



Analysis of RNA-sequencing data taken from microglial cells of *rTg4510 tau* transgenic and wild-type mice

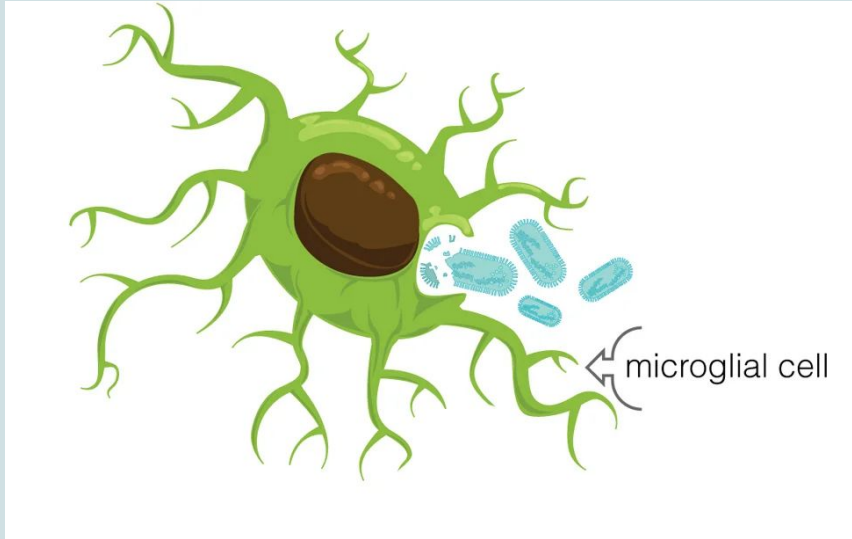
Maurizio Gilioli, 229322
QCB - Network based data analysis



Introduction



Microglial cells



➡ Guide **morphogenesis** and control **formation and depletion of synapses**

➡ **Homeostasis** of the tissue during adulthood

➡ **Immune innate responses** in the CNS

The Other Brain Cells.

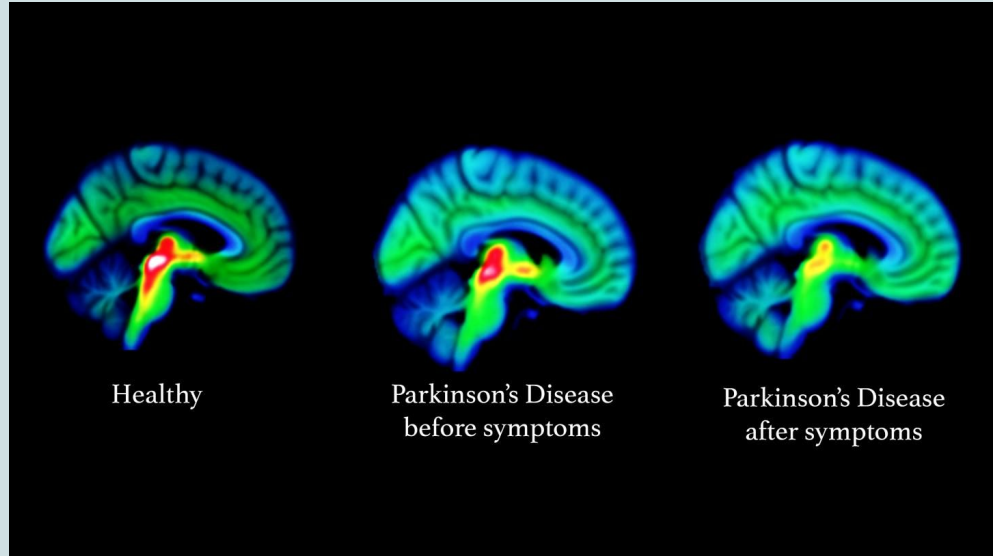
<https://learn.genetics.utah.edu/content/neuroscience/braincells/>.

Neurodegenerative diseases cause losses in the brain

Effect of neurodegenerative diseases on the brain

Microglial cells are generally considered to be able to assume two possible phenotypes:

- **M1/pro-inflammatory**
- **M2/tissue-repair**



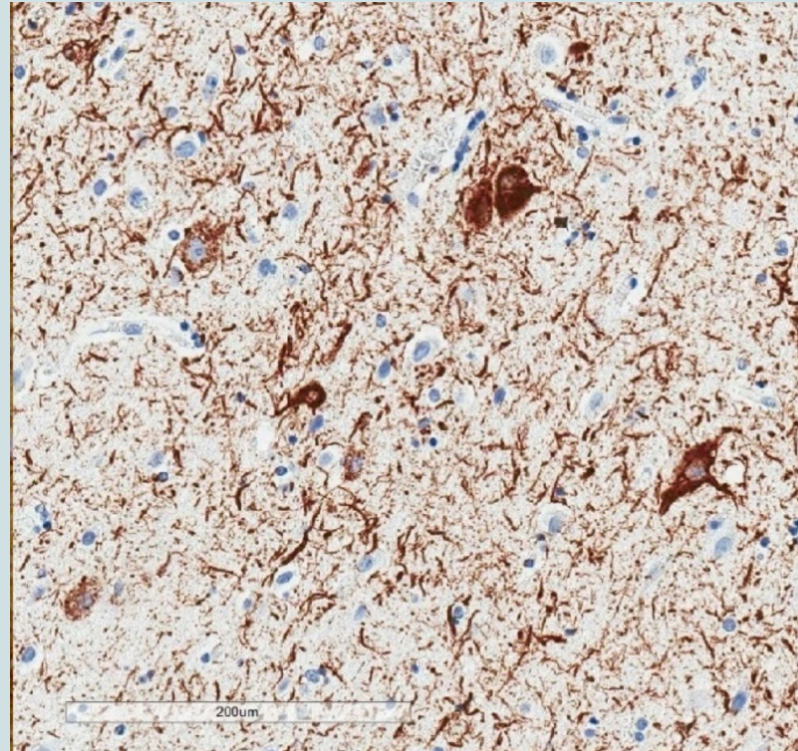
Parkinson's Disease Brain Malfunction Evident in Scans Potentially Years Before Symptoms.

<https://www.genengnews.com/news/parkinsons-disease-brain-malfunction-evident-in-scans-potentially-years-before-symptoms/>.

