The Expression of BRD9-containing BAF Complexes on Parkinson's Disease

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Abstract

Parkinson's Disease (PD), a progressive neurodegenerative disorder, affects millions of individuals globally and presents one of the fastest-growing neurological burdens. Despite extensive research, the mechanisms driving PD's progression remain elusive, particularly in relation to the role of chromatin remodeling complexes in gene expression. This study focuses on the BRD9-containing BAF complexes, hypothesizing that their dysregulation could contribute to PD pathogenesis, similar to findings in Huntington's Disease (HD). By analyzing mRNA sequencing data from post-mortem PD and control brain tissues, we explore the potential involvement of these complexes in PD. Through rigorous gene expression analysis and statistical testing, we aim to shed light on whether BRD9 and its associated subunits play a significant role in the disease and offer insight into potential therapeutic targets. While our findings suggest limited overexpression of these complexes, they open avenues for further investigation into their specific role in PD's molecular mechanisms and underscore the necessity to explore alternative pathways for novel therapeutic strategies.

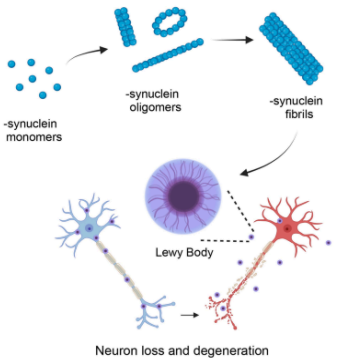
Keywords: Parkinson's Disease (PD), BAF complexes, neurodegeneration, gene expression

1. Background

1.1 Problem

Parkinson's Disease (PD) is an progressive neurodegenerative motor disease that affects over 10 million individuals worldwide and has the fastest increasing rates of disability and death than any other existing neurological condition.

The development of PD is primarily linked to mutations in the SNCA gene, which encodes the α-synuclein protein. These mutations cause malignant accumulations of α-synuclein in the substantia nigra region of the brain, called Lewy bodies. Thus, these abnormal protein aggregates block dopamine transmission, which is the neurotransmitter for muscle control, causing motor disorders such as limb tremors and impaired coordination to manifest in Parkinson's affected individuals.

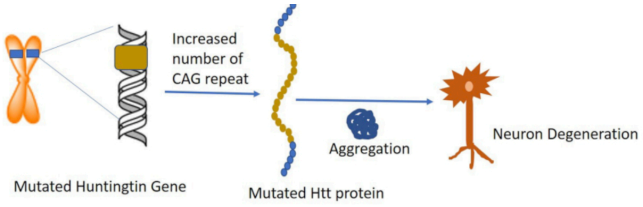


Development of Lewy Bodies in PD patient, (Lv et al., 2023)

Currently, there exists no cure for Parkinson's, other than symptom relief treatments such as physiotherapy, dopamine-inducing medication, and deep brain stimulation that can reduce tremors and improve movement. However, all

current solutions fail to address the underlying progression of the disease and can only relieve the symptoms that PD patients feel.

Huntington's Disease (HD) is a very similar neurodegenerative motor disorder. It is characterized by mutations in the HTT gene that result in the formation of toxic huntingtin protein clumps in the brain that kill nerve cells, causing muscle contractions and symptoms similar to PD. Recent studies in HD mouse models indicate that inhibiting the epigenetic reader bromodomain-containing 9 (BRD9), a component of the BAF chromatin remodeling complexes, reduces the toxic huntingtin clumps and improves mobility in the mice.



Development of toxic Huntingtin clumps in HD patient, (Kohli, n.d.)

2. Hypothesis

2.1 Rationale

Given that BRD9 is a therapeutic vulnerability in Huntington's, as well as the fact epigenetic readers and BAF complexes influence the expression of a wide array of genes, it is highly plausible that they could also impact gene expression in Parkinson's Disease as well. Especially as HD and PD share similarities in their pathogenesis, particularly regarding protein aggregation and neurodegeneration, it is conceivable that BRD9, in conjunction with other subunits within the BAF complexes, may be overexpressed in PD. This overexpression could potentially lead to increased transcription of genes implicated in PD, such as the genes encoding α-synuclein.

