

Transcriptomic signatures of brain regional vulnerability to Parkinson's disease

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HOPE 2020, Paris

January 30th, 2020



Leiden

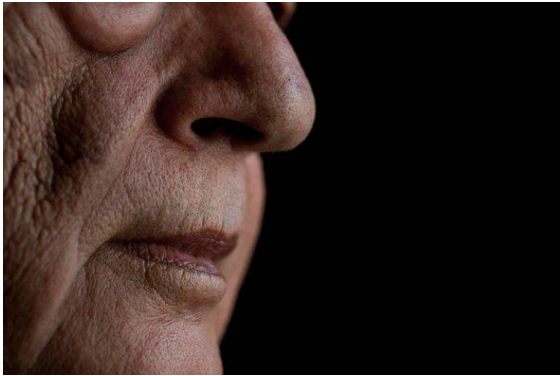
Computational Biology Center



Leiden University
Medical Center



Parkinson's disease (PD) progression



Loss of smell
Little or no facial expression
Tremor



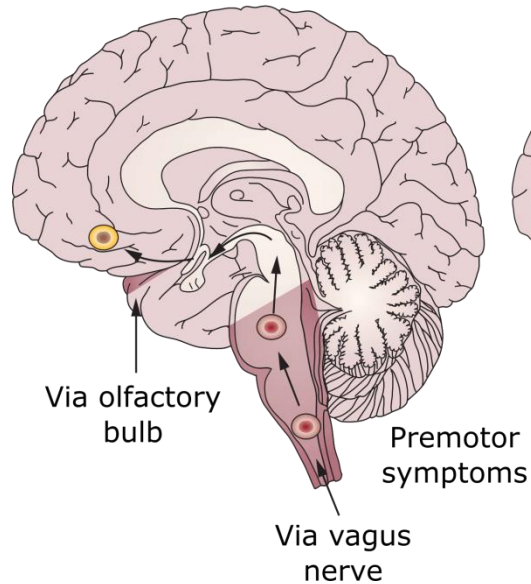
Bradykinesia
Rigidity



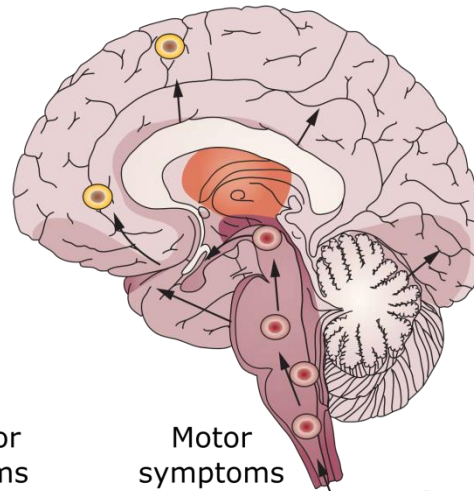
Cognitive impairment
Dementia

Progressive pathology described by Braak

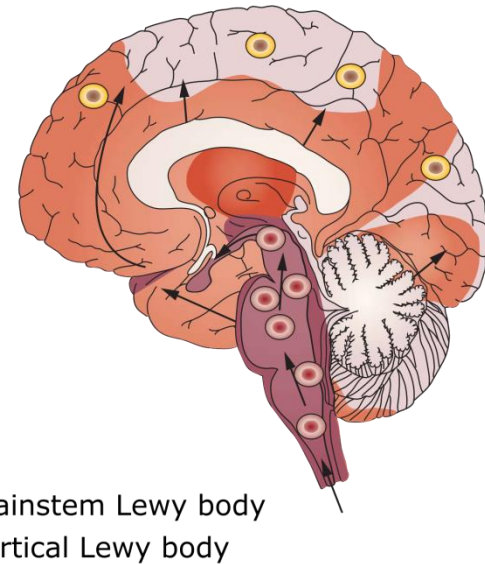
Braak stages 1 and 2



Braak stages 3 and 4

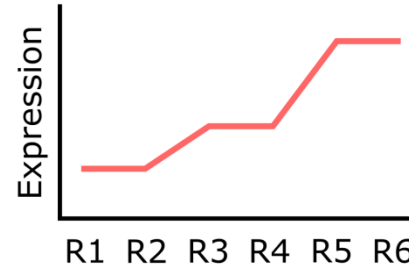
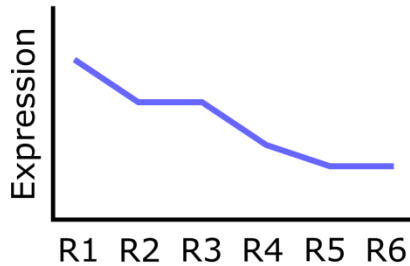


Braak stages 5 and 6



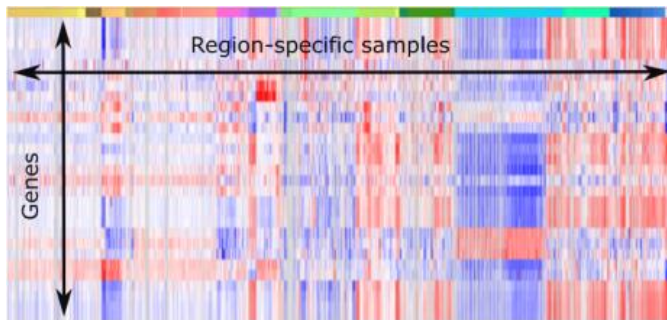
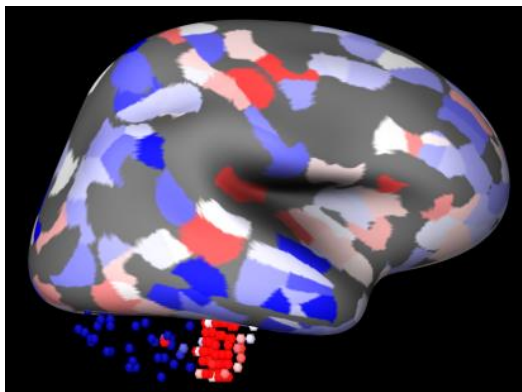
Hypothesis

- Spatial gene expression patterns
- Correlation with regions involved in Braak stages



Allen Human Brain Atlas (AHBA)

- Genome-wide microarray data of the healthy brain (20,017 genes)
- 6 Adult donors (5 males & 1 female, mean age 42, range 24-57 years)
- 3,702 Samples (363-946 per donor)
- Samples: MNI coordinates, anatomical annotation



Regions of interest (ROIs)

Brain regions involved in Braak stages

Myelencephalon (**R1**, N=279)

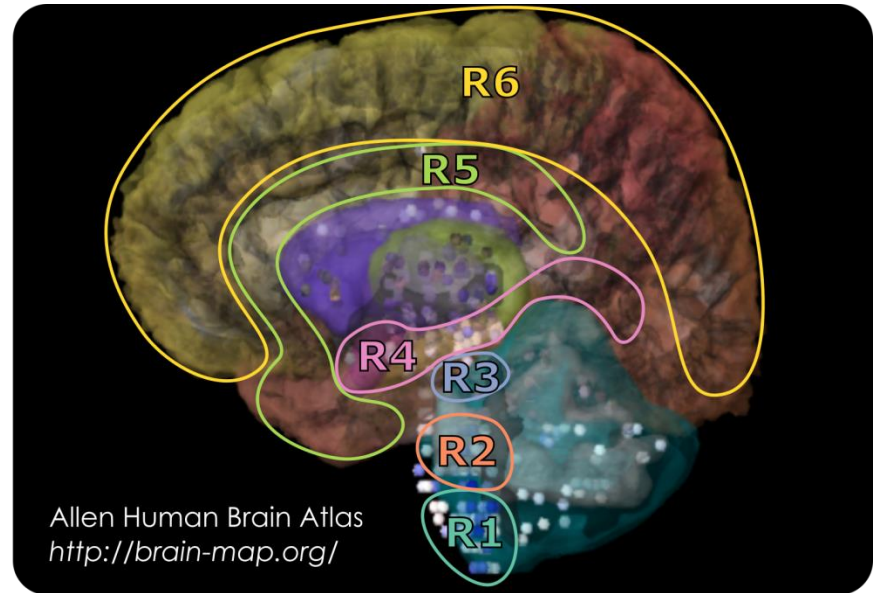
Pontine tegmentum (**R2**, N=414)

Substantia nigra, basal nucleus of Meynert,
CA2 field (**R3**, N=89)

Amygdala, occipito-temporal gyrus (**R4**, N=107)

Cingulate gyrus, temporal lobe (**R5**, N=618)

Frontal lobe, parietal lobe (**R6**, N=827)

