

Differentially expressed genes in three Brodmann areas in schizophrenia

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Introduction:

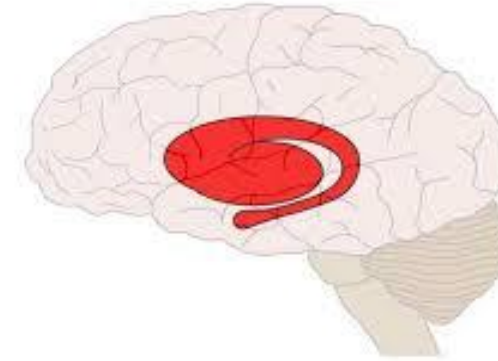
- Schizophrenia – neurodegenerative psychiatric disorder;
- Positive and negative symptoms;
- Approximately 23 million affected individuals (World Health Organization, April 2019) [1]
- Exact mechanism of pathogenesis is unknown;
- Neurobiological paradigm of pre-synaptic dopaminergic circuitry dysfunctions;
- G x E interplay;

[1] <https://www.who.int/news-room/fact-sheets/detail/schizophrenia>

- Dopaminergic dysfunctions:

1) Striatum regions (limbic, associative, sensorimotor) -> dopamine overproduction;

- Limbic : reward – motivation;
- Associative : behavioral flexibility;
- Sensorimotor : habit formation;

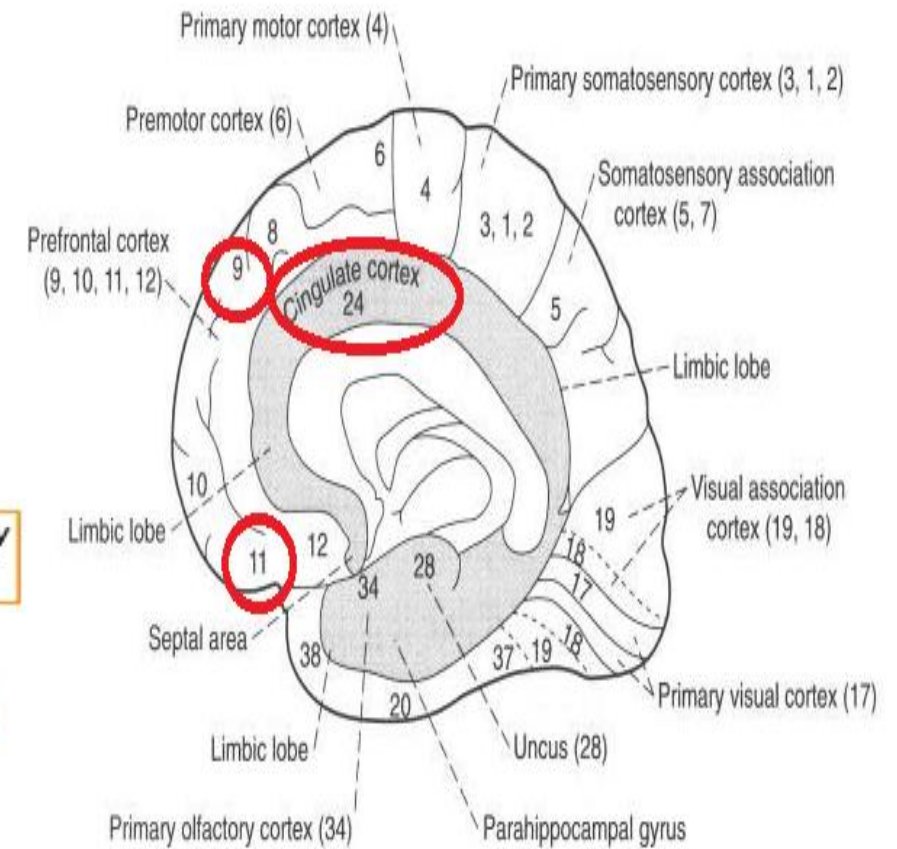
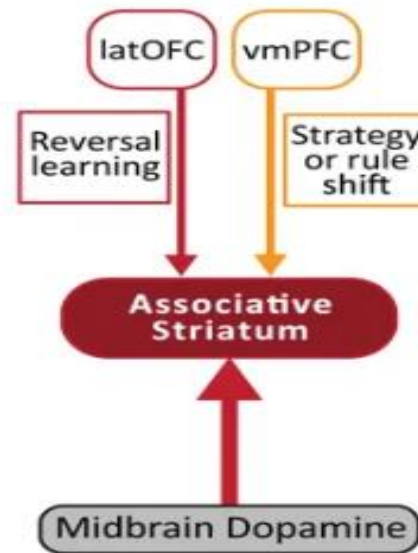
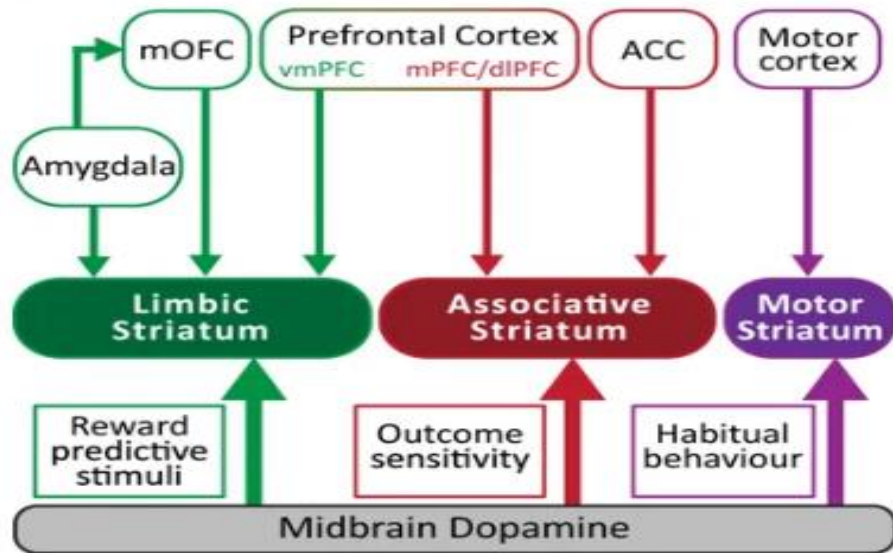