2.2 Hypothesis

I hypothesize that BRD9 - containing BAF complexes will be overexpressed in Parkinson's patients when compared to healthy controls.

3. Methodology

3.1 Overview

This study employs a case-control design to investigate the gene expression differences between Parkinson's disease (PD) patients and healthy controls. The primary aim is to analyze the involvement of BRD9-containing BAF chromatin remodeling complexes in PD. Using mRNA sequencing from post-mortem brain tissue, we examined differential gene expression and applied statistical tests to identify significant variations. The study followed standard protocols for sample preparation, sequencing, and statistical analysis to ensure reproducibility and accuracy.

3.2 Sample Selection

A gene expression profiling dataset with 16 post-mortem controls and experimental Parkinson's patients was retrieved. The dataset contained mRNA sequencing of post-mortem PD and control patient's substantia nigra, comprising 8 PD patients and 8 healthy controls. Pairing criteria were applied based on age and sex, ensuring that each PD sample was matched with a control sample of the same sex and a four-year age range. A total of 3 sets of samples were chosen, of which two were male and one was female.

Pairing	Sex	Age (Parkinson's)	Age (Control)
1	Male	86	88
2	Male	70	72
3	Female	77	73

Table 1. Age and sex of PD and control patients sample pairings.

3.3 Log_2 Fold Change Analysis

Log_2 fold change (Log2FC) analysis was employed to quantify the differences in gene expression between Parkinson's disease samples and control samples. This method normalizes the data by scaling large variations and converting fold changes into a symmetric scale, where equal upregulation and downregulation are represented as mirror values, facilitating more straightforward comparisons between genes. The log base 2 of the ratio of the average expression level of a gene in the Parkinson's samples to its average expression level in the control samples was calculated using the following equation:

$$log_2FC = log_2(\frac{average\ expression\ across\ PD\ samples}{average\ expression\ across\ controls})$$

3.3.1 Fold-Change Cut-Off

We used a fold-change cutoff of 1.5. A $\log_2$ fold change cutoff of 1.5 is commonly used in mRNA-seq research because it represents a nearly 3-fold change in gene expression, which is typically considered biologically significant and minimizes the inclusion of noise or small, irrelevant variations in genome datasets.

3.3.2 T-Test for Statistical Significance

After obtaining the FC values, a two-tailed unequal variance t-test (Welch's t-test) was used in the analysis to compare gene expression levels between Parkinson's disease patients and healthy controls. Since the variances between these two groups were not assumed to be equal, Welch's t-test was selected due to its ability to adjust for differences in variability, offering greater reliability than a standard t-test. Additionally, since we did not predict whether gene expression would be upregulated or downregulated, a two-tailed test was appropriate to detect significant differences in either direction. This allowed for a more accurate assessment of the significance of differences in gene expression between the populations, without making assumptions about variance or directionality.

P-values were calculated from the following two-tailed unequal variance equation T-test equation to determine the significance of gene expression differences between the two groups:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

Only genes that met both the Log2FC threshold and statistical significance (**p-value < 0.05**) were considered for further investigation.

3.4 Gene Selection

To test the hypothesis, the genes within BRD9-containing BAF complexes, gBAF (also referred to as ncBAF), npBAF, and nBAF were analyzed. Additionally, BRD9's splicing variants, BRD9-202, BRD9-204, and BRD9-207, were identified for analysis.