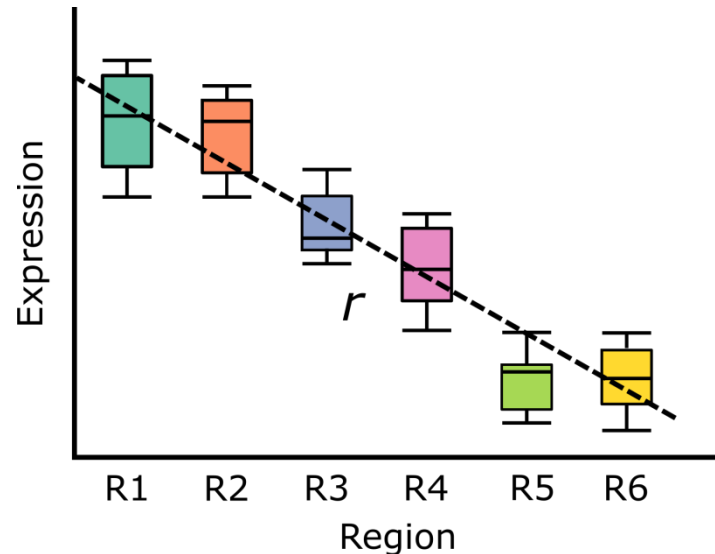


Correlation with Braak stages

Gene expression for one gene and one donor

← Vulnerability

**Correlation
between gene
expression and
Braak stage labels**

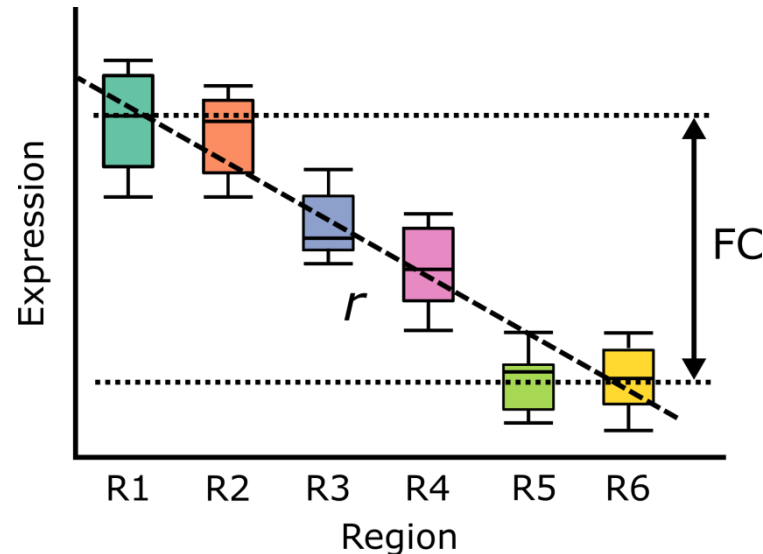


Fold-change (FC) between R1 and R6

Gene expression for one gene and one donor

← Vulnerability

**Differential gene
expression analysis**

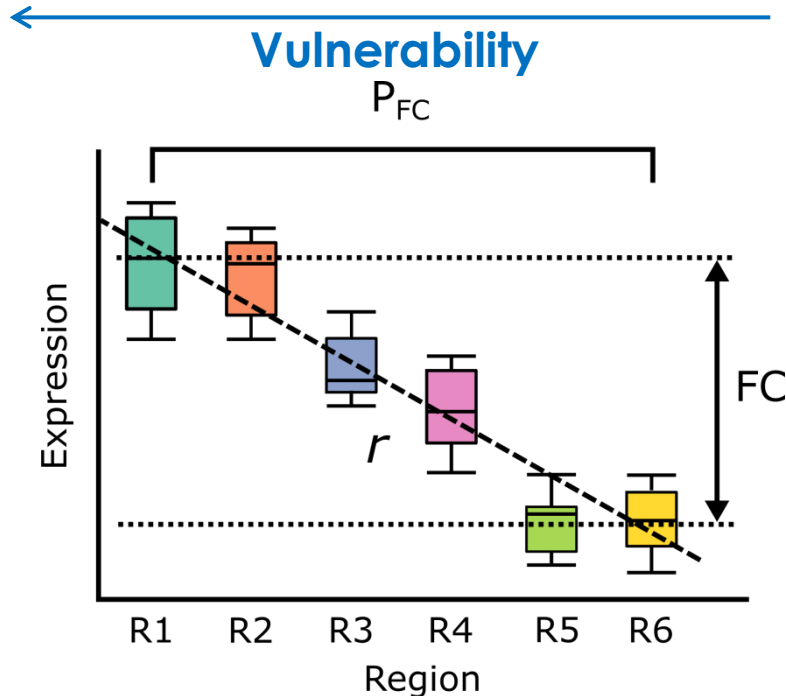


P-value of fold-change (P_{FC})

Gene expression for one gene and one donor

Differential gene expression analysis

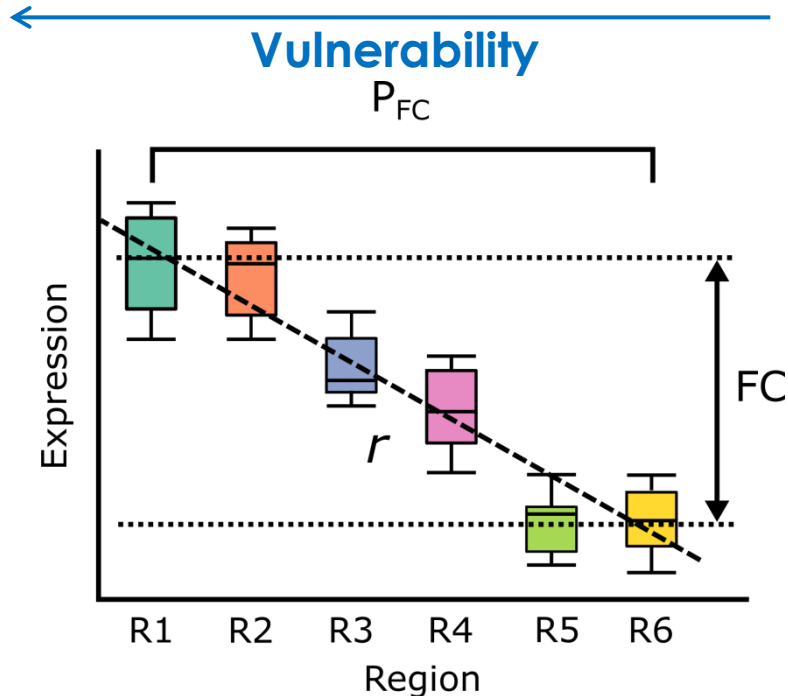
- T-test
- P -value corrected for multiple testing



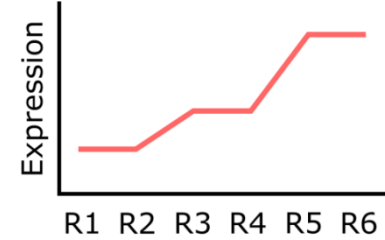
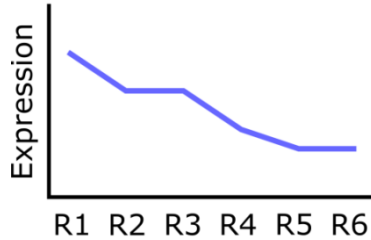
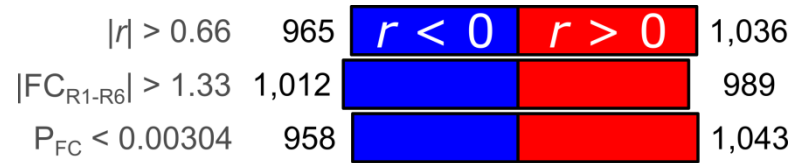
Select genes with 3 criteria

Gene expression for one gene and one donor

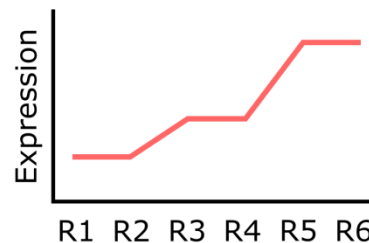
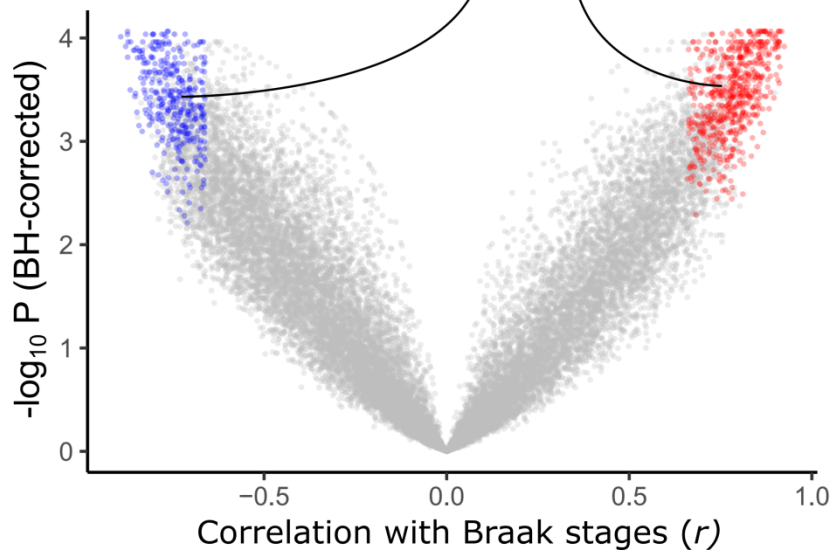
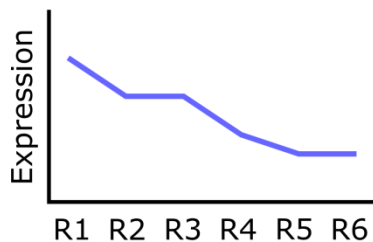
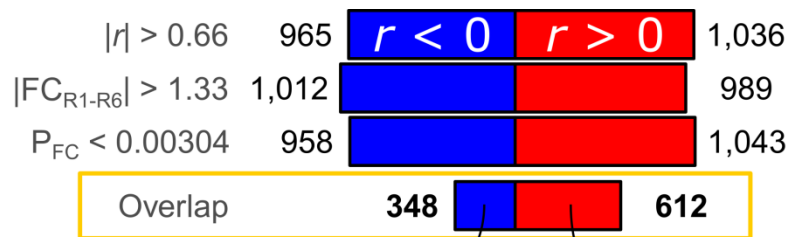
1. Correlation r with Braak stages
2. FC between R1 and R6
3. P-value of FC



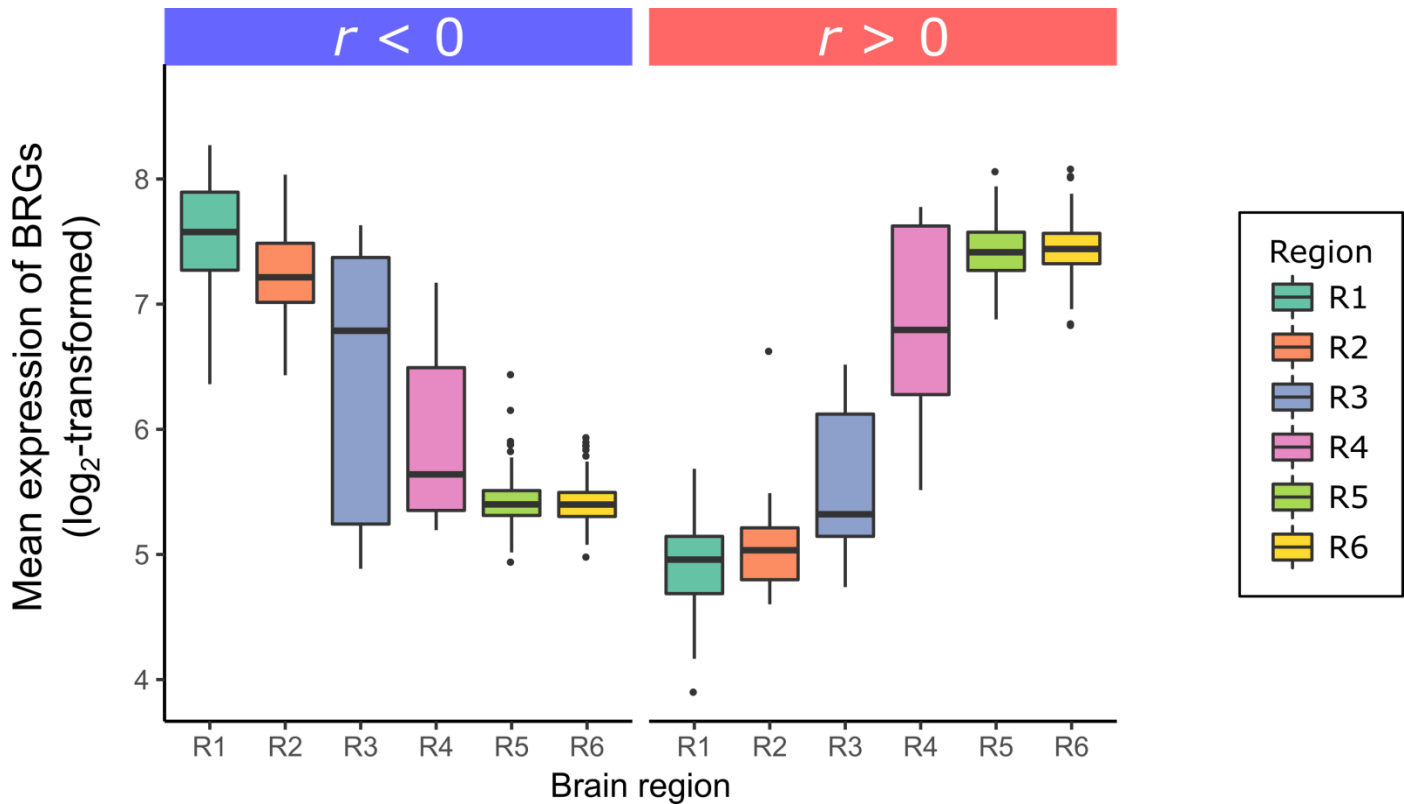
Braak stage-related genes (BRGs)



