κB and its subunits reside inactivated by the inhibitor IκB in the cytoplasm. Canonical and noncanonical pathways activate the NF-κB members to be translocated to the nucleus and exert their regulatory actions (51). However, inducible activation of NF-κB was proved to be involved in neuroinflammation and tissue damage in Parkinson’s and Alzheimer's diseases (49,52).

In our study, NF-κB activation was a notably significant pathway between the three diseases. NFKB1, NFKBIA, and RELA, a subunit of NF-κB, were significantly upregulated in the three diseases. RELA is involved in more than one pathway activating NF-κB. Furthermore, we found that two mediators activated NF- κB transcription factor: tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and transforming growth factor β-activated kinase 1 (TAK1). Interestingly, the pathways of these two mediators are mutually upregulated in the three diseases.

TRAFs, a protein family, and tumor necrosis factor (TNF) receptor superfamily signal the activation of NF-κB. TRAFs have E3 ligase activity that is ubiquitin-dependent. Once TRAFs lysine at position 63 is ubiquitinated, they form a scaffold to signal downstream kinases (53). TAK1 and the nuclear factor-κB (IκB) kinase (IKK) inhibitor are downstream kinases signaled by TRAF6 to activate NF-κB. TRAF6 was recently discovered to mediate both IL-1 and CD40 signaling (50). It plays a crucial role in the canonical activation of NF-κB so that other TRAF family members do not counteract its loss (53).

To further support the NF-κB activation, we found that the TNF signaling pathway is upregulated in both Parkinson’s and Huntington’s diseases. TNF cytokines additionally induce the genes regulating inflammatory processes when the NF-κB pathway is activated (54). It is worth mentioning that in patients with Alzheimer’s disease, the NF-κB pathway might be activated in areas surrounding the β-amyloid plaques and also stimulate the expression of TNF-α and IL-1ß cytokines. Activated NF-κB with the resultant neuroinflammation has been hypothesized as the leading cause of Alzheimer’s disease (49). Accordingly, inhibiting the NF-κB pathway may help prevent neuronal damage from the three diseases.

Toll-like receptors (TLR) are also involved in the signaling system of the NF-κB activation. Interleukin-1 receptor-associated kinase (IRAK1) is phosphorylated and dissociated from myeloid differentiation factor 88 (MyD88)-dependent TLR signaling pathway. Phosphorylated IRAK1 consequently reacts with TRAF6 and promotes NF-κB pathway activation. TLR5 was one of our study's upregulated genes of 274 mutual differentially expressed genes. TLR expression is enhanced in patients with Alzheimer's disease and also in mouse models of Parkinson's disease (44).

Based on the involvement of NF-κB activation in neurodegenerative diseases, multiple compounds have been tested and proposed to limit or inhibit its activation, especially in AD. Non-steroidal anti-inflammatory drugs were one of the key agents to halt neuroinflammation, yet their adverse effects interfere with their action in preventing brain damage (46). Antioxidants and bioflavonoids are other alternatives to alleviate neuroinflammation and improve disease prognosis. Sulforaphane, resveratrol, loganin, and icariside II are naturally occurring compounds that showed promising results interfering with the NF-κB activation (46,48,55,56). In rats, the antihyperlipidemic atorvastatin was proven to repress the expression of NF-κB together with TLR4 and TRAF6(57).

## Pathway: “RUNX3 Regulates BCL2L11 (BIM) Transcription”

Research studies have highlighted an intriguing inverse relationship between the progression of neurodegenerative diseases and cancer in which having one disease may decrease the risk of the other (58,59). In the three neurodegenerative diseases under study, we found that the BCL2L11 (BIM) transcription pathway modulated by RUNX3 is upregulated. BIM or BCL-2-like protein 11 (BCL2L11) belongs to the BCL-2 protein family that induces cellular apoptosis (60). RUNX3 is a runt domain-containing transcription factor that regulates the transforming growth factor (TGF- ß) pathway and upregulates BIM expression. TGF- ß pathway induces apoptosis through BIM and RUNX3 regulation. With activated SMADs and FOXO3A, RUNX3 enhances the transcription of BIM. SMAD4 is a tumor suppressor gene that inhibits epithelial cell proliferation and is upregulated in AD, PD, and HD in our study. Hence, the TGF- ß pathway can be a tumor suppressor pathway in which RUNX3 is considered a tumor suppressor gene, especially in gastric cancer (60,61). This can support the hypothesis of the relation between the three neurodegenerative diseases and cancer development. It should be noted that although RUNX3 is involved in the development of proprioceptive dorsal root ganglion neurons in mouse models and expressed in cranial and dorsal root ganglia in human, the exact role of RUNX3 in neuronal diseases is not fully understood yet (62,63). This may be correlated with aging as RUNX3 expression is observed to be elevated with age (64).