Tau accumulation in mice models

The mechanism causing neurodegeneration after tau accumulation is not well understood. **However, microglial cells are known to be involved**

➔ **Mice models,**
rTg4510 female mice transgenically modified to express an human tau containing the P301L mutation that has been linked with familial frontotemporal dementia





Dataset details

- Recount3 Database **SRP172787**
- **RNA sequencings of 96 micrgolial cells, 12 for each age and genotype combination, (Wild-type/Transgenic; 2-, 4-, 6-, and 8-months old).**
- **Brain cortex**



Aim of the analysis

Make **differential gene expression (DGE)** analysis to

- Compare samples of all ages (***all-ages case***)
- Compare samples 2-4 months old (***young-samples case***)

Methods

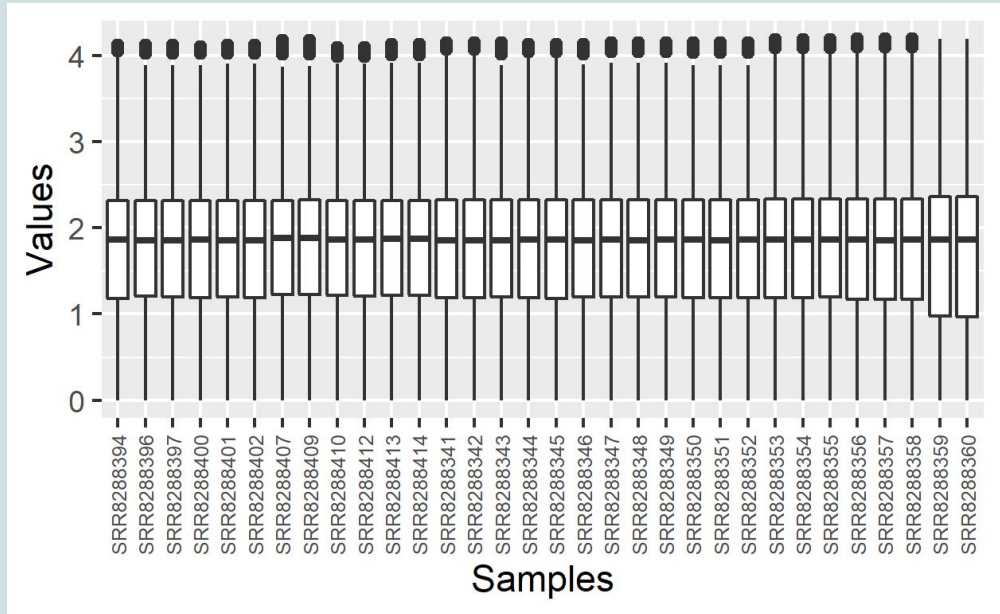
- Data pre-processing
- Main Analysis Procedure



Data pre-processing

initial n. of genes = **55421 genes**

1. Conversion into **read counts**.
2. (**First filtering**) Filtering of genes with zero mapped reads for more the 20% of the samples. → **30351**
3. **Normalization** (GeTMM method)..
4. (**Second filtering**) Filtering of genes with cpm values below 0.5 for more than the 20% of the samples. → **22472**
5. **Two consecutive log₂ transformations.**





Main Analysis Procedure - Unsupervised and Supervised methods

1. Assign **labels** to samples (Transgenic / Wild-type)
2. Principal Components Analysis (**PCA**)
3. **Unsupervised Learning**
 - **Kmeans clustering**
 - **Hierarchical clustering**
4. Testing of the genes with **Wilcoxon rank sum tests**
5. **Heatmap** of the 25 most important genes
6. **Supervised learning**
 - **cross-validated Random Forest, LDA and Lasso regression** (10 partitions).
 - **repeated cross-validated Random Forest and LDA** (10 partitions; 10 repetitions).
 - **bootstrapping Random Forest and LDA** (10 iterations).



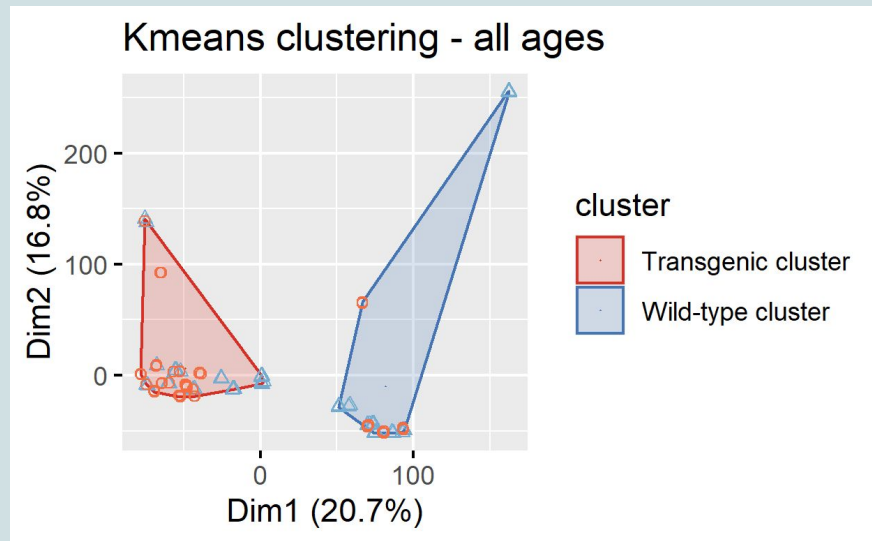
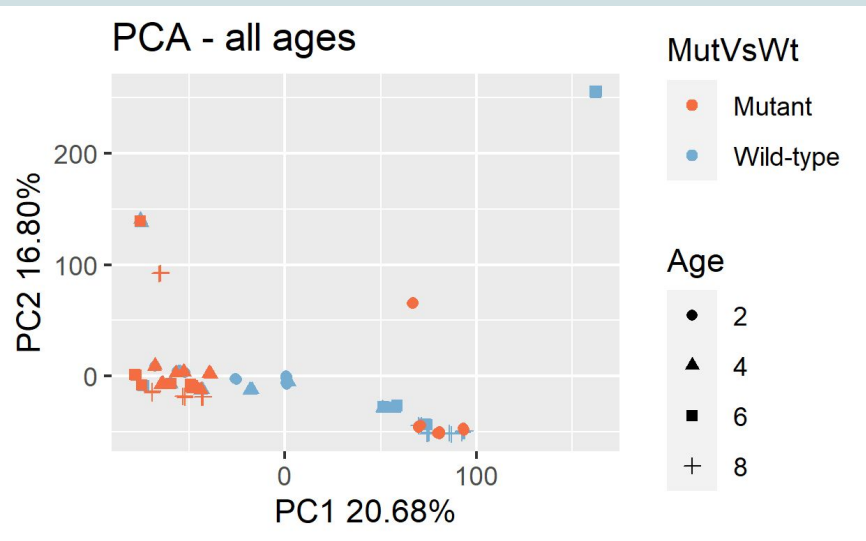
Main Analysis Procedure - Network generation and Enrichment analysis