4. Results

4.1 Tables

4.1.1 GBAF Complex

Figure 1. LOG2FC values of GBAF complex

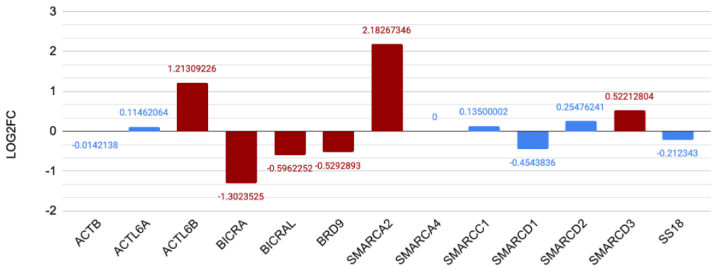
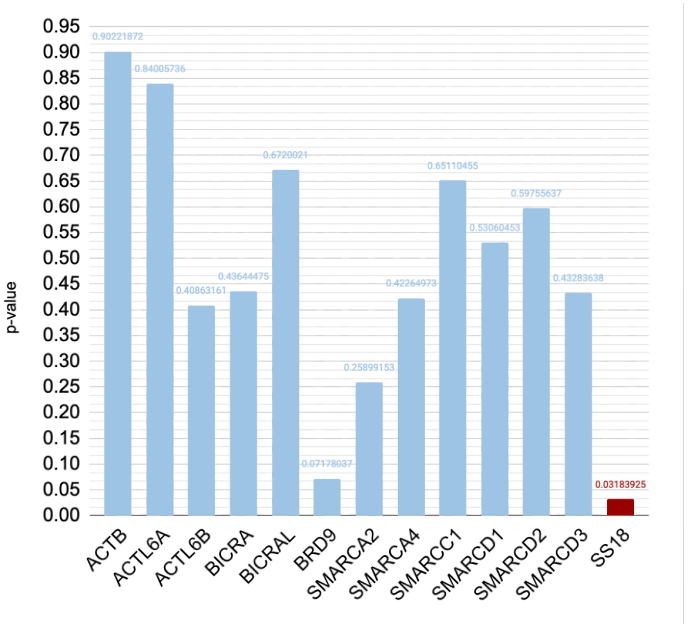


Figure 2. Statistical significance of GBAF complex



4.1.2 npBAF Complex

Figure 3. LOG2FC values of npBAF complex not shared with GBAF complex

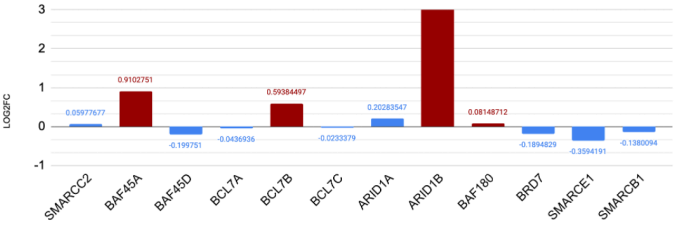
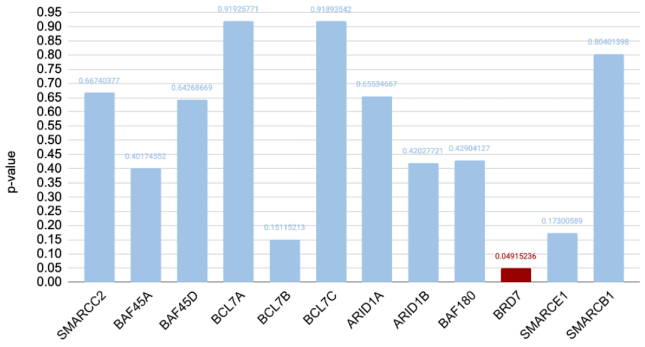


Figure 4. Statistical significance of unique npBAF complex genes not found in GBAF complex