Runt-related transcription factors, RUNX3 is expressed in multiple hematopoietic lineages as well as in numerous tissues, including cranial and dorsal root ganglia, thymus, chondrocytes, and the mesenchyme of epidermal appendages. RUNX3 is required to properly develop cytotoxic T-lymphocytes and Langerhans cells (63).

## Epidermal growth factor receptor (EGFR)

Although epidermal growth factor receptor (EGFR) signaling pathways are related to neuron survival and protection from stress and insults, upregulation of EGFR has been associated with multiple neurodegenerative diseases (65). In our study, EGFR was overexpressed in Huntington's and Parkinson's diseases, while EGFR antisense RNA 1 was overexpressed in Alzheimer's. EGFR inhibitors are now being studied for repurposing to treat neurodegenerative diseases (66).

# **Methods**

## Datasets Description ‎

‎ GEO database[[1]](#footnote-1) was searched for genome-wide expression datasets (67). We aimed to include datasets with comparable sample sizes and experimental design where raw expression counts were available. The search strategy was <name of disease> AND “RNA-seq” AND “human”, where the name of the disease is either “Parkinson”, “Alzheimer's”, or “Huntington”. The inclusion criteria ‎were as follows: ‎(a) all datasets were genome-wide, (b) the GEO series type was expression profiling by high throughput sequencing, (c) raw data files were available, with control and disease samples of the human brain, (d) all samples were tissue samples and not blood-derived, and (e) for each study, the total number of available samples was ≥ 20. Hence, the three datasets that have been retrieved were: GSE153873 for AD, GSE68719 for PD, and GSE64810‎‎ ‎for HD.

The AD dataset (GSE153873) has three groups: the AD group, the old control group, and ‎the young control group. The AD group is 12 samples. The healthy controls were divided into the old control group (10 ‎samples) and the young control group (8 samples). The age of young controls ranged from 42 ‎years to 60 years, while the old control and AD groups ranged from 61-79 years. The brain ‎tissue samples were obtained from postmortem human brain samples from the lateral temporal ‎lobe (Brodmann area 21 or 20) (68). The AD and old age control were included in the ‎analysis to compare AD patients to comparable age controls.‎

The PD dataset (GSE68719) was divided into the PD and neurologically normal ‎control groups. The PD group comprised 29 samples, and the healthy control group included ‎‎44. The age of healthy controls ranged from 46 to 97 years, while the patients with PD ranged from 64 to 95 years. The brain tissue samples were obtained from postmortem ‎human brain samples from the prefrontal cortex Brodmann area 9 (69).‎

The HD dataset (GSE64810) had two groups: the HD group (20 samples) and the healthy control ‎group (49 ‎samples). The age of the healthy control group ranged from 36 to 106 years, while the HD patients’ ‎age ranged from 40 to 75 years. The brain tissue samples were obtained from postmortem ‎human brain samples from the prefrontal cortex Brodmann area 9 (70).

## Pathway Enrichment Analysis

Pathway enrichment analysis was conducted on the RNA-Seq raw counts for each disease. A pathway was considered enriched when the maximum FDR was below 0.05. RNA-Seq raw counts were analyzed separately using the Reactome ‎Pathway Browser Tool[[2]](#footnote-2) (71), where the overall lfc for all genes involved in a pathway is calculated. Using raw gene RNA-Seq counts, enriched pathways from each disease were reported. ‎The Venny online tool Field (73) was used to highlight the mutual pathways from the raw counts. Three distinct pathways were found to be mutual between the NDs under study.

