

Underlying regulatory expression mechanisms of Parkinson's disease

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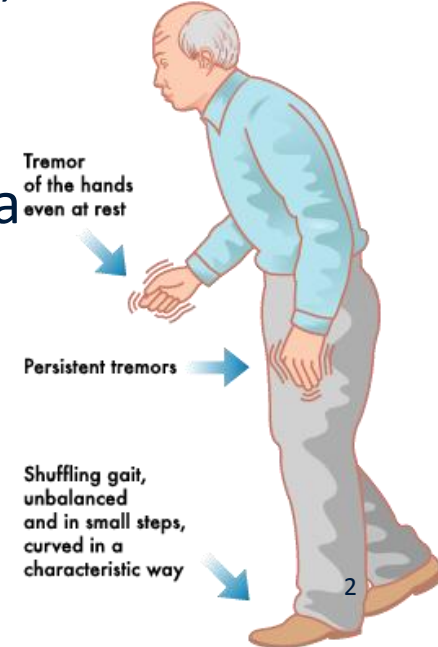
Date: 03/07/2019

Supervisor: Lars Eijssen



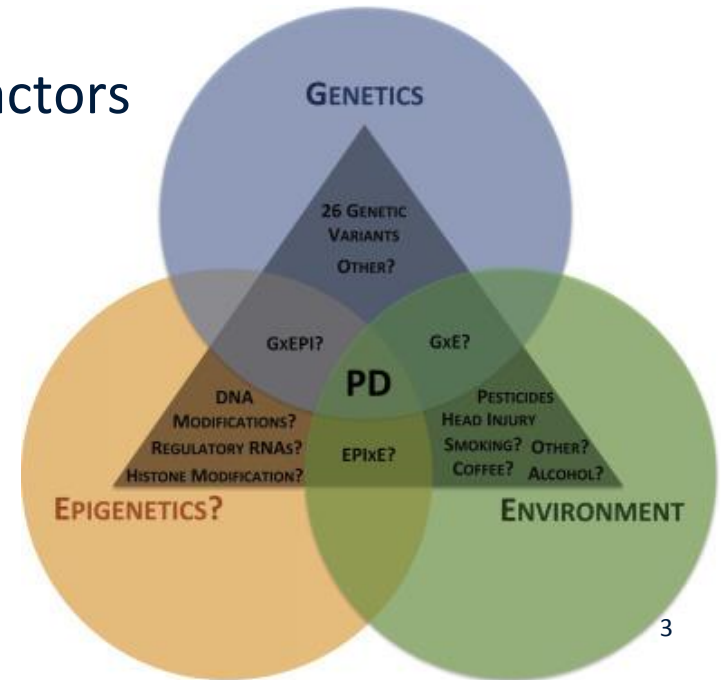
Introduction

- Parkinson's is the second most common neurodegenerative disorder ^[1]
- Risk factors: advanced age, exposure to chemicals, familial history^[2]
- Symptoms: tremors, trouble walking, depression^[2]
- Death of dopaminergic neurons in substantia nigra pars compacta is the main cause of symptoms



Background

- Only 10% of cases can be explained through genetics^[3]
- Large influence of environmental factors and epigenetics^[3]
- May not represent a single disease
- Genetic expression affected by all factors



Aim

My goal is to look at the expression differences of different genes to better understand the mechanisms of Parkinson's disease.

Methods: dataset

- Dataset was obtained from the NCBI GEO database
- keywords in search: RNA, epigenetic, Parkinson, genetic
- dataset was selected based on the vast amount of different data types and cell types present

Methods: dataset

- 17 subjects, 9 pd and 8 control
- mRNA, miRNA and RRBS (epigenetic) data
- Cingulate gyrus cells, dermal fibroblasts, induced pluripotent stem cells (iPSCs) and neurons

Series GSE110720

[Query DataSets for GSE110720](#)

Status	Public on Oct 08, 2018
Title	RNA-seq, RRBS-seq and miRNA-seq study of Parkinson's disease patients
Organism	Homo sapiens
Experiment type	Expression profiling by high throughput sequencing Methylation profiling by high throughput sequencing Non-coding RNA profiling by high throughput sequencing
Summary	This SuperSeries is composed of the SubSeries listed below.
Overall design	Refer to individual Series



Methods: R analysis and quality control

- R studio V1.2.1335
- Statistical analysis template from BiGCaT
- Own code written for direct comparison