1. Network generation with RScudo and Cytoscape
2. Enrichment analysis with
 - gprofiler2
 - enrichGO (clusterProfiler)
 - pathfindR

Results

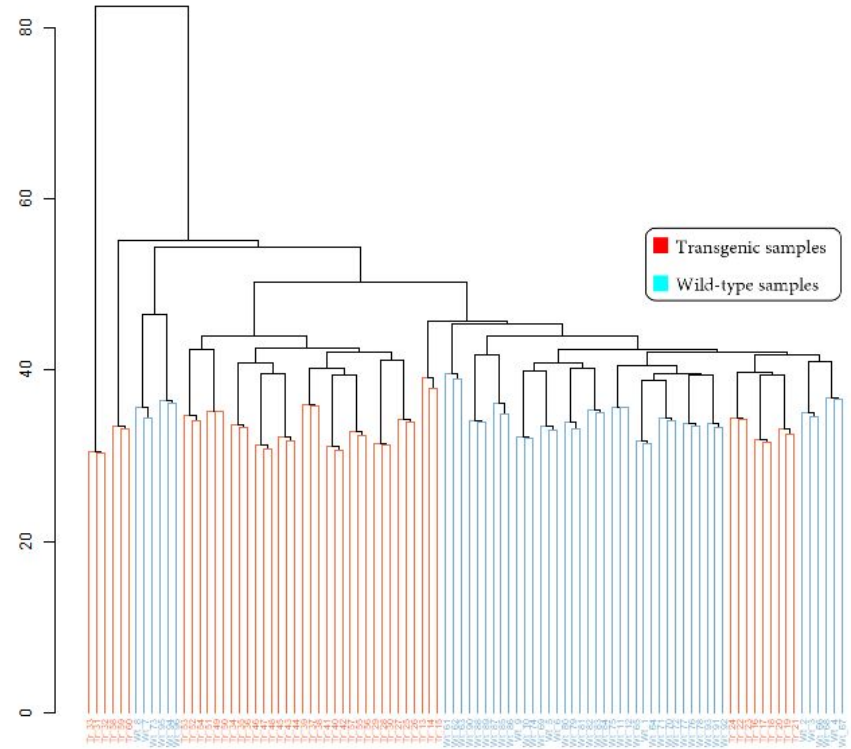


PCA and Kmeans clustering - *all-ages case*



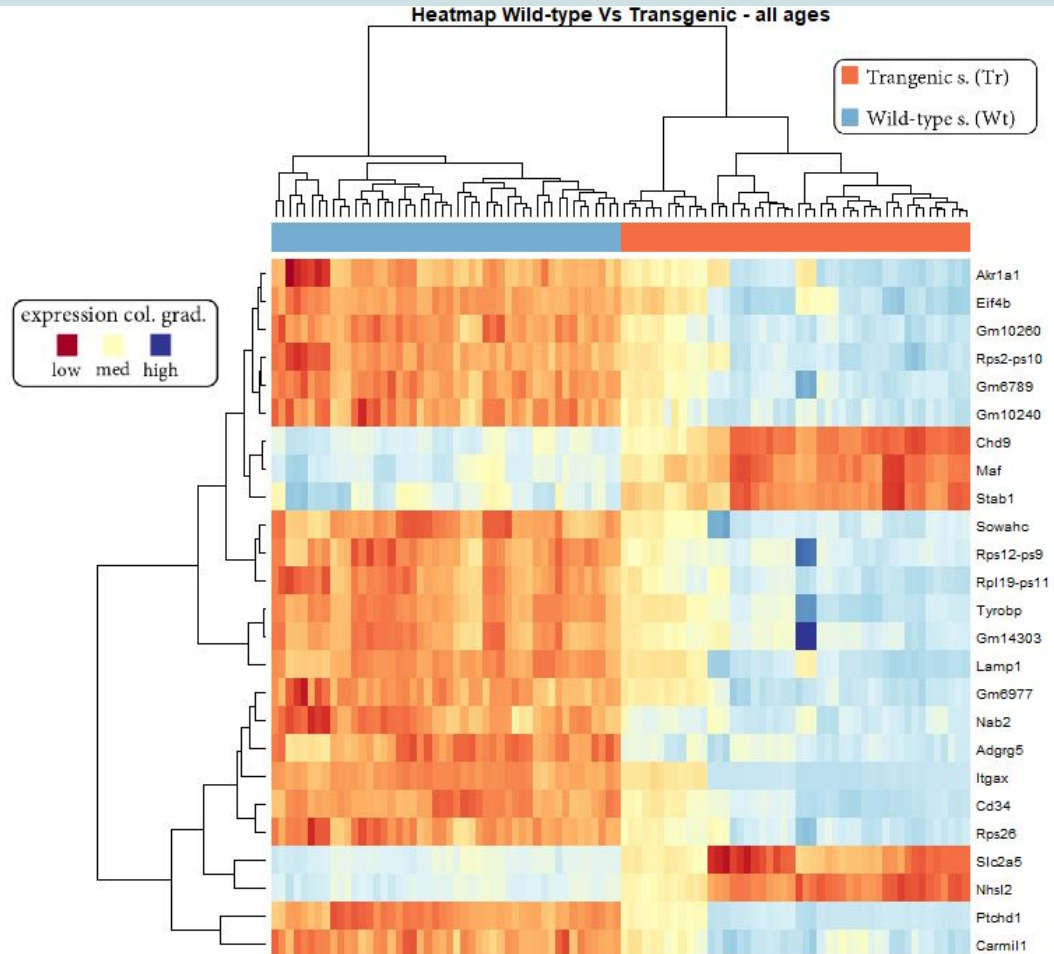
Hierarchical clustering - *all-ages case*