## Differential Gene Expression Analysis and Functional Pathway Analysis

The differential expression analysis was performed using DESeq2 R package v.1.30.0 (72). Genes with adjusted P-values less than 0.05 are defined to be significantly differentially expressed. After matching the DEGs with their Ensembl IDs, the org.Hs.eg.db R package (version 3.12.0) was ‎used to convert Ensembl IDs to gene symbols (73). This step was performed in two datasets, HD and PD ‎, because gene symbols were already provided in the AD dataset.‎ Volcano plots visualized gene expression in each disease using the EnhancedVolcano R package[[3]](#footnote-3) after applying log fold change shrinkage on counts using ashr(74). To conclude the mutual DEGs between the three NDs, Venny online tool (75) was used, and a Venn diagram was plotted. A set of 274 genes was identified as differentially expressed mutually between AD, PD, and HD.

KEGG Brite database was used to get functional classification systems for the mutual DEGs between ‎the three diseases (76). The Search & Color Pathway tool 3 was used to access the KEGG Brite database. Search & Color ‎Pathway tool searched mutual DEGs against KEGG pathway maps, ‎KEGG Brite hierarchies, and KEGG modules.

Drug Discovery

To identify potential drugs that can reverse the 5 genes of interest from the mutual pathways, the Connectivity map [1] (CMAP)[[4]](#footnote-4) data from the broad institute was used (available on GEO: GSE70138). The dataset provides transcriptomic profiles for different perturbagens (e.g., drugs, chemical compounds and reagents) after being introduced to human cell lines. The dataset was filtered to only include FDA-approved drugs, and the five genes: NFKB1, NFKBIA, RELA, TRIM4, and SMAD4. Only four genes were found to have a measured gene expression in the CMAP dataset: NFKB1, NFKBIA, RELA, and SMAD4. The drugs were then filtered to only keep the ones with the ability to downregulate all four genes at once. Hence, they showed an upregulated status in AD, PD, and HD.

**Conclusion**

Based on the current study, the significant pathways between AD, PD, and HD are inflammation-mediated pathways that activate the NF-κB transcription factor. Targeting the cycle of NF-κB activation may serve as a potential therapy for the three NDs simultaneously. The analysis also provides further evidence for the decreased risk of patients with NDs having cancer. Both conclusions can be further supported by experimental validation by knocking out targets in these pathways and studying the impact in vivo. While in silico validation is another approach, the availability of data with a similar study design for each neurodegenerative disease and the suitable sample size was quite challenging, especially with autopsy samples from the brain tissue.

# ‎**Abbreviations**

AD: Alzheimer’s disease

DEGs: Differentially expressed genes

EGFR: Epidermal growth factor receptor

FDR: False discovery rate

GEO: Gene Expression ‎Omnibus

GO: Gene ontology

HD: Huntington’s disease

IκB: Inhibitor of nuclear factor-κB

IL-1β: Interleukin-‎‎1β

IRAK1: Interleukin-1 receptor associated kinase

KEGG: Kyoto Encyclopedia of Genes and Genomes

Lfc: Log fold change

NF-κB: Nuclear factor kappa B

NDs: Neurodegenerative diseases

PD: Parkinson’s disease

TGF- ß: Transforming growth factor

TLR: Toll-like receptors

TNF: Tumor necrosis factor

TRAF6: Tumor necrosis factor receptor-associated factor 6

# **Declarations**

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

# **Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets included in the analysis are publicly available on the GEO database under the accessions of GSE153873, GSE68719, and GSE64810‎‎. The codes used to develop this article were written and run with R Notebook and are available online on GitHub (<https://github.com/melsadany/Transcriptome-Analysis-for-Three-Neurodegenerative-Diseases-AD-PD-and-HD> ).

# **Competing interests**

The authors declare that they have no competing interests

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# **Authors' contributions**

M.E. developed the pipeline, N.A. performed the biological analysis, E.B. conducted the project administration, M.E., N.A., and E.B. worked on the methodology, N.A., M.E. worked on the original draft preparation, E.B. conducted the manuscript review and editing.

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# References

1. Feigin VL, Nichols E, Alam T, Bannick MS, Beghi E, Blake N, et al. Global, regional, and national burden of neurological disorders, 1990–2016: A systematic analysis for the global burden of disease study 2016. Lancet Neurol. 2019 May;18(5):459–80.