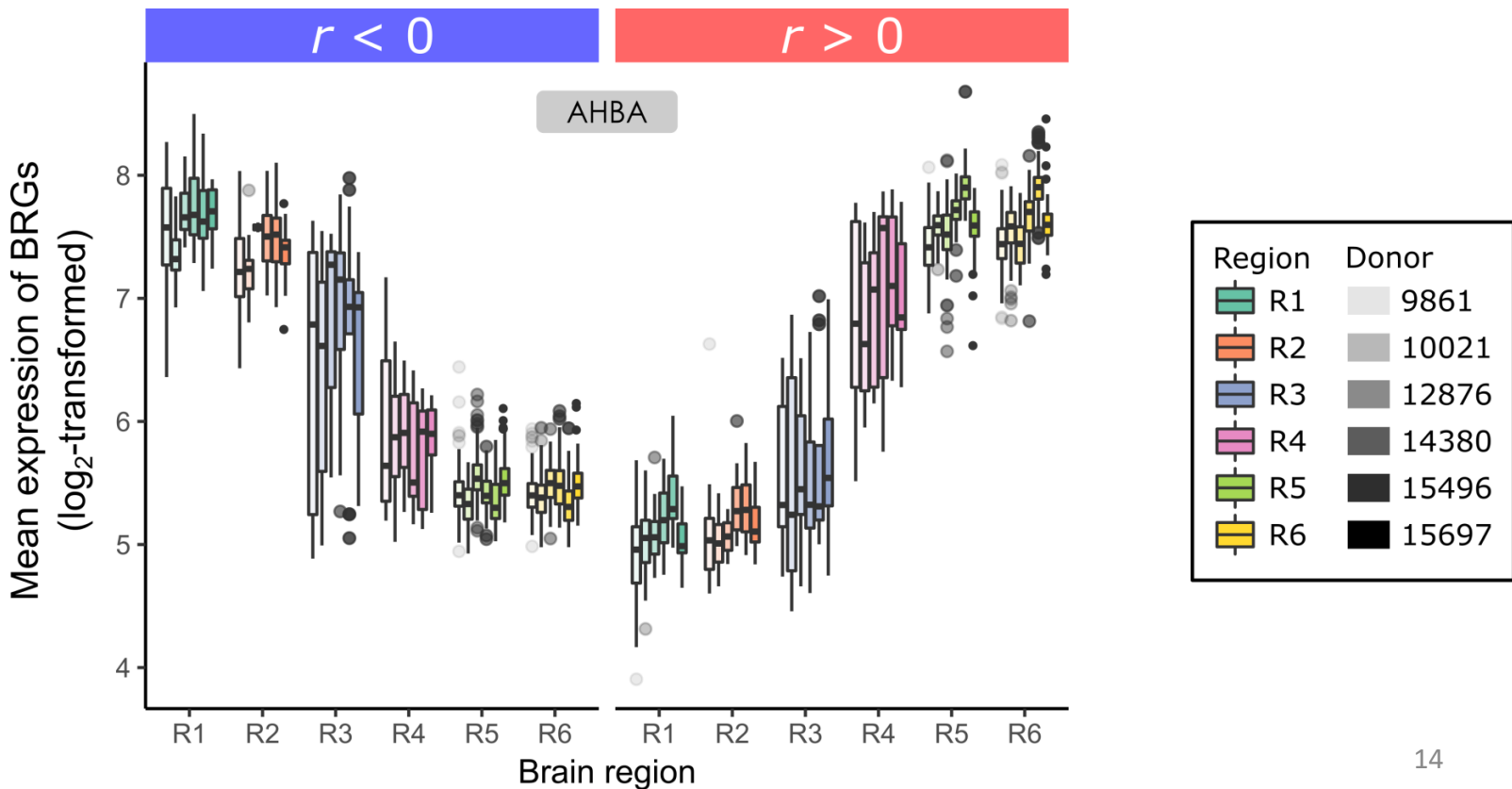
Braak stage-related genes (BRGs)



Mean expression across BRGs for one donor



Replicated across donors



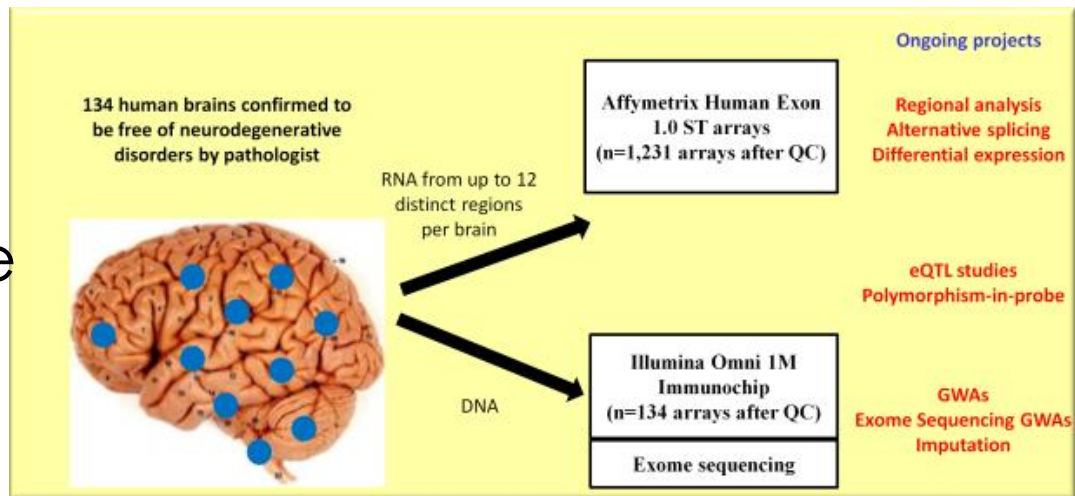
Validation in non-neurological brains



UK Brain Expression Consortium (UKBEC)

- Microarray data
- 134 Brain donors (mean age 59, range 16-102 years)

<http://www.braineac.org/>



- | | |
|---|---|
| <input type="checkbox"/> Occipital cortex | <input type="checkbox"/> Cerebellar cortex |
| <input type="checkbox"/> Frontal cortex | <input type="checkbox"/> Thalamus |
| <input type="checkbox"/> Temporal cortex | <input type="checkbox"/> Putamen |
| <input type="checkbox"/> Hippocampus | <input type="checkbox"/> Substantia nigra |
| <input type="checkbox"/> Intralobular white matter | <input type="checkbox"/> Medulla (inf. olivary nucleus) |
| <input type="checkbox"/> Hypothalamus (limited samples) | <input type="checkbox"/> Spinal Cord (limited samples) |
- R6
- R5
- R3
- R1

Genotype-Tissue Expression (GTEx) project

- RNA-seq data
- 751 donors
- Range 20-79y
- 88-129 samples

Configuration back

Label: gene symbol accession both Show GTEx gene model:

Log10 transform: View limits maximum: RPKM (range 0-711778)

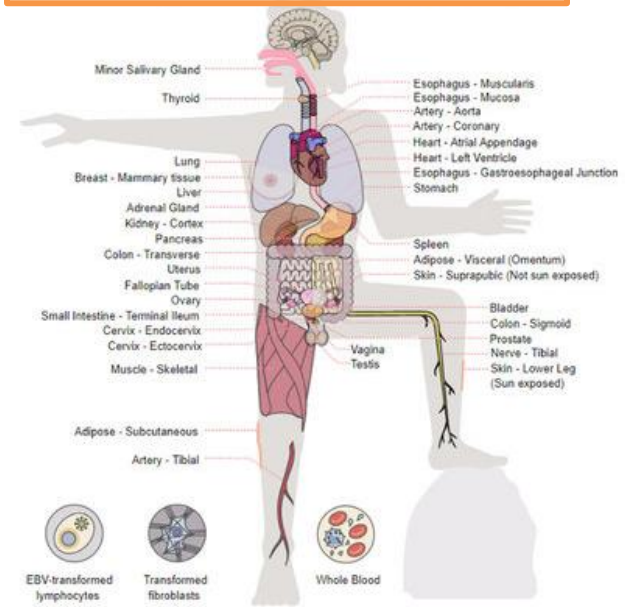
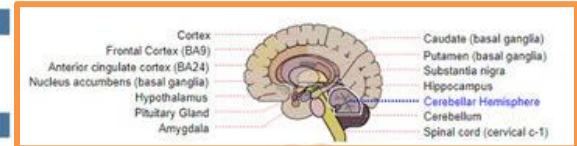
Limit to protein coding genes: Limit to genes scored at or above: (range 0-1000)