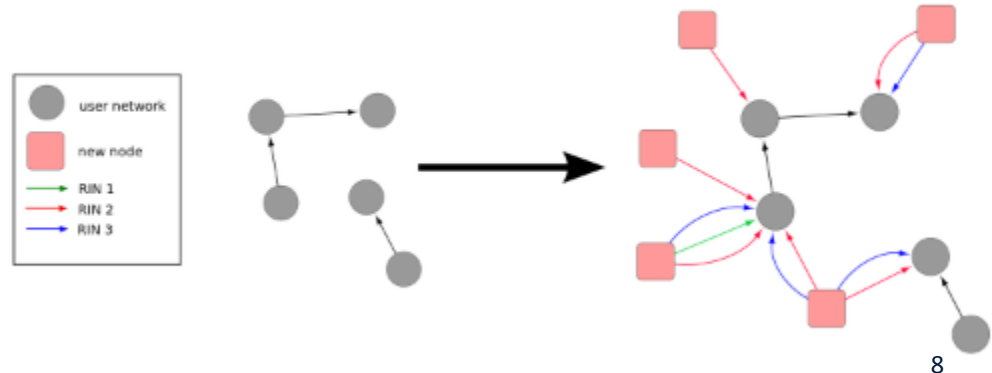
```

11 desc$age2 <- factor(c(1:10, desc$age<70, young, old))
12 desc$age2 <- relevel(desc$age2, "young")
13 rownames(desc) <- desc$sampleName
14
15 #load the data
16 # mrna
17
18 FileNames <- list.files(path="C:\\Users\\larsr\\Documents\\R\\data\\mrna\\")
19
20 CountTablemrna <- read.delim(file=paste("C:\\Users\\larsr\\Documents\\R\\data\\mrna\\", FileNames[1], sep=
21 CountTablemrna <- CountTablemrna[, -dim(CountTablemrna)[2]]
22 colnames(CountTablemrna)[length(colnames(CountTablemrna))] <- sub(".txt","",paste(unlist(strsplit(FileNames
23
24 RPKMTablemrna <- read.delim(file=paste("C:\\Users\\larsr\\Documents\\R\\data\\mrna\\", FileNames[1], sep=
25 RPKMTablemrna <- RPKMTablemrna[, -(dim(RPKMTablemrna)[2]-1)]
26 colnames(RPKMTablemrna)[length(colnames(RPKMTablemrna))] <- sub(".txt","",paste(unlist(strsplit(FileNames
27
28 for(i in 2:(length(FileNames))) {
29
30

```

Methods: Cytoscape

- Two different networks created by coupling miRNA to genes using cytargetlinker
- Networks filtered by either P-value and interconnectedness or adjusted P-value



Methods: Pathvisio

- Genes from data were coupled to wikipathways using hs-derby-ensemble-91 mapping database
- Pathways filtered on: Permutation P- value < 0.05 , Z-score > 1.96 and a minimum of 4 positive genes per pathway



Results: R

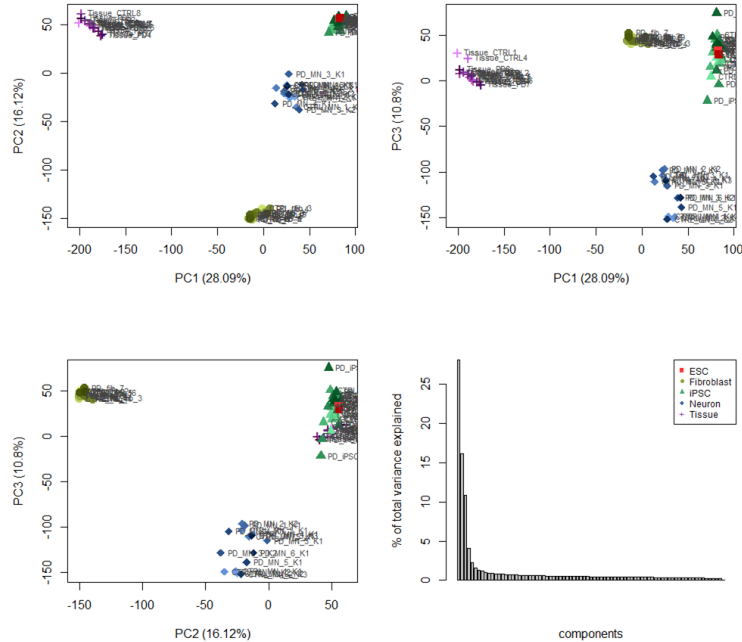
- Greatest miRNA correlations between brain tissue and neurons derived from stem cells
- mRNA tissue difficult to explain, degradation?

correlation	Fibroblast & Neuron	Tissue & Neuron	Fibroblast & Tissue
mRNA	0.1977	-0.1001	0.0351
miRNA	0.1899	0.5713	0.2865

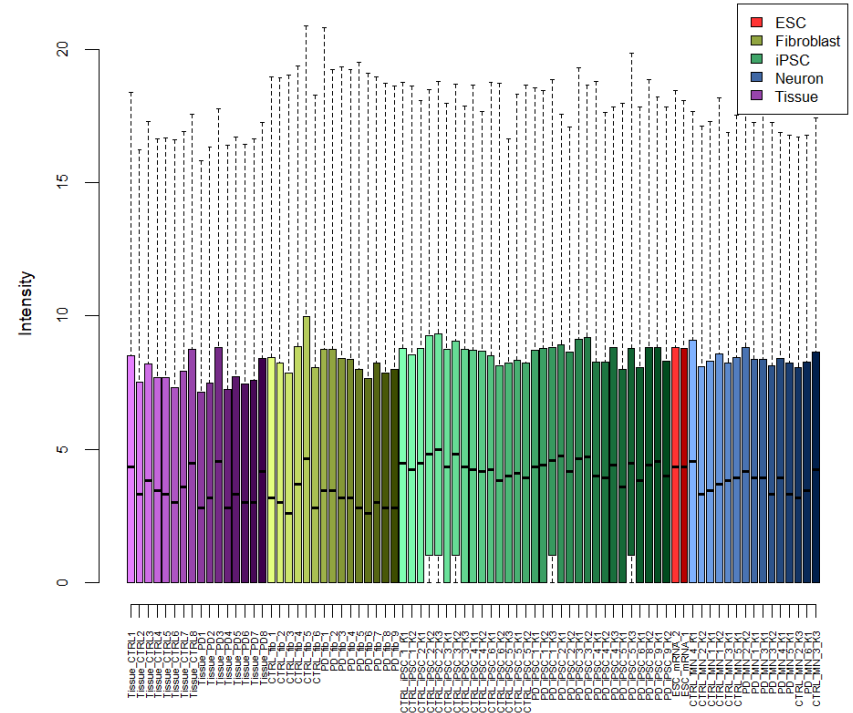
Tissue = brain tissue and Neuron = neurons generated from fibroblast-derived iPSCs.