2. Hussain R, Zubair H, Pursell S, Shahab M. Neurodegenerative diseases: Regenerative mechanisms and novel therapeutic approaches. Brain Sci. 2018 Sep 15;8(9):177.

3. Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol. 2019 Oct 9;15(10):565–81.

4. Ramanan VK, Saykin AJ. Pathways to neurodegeneration: Mechanistic insights from GWAS in Alzheimer’s disease, Parkinson’s disease, and related disorders. Am J Neurodegener Dis. 2013 Sep;2(3):145.

5. Guo F, Liu X, Cai H, Le W. Autophagy in neurodegenerative diseases: pathogenesis and therapy. Brain Pathol. 2018 Jan;28(1):3–13.

6. Briston T, Hicks AR. Mitochondrial dysfunction and neurodegenerative proteinopathies: Mechanisms and prospects for therapeutic intervention. Biochem Soc Trans. 2018 Aug;46(4):829–42.

7. Cairns NJ, Lee VM-Y, Trojanowski JQ. The cytoskeleton in neurodegenerative diseases. J Pathol. 2004 Nov;204(4):438–49.

8. Bredesen DE, Rao R V., Mehlen P. Cell death in the nervous system. Nature. 2006 Oct 18;443(7113):796–802.

9. Esteves AR, Arduíno DM, Silva DF, Viana SD, Pereira FC, Cardoso SM. Mitochondrial metabolism regulates microtubule acetylome and autophagy trough sirtuin-2: Impact for Parkinson’s disease. Mol Neurobiol. 2018 Feb 6;55(2):1440–62.

10. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem. 2010 Dec 22;345(1–2):91–104.

11. Smith DM. Could a common mechanism of protein degradation impairment underlie many neurodegenerative diseases? J Exp Neurosci. 2018 Jan 19;12:117906951879467.

12. Thibaudeau TA, Anderson RT, Smith DM. A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers. Nat Commun. 2018 Dec 15;9(1):1097.

13. Elsadany M, Elghaish RA, Khalil AS, Ahmed AS, Mansour RH, Badr E, et al. Transcriptional analysis of nuclear-encoded mitochondrial genes in eight neurodegenerative disorders: The analysis of seven diseases in reference to Friedreich’s ataxia. Front Genet. 2021;12:749792.

14. Montero-Odasso M, Pieruccini-Faria F, Bartha R, Black SE, Finger E, Freedman M, et al. Motor phenotype in neurodegenerative disorders: Gait and balance platform study design protocol for the Ontario Neurodegenerative Research Initiative (ONDRI). J Alzheimers Dis. 2017;59(2):707–21.

15. Kovacs G. Molecular pathological classification of neurodegenerative diseases: Turning towards precision medicine. Int J Mol Sci. 2016 Feb 2;17(2):189.

16. Bloom GS. Amyloid-β and tau: The trigger and bullet in Alzheimer disease pathogenesis. JAMA Neurol. 2014 Apr;71(4):505–8.

17. Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, et al. Single-cell transcriptomic analysis of Alzheimer’s disease. Nature. 2019 Jun 20;570(7761):332–7.

18. Van der Perren A, Gelders G, Fenyi A, Bousset L, Brito F, Peelaerts W, et al. The structural differences between patient-derived α-synuclein strains dictate characteristics of Parkinson’s disease, multiple system atrophy and dementia with Lewy bodies. Acta Neuropathol. 2020 Jun 30;139(6):977–1000.

19. Miller DW, Hague SM, Clarimon J, Baptista M, Gwinn-Hardy K, Cookson MR, et al. α-Synuclein in blood and brain from familial Parkinson disease with SNCA locus triplication. Neurology. 2004 May;62(10):1835–8.

20. Farrer M, Kachergus J, Forno L, Lincoln S, Wang D-S, Hulihan M, et al. Comparison of kindreds with parkinsonism and α-synuclein genomic multiplications. Ann Neurol. 2004 Feb;55(2):174–9.

21. Wang C, Chen L, Yang Y, Zhang M, Wong G. Identification of potential blood biomarkers for Parkinson’s disease by gene expression and DNA methylation data integration analysis. Clin Epigenetics. 2019 Dec 11;11(1):24.