Tissues set all clear all

Click label below or in Body Map to set or clear a tissue

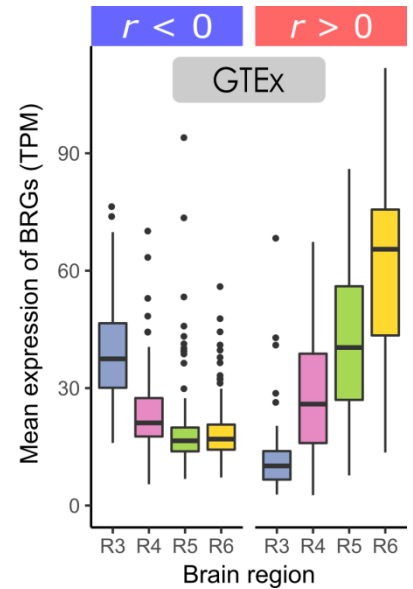
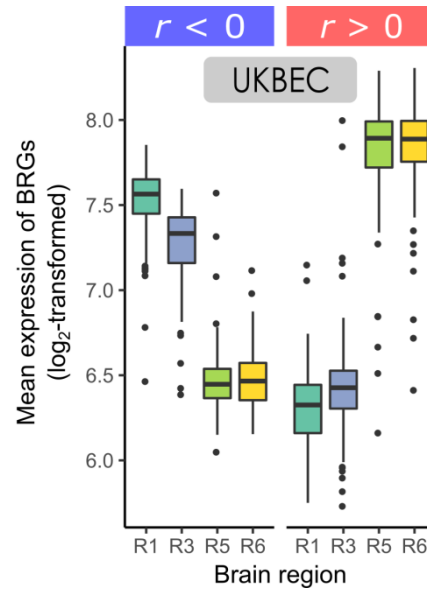
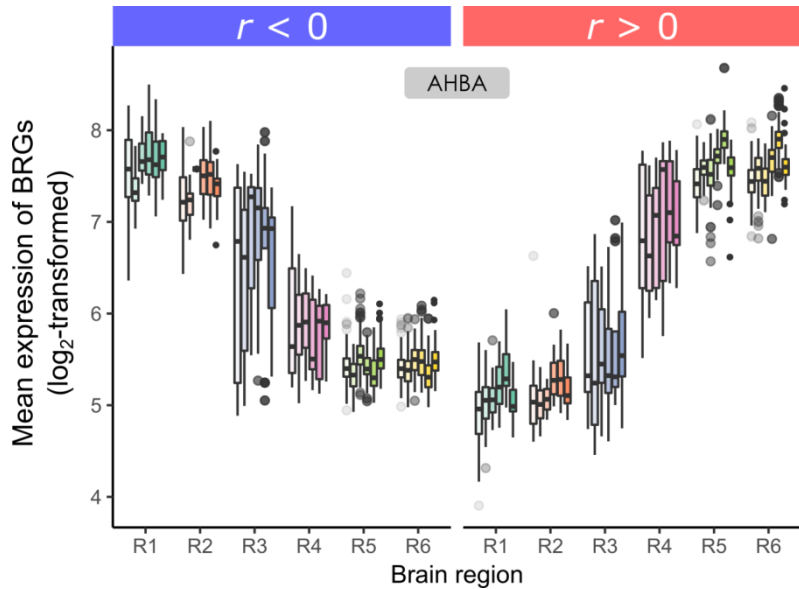
- | | |
|--|--|
| <input type="checkbox"/> Adipose - Subcutaneous | <input type="checkbox"/> Esophagus - Gastroesophageal Junction |
| <input type="checkbox"/> Adipose - Visceral (Omentum) | <input type="checkbox"/> Esophagus - Mucosa |
| <input type="checkbox"/> Adrenal Gland | <input type="checkbox"/> Esophagus - Muscularis |
| <input type="checkbox"/> Artery - Aorta | <input type="checkbox"/> Fallopian Tube |
| <input type="checkbox"/> Artery - Coronary | <input type="checkbox"/> Heart - Atrial Appendage |
| <input type="checkbox"/> Artery - Tibial | <input type="checkbox"/> Heart - Left Ventricle |
| <input type="checkbox"/> Bladder | <input type="checkbox"/> Kidney - Cortex |
| <input type="checkbox"/> Brain - Amygdala | <input type="checkbox"/> Liver |
| <input type="checkbox"/> Brain - Anterior cingulate cortex (BA24) | <input type="checkbox"/> Lung |
| <input type="checkbox"/> Brain - Caudate (basal ganglia) | <input type="checkbox"/> Minor Salivary Gland |
| <input type="checkbox"/> Brain - Cerebellar Hemisphere | <input type="checkbox"/> Muscle - Skeletal |
| <input type="checkbox"/> Brain - Cerebellum | <input type="checkbox"/> Nerve - Tibial |
| <input type="checkbox"/> Brain - Cortex | <input type="checkbox"/> Ovary |
| <input type="checkbox"/> Brain - Frontal Cortex (BA9) | <input type="checkbox"/> Pancreas |
| <input type="checkbox"/> Brain - Hippocampus | <input type="checkbox"/> Pituitary |
| <input type="checkbox"/> Brain - Hypothalamus | <input type="checkbox"/> Prostate |
| <input type="checkbox"/> Brain - Nucleus accumbens (basal ganglia) | <input type="checkbox"/> Skin - Not Sun Exposed (Suprapubic) |
| <input type="checkbox"/> Brain - Putamen (basal ganglia) | <input type="checkbox"/> Skin - Sun Exposed (Lower leg) |
| <input type="checkbox"/> Brain - Spinal cord (cervical c-1) | <input type="checkbox"/> Small Intestine - Terminal Ileum |
| <input type="checkbox"/> Brain - Substantia nigra | <input type="checkbox"/> Spleen |
| <input type="checkbox"/> Breast - Mammary Tissue | <input type="checkbox"/> Stomach |
| <input type="checkbox"/> Cells - EBV-transformed lymphocytes | <input type="checkbox"/> Testis |
| <input type="checkbox"/> Cells - Transformed fibroblasts | <input type="checkbox"/> Thyroid |
| <input type="checkbox"/> Cervix - Ectocervix | <input type="checkbox"/> Uterus |
| <input type="checkbox"/> Cervix - Endocervix | <input type="checkbox"/> Vagina |
| <input type="checkbox"/> Colon - Sigmoid | <input type="checkbox"/> Whole Blood |
| <input type="checkbox"/> Colon - Transverse | |

R4
R5
R6
R3

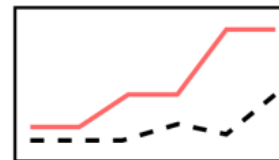
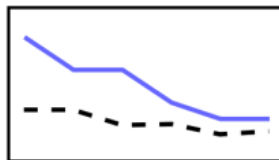
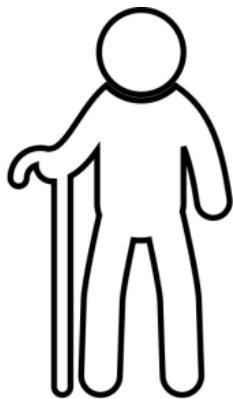


<https://gtexportal.org/>

BRGs: Replication in UKBEC & GTEx



Validation in PD brains



Microarray dataset of PD brains

- Data from PD- and, iLBD patients, and age-matched non-demented controls
- Samples taken from the medulla oblongata (**R1**), locus ceruleus (**R2**), and substantia nigra (**R3**)



PD

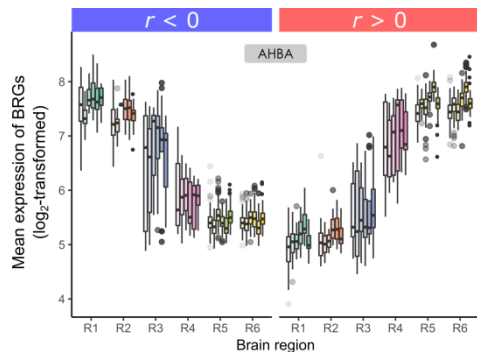


iLBD

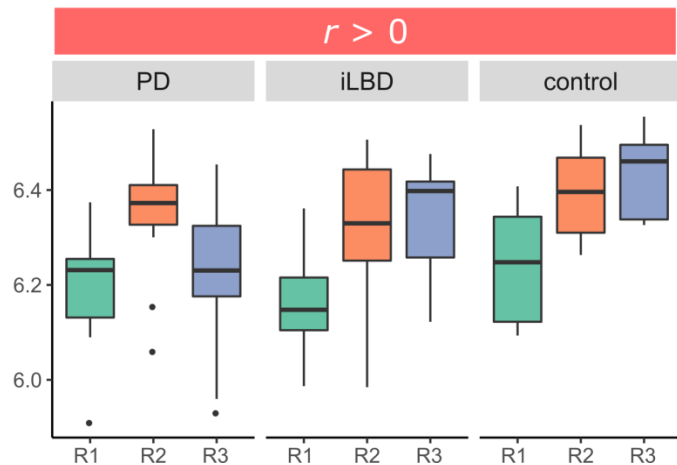
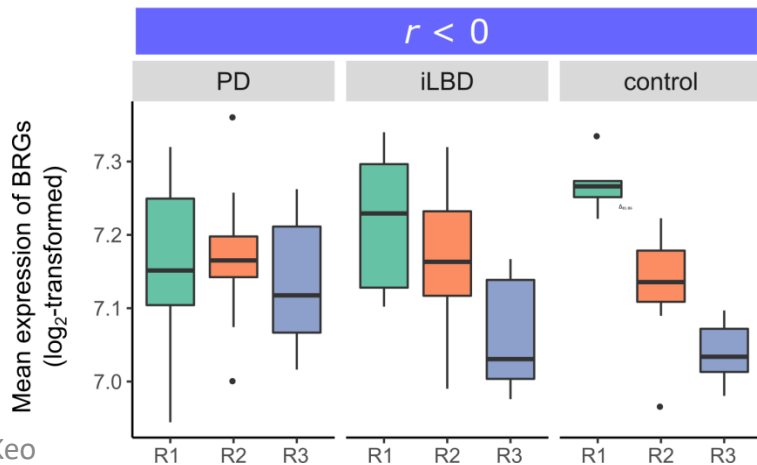


Control

BRGs: Replication in PD brains

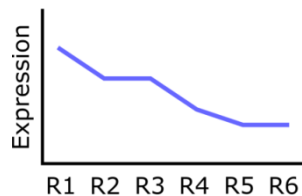


PD microarray dataset



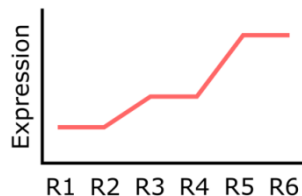
BRGs: PD-variant associated genes

Negative correlated BRGs



Gene	Braak correlation	Fold-change	P-value (BH-corrected)	Reference
<i>SCARB2</i>	-0.78	-1.44	1.7E-03	Nalls et al. 2014
<i>ELOVL7</i>	-0.67	-1.35	1.4 E-03	Chang et al. 2017