4.1.3 nBAF Complex

Figure 5. LOG2FC values of nBAF complex not shared with GBAF complex

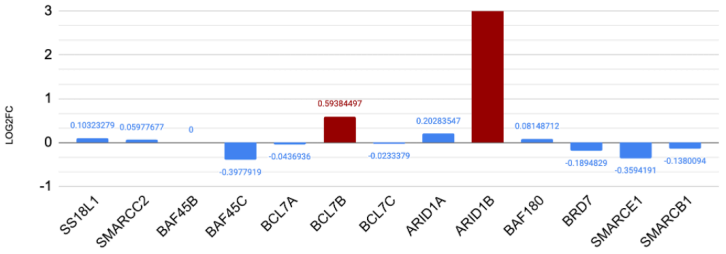
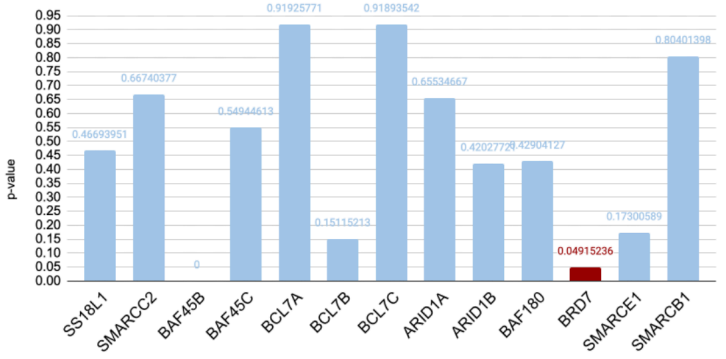


Figure 6. Statistical significance of unique nBAF complex genes not found in GBAF complex



This gene expression analysis of BRD9-containing BAF complexes in Parkinson's Disease (PD) yielded several notable findings:

4.2 BRD9 Expression

Contrary to the initial hypothesis that BRD9 might be overexpressed in PD due to its role in Huntington's Disease (HD), the analysis revealed that BRD9 was downregulated in PD patients by a factor of 1.5-fold (Fig. 1). However, this reduction did not reach statistical significance (Fig. 2), meaning that the expression levels of BRD9 did not differ substantially between PD patients and healthy controls in a statistically meaningful way.

4.3 SS18 and BRD7

Among the BAF complex subunits analyzed, SS18 and BRD7 emerged as statistically significant. While both genes were also downregulated, their p-values were below the threshold for statistical significance ($p < 0.05$), indicating that their decreased expression in PD samples could be biologically relevant. These findings suggest that SS18 and BRD7 might play a specific role in PD, possibly related to altered chromatin remodeling mechanisms.

4.4 BAF Complex Subunits Meeting Log2FC Cutoff

Several other subunits of the BAF complex, including ACTL6B, BICRA, SMARCA2, and BCL7B, met the Log2 Fold Change (Log2FC) cut off of 0.5, implying a noticeable change in their expression levels. However, none of these genes were statistically significant, limiting the conclusions that can be drawn about their role in PD without further investigation.

4.5 Gene Expression Patterns in PD:

The overall expression pattern of genes involved in the BRD9-containing BAF complexes did not indicate overexpression in PD. Most genes that were up- or downregulated did not meet both the fold-change threshold and statistical significance, suggesting that the mechanisms of chromatin remodeling in PD may involve other factors or may operate through more subtle regulatory changes.

4.6 Therapeutic Implications

Although BRD9 and its associated BAF complexes did not appear to be overexpressed in this cohort of PD patients, the downregulation of SS18 and BRD7 presents potential areas for future research. Understanding how these subunits contribute to chromatin remodeling in neurodegeneration could help identify new therapeutic strategies that target epigenetic readers in PD.

5. Conclusions

This research assesses the expression levels of genes within BRD9-containing BAF complexes in the context of Parkinson's disease. Our findings dictate that BRD9 was downregulated by a factor of 1.5-fold compared to control subjects, lacking statistical significance. Similarly, SS18 and BRD7, despite being statistically significant, were also downregulated. Other genes in the complex, namely ACTL6B, BICRA, BICRAL, SMARCA2, SMARCD3, BCL7B, ARID1B, BAF180, and BAF45B, met the log2 fold change threshold but did not show statistical significance.

This indicates that these complexes are not overexpressed in Parkinson's disease within the parameters of our study, because none of the genes in the BRD9-containing BAF complexes were both statistically significant and met fold change cut - offs. However, it is premature to conclusively exclude these complexes as potential therapeutic targets solely based on expression levels. They may still play a role in the disease's pathophysiology that overexpression does not capture. To clarify the potential therapeutic relevance of BRD9-containing BAF complexes, further data analysis and allocation of more resources are necessary.

