A Systematic Approach to Assessing the Link between Parkinson's Disease, Neuromelanin, and Aging using Gene Expression Data

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Objective

Parkinson's Disease

- Incidence of Parkinson's disease (PD) expected to increase by 30% by 2030 (Kouli, Torsney, & Kuan, 2018)
- Caused by death of dopaminergic (DA) neurons of the **substantia nigra pars compacta (SNpc**) linked to the aggregation of **α-synuclein** (Vila, 2019)
- **Age is the greatest risk factor** for PD
- **Only melanized DA neurons significantly lost** in PD [2,3] (Vila, 2019; Haining & Achat-Mendes, 2017)
- DA neurons in SNpc contain high levels of neuromelanin (are melanized)
- DA neurons in **ventral tegmental area (VTA**), lack neuromelanin, not lost in PD (Vila, 2019)

Unknowns about Parkinson's

- **Molecular mechanism** linking **PD** with **age** and **Neuromelanin** (**NM**)
- Initial trigger of neurotoxic mechanisms/neuron loss in PD (could be related to NM)
- **Drug targets for disease-modifying therapies** involving NM

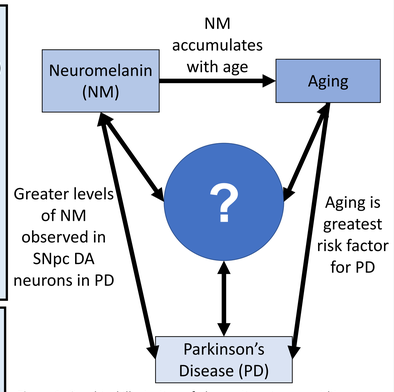


Figure 1. Graphical illustration of observations in current literature that suggest an as-of-yet unknown link between neuromelanin (NM), aging, and Parkinson's disease (PD) exists.

Research Question: By which genes and biological processes does neuromelanin contribute to the pathogenesis of Parkinson's disease in an age-dependent manner?

Abstract

Parkinson's disease (PD), the second most common neurodegenerative disease in the world, is characterized by the loss of dopaminergic neurons in the neuromelanin-containing substantia nigra pars compacta (SNpc). Despite observations that melanized neurons are selectively vulnerable to degeneration in PD in an age-dependent manner, the relationship between aging, neuromelanin, and PD remains poorly understood. This study identified novel gene expression signatures of age-dependent and cell type-specific neuromelanin-linked neurotoxicity that may be associated with the pathogenesis of PD. Differential expression analysis between the melanized dopaminergic SNpc and the non-melanized dopaminergic ventral tegmental area (VTA) and correlations of gene expression in the substantia nigra (SN) with age were performed in R. These analyses were validated by studying the selected genes' differential expression in the SN of PD brains. Population-specific expression analysis of the SNpc relative to the VTA revealed that microglia-specific genes were enriched for aging (fold enrichment = 1.51, p = 0.0237) and PD (fold enrichment = 2.36, p = 0.0188), indicating that microglia may provide the link between neuromelanin, aging, and PD. Overall, 37 genes were both differentially expressed in the SNpc relative to the VTA and significantly correlated with age (p < 0.05). In particular, the proteins corresponding to *RAB9A, YWHAZ, DTNBP1*, and *CAT* were found to interact with PD-implicated proteins (mean interaction scores 0.493, 0.717, 0.902, 0.581, respectively). These genes could serve as novel preventative and therapeutic drug targets for PD and also be used to develop more accurate rodent models of PD based on neuromelanin-linked neurotoxicity.

Materials and Methodology

1. **Expression-Age Correlations** → address age factor
   - ➢ Identify genes significantly correlated with age (α = 0.05)
2. **Melanized Dopaminergic Brain Region analysis** (**mDBR**) → address NM factor
   - ➢ Identify genes differentially expressed in presence of NM (α = 0.05)
   - ➢ Differential expression analysis between melanized SNpc and non-melanized VTA
   - ➢ Observe cell-type specific expression changes using population-specific expression analysis (PSEA)
3. **Overlap and Validation in PD**
   - ➢ Overlap: Identify genes that are both correlated with age and differentially expressed in SNpc
   - ➢ Confirm overlapping genes also differentially expressed in SN of PD brains (α = 0.05)

Differential Expression Analysis:

- Identify genes exhibiting expression changes in SNpc relative to VTA (**mDBR**) and in PD relative to controls
- T-test, Benjamini-Hochberg (BH) procedure to correct p-values → minimize false discovery rate (FDR)