Results: R mRNA

PCA analysis of sample data
mRNA RPKM

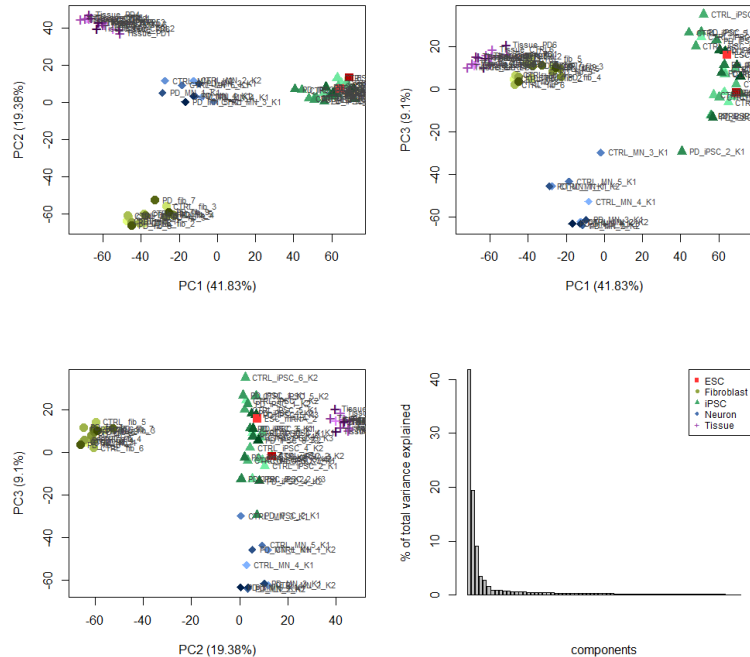


Boxplot of signal intensities
mRNA counts

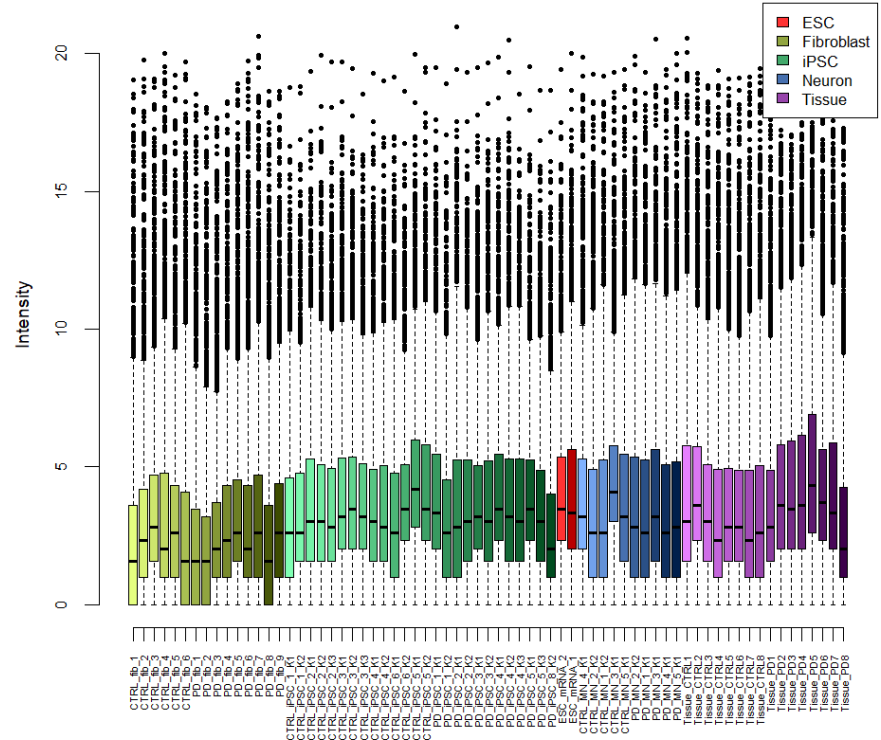


Results: R miRNA

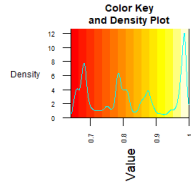
PCA analysis of sample data
miRNA norm counts



Boxplot of signal intensities
miRNA counts

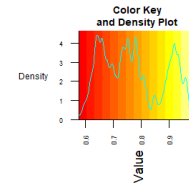
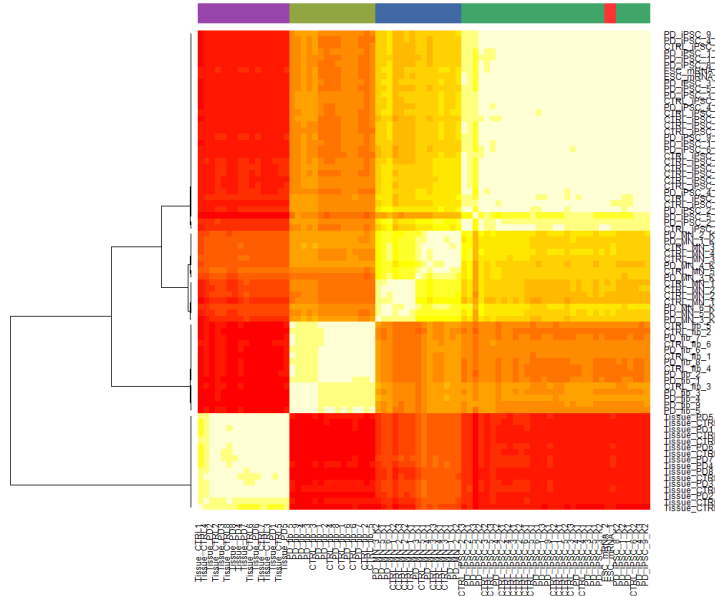