- Frontal lobe structures dysfunctions:

- Gray and white matter reduction;
- Constant communication with associative and limbic striatum;
- Impaired communication due to dysfunctions;

- Brain structures investigated:

- 1) Brodmann area 9 – dorsolateral and medial prefrontal cortex;
- 2) Brodmann area 11 – orbitofrontal cortex;
- 3) Brodmann area 24 – anterior cingulate cortex;



<https://www.nature.com/articles/s41398-017-0071-9/figures/5>

<https://www.pinterest.com/pin/532269249686058151/>

- Hypothesis:

- Different expression profiles of genes in the three Brodmann areas lead to abnormal gene product formation that play a role in schizophrenia symptoms manifestation:

- Aim:


- Establish the presence of differentially expressed genes in the three Brodmann areas, as well as their expression profiles;


- Expectations:

- Comparisons for all brain regions in diseased samples to yield genes with differential expression as an outcome;

- Due to reported deficits in the frontal lobe structures, mainly findings of underexpression;

Methods:

- ArrayExpress: 
 - RNA-seq with a count table;
 - Schizophrenia samples;
 - E-GEOD-78936;
- Sample information:
 - 82 post-mortem brain tissues: 44 BA11, 19 BA9, 19 BA24;
 - BA9 and BA24 come from the same individuals;
 - Schizophrenia, bipolar disorder and control samples;

- R (version 3.5.2): 
 - DESeq2 package for differential expression analysis;
 - DESeqDataSet object created from the count table, metadata and design formula;
 - Count table: 27112 genes, 82 samples -> filtered to 23688 genes;
 - Metadata modified and reordered to match count table;

```
> head(meta)
```

	Name	Disorder	Area	Individual	Code	Group
BA11_1	BD_BA11_1	BD	BA11	A_I1	BA11_1	BD_BA11
BA11_2	BD_BA11_2	BD	BA11.	A_I2	BA11_2	BD_BA11
BA11_3	BD_BA11_3	BD	BA11	A_I3	BA11_3	BD_BA11
BA11_4	BD_BA11_4	BD	BA11	A_I4	BA11_4	BD_BA11
BA11_5	BD_BA11_5	BD	BA11	A_I5	BA11_5	BD_BA11
BA11_6	BD_BA11_6	BD	BA11	A_I6	BA11_6	BD_BA11

- DESeqDataSet:

- *dds <- DESeqDataSetFromMatrix(countData = cts,
colData = meta,
design = ~ Group)*

- Quality Control:

- 1) Raw Data;

- 2) Normalized Data;

- 3) Normalized without outliers;

- Statistics:
 - *results* function of the DESeq2 package;
 - Performed on the data with removed outliers;
 - Six comparisons:

```
res <- results(dds_NO, contrast=c("Group", "SZ_BA24", "Control_BA24"))
```

```
resSig <- res[ which(res$padj < 0.05), ]
```

```
resSig <- resSig[ order( resSig$log2FoldChange ), ]
```

```
resSig
```

Comparison
SZ_BA24 vs Control BA24
SZ_BA11 vs Control BA11
SZ_BA9 vs Control BA9
BD_BA24 vs Control BA24
BD_BA11 vs Control BA11
BD_BA9 vs Control BA9

Pathway analysis

- PathVisio (version 3.30)



- Comparison for SZ_BA24 – Control_BA24 was used;
- Genes with Entrez and Ensembl ID's:

```
sysCodes <- rep("L",dim(res)[1])
```

```
sysCodes[grep("ENSG",rownames(res))] <- "En"
```