Hierarchical clustering Transgenic vs Wild-type - all ages



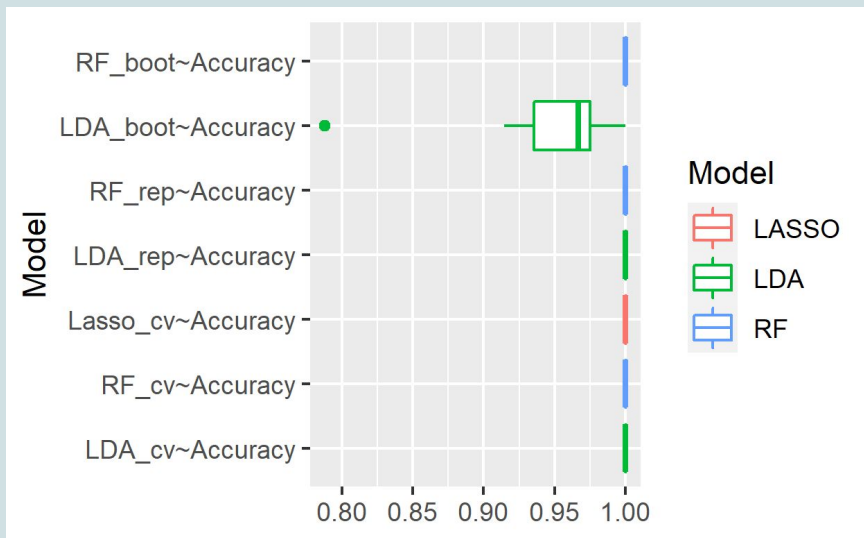
Heatmap - *all-ages case*

Samples were divided into two main partitions, corresponding to the **Wild-type** and the **transgenic** phenotype

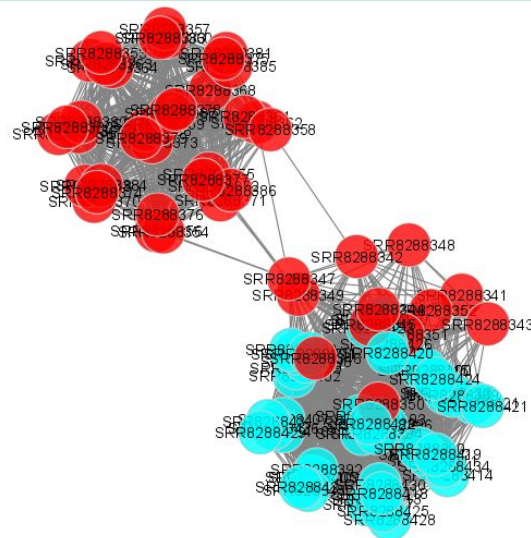


Accuracy supervised models and Network - *all-ages*

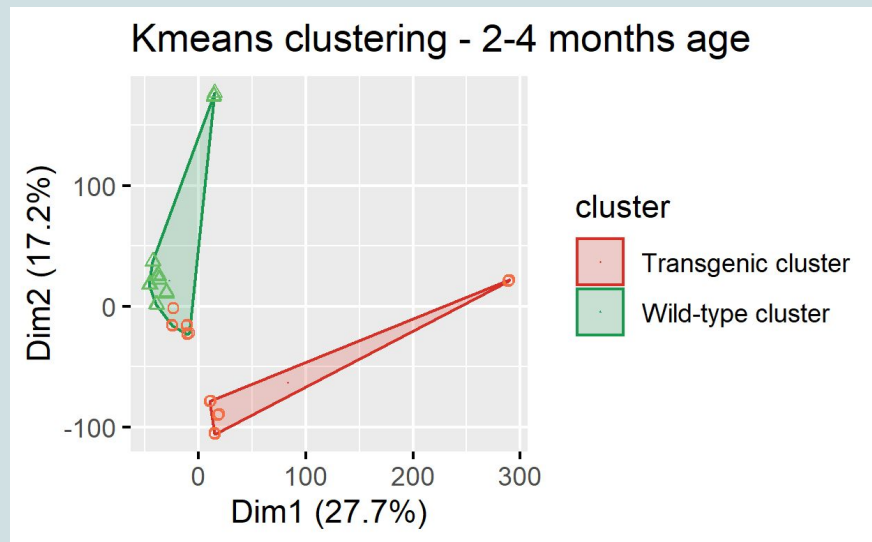
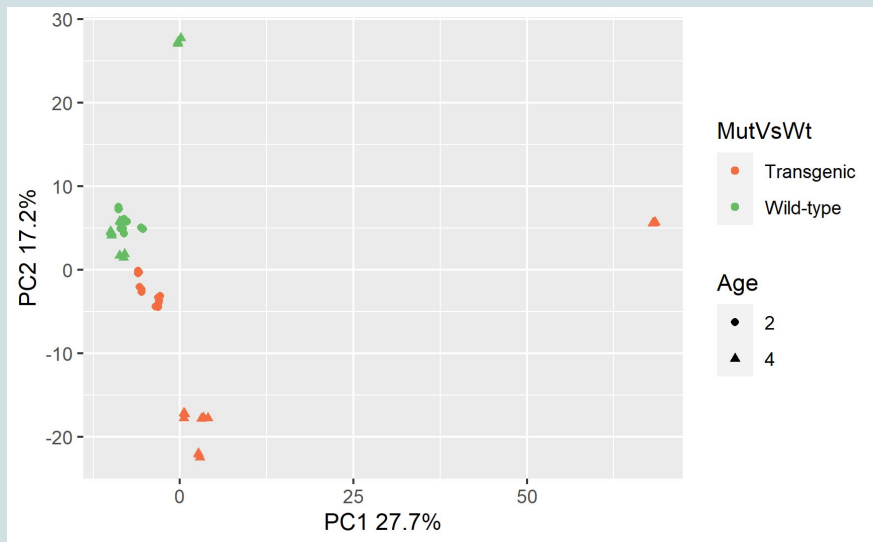
Accuracy distribution of the supervised learning methods