Results: R



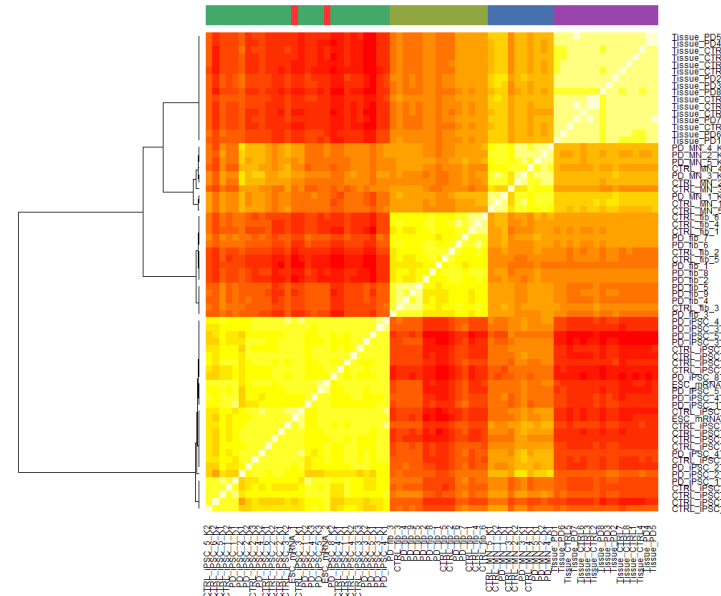
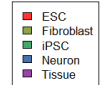
Sample data correlation plot
correlation method: pearson
cluster method: ward.D2

mRNA RPKM

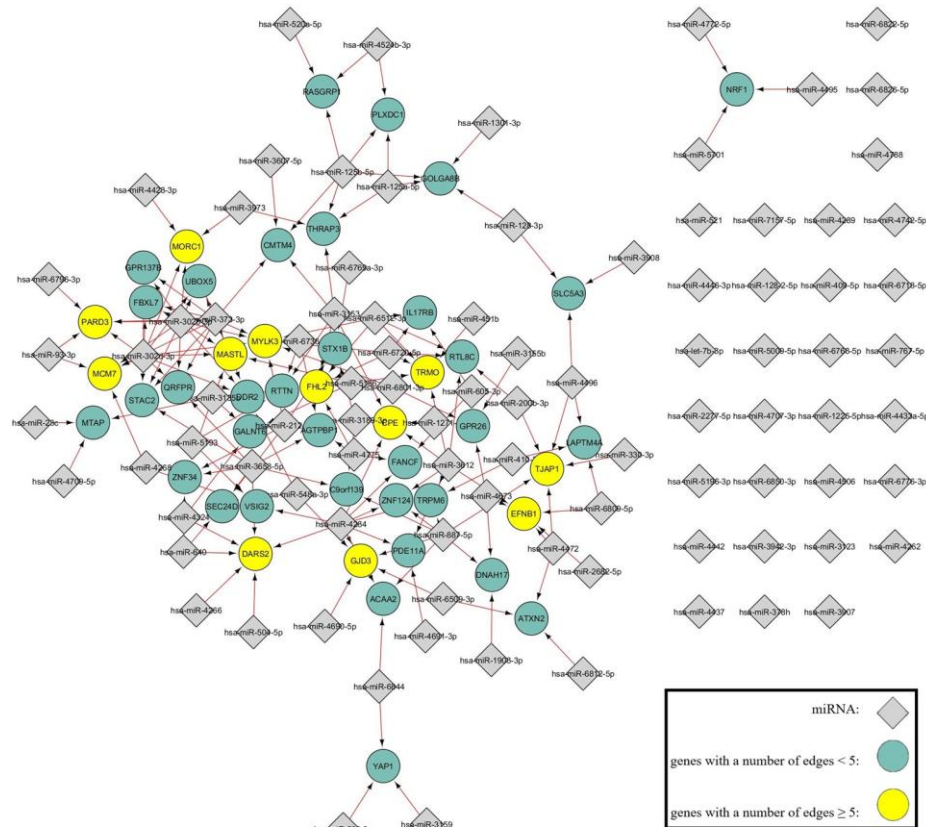
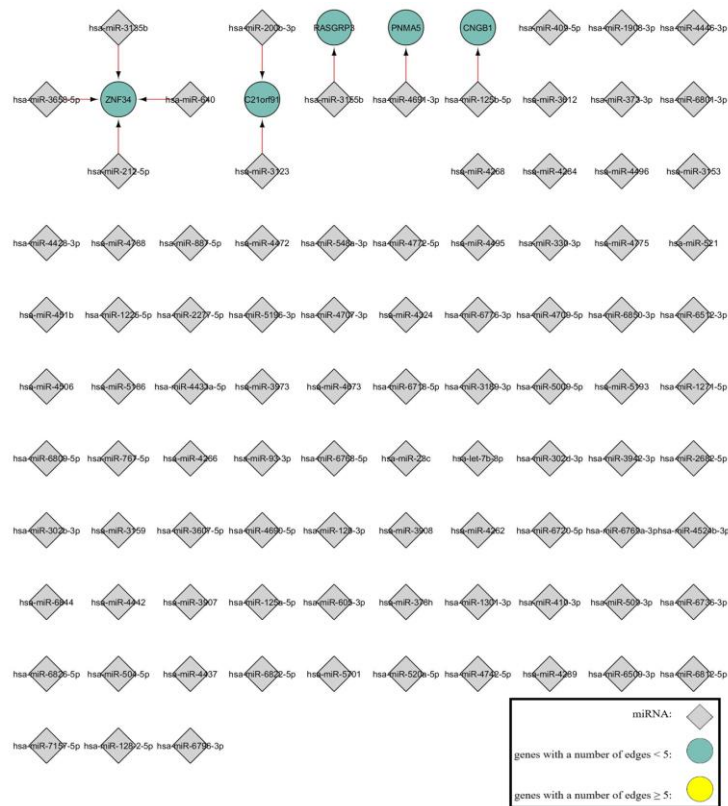