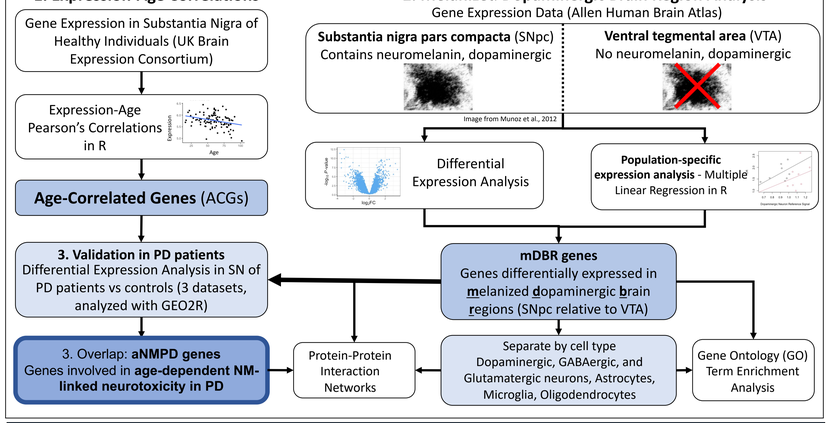
Population-Specific Expression Analysis (PSEA)

- Correct for cell-type population size differences between SNpc and VTA (**mDBR**)
- Dopaminergic neurons, GABAergic neurons, Glutaminergic neurons, Oligodendrocytes, Microglia, Astrocytes
- Regress gene expression on expression of reference genes, using interaction regressor for brain region

Gene Ontology Term Enrichment and Protein-Protein Interaction Networks:

- Identify biological processes, molecular functions, and cellular components associated with gene sets
- Characterize interactions of proteins with PD-implicated genes previously noted in the literature

Materials and Methodology (continued)



Results and Interpretations

Microglia-specific genes exhibited enrichment for aging and Parkinson's disease

PSEA: Applying linear models to gene expression data to identify genes whose expression is different in certain cell types of SNpc compared to the same cell type in the VTA

- Takes gene expression data of a whole tissue (not single-cell) and splits up by cell type

Population-specific expression changes in microglia of SNpc relative to VTA (Fig. 2)

- GO term enrichment of genes exhibiting microglia-specific expression changes in SNpc:
  - **Aging** (Fold enrichment = 1.51, BH-corrected p = 0.0237)
  - **Parkinson's disease** (Fold enrichment = 2.36, BH-corrected p = 0.0188)
  - **Response to iron(II) ion** (Fold enrichment = 4.17, BH-corrected p = 0.0128)
  - Included PD-implicated genes **SNCA** (codes for α-synuclein), **PINK1**, HTRA2, CHCHD2

SNCA
p = 5.01e-07, Fold Change = 1.50, adj. R^2 = 0.86

PINK1
p = 6.34e-06, Fold Change = 1.54, adj. R^2 = 0.79

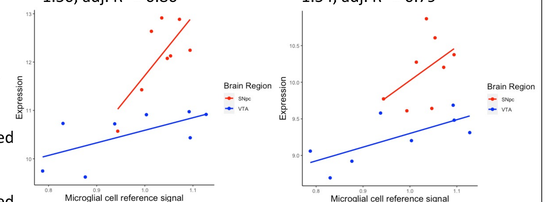


Figure 2. Scatterplots of microglial cell-specific expression of SNCA and PINK1 in the SNpc and VTA. Red indicates expression in the SNpc, and blue indicates expression in the VTA. The greater slopes of the SNpc lines relative to the VTA lines indicate that SNCA and PINK1 are upregulated in the microglia of the SNpc. The high adjusted R^2 values indicate that the microglia-specific expression accounts for the majority of the variation in expression of these genes. Data from Allen Human Brain Atlas, graphs produced by student researcher.

Results and Interpretations (continued)

37 genes identified that may contribute to age-dependent NM-linked neurotoxicity (Fig. 3)

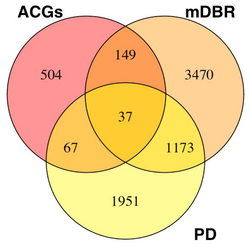


Figure 3. Venn diagram of genes that were found to be age-correlated in the SN (ACGs), differentially expressed in the SNpc (mDBR), and differentially expressed in PD. The 37 genes in the center are aNMPD genes that may contribute to age-dependent neuromelanin-linked neurotoxicity in PD.

Patterns of Differential Expression and Correlation with Age (Fig. 4)

- ➢ Most aNMPD genes exhibited similar patterns of differential expression in SNpc and PD
- ➢ Few aNMPD genes exhibited similar patterns of differential expression and correlation with age
- ➢ aNMPD genes enriched for identical protein binding molecular function (fold enrichment = 1.44, p = 0.0399)

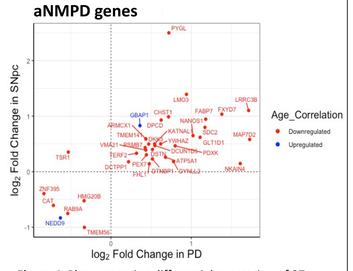


Figure 4. Plot comparing differential expression of 37 aNMPD genes in the SNpc and in PD and their correlations with age in the SN. Red indicates genes that were downregulated with age; blue indicates genes that were upregulated with age. Genes in first quadrant were upregulated in both the SNpc and the PD, and genes in the third quadrant were downregulated in both the SNpc and PD. Graph produced by student researcher.