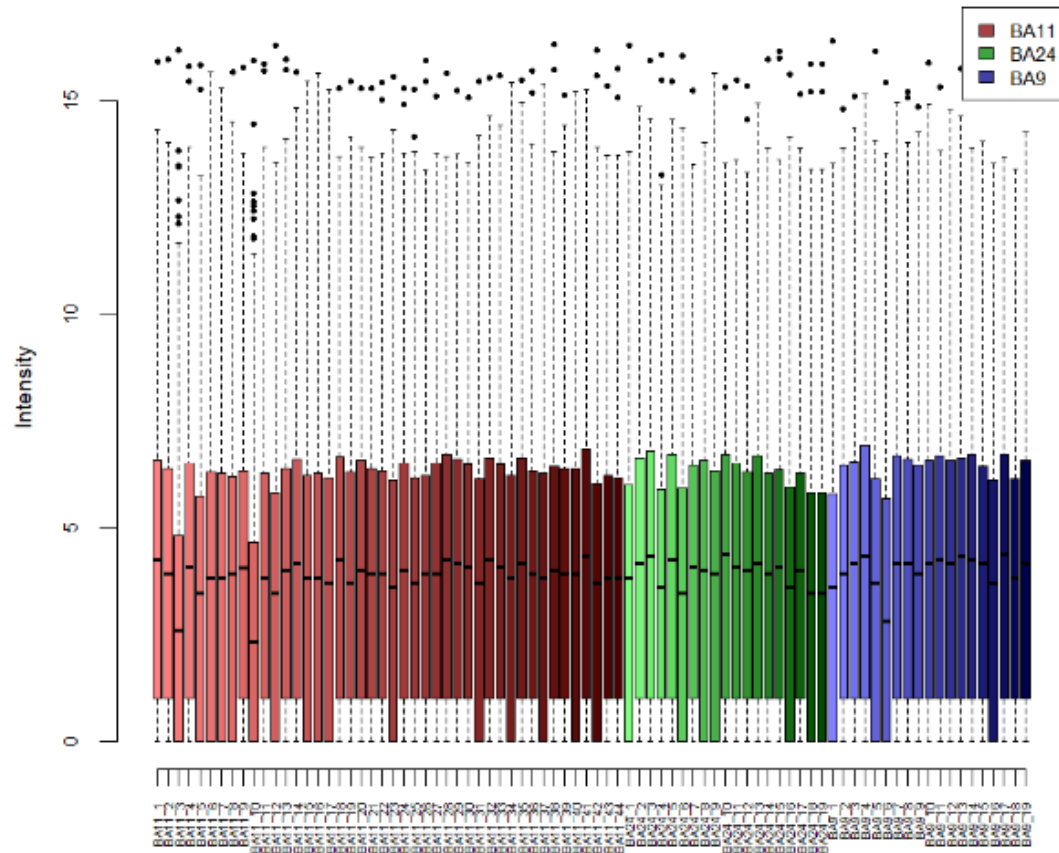
- Imported data mapped to *Hs_Derby_Ensembl_91* (Homo Sapiens database)
- *wikipathways_Homo_sapiens_Curation-AnalysisCollection* pathway collection;

<https://www.pathvisio.org/>

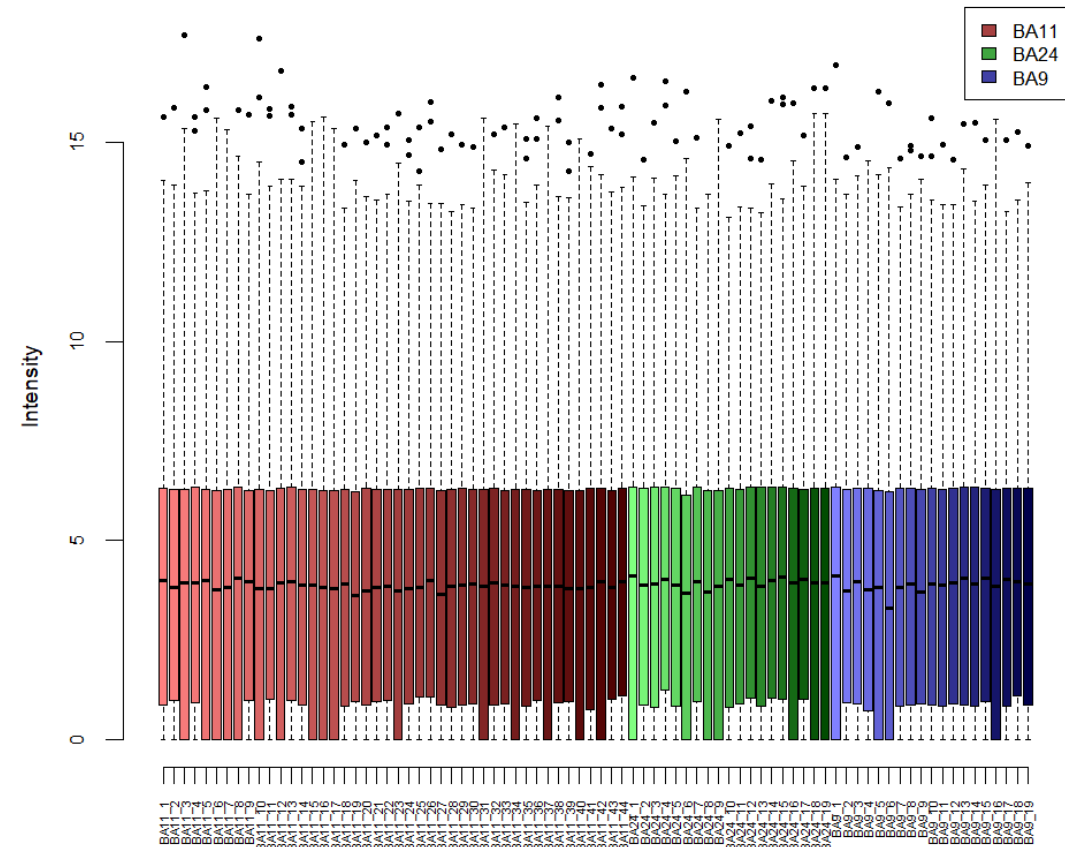
Results:

Quality Control

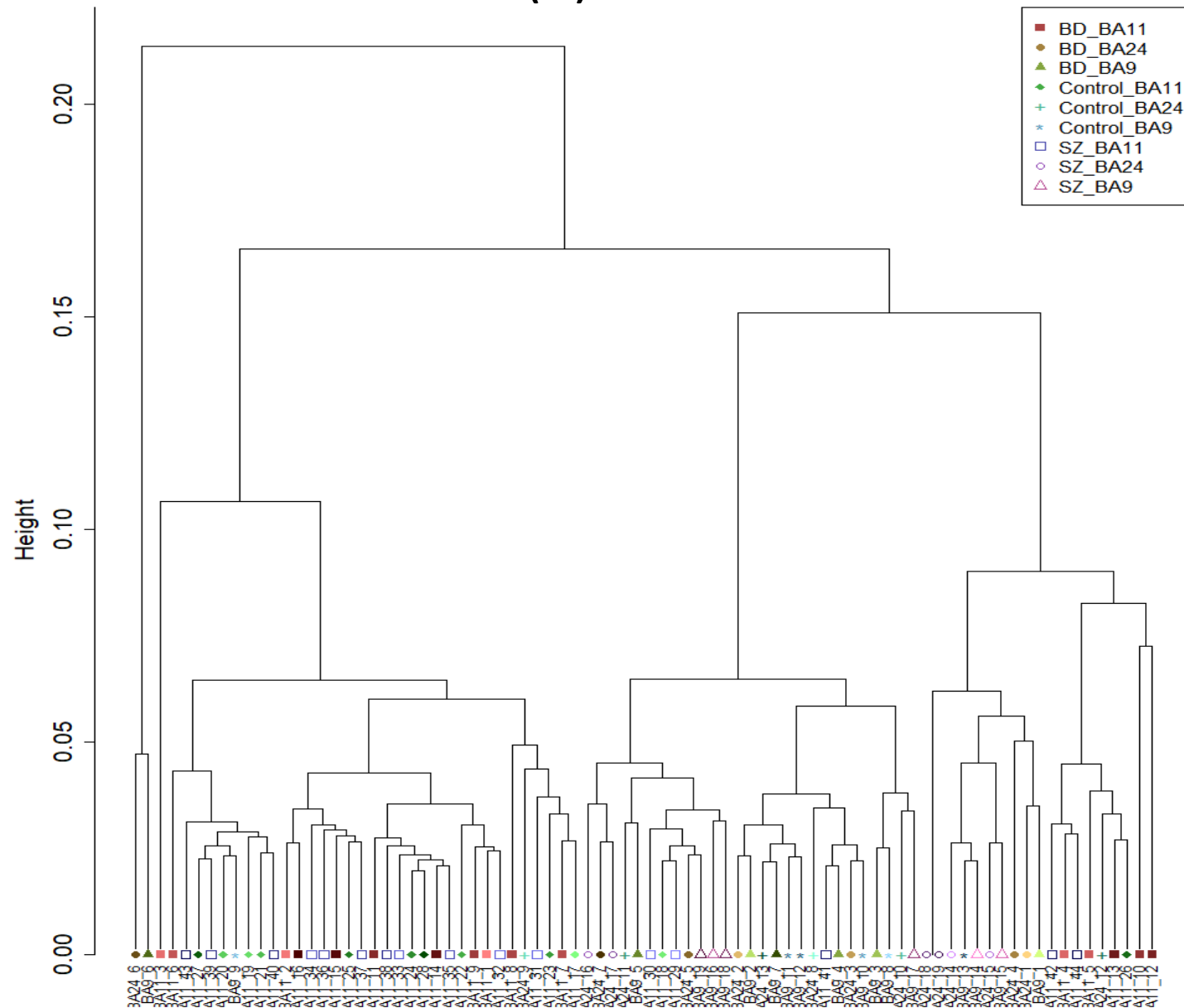
Raw Data Boxplot



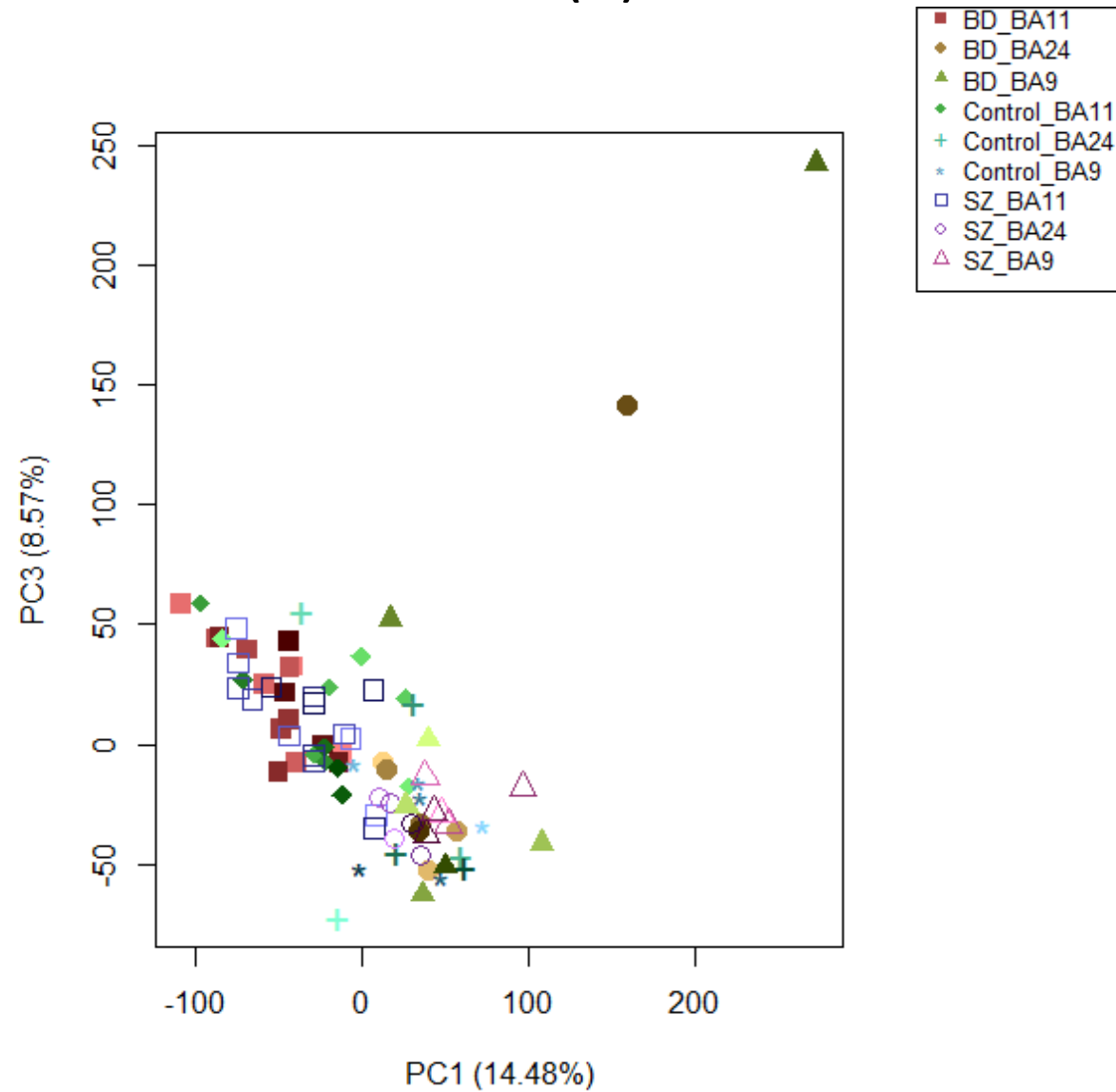
First Normalization boxplot