Positive correlated BRGs

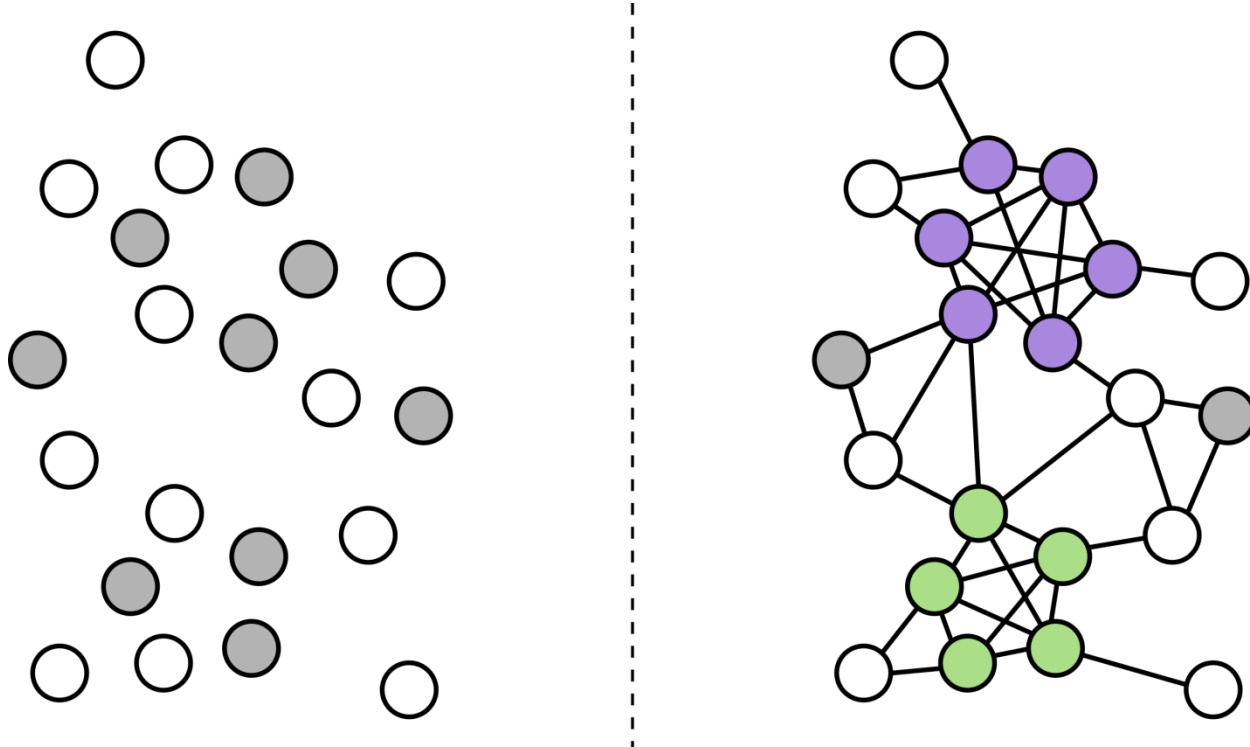


<i>SH3GL2</i>	0.70	1.40	2.3E-03	Chang et al. 2017
<i>SNCA</i>	0.70	1.75	4.3E-04	Bonifati et al. 2014, Chang et al. 2017, Nalls et al. 2014
<i>BAP1</i>	0.77	1.99	1.6E-03	Chang et al. 2017
<i>ZNF184</i>	0.81	2.34	2.9E-03	Chang et al. 2017

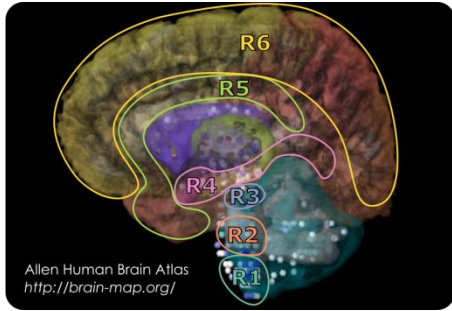
Summary so far...

- AHBA to identify Braak stage-related genes (BRGs)
- Expression patterns are replicated in non-neurological brains, and diminished in PD brains
- BRGs include known genetic risk factors for PD
- BRGs may influence vulnerability at regional level as well as between PD patients and controls

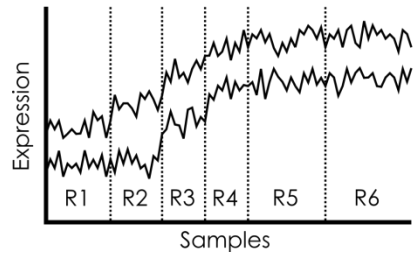
Single genes -> groups of genes (modules)



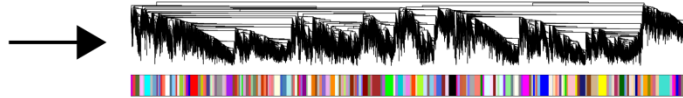
Clustering 20,017 genes



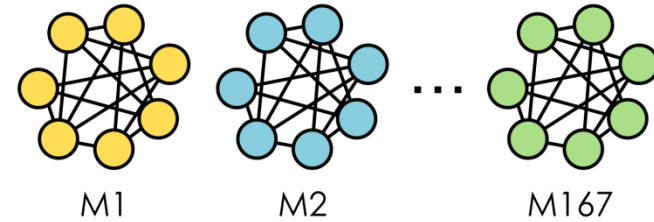
Co-expression



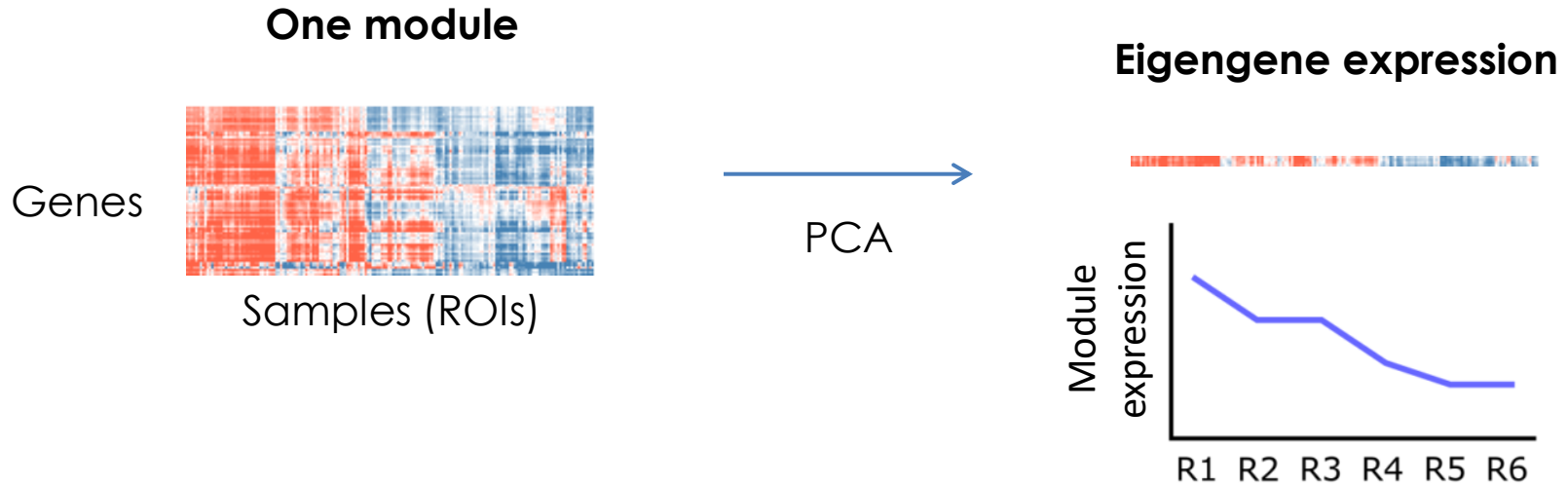
Clustering



Modules

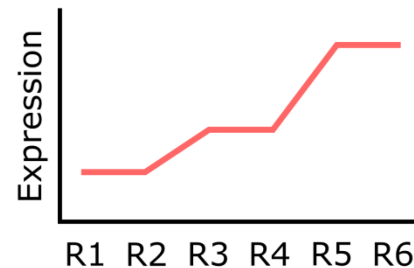
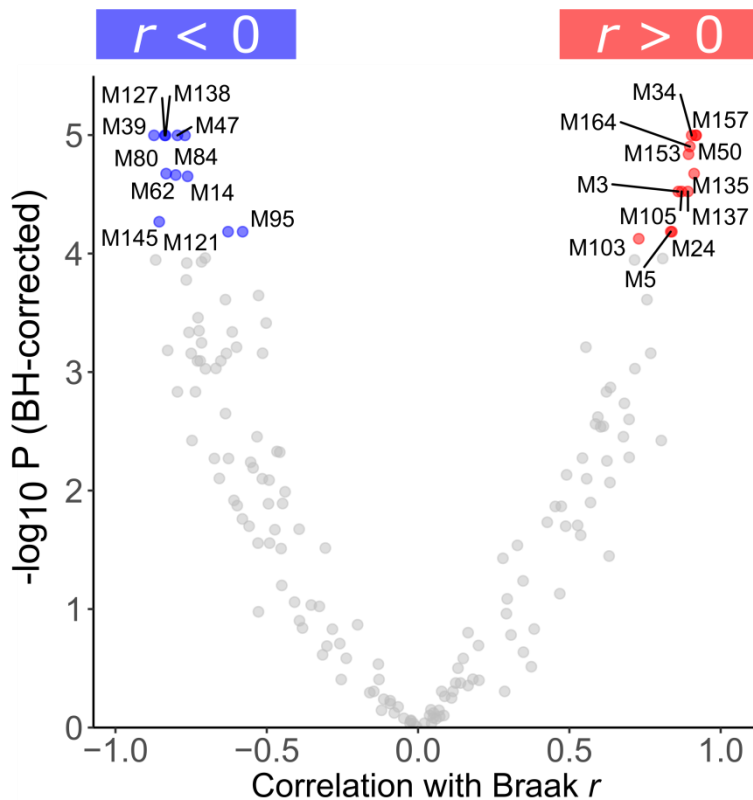
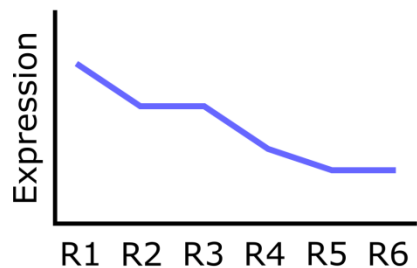