Results and Interpretations (Continued)

RAB9A, YWHAZ, DTNBP1, CAT exhibited significant interactions with PD-implicated genes

- ➢ aNMPD genes: RAB9A, YWHAZ, DTNBP1, CAT (Table 1)
  - ➢ Interactions with PD-implicated proteins (mean interaction score > 0.4, STRING DB)
  - ➢ Associated with NM and PD-related GO terms (melanosome, reactive oxygen species metabolic process)
- ➢ RAB9A is required for trafficking of melanogenic enzymes to the melanosome
- ➢ CAT protects against hydrogen peroxide oxidative stress

Table 1. Statistics from analyses of RAB9A, YWHAZ, DTNBP1, CAT. All p-values listed are BH-corrected.

Gene	Correlation with Age (*r*)	P-value of Correlation with Age	Fold change in SNpc	P-value of fold change in SNpc	Fold change in PD	P-value of fold change in PD	Mean Interaction Score*	Gene Ontology (GO) terms
RAB9A	–0.39	0.0087	0.59	0.0018	0.69	0.028	0.493	Melanosome
YWHAZ	–0.32	0.042	1.4	0.0080	1.54	0.0038	0.717	Melanosome
DTNBP1	–0.33	0.032	1.17	0.0039	1.43	0.018	0.902	Melanosome membrane
CAT	–0.31	0.044	0.66	0.0064	0.61	0.038	0.581	Aging, Reactive oxygen species metabolic process

*Mean Interaction Score is the average interaction score with proteins previously implicated in PD in the literature as provided by STRING DB.

Conclusions

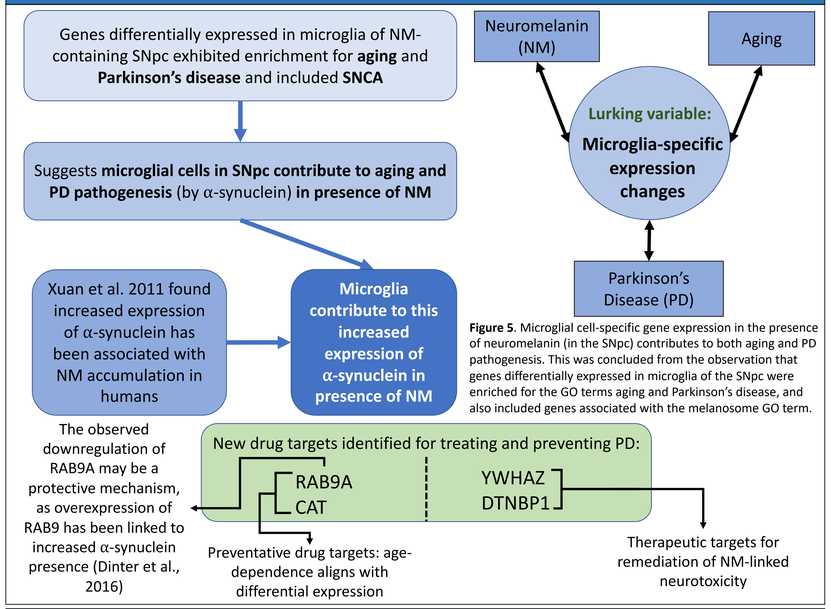


Figure 5. Microglial cell-specific gene expression in the presence of neuromelanin (in the SNpc) contributes to both aging and PD pathogenesis. This was concluded from the observation that genes differentially expressed in microglia of the SNpc were enriched for the GO terms aging and Parkinson's disease, and also included genes associated with the melanosome GO term.

Relevant Applications to Biotechnology

- ➢ Currently a rat model of PD based on NM neurotoxicity does exist (Carballo-Carbajal et al., 2019), although it is inaccurate because it is based on the injection of human tyrosinase into rat SNs to induce NM formation
- ➢ **Using the genes identified in study to construct animal models of PD based on natural NM-linked neurotoxicity will increase model accuracy (Fig. 5)**

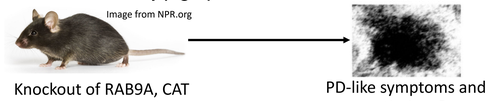


Figure 6. Theoretical construction of a future study that would evaluate the efficacy of using select aNMPD genes to generate to more accurate rodent model of PD. This model would be more pathologically accurate because it is based off neuromelanin-linked neurotoxicity rather than using external neurotoxins or genes to induce DA neuron death Such a study would involve altering the expression of RAB9A, CAT, YWHAZ, and DTNBP1 in accordance with their expression differences observed with age, in the SNpc, and in PD patients in this study.

Acknowledgements

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