22. Roos RA. Huntington’s disease: a clinical review. Orphanet J Rare Dis. 2010 Dec 20;5(1):40.

23. Walker FO. Huntington’s disease. Lancet. 2007 Jan;369(9557):218–28.

24. Colpo GD, Rocha NP, Furr Stimming E, Teixeira AL. Gene expression profiling in Huntington’s disease: Does comorbidity with depressive symptoms matter? Int J Mol Sci. 2020 Nov 11;21(22):8474.

25. Durães F, Pinto M, Sousa E. Old drugs as new treatments for neurodegenerative diseases. Pharmaceuticals. 2018 May 11;11(2):44.

26. Chahrour M, Jung SY, Shaw C, Zhou X, Wong STC, Qin J, et al. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science. 2008 May 30;320(5880):1224–9.

27. Wittrahm R, Takalo M, Marttinen M, Kuulasmaa T, Mäkinen P, Kemppainen S, et al. MECP2 increases the pro-inflammatory response of microglial cells and phosphorylation at serine 423 regulates neuronal gene expression upon neuroinflammation. Cells. 2021;10(4).

28. Maphis NM, Jiang S, Binder J, Wright C, Gopalan B, Lamb BT, et al. Whole genome expression analysis in a mouse model of tauopathy identifies MECP2 as a possible regulator of tau pathology. Front Mol Neurosci. 2017;10:69.

29. Zimmermann H. ATP and acetylcholine, equal brethren. Neurochem Int. 2008 Mar;52(4–5):634–48.

30. Behl C. Apoptosis and Alzheimer’s disease. J Neural Transm. 2000 Nov 8;107(11):1325–44.

31. Gupta S, You P, SenGupta T, Nilsen H, Sharma K. Crosstalk between different DNA repair pathways contributes to neurodegenerative diseases. Biology (Basel). 2021 Feb 19;10(2):163.

32. Mueller KA, Glajch KE, Huizenga MN, Wilson RA, Granucci EJ, Dios AM, et al. Hippo signaling pathway dysregulation in human Huntington’s disease brain and neuronal stem cells. Sci Rep. 2018 Dec 27;8(1):11355.

33. Ito T, Matsubara D, Tanaka I, Makiya K, Tanei Z, Kumagai Y, et al. Loss of YAP1 defines neuroendocrine differentiation of lung tumors. Cancer Sci. 2016 Oct;107(10):1527.

34. Chang JR, Ghafouri M, Mukerjee R, Bagashev A, Chabrashvili T, Sawaya BE. Role of p53 in neurodegenerative diseases. Neurodegener Dis. 2012;9(2):68–80.

35. Freitas AE, Neis VB, Rodrigues ALS. Agmatine, a potential novel therapeutic strategy for depression. Eur Neuropsychopharmacol. 2016 Dec;26(12):1885–99.

36. Morris BJ, Willcox DC, Donlon TA, Willcox BJ. FOXO3: A major gene for human longevity--A mini-review. Gerontology. 2015;61(6):515–25.

37. Du S, Zheng H. Role of FoxO transcription factors in aging and age-related metabolic and neurodegenerative diseases. Cell Biosci. 2021;11(1):1–17.

38. Zhang Y-L, Wang R-C, Cheng K, Ring BZ, Su L. Roles of Rap1 signaling in tumor cell migration and invasion. Cancer Biol Med. 2017 Feb;14(1):90–9.

39. McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. J Neuroinflammation. 2008 Oct 17;5:45.

40. Martin R. Targeting fibrin in neurodegeneration. Nat Immunol. 2018;19(11):1149–50.

41. Hu W, Yang Z, Yang W, Han M, Xu B, Yu Z, et al. Roles of forkhead box O (FoxO) transcription factors in neurodegenerative diseases: A panoramic view. Prog Neurobiol. 2019;181:101645.

42. Cymerman IA, Chung I, Beckmann BM, Bujnicki JM, Meiss G. EXOG, a novel paralog of Endonuclease G in higher eukaryotes. Nucleic Acids Res. 2008 Mar;36(4):1369–79.

43. Cooper PC, Hill M, Maclean RM. The phenotypic and genetic assessment of protein C deficiency. Int J Lab Hematol. 2012 Aug;34(4):336–46.

44. Okun E, Griffioen KJ, Lathia JD, Tang S-C, Mattson MP, Arumugam T V. Toll-like receptors in neurodegeneration. Brain Res Rev. 2009;59(2):278–292.