Module -> module expression



Braak co-expression modules

Corrected P -value for testing multiple modules



Modules enriched for specific genes

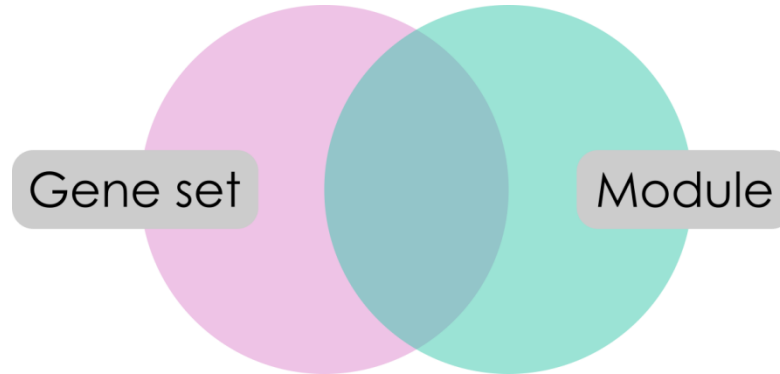
Biological functions

Diseases

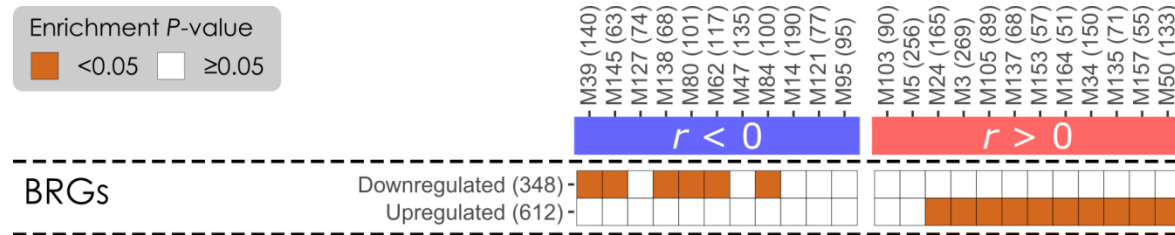
Cell-types

BRGs

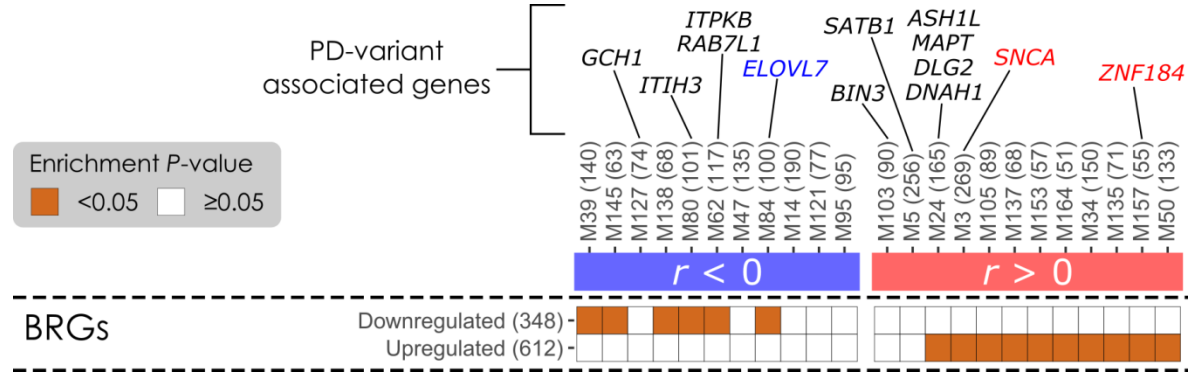
Co-expression
modules



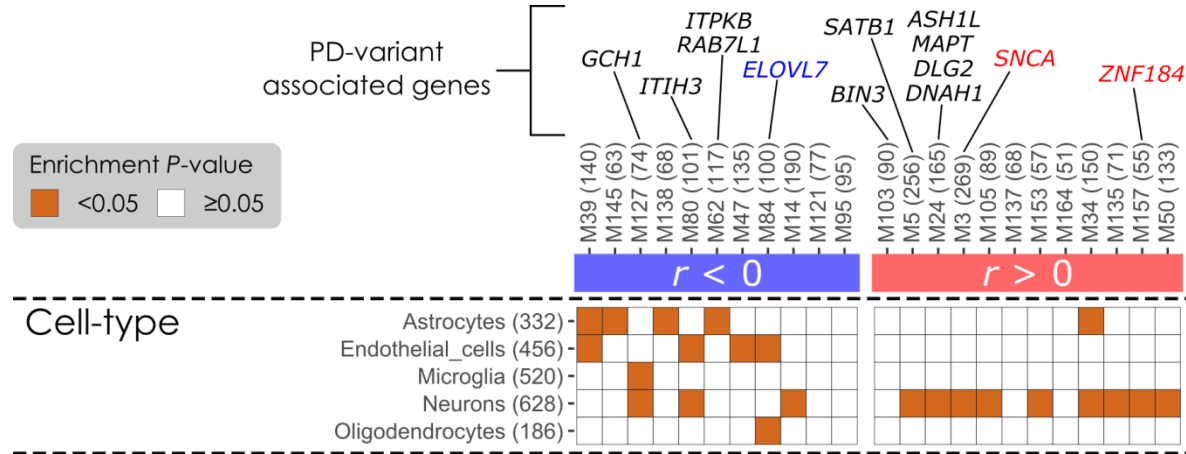
Module enrichment: BRGs



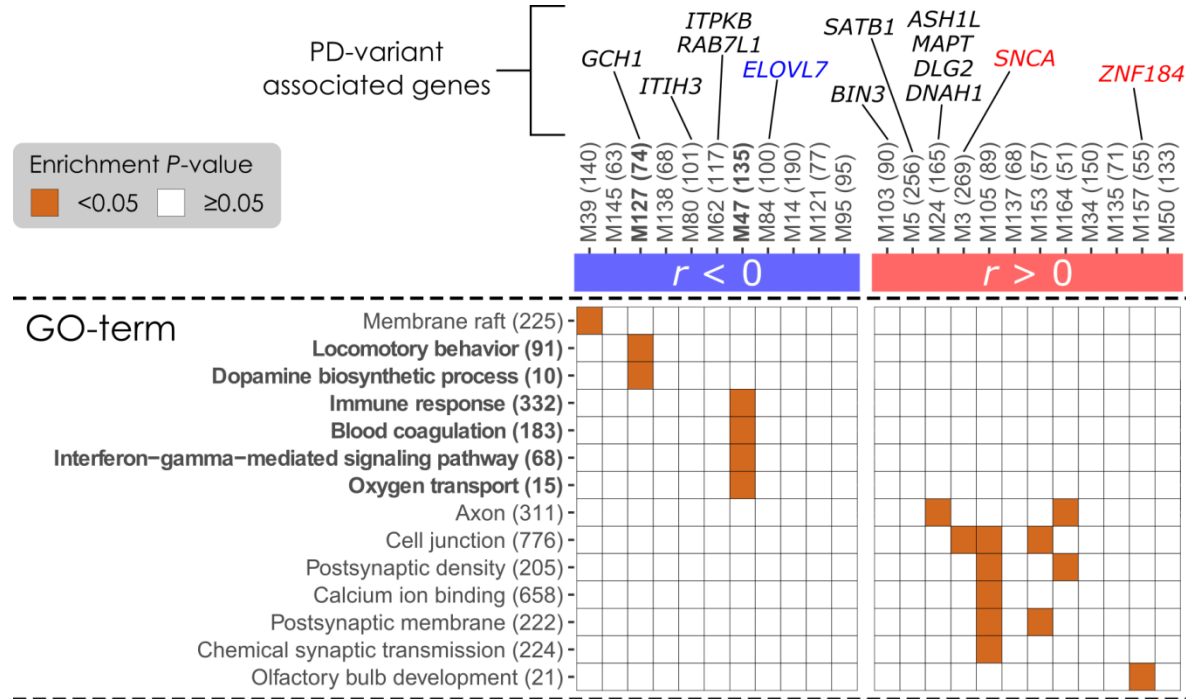