Network → Not separate clusters

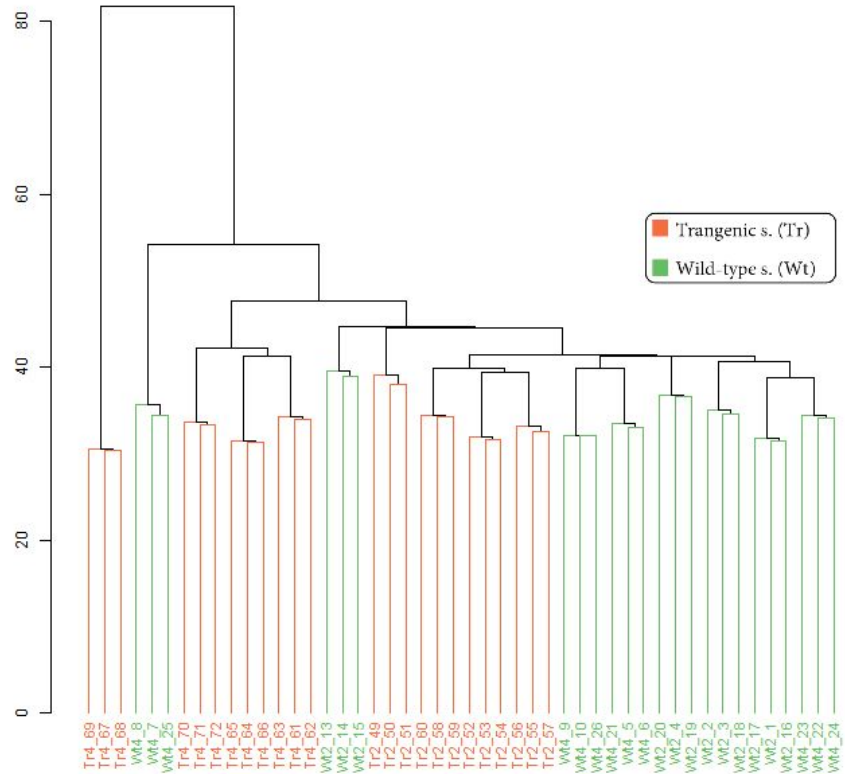


PCA and Kmeans clustering - *young-samples case*



Hierarchical clustering - *young-samples case*

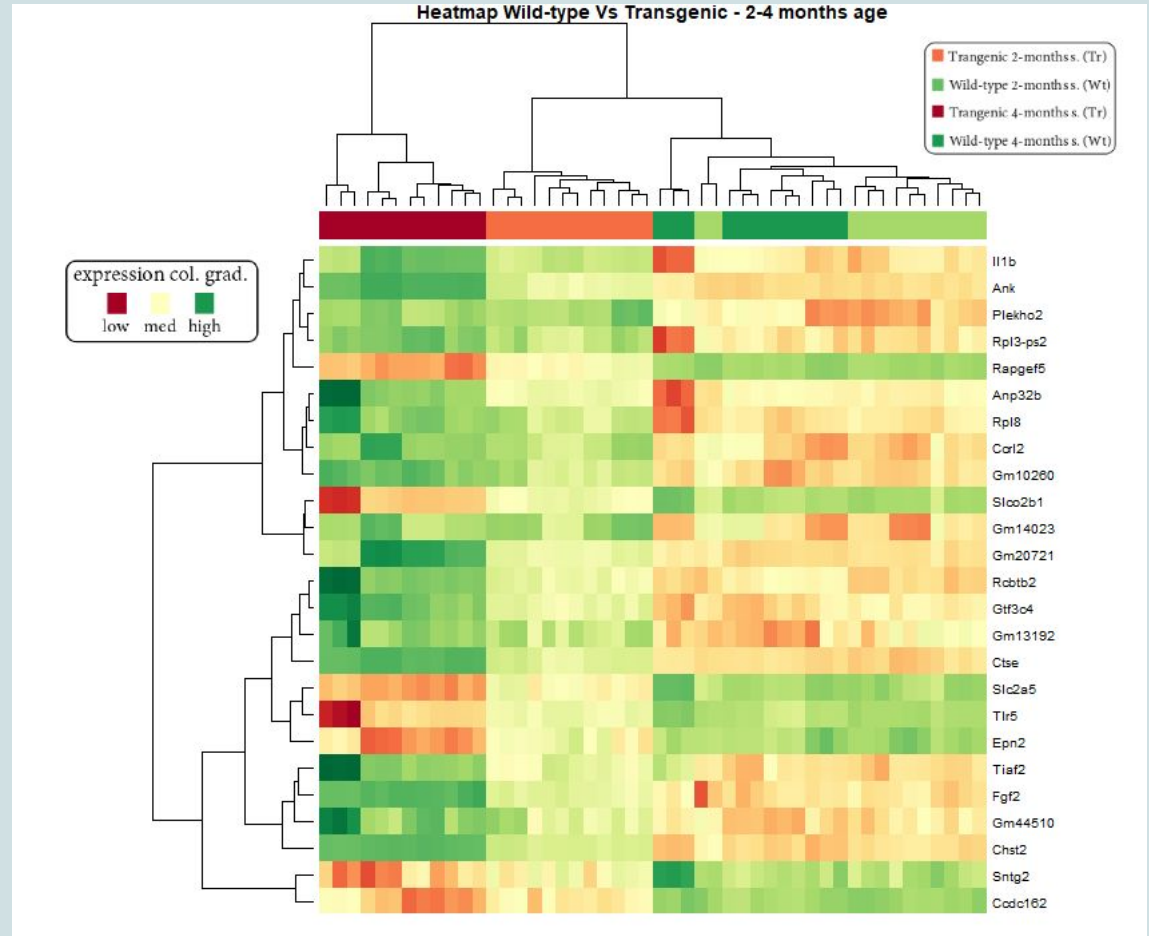
Hierarchical clustering Transgenic vs Wild-type - 2-4 months age