6. Limitations

The conclusions of this study should be interpreted within the context of several potential sources of error:

6.1 Sample Size

With a total of 16 samples and only 6 selected for detailed analysis, this study is constrained by a small sample size, which inherently limits the generalizability of the findings. These samples may not be representative of the broader Parkinson's patient and control populations.

6.2 Matching Criteria

This study's matching approach was based solely on age and sex. However, Parkinson's disease is a multifaceted condition where stages of progression can significantly affect biological markers. The absence of matching for disease stage means that variations in the expression of epigenetic readers may be due to different disease stages rather than the presence of the disease itself.

6.3 SAMPLE GENDERS

Data availability limited my access to a balanced gender distribution within the samples. Consequently, I obtained only one female sample compared to two male samples, which may introduce a bias in the sex-based analysis and affect the applicability of my results.

7. Future Work

7.1 FLUORODOPA (F-DOPA) PET SCANNING

I plan to expand my sample size by leveraging F-DOPA PET scans to visualize dopaminergic activity in living patients with PD. This method will allow me to expand my current dataset, which is limited to post-mortem patients, by including live imaging data. I plan to reach out to local institutions to collaborate and gain access to F-DOPA PET scanning facilities. Additionally, I plan to sample diversely to achieve a balanced distribution of each sex.

7.2 REFINE MATCHING CRITERIA

I also plan to create an improved matching strategy that carefully considers disease severity, treatment history, and other pertinent clinical factors.

7.3 CHROMATIN IMMUNOPRECIPITATION (ChIP)

Future experiments can utilize ChIP assays to pinpoint the exact genomic regions BRD9 –containing BAF complexes target within neuronal cells for further investigation.

7.4 BRD9 IN OTHER TISSUES AFFECTING PD

BRD9's role in lipid transcription for myelination in dopamine neurons might lead to neural short-circuits, suggesting that studying lipid levels in fat tissues could be future areas for investigating how BRD9 might indirectly affect PD.

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References

[1] Joon Yan Selene Lee, Jing Han Ng, Seyed Ehsan Saffari, Eng-King Tan. Parkinson's disease and cancer: a systematic review and meta-analysis on the influence of lifestyle habits, genetic variants, and gender. AGING 2022, Vol. 14, No. 5 https://pubmed.ncbi.nlm.nih.gov/35247252/

[2] Helena Xicoy , Bé Wieringa and Gerard J. M. Martens.The Role of Lipids in Parkinson's Disease. Cells https://pubmed.ncbi.nlm.nih.gov/30621069/

[3] Omar de Faria Jr ,David G Gonsalvez, Madeline Nicholson, Junhua Xiao. Activity-dependent central nervous system myelination throughout life. Journal of Neurochemtry Feb 2019. https://pubmed.ncbi.nlm.nih.gov/30225984/

[4] Xiuzuo Zhu,Yi Liao,Liling Tang.Targeting BRD9 for Cancer Treatment: A New Strategy. OncoTargets and Therapy Dec 2020. https://pubmed.ncbi.nlm.nih.gov/33380808/

[5] Sola Han, Siin Kim, Hyungtae Kim, Hae-Won Shin, Kyoung-Sae Na and Hae Sun Suh. Prevalence and incidence of Parkinson's disease and drug-induced parkinsonism in Korea.BMC Publib Health 2019 https://doi.org/10.1186/s12889-019-7664-6

[6] Alfert, A., Moreno, N., & Kerl, K. (2019, March 21). The BAF complex in development and disease - Epigenetics & Chromatin. Epigenetics & Chromatin. Retrieved March 31, 2024. https://epigeneticsandchromatin.biomedcentral.com/articles/10.1186/s13072-019-0264-y

[7] Birkett, A. (2023, November 11). One-Tailed vs. Two-Tailed Tests (Does It Matter?). CXL. Retrieved March 20, 2024. https://cxl.com/blog/one-tailed-vs-two-tailed-tests/

