# Selective Neurodegeneration in Alzheimer's Disease and Parkinson's Disease

Juelu Wang, [§], Huiting Ma[#], Yifan Zhang[#], Wen Si (Sibyl) Gao *, and Mang Zhu[&]

[§] Graduate Program in Neuroscience, [#]Department of Statistics, * Undergraduate Program in Computer Science and Microbiology, [&] Genome Science and Technology Graduate Program

The University of British Columbia, Vancouver, BC, V6T 1Z3, Canada

## Introduction

- Alzheimer's disease (AD) is the most common neurodegenerative disorder, accounting for 64% of dementia in Canada. Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting nearly 100,000 Canadians.
- Neuropathological features of AD include extracellular neuritic plaques of deposited Aβ, intracellular neurofibrillary tangles and profound deficiency within cholinergic system.
- The neuropathology of PD is characterized by intercellular Lewy bodies, atrophic Lewy neurites and severe dopaminergic neurodegeneration.
- Dysfunction of certain neurotransmitter system is involved in both AD and PD. Dementia severity of AD is correlated with cholinergic deficiency and progressive dopaminergic deficiency is related to motor symptoms in PD patients.
- Swedish APP mutation was discovered in 1992 , promoting Aβ generation and neuronal loss. SNCAA53T mutation was identified in rare familial PD, making αSyn more prone to aggregate.
- The mutations is ubiquitously existed in AD and PD, but results in neuronal death of specific groups. The aim of this study is to test the hypothesis that APP and SNCA mutations change gene expression in a cell type-dependent way, which contributes to selective neurodegeneration in AD and PD.

## Methods

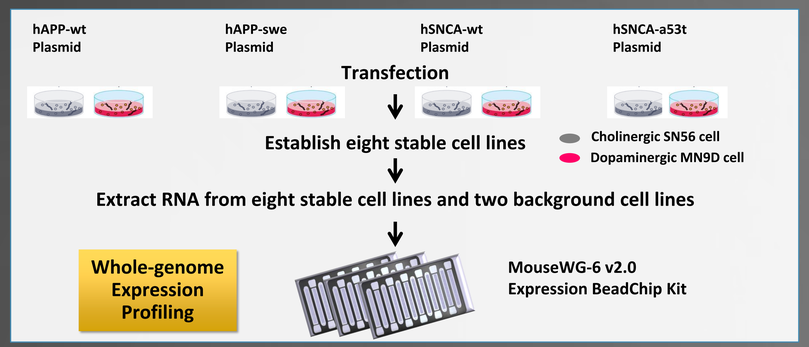


**Figure 1. Whole-genome expression profiling for eight stable cell lines and two background cell lines.** Cholinergic SN56 cells and dopaminergic MN9D cells from mouse were stably overexpressed wild-type (WT) or mutant APP gene to generate four stable cell lines. The other four stable cells were established by overexpressing WT or mutant SNCA gene in two background cell lines. RNA extracted from ten cell lines were performed whole-genome expression profiling by Illumina MouseWG-6 v2.0 expression beadChip Kit. There were two independent replicate for each cell line.

## Objectives and datasets

- **To examine the overall difference in gene expression between SN56 and MN9D cell lines.**
- **To explore the effect of APP Swedish mutation on gene expression within each background cell line and the effect of SNCA A53T mutation on gene expression within each background cell line.**
- **To study the effect of the same mutation on gene expression between two cell lines**
- **To find out genes identified in third objective involved in cell death or cell survival pathways.**

| Overexpressed gene \ Cell line | SN56 | MN9D | Differential expression analysis (DEA) |
|---|---|---|---|
| NA | SN56-INI | MN9D-INI | SN56 vs. MN9D |
| APPwt | SN56-APPwt | MN9D-APPwt | SN56-APPwt vs. SN56-APPswe; MN9D-APPwt vs. MN9D-APPswe;Interaction of mutation and cell line |
| APPswe | SN56-APPswe | MN9D-APPswe | |
| SNCAwt | SN56-SNCAwt | MN9D-SNCAwt | SN56-SNCAwt vs. SN56-SNCAa53t; MN9D-SNCAwt vs. MN9D-SNCAa53t;Interaction of mutation and cell line |
| SNCAa53t | SN56-SNCAa53t | MN9D-SNCAa53t | |

## Results

### Exploratory data analysis

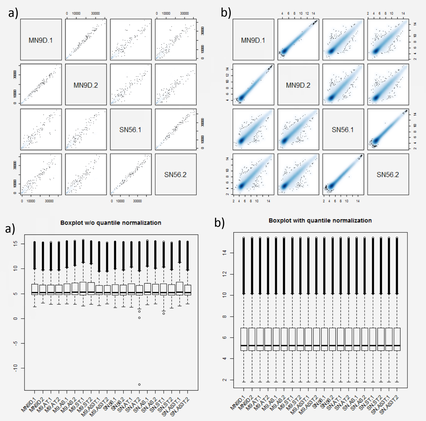


Figure1. Log2 transformation was performed on 20 expression. Sample-to-sample correlation scatterplot matrix before a) and after b) log2 transformation for SN56 and MN9D cell lines.




Figure2. Quantile normalization for 20 datasets to correct for between-array variance. Boxplot diagram shows the distribution of probes reading for each of the 20 arrays before a) and after b) quantile normalization.

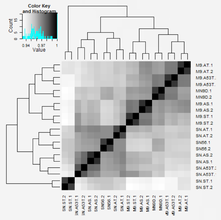


Figure3. Heatmap of sample-to-sample correlation after log2 transformation and quantile normalization. It shows high correlation within sample replicate. Samples of the same background cell line are clustered together.