Sample data correlation plot
correlation method: pearson
cluster method: ward.D2

miRNA norm counts

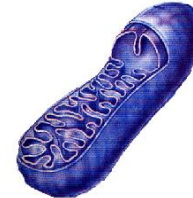


Results: cytoscape



Discussion: Cytoscape

- Multiple genes related to prenatal brain development (c21orf91) or cell division (PARD3 and MCM7)
- Several genes related to cell adhesion methods: TJAP1, GJD3, EFNB1
- Mitochondrial genes: p450c11, Dars2



Results: Pathvisio

Pathway	positive (r)	measured (n)	total	%	Z Score	p-value (permuted)
Glial Cell Differentiation	4	7	9	57.14%	4.74	0
GABA receptor Signalling	10	34	57	29.41%	4.53	0
miR-509-3p alteration of YAP1/ECM axis	6	17	19	35.29%	4.09	0
Oligodendrocyte Specification and differentiation (including remyelination), leading to Myelin Components for CNS	8	31	46	25.81%	3.59	0
Classical pathway of steroidogenesis, including diseases	5	15	49	33.33%	3.56	0.002
Sudden Infant Death Syndrome (SIDS) Susceptibility Pathways	24	163	182	14.72%	3.09	0.003
GPCRs, Class A Rhodopsin-like	34	256	272	13.28%	3.04	0.003
Arrhythmogenic Right Ventricular Cardiomyopathy	13	74	81	17.57%	2.97	0.004
Monoamine GPCRs	7	33	43	21.21%	2.74	0.009