Module enrichment: BRGs



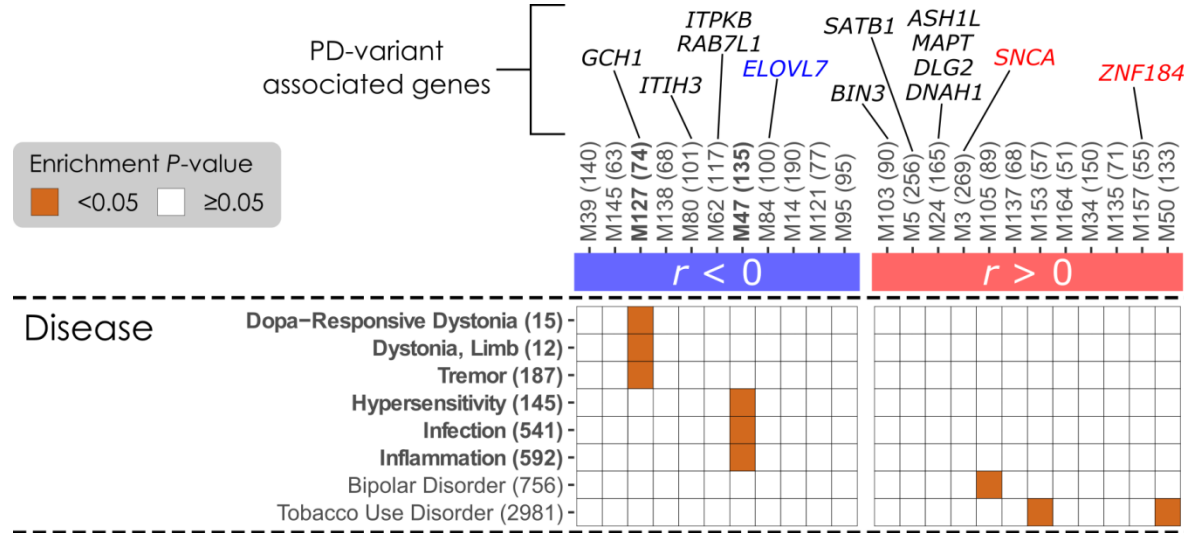
Module enrichment: Cell-types



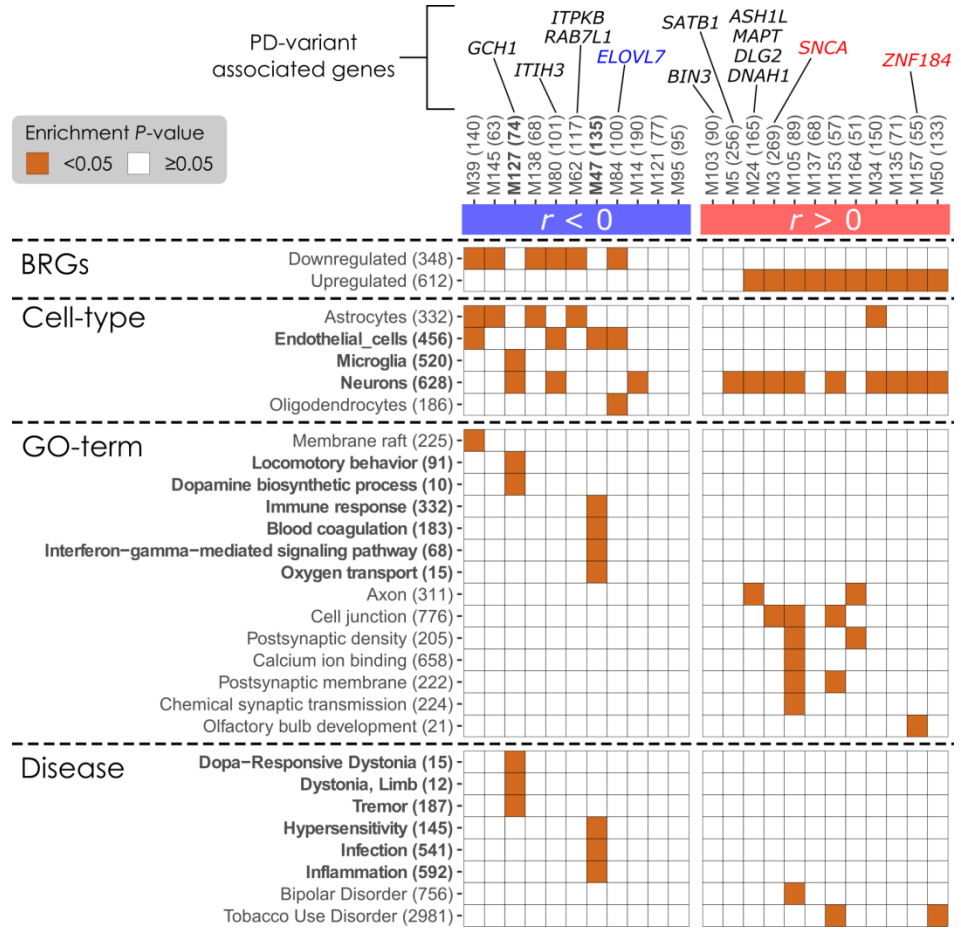
Module enrichment: GO-terms



Module enrichment: Diseases



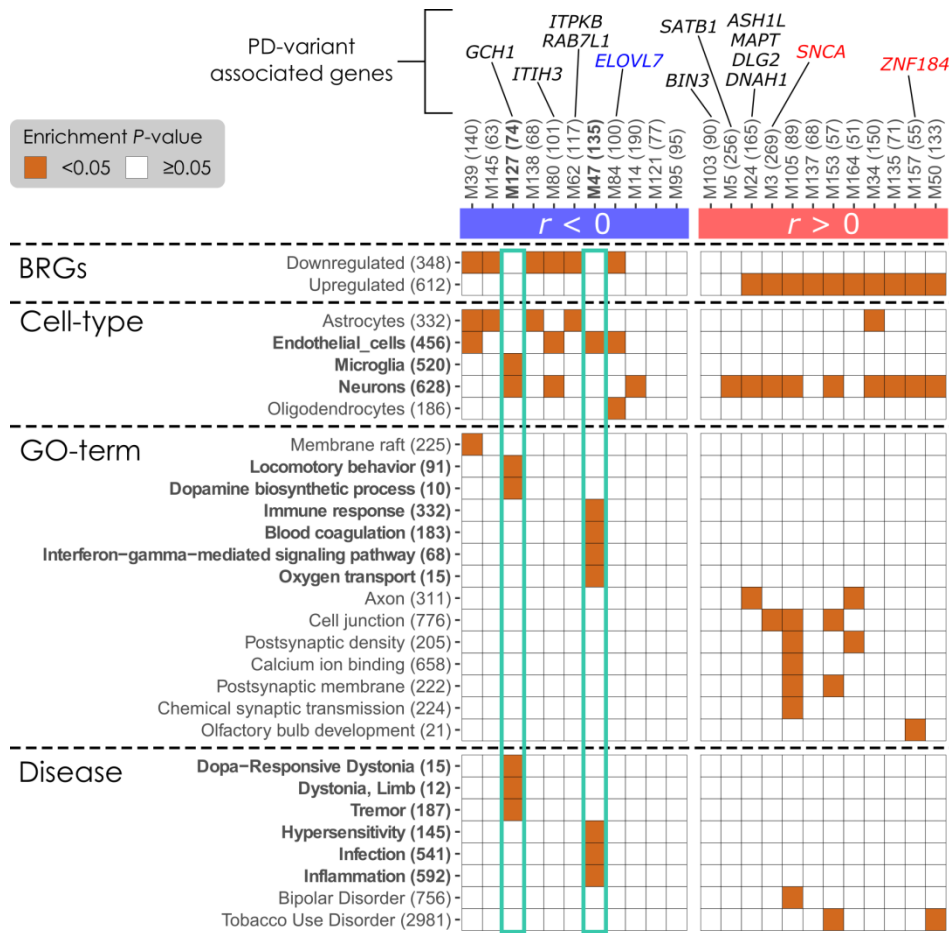
Module enrichment



Zoom in on module

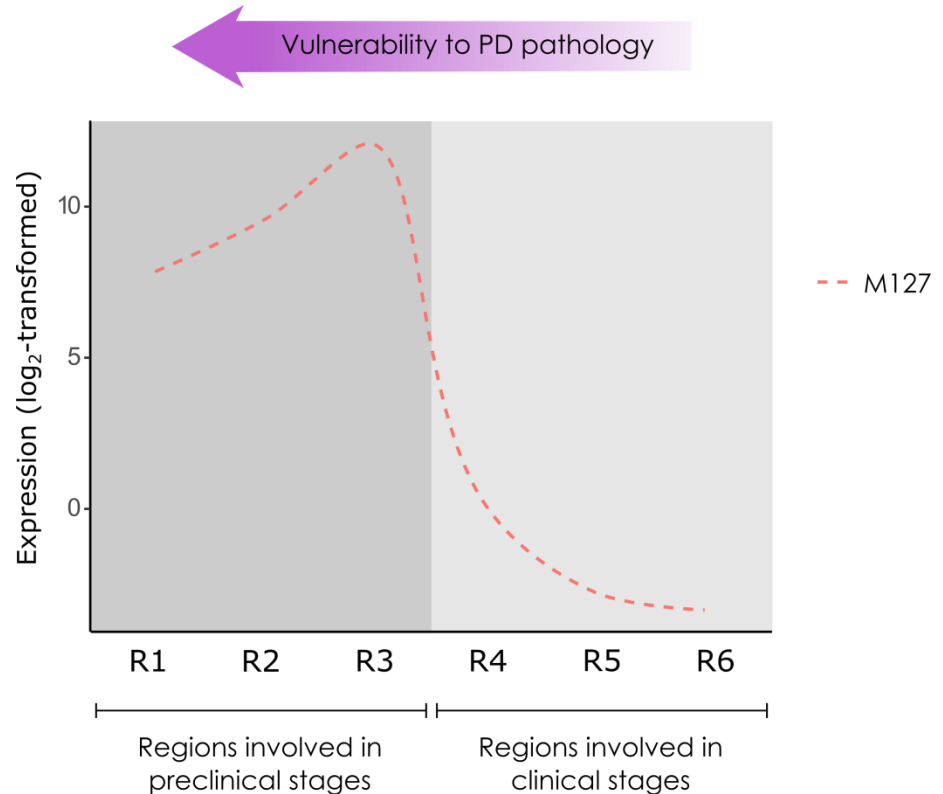
M127: Dopamine synthesis

M47: Blood oxygen transport



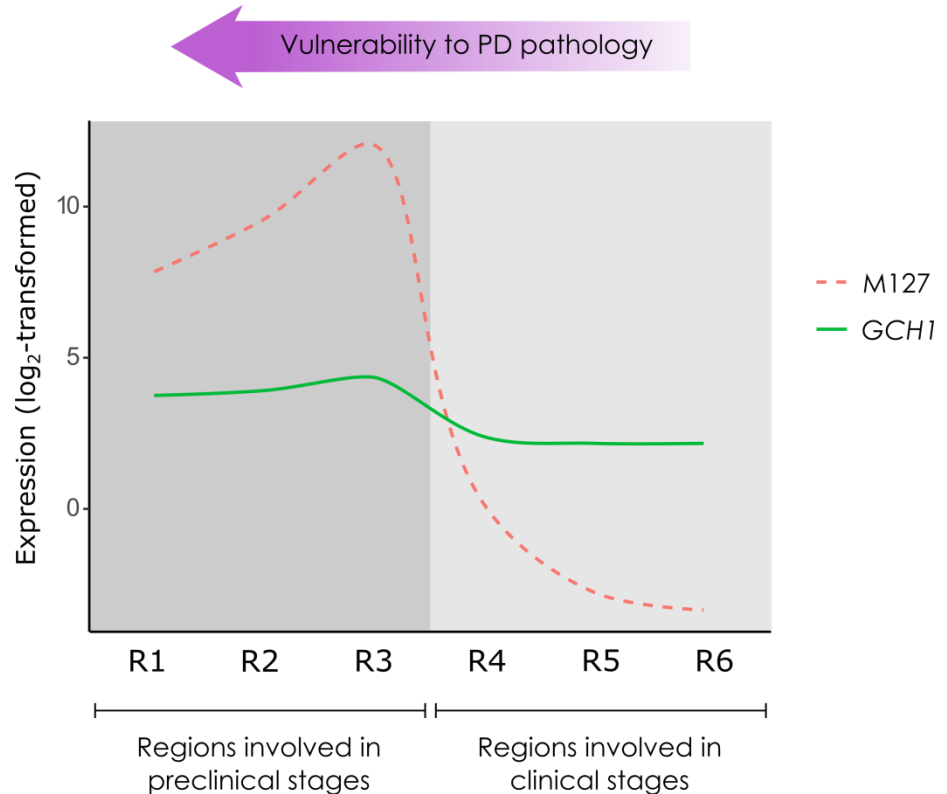
Expression patterns of dopaminergic genes