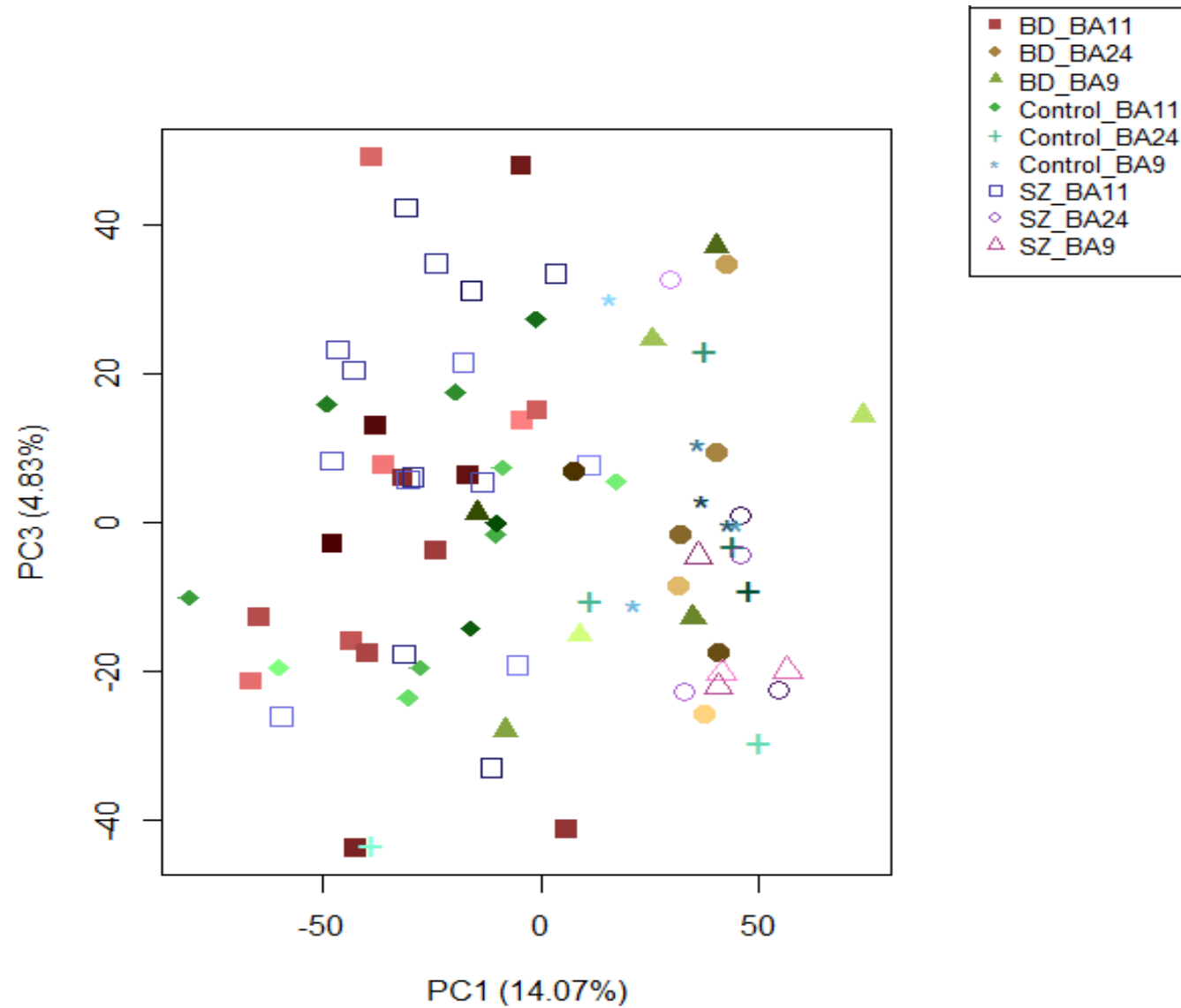
- First Normalization (1):



- First Normalization (2):



- Second Normalization with removed outliers:

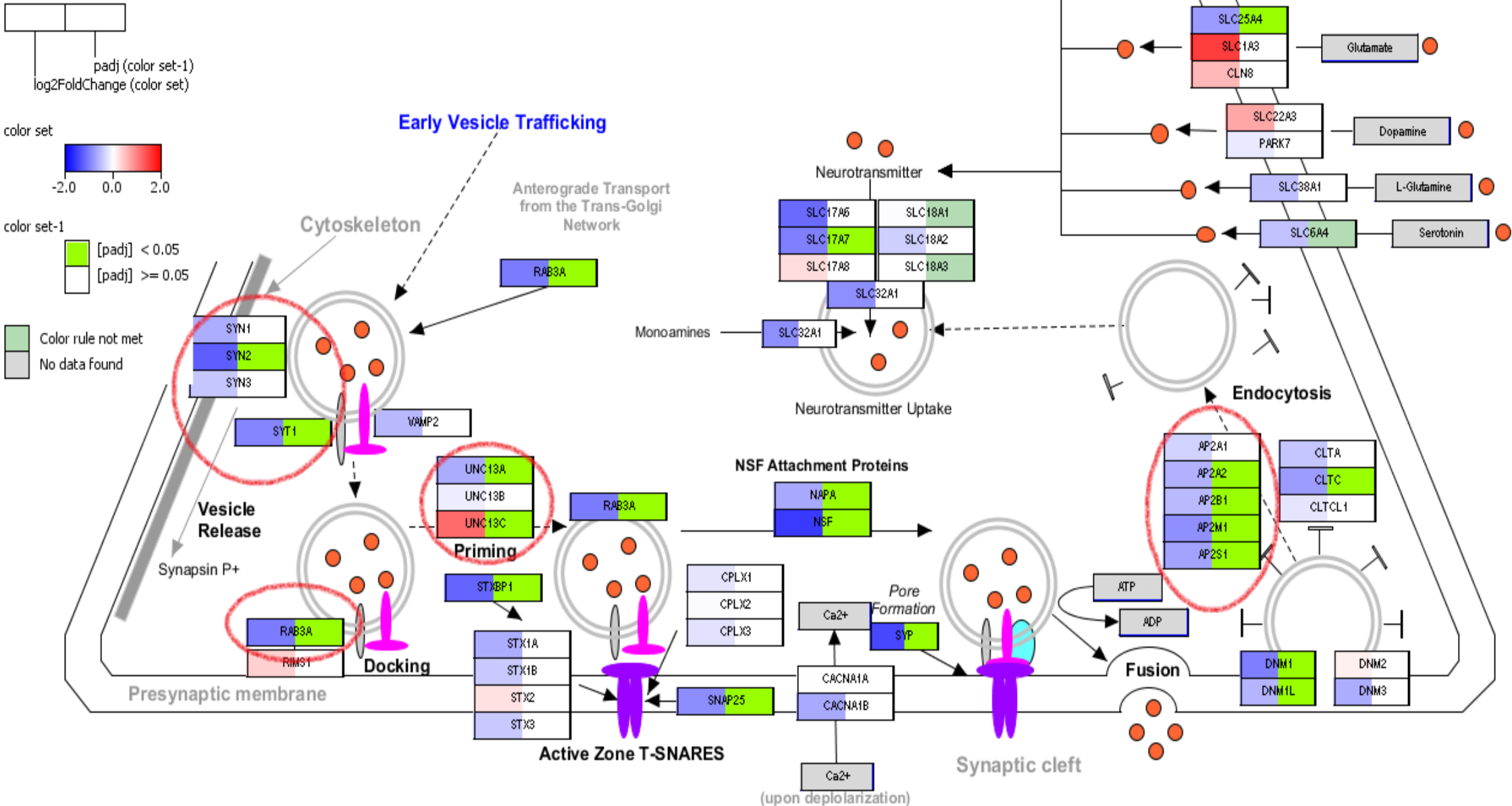


- Statistics:

Comparison	Number of genes detected significantly ($p_{adj} < 0.05$)	Underexpressed	Overexpressed
SZ_BA24 vs Control BA24	2047	1276	771
SZ_BA11 vs Control BA11	0	0	0
SZ_BA9 vs Control BA9	2	1	1
BD_BA24 vs Control BA24	2	0	2
BD_BA11 vs Control BA11	400	169	231
BD_BA9 vs Control BA9	0	0	0

- Pathway analysis:

Pathway	positive (r)	measured (n)	total	%	Z Score	p-value ...
Glycolysis and Gluconeogenesis	18	44	69	40.91%	5.48	0.000
Synaptic Vesicle Pathway	19	51	59	37.25%	5.13	0.000
Brain-Derived Neurotrophic Factor (BDNF) signaling pathway	39	144	150	27.08%	5.02	0.000
Calcium Regulation in the Cardiac Cell	39	147	164	26.53%	4.87	0.000
Amino Acid metabolism	25	90	205	27.78%	4.15	0.000
Cori Cycle	7	14	53	50.00%	4.10	0.000
Splicing factor NOVA regulated synaptic proteins	14	41	44	34.15%	4.00	0.001
EGF/EGFR Signaling Pathway	37	161	163	22.98%	3.75	0.000
miRs in Muscle Cell Differentiation	10	27	42	37.04%	3.69	0.000