[8] Faria Jr, O. d., Gonsalvez, D. G., Nicholson, M., &Xiao, J. (n.d.). Activity-dependent central nervous system myelination throughout life. PubMed. Retrieved April 3, 2024. https://pubmed.ncbi.nlm.nih.gov/30225984/

[9] The Genetic Link to Parkinson's Disease, (n.d.). Johns Hopkins Medicine. Retrieved March 19, 2024. https://www.hopkinsmedicine.org/health/conditions-and-diseases/parkinsons-disease/the -genetic-link-to-parkinsons-disease

[10] Hammond, N. (2021, March 30). Parkinson's life expectancy: Stages and treatment options. Medical News Today. Retrieved March 11, 2024, from https://www.medicalnewstoday.eom/articles/parkinsons-life-expectancy#treatment

[11] Huntington's disease - Symptoms and causes. (2022, May 17). Mayo Clinic. Retrieved March 19, 2024, from https://www.mayoclinic.Org/diseases-conditions/huntingtons-disease/symptoms-causes/syc-20356117

[12] Kohli, H. (n.d.). In silico designing of putative peptides for targeting pathological protein Htt in Huntington's disease. - Abstract. Europe PMC. Retrieved March 19, 2024, from https://europepmc.org/article/pmc/pmc7890153

[13] Launch of WHO's Parkinson disease technical brief. (2022, June 14). World Health Organization (WHO). Retrieved March 19, 2024, from https://www.who.int/news/item/14-06-2022-launch-of-who-s-parkinson-disease-technical-brief

[14] Lee, S. W, Ruwa, R., & Kubala, J. (2024, January 18). Parkinson's vs. Huntington's: Symptoms, Causes, and Outlook. Healthline. Retrieved March 11, 2024, from https://www.healthline.eom/health/parkinsons/parkinsons-vs-huntingtons#symptoms

[15] Lewy Body Dementia (LBD). (n.d.). Johns Hopkins Medicine. Retrieved March 19, 2024, from https://www.hopkinsmedicine.org/health/conditions-and-diseases/dementia/dementia-wit h-lewy-bodies

[16] Lv, Q.-K., Tao, K.-X., & Wang, X.-B. (2023, January 6). , - YouTube. Retrieved March 19, 2024, from https://link.springer.eom/artide/10.1007/s00011-022-01676-x

[17] Maia, M., & Briceno, C. (2023, December 19). Partners' goal: Huntington's disease research to stop progression. Huntington's Disease News. Retrieved March 19, 2024, from https://huntingtonsdiseasenews.com/news/partners-goai-huntingtons-disease-research- halt-progression/

[18] Overview of Chromatin Immunoprecipitation (ChIP). (n.d.). Cell Signaling Technology. Retrieved April 3, 2024, from https://www.cellsignal.com/applications/chip-and-chip-seq/regulation-expression-in-cell-and-tissue

[19] Parkinson disease. (2023, August 9). World Health Organization (WHO). Retrieved March 11, 2024, from https://www.who.int/news-room/fact-sheets/detail/parkinson-disease

[20] Parkinson's Disease: Causes, Symptoms, and Treatments | National Institute on Aging. (2022,April 14). National Institute on Aging. Retrieved March 11, 2024, from https://www.nia.nih.gov/health/parkinsons-disease/parkinsons-disease-causes-symptoms-and-treatments

[21] Tranchevent, L.-C., Halder, R., & Glaab, E. (2023, January 21). Wikipedia. Retrieved March 20,2024, from https://doi.org/10.1038/s41531-023-00446-8

[22] Varga, J., & Kube, M. (2021, August 27). The BAF chromatin remodeling complexes: structure, function, and synthetic lethalities. PubMed. Retrieved March 31, 2024, from https://pubmed.ncbi.nlm.nih.gov/34431497/

[23] What is Parkinson's? (n.d.). Parkinson's Foundation. Retrieved March 11, 2024, from https://www.parkinson.org/understanding-parkinsons/what-is-parkinsons