- M127: co-expression module involved in **dopamine biosynthetic process**



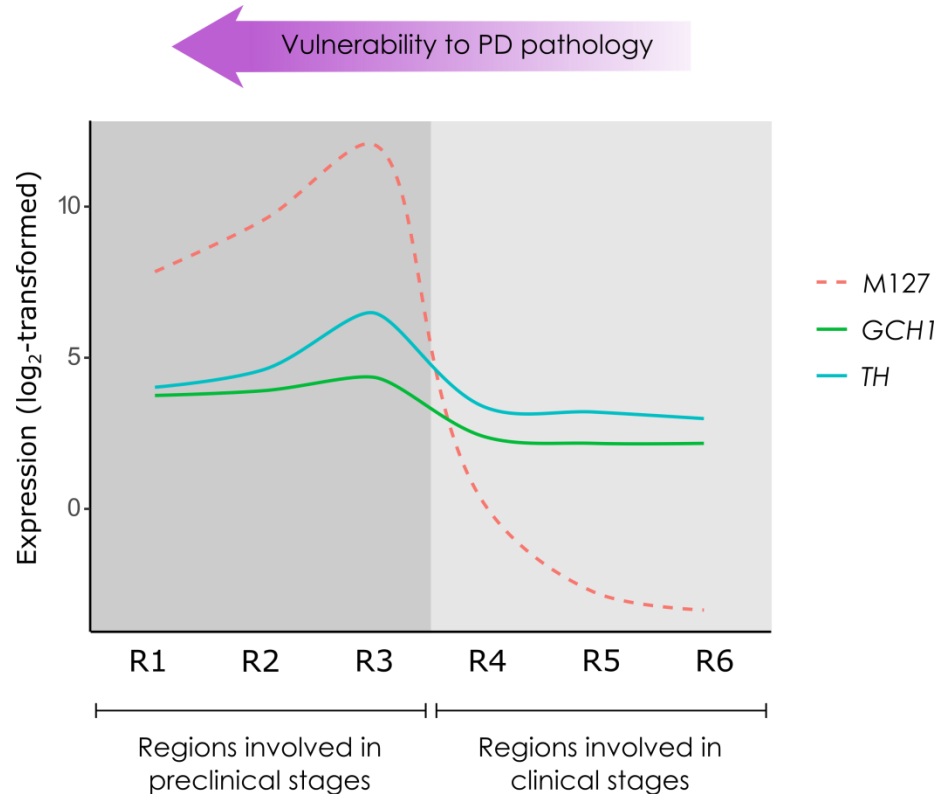
Expression patterns of dopaminergic genes

- M127: co-expression module involved in **dopamine biosynthetic process**
- *GCH1*: together with *TH* required for **production of dopamine**



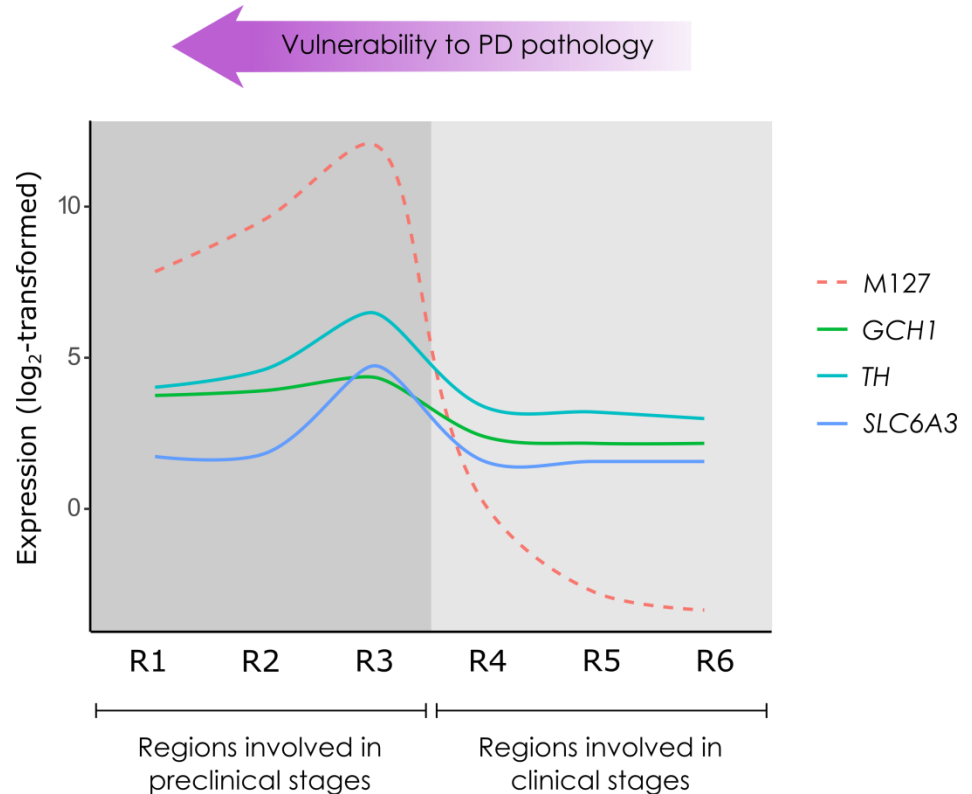
Expression patterns of dopaminergic genes

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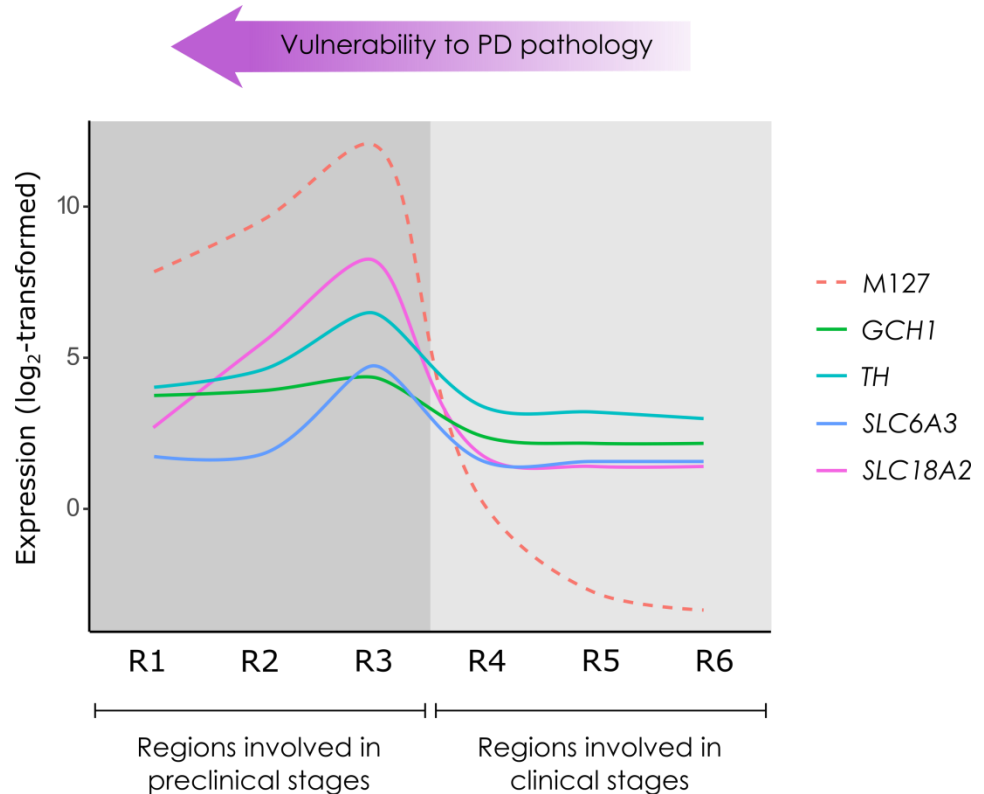
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- *SLC6A3/DAT*: **transports dopamine** from the synaptic cleft back to the cytosol



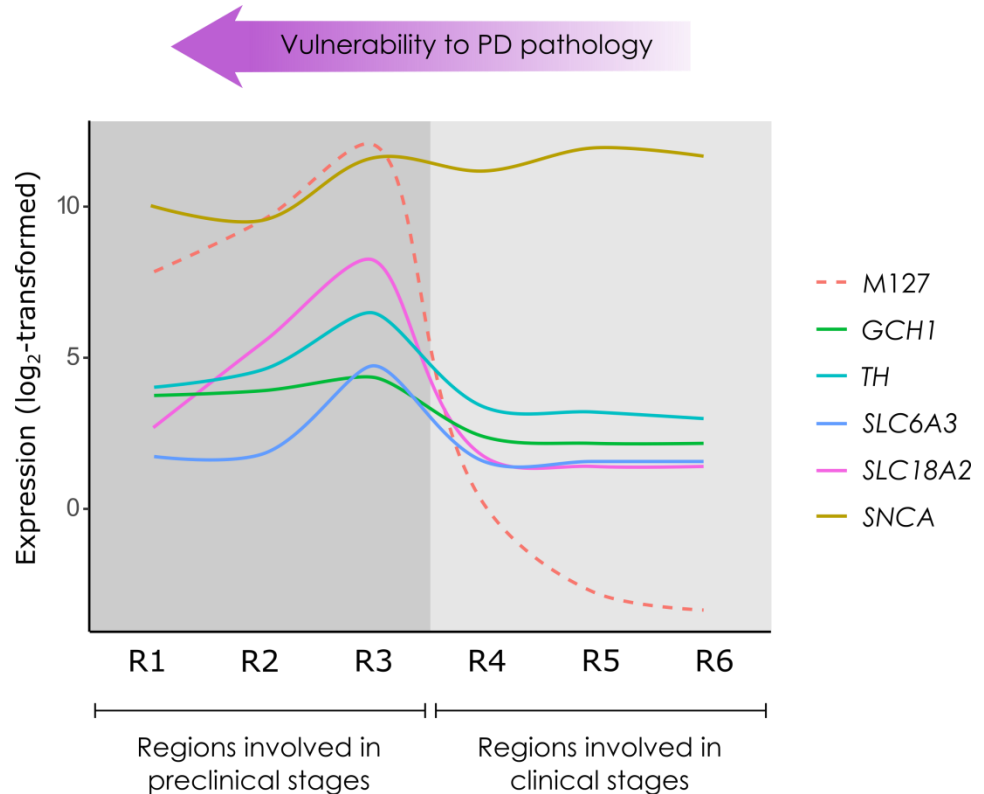
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- *SLC18A2/VMAT2*: **stores dopamine** into synaptic vesicles.



Expression patterns of dopaminergic genes

- M127: co-expression module involved in **dopamine biosynthetic process**
- *GCH1*: together with *TH* required for **production of dopamine**
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- *SLC6A3/DAT*: **transports dopamine** from the synaptic cleft back to the cytosol
- *SLC18A2/VMAT2*: **stores dopamine** into synaptic vesicles.
- *SNCA*: responsible for **dopamine release/dopamine homeostasis**



Take-home message

- We found genes and modules with expression patterns that correlated with Braak staging
- We found associations with known pathways and reveal spatial information
- Gene (co-)expression patterns need further validation in lab experiments

Summary

- Modules implicated in the dopaminergic and blood oxygen pathways related to Braak staging
- Found risk factors associated with PD
- BRGs and modules remain differentially expressed after correcting for cell-type abundance

Acknowledgements

Sjoerd M. H. Huisman

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Computational Biology Center

Arlin Keo

Jean-Pascal Meneboo

Celine Villenet

Eugénie Mutez

Thomas Comptdaer

Martin Figeac

Marie-Christine Chartier-Harlin



Ahmed Mahfouz

Boudewijn P.F. Lelieveldt

Marcel J.T. Reinders



connecting innovators

Braak stage-related gene *ADCY1*

AHBA