Urea cycle and associated pathways	8	21	78	38.10%	3.40	0.001
Myometrial Relaxation and Contraction Pathways	34	153	161	22.22%	3.37	0.002
GABA receptor Signaling	11	34	57	32.35%	3.33	0.006
Sudden Infant Death Syndrome (SIDS) Susceptibility Pathways	35	160	182	21.88%	3.32	0.000
Cell migration and invasion through p75NTR	10	30	31	33.33%	3.29	0.002
Pathogenic Escherichia coli infection	15	54	79	27.78%	3.21	0.001
Mechanoregulation and pathology of YAP/TAZ via Hippo and non-Hippo mechanisms	13	45	47	28.89%	3.15	0.003
Cholesterol Biosynthesis Pathway	6	15	32	40.00%	3.09	0.005
Common Pathways Underlying Drug Addiction	12	41	50	29.27%	3.08	0.003
BDNF-TrkB Signaling	10	33	38	30.30%	2.94	0.004
Pathways in clear cell renal cell carcinoma	20	84	92	23.81%	2.92	0.005
MAPK Signaling Pathway	47	245	259	19.18%	2.86	0.004
G Protein Signaling Pathways	21	91	97	23.08%	2.84	0.005
Dopamine metabolism	5	13	48	38.46%	2.71	0.008
Translation inhibitors in chronically activated PDGFRA cells	12	45	49	26.67%	2.71	0.006