Heatmap - young-samples case

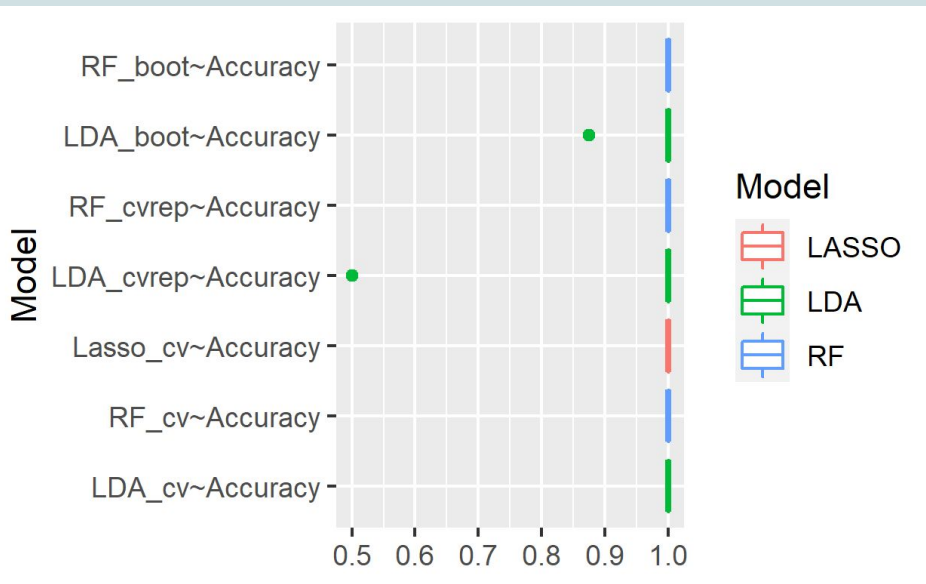
3 main clusters

- **Transgenic** samples 4 months old
- **Transgenic** samples 2 months old
- **Control** samples

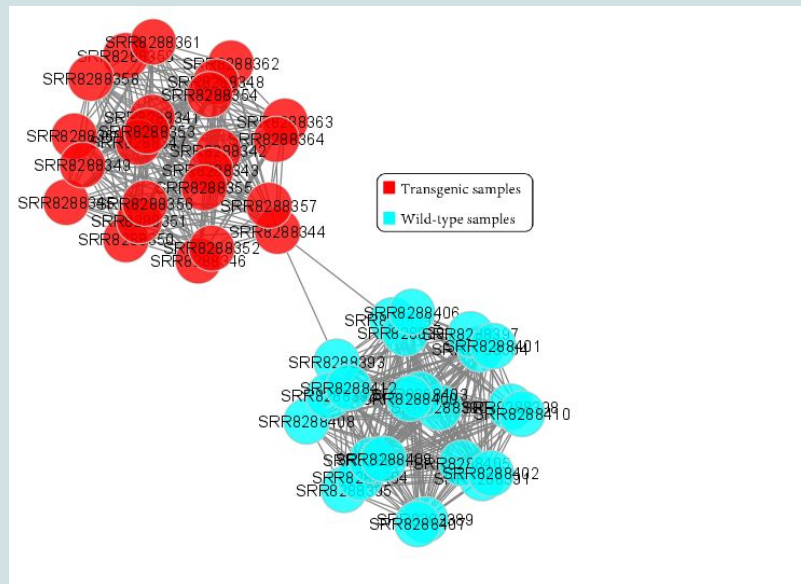


Accuracy supervised models and Network - *young-samples*

Accuracy distribution of the supervised learning methods



Network



Young-samples case

1. **"Ribosome"** ($3.8e-25$),
2. **"Lysosome"** ($1.2e-07$),
3. **"Toll-like receptor signaling pathway"** ($1.5e-6$)
4. **"Cytokine-cytokine receptor interaction"** ($4.7e-6$),
5. **"Chemokine signaling pathway"** ($1.1e-5$),
6. **"TNF signaling pathway"** ($3.0e-04$),
7. **"Oxidative phosphorylation"** ($9.1e-4$),
8. **"Apoptosis"** ($1.3e-03$),
9. **"Parkinson disease"** ($8.1e-3$),
10. **"Prion disease"** ($8.6e-3$).