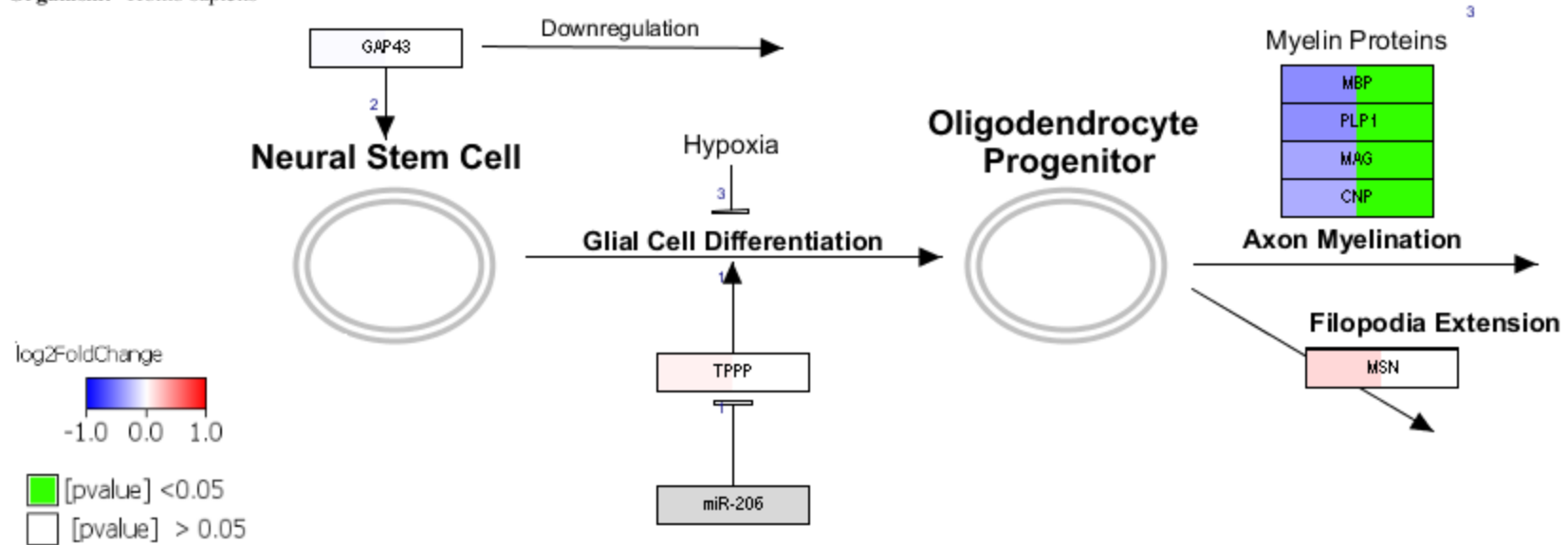
Results: Pathvisio

Hair Follicle Development: Cytodifferentiation (Part 3 of 3)	14	87	92	16.09%	2.72	0.01
Rett syndrome causing genes	9	48	60	18.75%	2.69	0.004
Calcium Regulation in the Cardiac Cell	21	149	164	14.09%	2.67	0.011
GPCRs, Class B Secretin-like	4	15	24	26.67%	2.62	0.011
The alternative pathway of fetal androgen synthesis	4	15	48	26.67%	2.62	0.013
Spinal Cord Injury	17	115	127	14.78%	2.61	0.012
Dopaminergic Neurogenesis	6	30	32	20.00%	2.37	0.022
GPCRs, Other	13	86	118	15.12%	2.37	0.017
G13 Signalling Pathway	7	38	39	18.42%	2.32	0.026
Pathways Regulating Hippo Signalling	14	98	104	14.29%	2.23	0.01
Differentiation Pathway	8	50	64	16.00%	2.03	0.037
Splicing factor NOVA regulated synaptic proteins	7	42	44	16.67%	2.02	0.032