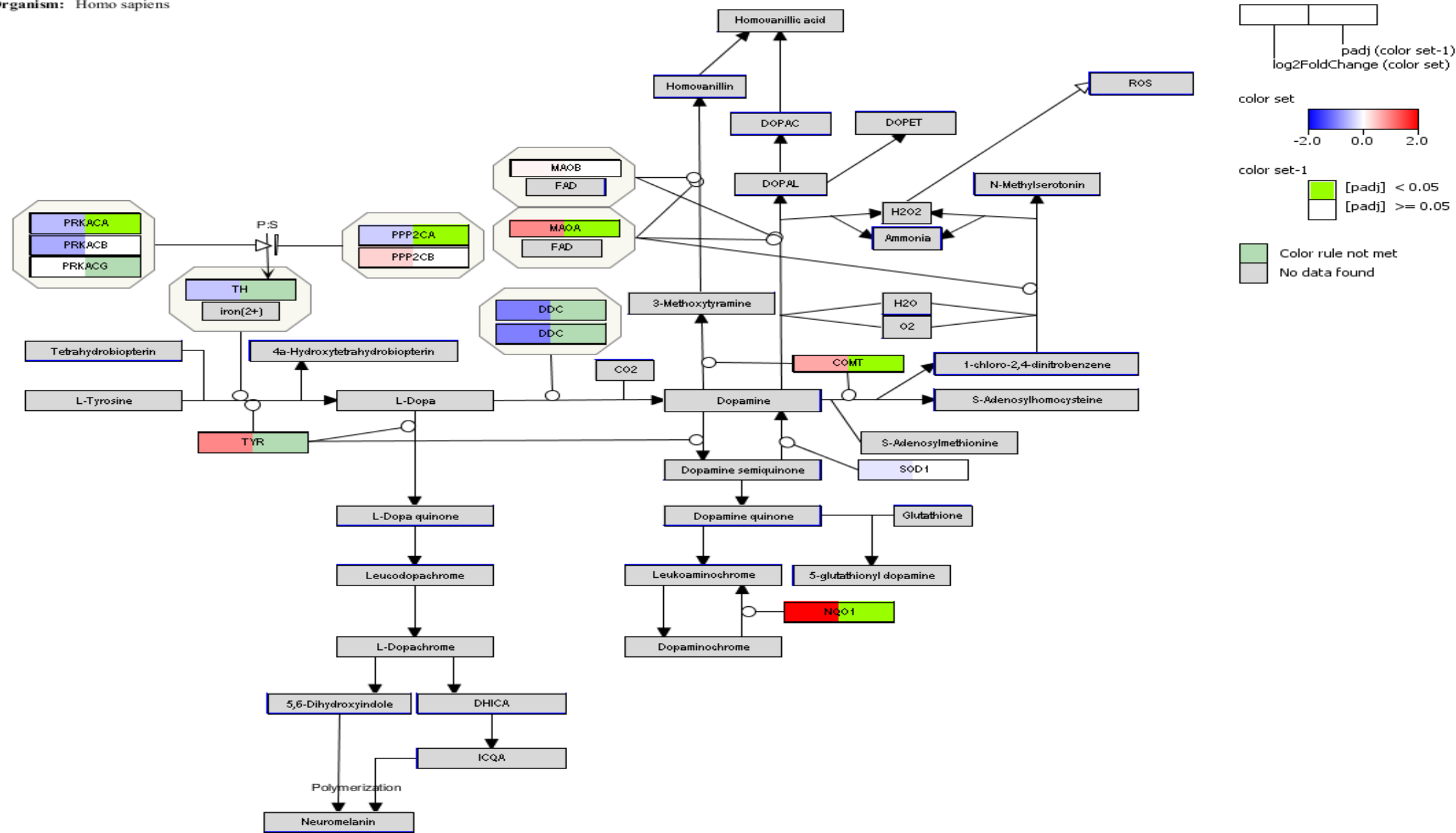


Discussion:

- Synaptic Vesicle Pathway:

- 1) Early Vesicle Trafficking -> SYN2 and SYT1 underexpression;
- 2) Docking -> RAB3A underexpression;
- 3) Priming -> UNC13A underexpressed, UNC13C overexpressed;
- 4) Endocytosis following fusion -> AP2 adaptor protein genes show underexpressed profiles; (AP2A2, AP2B2, AP2M1, AP2S1)

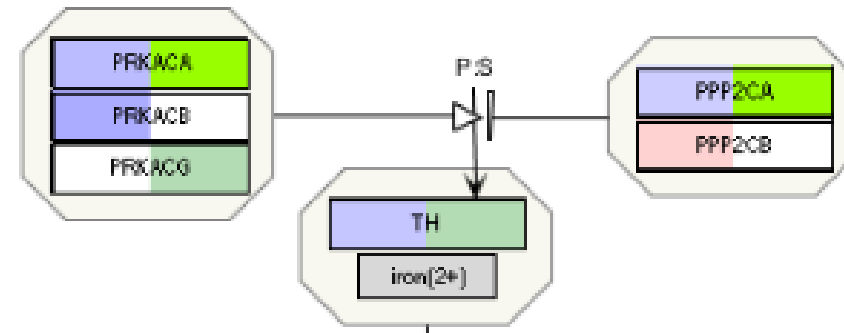
- Inferred impairment of dopamine transport and release does not dismiss the overproduction dogma -> even with a deficit in these processes, overproduction can still gain the upper hand in the balance.



- Dopamine Metabolism pathway:

1) Initializing the metabolic pathway: competitive loop between protein kinase A genes and PPP2CA, both underexpressed;

2) Monoamine Oxidase A -> overexpression



3) No in-depth literature has investigated this relationship; however certain speculations can be made:

- Decreased targeting for metabolism -> increased levels of active compound;
- Upregulated degradation by MAOA -> increased ROS levels and neurotoxicity;

- Study strengths:

- 1) Samples taken from direct sources of interest;
- 2) Researches gene expression of the anterior cingulate in relation to schizophrenia;

- Study limitations:

- 1) Samples coming from different cohorts;
- 2) No protein expression and activation investigation;

Conclusions:

- Differential gene expression analysis in three Brodmann regions;
- R and PathVisio tools;
- Two/Six comparisons returned substantial outputs, contrary to the initial expectations;
- The project findings suggest genetic changes in a multitude of pathways in neurons from the anterior cingulate cortex, with the main focus being on the Synaptic Vesicle Pathway;

Acknowledgements

- Lars Eijssen
- The BigCat department

Questions?