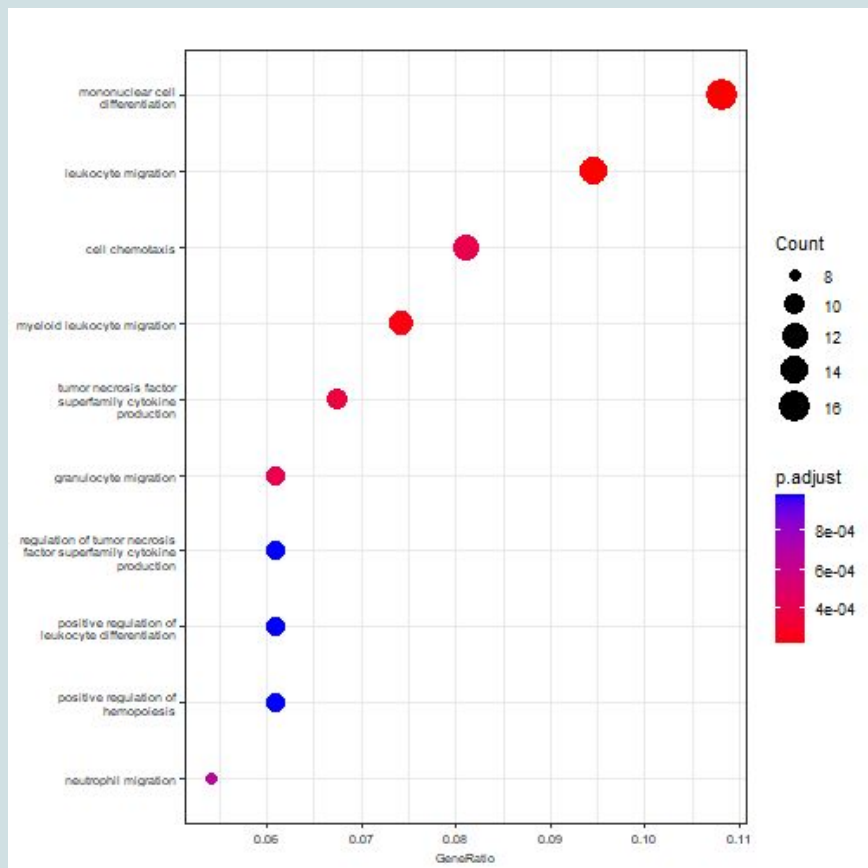


All-ages case

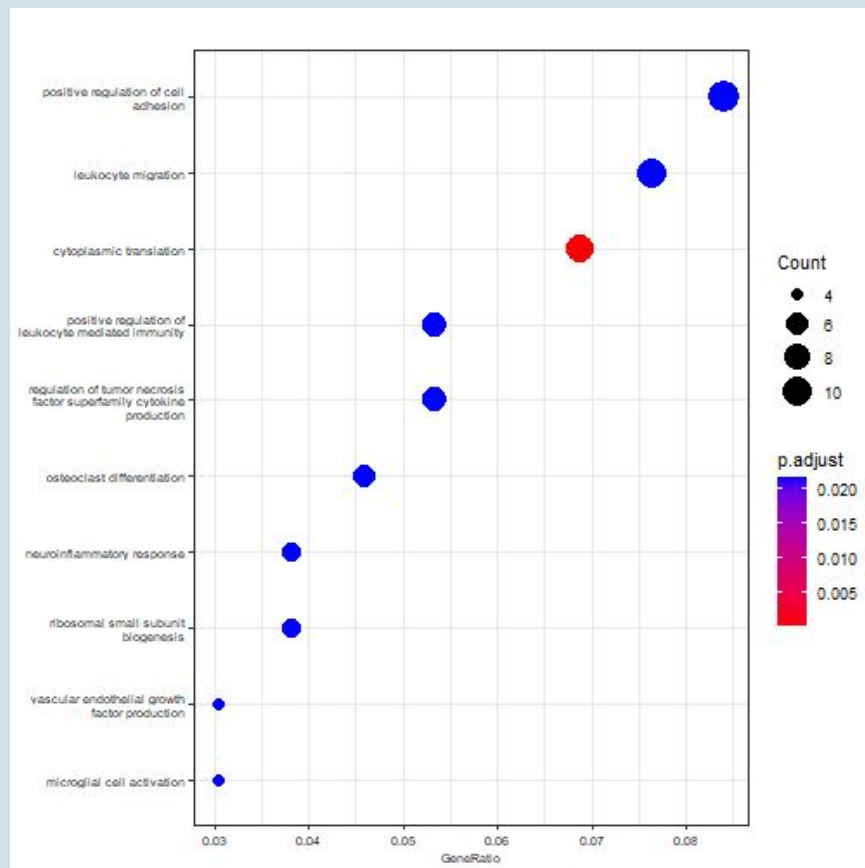
1. **"Ribosome"** ($2.2e-42$),
2. **"Lysosome"** ($1.6e-12$),
3. **"Apoptosis"** ($1.6e-05$),
4. **"Toll-like receptor signaling pathway"** ($4.8e-04$),
5. **"Antigen processing and presentation"** ($5.0e-04$),
6. **"TNF signaling pathway"** ($7.2e-04$),
7. **"Oxidative phosph."** ($9.1e-4$),
8. **"Parkinson disease"** ($8.1e-3$),
9. **"Prion disease"** ($8.6e-3$).

Enrichment results - enrichGO

Young-samples case



All-ages case



Conclusions





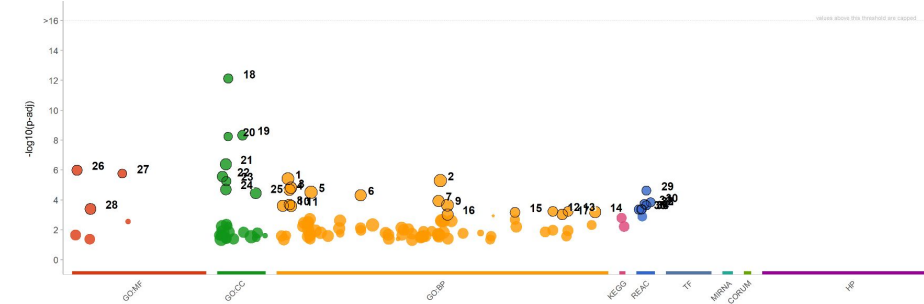
Conclusions

- it was possible to generate **highly accurate models** for performing classifications. All of them gave accuracies ranging around 1, however, LDA generally retrieved lower results.
- Alterations of multiple terms (such as **Lysosomes**, **Ribosomes**, and **Apoptosys**) were observed in both the cases of study
- Genes related to **Parkinson** were found overexpressed
- **Chemokines**, such as *Cxcl10*, were found to be mostly altered between young samples
- **The role of cytokines and chemokines** should be further explored as the genes related were found overexpressed in the analysis involving **young samples**.

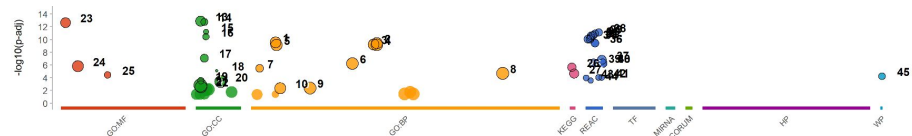


Thank you for your attention!