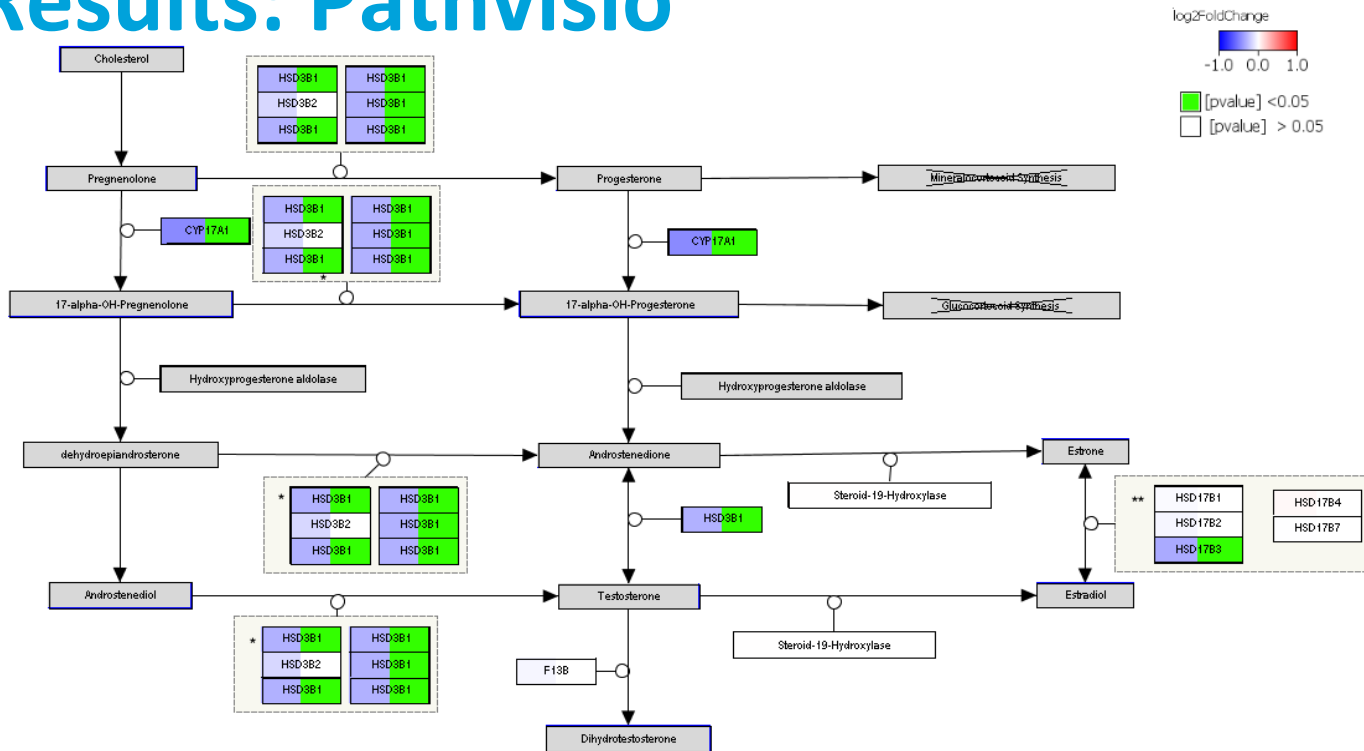
Results: Pathvisio

Title: Glial Cell Differentiation

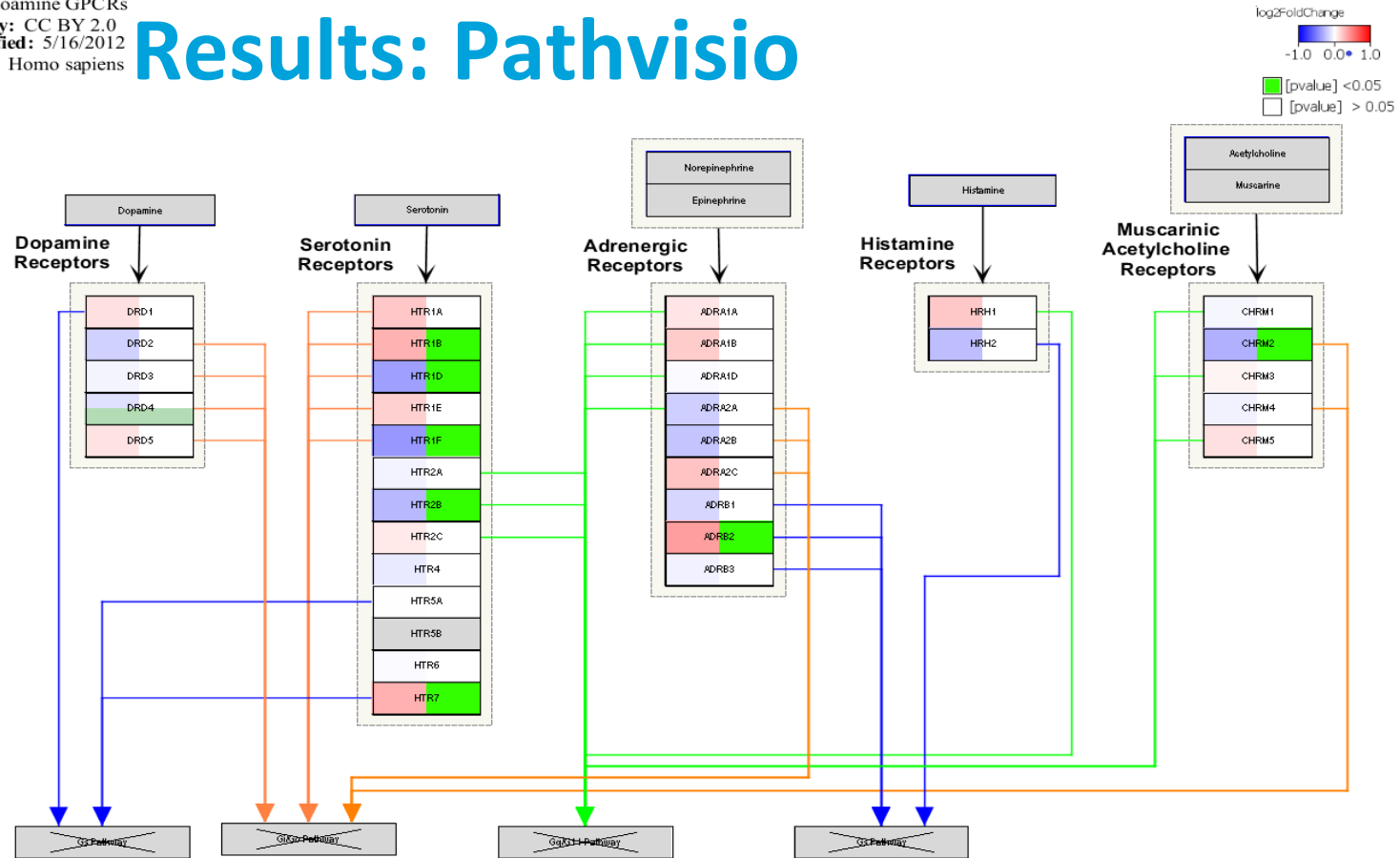
Organism: Homo sapiens



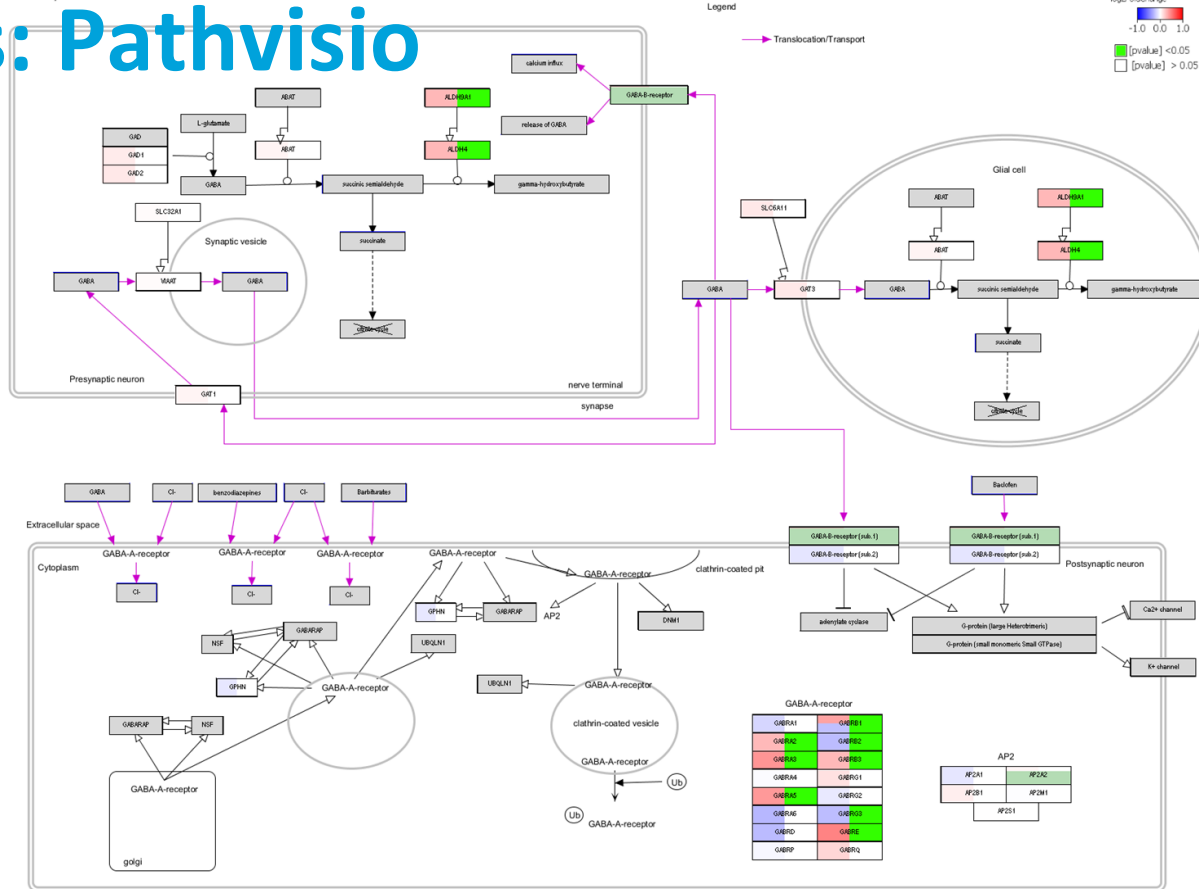
Results: Pathvisio



Results: Pathvisio

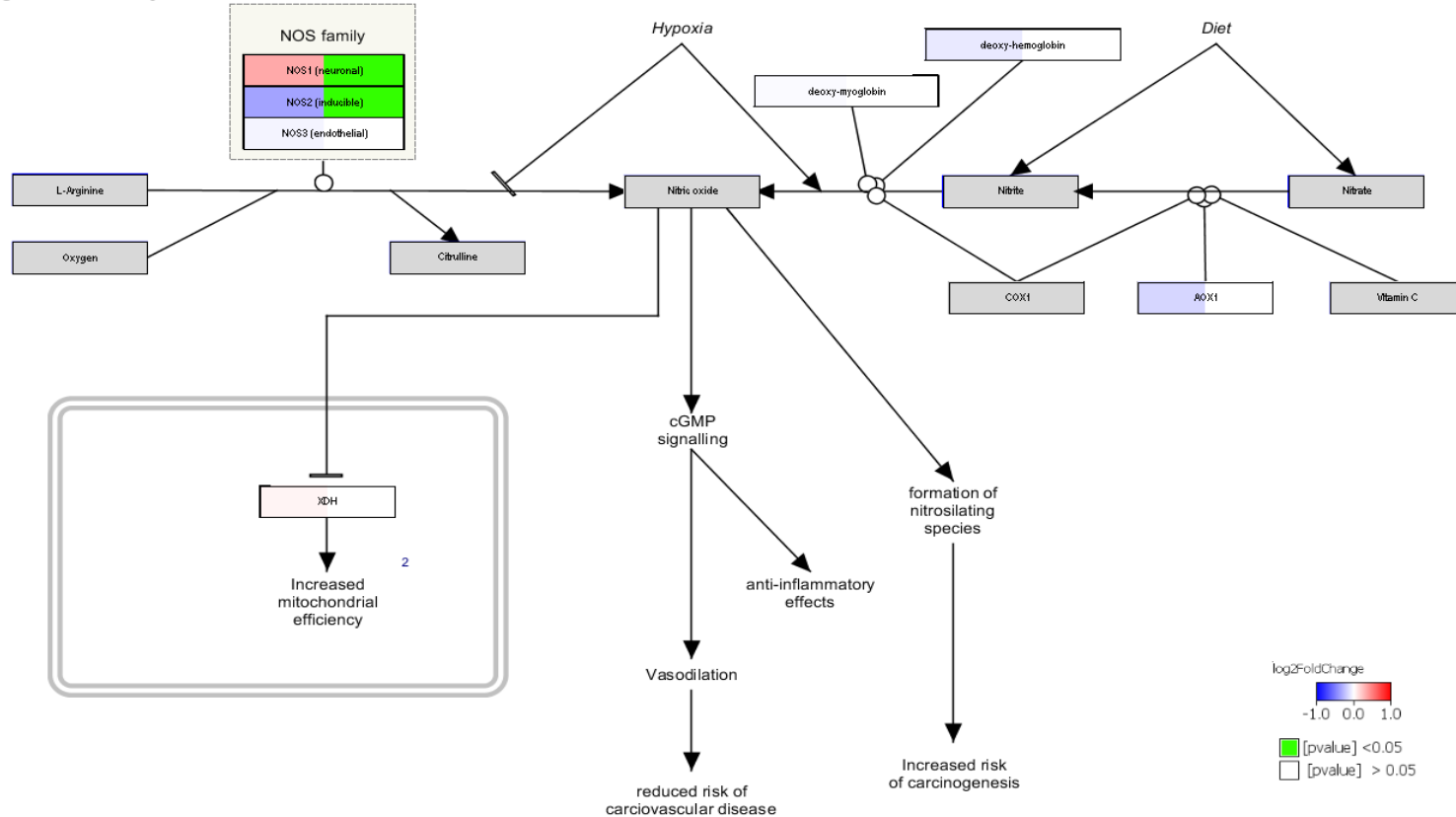


Results: Pathvisio



Title: Effects of Nitric Oxide
Last modified: 2/22/2013
Organism: Homo sapiens

Results: Pathvisio



Discussion

Connecting factor: Nitric Oxide

- Component neuronal cell death pathway^[4]
- Free radical
- excitotoxicity^[5]
- Dopaminergic neurons especially sensitive to NO^[6]



nitric oxide

Conclusion

- Data confirmed the involvement of several processes: Myelin degradation, mitochondria dysfunction
- Strengthened the GABA-collapse hypothesis and NO-hypothesis
- Suggested involvement of: TJAP1, TRMO, Znf34, DARS2 MCM7 and many more
- Most likely not a single disease

Thank you for your attention

Questions?