45. Di Pardo A, Ciaglia E, Cattaneo M, Maciag A, Montella F, Lopardo V, et al. The longevity-associated variant of BPIFB4 improves a CXCR4-mediated striatum–microglia crosstalk preventing disease progression in a mouse model of Huntington’s disease. Cell Death Dis. 2020;11(7).

46. Zhou J, Deng Y, Li F, Yin C, Shi J, Gong Q. Icariside II attenuates lipopolysaccharide-induced neuroinflammation through inhibiting TLR4/MyD88/NF-κB pathway in rats. Biomed Pharmacother. 2019 Mar;111:315–24.

47. Wu Y, Hu Y, Wang B, Li S, Ma C, Liu X, et al. Dopamine uses the DRD5-ARRB2-PP2A signaling axis to block the TRAF6-mediated NF-κB pathway and suppress systemic inflammation. Mol Cell. 2020;78(1):42-56.e6.

48. Wang Z, Chen Q, Wang J, Yu L, Chen L. Sulforaphane mitigates LPS-induced neuroinflammation through modulation of Cezanne/NF-κB signalling. Life Sci. 2020 Dec;262:118519.

49. Jha NK, Jha SK, Kar R, Nand P, Swati K, Goswami VK. Nuclear factor-kappa β as a therapeutic target for Alzheimer’s disease. J Neurochem. 2019;150(2):113–37.

50. Dainichi T, Matsumoto R, Mostafa A, Kabashima K. Immune control by TRAF6-mediated pathways of epithelial cells in the EIME (Epithelial Immune Microenvironment). Front Immunol. 2019;10:1107.

51. Shi J-H, Sun S-C. Tumor necrosis factor receptor-associated factor regulation of nuclear factor κB and mitogen-activated protein kinase pathways. Front Immunol. 2018;9:1849.

52. Pal R, Tiwari PC, Nath R, Pant KK. Role of neuroinflammation and latent transcription factors in pathogenesis of Parkinson’s disease. Neurol Res. 2016;38(12):1111–22.

53. Yamamoto M, Gohda J, Akiyama T, Inoue JI. TNF receptor-associated factor 6 (TRAF6) plays crucial roles in multiple biological systems through polyubiquitination-mediated NF-қB activation. Proc Japan Acad Ser B Phys Biol Sci. 2021;37(4):145–60.

54. Hayden M, Ghosh S. Regulation of NF-κB by TNF family cytokines. Semin Immunol. 2014;26(3):253–266.

55. Cui Y, Wang Y, Zhao D, Feng X, Zhang L, Liu C. Loganin prevents BV-2 microglia cells from Aβ1-42-induced inflammation via regulating TLR4/TRAF6/NF-κB axis. Cell Biol Int. 2018;42(12):1632–42.

56. Ge YT, Zhong AQ, Xu GF, Lu Y. Resveratrol protects BV2 mouse microglial cells against LPS-induced inflammatory injury by altering the miR-146a-5p/TRAF6/NF-κB axis. Immunopharmacol Immunotoxicol. 2019;41(5):549–57.

57. Wang S, Zhang X, Zhai L, Sheng X, Zheng W, Chu H, et al. Atorvastatin attenuates cognitive deficits and neuroinflammation induced by Aβ1–42 involving modulation of TLR4/TRAF6/NF-κB pathway. J Mol Neurosci. 2018;64(3):363–73.

58. Houck AL, Seddighi S, Driver JA. At the crossroads between neurodegeneration and cancer: A review of overlapping biology and its implications. Curr Aging Sci. 2018;11(2):77–89.

59. Shafi O. Inverse relationship between Alzheimer’s disease and cancer, and other factors contributing to Alzheimer’s disease: A systematic review. BMC Neurol. 2016;16(1):1–17.

60. Yano T, Ito K, Fukamachi H, Chi X-Z, Wee H-J, Inoue K, et al. The RUNX3 tumor suppressor upregulates Bim in gastric epithelial cells undergoing transforming growth factor beta-induced apoptosis. Mol Cell Biol. 2006 Jun;26(12):4474–88.