id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0002376	immune system process	2849	3.9e-06
2	GO:BP	GO:0048731	system development	4533	5.1e-06
3	GO:BP	GO:0002682	regulation of immune system process	1507	1.6e-05
4	GO:BP	GO:0002520	immune system development	1080	2.1e-05
5	GO:BP	GO:0007275	multicellular organism development	5045	3.1e-05
6	GO:BP	GO:0030097	hemopoiesis	970	4.9e-05
7	GO:BP	GO:0048534	hematopoietic or lymphoid organ development	1016	1.2e-04
8	GO:BP	GO:0002521	leukocyte differentiation	605	2.1e-04
9	GO:BP	GO:0051239	regulation of multicellular organismal process	2854	2.3e-04
10	GO:BP	GO:0001775	cell activation	1165	2.4e-04
11	GO:BP	GO:0002684	positive regulation of immune system process	994	2.5e-04
12	GO:BP	GO:1902107	positive regulation of leukocyte differentiation	184	5.8e-04
13	GO:BP	GO:1903708	positive regulation of hemopoiesis	184	5.8e-04
14	GO:BP	GO:2000026	regulation of multicellular organismal development	1494	6.6e-04
15	GO:BP	GO:0097529	myeloid leukocyte migration	221	6.8e-04
16	GO:BP	GO:0051241	negative regulation of multicellular organismal process	1078	9.5e-04
17	GO:BP	GO:1903131	mononuclear cell differentiation	468	9.6e-04
18	GO:CC	GO:0022626	cytosolic ribosome	111	7.5e-13
19	GO:CC	GO:0044391	ribosomal subunit	200	4.7e-09
20	GO:CC	GO:0022625	cytosolic large ribosomal subunit	63	5.7e-09
21	GO:CC	GO:0009986	cell surface	1117	4.1e-07
22	GO:CC	GO:0005840	ribosome	419	2.7e-06
23	GO:CC	GO:0015934	large ribosomal subunit	125	5.5e-06
24	GO:CC	GO:0009897	external side of plasma membrane	594	2.0e-05
25	GO:CC	GO:0098552	side of membrane	792	3.6e-05
26	GO:MF	GO:0003735	structural constituent of ribosome	351	1.0e-06
27	GO:MF	GO:0019843	rRNA binding	70	1.7e-06
28	GO:MF	GO:0005198	structural molecule activity	790	4.1e-04
29	REAC	REAC:R-MMU-975956	Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	90	2.4e-05
30	REAC	REAC:R-MMU-1799339	SRP-dependent cotranslational protein targeting to membrane	88	1.5e-04
31	REAC	REAC:R-MMU-156827	L13a-mediated translational silencing of Ceruloplasmin expression	107	1.9e-04
32	REAC	REAC:R-MMU-975957	Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)	109	2.3e-04
33	REAC	REAC:R-MMU-927602	Nonsense-Mediated Decay (NMD)	109	2.3e-04
34	REAC	REAC:R-MMU-72689	Formation of a pool of free 40S subunits	97	4.2e-04
35	REAC	REAC:R-MMU-72737	Cap-dependent Translation Initiation	115	4.4e-04



id	source	term_id	term_name	term_size	p_value
1	GO:BP	GO:0006412	translation	716	2.7e-10
2	GO:BP	GO:0043604	amide biosynthetic process	857	3.8e-10
3	GO:BP	GO:0043043	peptide biosynthetic process	739	5.6e-10
4	GO:BP	GO:0043603	cellular amide metabolic process	1170	7.1e-10
5	GO:BP	GO:0006518	peptide metabolic process	910	7.2e-10
6	GO:BP	GO:0034645	cellular macromolecule biosynthetic process	1174	6.0e-07
7	GO:BP	GO:0002181	cytoplasmic translation	115	3.5e-06
8	GO:BP	GO:1901566	organonitrogen compound biosynthetic process	1659	2.1e-05
9	GO:BP	GO:0016477	cell migration	1505	4.1e-03
10	GO:BP	GO:0006954	inflammatory response	745	4.5e-03
11	GO:CC	GO:0022626	cytosolic ribosome	111	1.1e-21
12	GO:CC	GO:0044391	ribosomal subunit	200	4.6e-20
13	GO:CC	GO:0005840	ribosome	419	1.3e-13
14	GO:CC	GO:0015935	small ribosomal subunit	80	1.7e-13
15	GO:CC	GO:0022627	cytosolic small ribosomal subunit	46	6.2e-12
16	GO:CC	GO:0022625	cytosolic large ribosomal subunit	63	3.8e-11
17	GO:CC	GO:0015934	large ribosomal subunit	125	8.3e-08
18	GO:CC	GO:0042788	polysomal ribosome	34	7.9e-06
19	GO:CC	GO:0005844	polysome	72	2.7e-04
20	GO:CC	GO:0045202	synapse	1499	4.1e-04
21	GO:CC	GO:0005829	cytosol	4082	1.6e-03
22	GO:CC	GO:0005887	integral component of plasma membrane	1515	2.7e-03
23	GO:MF	GO:0003735	structural constituent of ribosome	351	2.1e-13
24	GO:MF	GO:0005198	structural molecule activity	790	1.6e-06
25	GO:MF	GO:0019843	rRNA binding	70	3.6e-05
26	KEGG	KEGG:03010	Ribosome	164	2.3e-06
27	KEGG	KEGG:05171	Coronavirus disease - COVID-19	233	2.3e-05
28	REAC	REAC:R-MMU-1799339	SRP-dependent cotranslational protein targeting to membrane	88	7.7e-12
29	REAC	REAC:R-MMU-975956	Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	90	1.2e-11
30	REAC	REAC:R-MMU-156827	L13a-mediated translational silencing of Ceruloplasmin expression	107	2.2e-11
31	REAC	REAC:R-MMU-72706	GTP hydrolysis and joining of the 60S ribosomal subunit	108	2.6e-11
32	REAC	REAC:R-MMU-72689	Formation of a pool of free 40S subunits	97	4.6e-11
33	REAC	REAC:R-MMU-72613	Eukaryotic Translation Initiation	115	8.6e-11
34	REAC	REAC:R-MMU-72737	Cap-dependent Translation Initiation	115	8.6e-11
35	REAC	REAC:R-MMU-927602	Nonsense-Mediated Decay (NMD)	109	3.8e-10
36	REAC	REAC:R-MMU-975957	Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)	109	3.8e-10
37	REAC	REAC:R-MMU-72766	Translation	216	1.5e-07
38	REAC	REAC:R-MMU-72312	rRNA processing	166	5.0e-07
39	REAC	REAC:R-MMU-6791226	Major pathway of rRNA processing in the nucleolus and cytosol	166	5.0e-07
40	REAC	REAC:R-MMU-8668773	rRNA processing in the nucleus and cytosol	166	5.0e-07
41	REAC	REAC:R-MMU-72649	Translation initiation complex formation	55	9.3e-05
42	REAC	REAC:R-MMU-72702	Ribosomal scanning and start codon recognition	55	9.3e-05
43	REAC	REAC:R-MMU-72662	Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S	56	1.1e-04
44	REAC	REAC:R-MMU-72695	Formation of the ternary complex, and subsequently, the 43S complex	48	2.8e-04