### Differential expression analysis between SN56 and MN9D cell lines

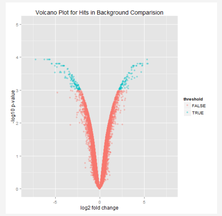


Figure4. Comparison of SN56 and MN9D cell lines by log2FC and p-value (-log base 10) for each probereading. 212 hits (p-value<10e-4 and log2FC>1) are coloured blue, accounting for 0.047% of all tested probes

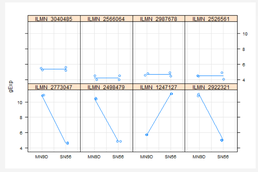


Figure5. Examples of probes not significantly(upper panel) or significantly (lower panel) differential expression between SN56 and MN9D cell lines

### Differential expression analysis for APP and SNCA genes

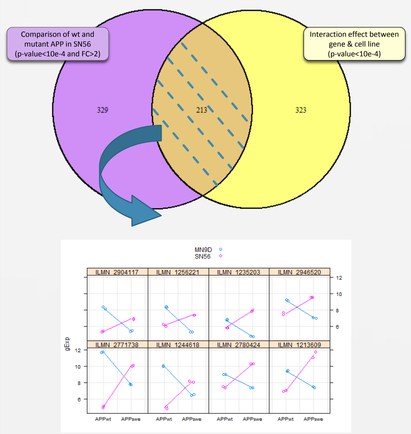


Figure 6. Illustration of differentially expressed probes in SN56 and MN9D cell lines overexpressing wt or mutant APP gene.

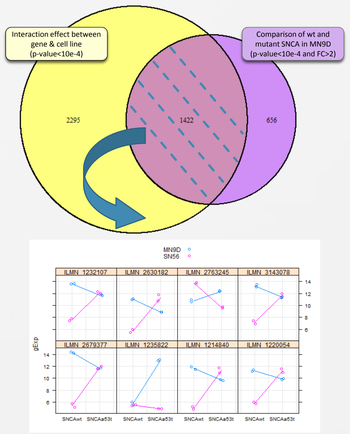


Figure 7. Illustration of differentially expressed probes in SN56 and MN9D cell lines overexpressing wt or mutant SNCA gene.

### Gene group enrichment analysis

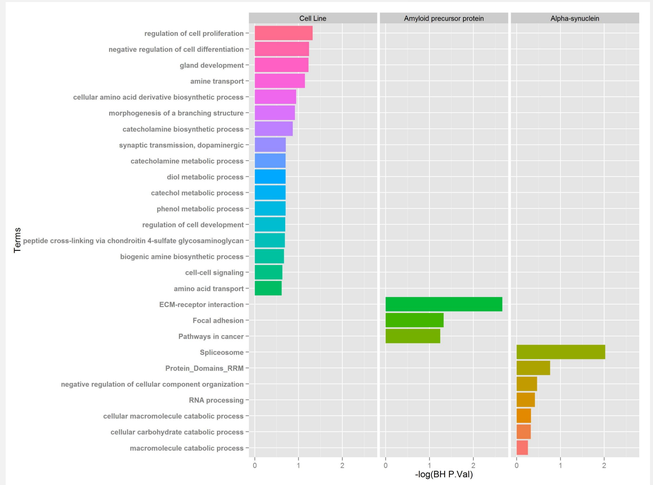


Figure8. Gene group enrichment analysis by DAVID using gene annotation GOTERM_BP_FAT, KEGG_PATHWAY, INTERPRO and SMART with FDR of 5%. "Cell line" column shows differential expressed gene groups between SN56 and MN9D cell lines; "Amyloid precursor protein" column shows differential expressed gene groups between cells overexpressing wt or mutant APP gene; "Alpha-synuclein" column shows differential expressed gene groups between cells overexpressing wt or mutant SNCA gene. X-axis shows the –log10 of Benjamini-Hochberg adjusted P-value.

### Cluster analysis

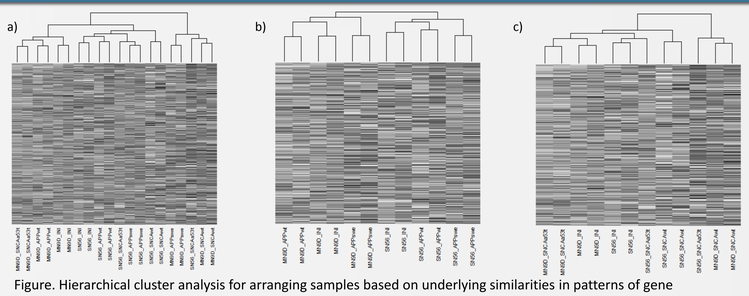


Figure. Hierarchical cluster analysis for arranging samples based on underlying similarities in patterns of gene expression. The attributes in the three cluster analyses are based on intensity reading from all tested probes after transformation and normalization in an unsupervised way. Darker cells indicate higher expression and lighter cells indicate lower expression. a) Unsupervised cluster analysis for 20 datasets from 10 samples (n=2). b) Unsupervised cluster analysis for two background cell lines and wt or mutant APP overexpressing cell lines (n=2). c) Unsupervised cluster analysis for two background cell lines and wt or mutant SNCA overexpressing cell lines (n=2).

## Conclusion

- The overall expression pattern for all tested probes did not significantly different between SN56 and MN9D cell lines.
- There are 213 probes identified as having an interaction effect between APP gene and cell line and 1422 probes having an interaction effect between SNCA gene and cell line (BH pvalue< 10e-4).
- Overexpression of APP gene has the strongest effect on ECM-receptor gene expression; overexpression of SNCA gene has the strongest effect on spliceosome gene expression.
- Overexpression of APP gene in SN56 and MN9D cell lines did not have a strong effect to change their gene expression pattern, but overexpression of SNCA gene changed two background cell lines' gene expression pattern.

## Acknowledgement

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