61. Tong DD, Jiang Y, Li M, Kong D, Meng XN, Zhao YZ, et al. RUNX3 inhibits cell proliferation and induces apoptosis by TGF-β-dependent and-independent mechanisms in human colon carcinoma cells. Pathobiology. 2009;76(4):163–9.

62. Inoue K ichi, Ozaki S, Shiga T, Ito K, Masuda T, Okado N, et al. Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. Nat Neurosci. 2002;5(10):946–54.

63. Boto P, Csuth TI, Szatmaria I. RUNX3-mediated immune cell development and maturation. Crit Rev Immunol. 2018;38(1):63–78.

64. Meng G, Zhong X, Mei H. A systematic investigation into aging related genes in brain and their relationship with Alzheimer’s disease. PLoS One. 2016;11(3):1–17.

65. Habib R, Noureen N, Nadeem N. Decoding common features of neurodegenerative disorders: From differentially expressed genes to pathways. Curr Genomics. 2018;19(4):300–12.

66. Tavassoly O, Sato T, Tavassoly I. Inhibition of brain epidermal growth factor receptor activation: A novel target in neurodegenerative diseases and brain injuries. Mol Pharmacol. 2020;98(1):13–22.

67. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002 Jan 1;30(1):207–10.

68. Nativio R, Lan Y, Donahue G, Sidoli S, Berson A, Srinivasan AR, et al. An integrated multi-omics approach identifies epigenetic alterations associated with Alzheimer’s disease. Nat Genet. 2020 Oct 28;52(10):1024–35.

69. Dumitriu A, Golji J, Labadorf AT, Gao B, Beach TG, Myers RH, et al. Integrative analyses of proteomics and RNA transcriptomics implicate mitochondrial processes, protein folding pathways and GWAS loci in Parkinson disease. BMC Med Genomics. 2015 Dec 21;9(1):5.

70. Labadorf A, Hoss AG, Lagomarsino V, Latourelle JC, Hadzi TC, Bregu J, et al. RNA sequence analysis of human Huntington disease brain reveals an extensive increase in inflammatory and developmental gene expression. PLoS One. 2015 Dec 4;10(12):e0143563.

71. Fabregat A, Sidiropoulos K, Garapati P, Gillespie M, Hausmann K, Haw R, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2016;44(D1):D481–7.

72. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014 Dec 5;15(12):550.

73. Carlson M. org.Hs.eg.db: Genome wide annotation for Human. R package version 3.8.2. 2019.

74. Stephens M. False discovery rates: a new deal. Biostatistics. 2016 Oct 17;kxw041.

75. Oliveros JC. An interactive tool for comparing lists with Venn’s diagrams (2007–2015) [Internet]. 2018. Available from: https://bioinfogp.cnb.csic.es/tools/venny/index.html

76. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. Protein Sci. 2019 Nov;28(11):1947–51.

**Supplementary Information**

## Additional File 1.

Excel Additional File 1.xls

Table S1: Differentially expressed genes in AD, PD, and HD

Differentially expressed genes in three neurodegenerative diseases. The file includes log fold change and adjusted P values for differentially expressed genes in Alzheimer’s, Parkinson’s, and Huntington’s diseases. It also has mutual differentially expressed genes between the three diseases. For the list of mutual DEGs, the classification of these genes is described according to the KEGG database.

## Additional File 2

Additional File 2.xls

Table S2: Pathways enriched in AD, PD, and HD

Enriched pathways in three neurodegenerative diseases with FDR below 0.05. The file includes regulation of the enriched pathways (upregulated or downregulated), FDR, P values, and average fold change for Alzheimer’s, Parkinson’s, and Huntington’s diseases. It also includes mutual pathways between the three diseases and the genes involved in these pathways for each disease.

[1] A. Subramanian *et al.*, "A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles," *Cell,* vol. 171, no. 6, pp. 1437-1452 e17, Nov 30 2017, doi: 10.1016/j.cell.2017.10.049.

1. <https://www.ncbi.nlm.nih.gov/geo/> [↑](#footnote-ref-1)
2. <https://reactome.org/PathwayBrowser/#TOOL=AT> [↑](#footnote-ref-2)
3. <https://github.com/kevinblighe/EnhancedVolcano> [↑](#footnote-ref-3)
4. <https://www.broadinstitute.org/connectivity-map-cmap> [↑](#footnote-ref-4)