Shared alterations in the human brain transcriptome during adult aging and in Parkinson's disease

Enrico Glaab and Reinhard Schneider - Contact: enrico.glaab@uni.lu

PARKINSON National Centre of Excellence in Research

Fonds National de la Recherche Luxembourg

1 Introduction

Natural aging of the human brain is considered as one of the main risk factors for Parkinson's disease (PD) and other neurodegenerative disorders. To evaluate previous hypotheses on shared molecular changes and **identify new significant associations between cellular process alterations in sporadic PD and during natural aging of the human brain**, we have investigated brain transcriptomics data from PD patients and unaffected individuals from different adult age groups using a **statistical meta-analysis** and a recently published **pathway and network analysis** approach [1].
Our analyzes provide statistical evidence for specific functional associations between molecular network changes in PD and aging, identify new significant joint pathway deregulations and suggest mechanistic explanations for the age-dependence of PD risk [2].

2 Gene-level analysis

Method: Differential gene expression was scored in transcriptomics data from *post mortem* brain samples derived from **8 published PD case-control microarray datasets** using a meta-analysis statistic [3]. Similarly, differential expression in **brain samples from the Human Brain Transcriptome (HBT) project [4] across 3 age periods (20 to 40 years, 40 to 60 years and 60 years onwards)** was quantified. Finally, genes with shared significant expression alteration in PD vs. controls and across groups of increasing age were determined [2].

Results: We identified 120 significant shared genes (false-discovery rate < 0.05, see Fig. 1). Their **PD- and aging-associated changes were significantly correlated** ($r = 0.43$, $p < 0.001$). Four of the shared genes, *NR4A2*, *CALB1*, *GRIA1* and *MAPT*, have been associated previously with PD and aging in independent studies. The transcription factor ***NR4A2* (*NURR1*)**, for which **polymorphisms and mutations in familial cases of PD** have been reported, stands out as **the most significant altered gene** (Z-score: -7.43, see Fig. 2a and 3) and for a **high negative correlation with adult brain aging** ($r = -0.53$, see Fig. 2b). A downstream analysis for *NR4A2* shows that it reduces the expression of dopamine transporters and mitochondrial genes in PD, suggesting similar effects for brain aging [2].

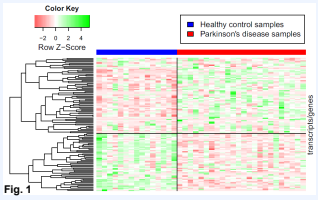


Fig. 1

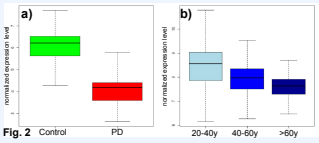
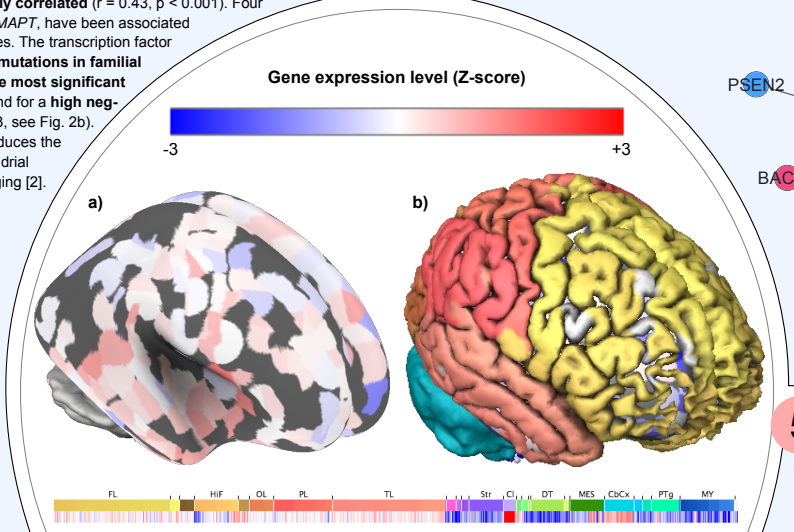


Fig. 2



Visualization of gene expression levels for the brain-specific transcription factor *nuclear receptor subfamily 4, group A, member 2* (***NR4A2***, ***NURR1***) from human post-mortem brain transcriptomics data, using the Brain Explorer Software from the Allen Brain Atlas [6]: **a)** inner brain structures; **b)** outer brain structures. NR4A2 is the gene with the most significant shared alterations in PD and during adult brain aging.

Fig. 3

3 Pathway analysis

Method: To assess shared transcriptome changes in aging and PD at the level of cellular pathways, we mapped significantly differentially expressed genes from the PD meta-analysis and from the aging data set onto a genome-scale human protein-protein interaction network and scored their associations with pathways from public databases mapped onto the same network. The pathway association scores were obtained using our previously developed **graph-based statistic *EnrichNet***, an extension of classical pathway enrichment analysis, exploiting molecular network data as an additional information source [1].

Results: Among the biological processes in the Gene Ontology (GO) database, seven were identified to have statistically significant network association scores with the altered genes in PD and aging (see top entries in Tab. 1), and one process was identified as significant using a classical pathway enrichment analysis (Fisher's exact test, see last entry in Tab. 1). Five **synaptic processes** are included in the significant results, suggesting that synaptic signaling is profoundly affected by both PD- and aging-related gene alterations. Moreover, two of the identified significant GO terms point to associations of the deregulated genes with **endocytosis**, in line with previously observed mutations in familial PD, affecting genes associated with endocytic processes (e.g. LRRK2 and DNAJC13). Similarly, the top-ranked term **dopamine metabolic process** is in agreement with prior observations on the decline of dopamine synthesis during natural aging and PD pathogenesis. Finally, the significant network association of the altered genes with the GO term **phosphatidylinositol metabolic process** matches with an aging-related decline in the activity of this pathway observed in rats, and with the known role of the phosphatidylinositol3-kinase (PI3K)/Akt pathway in controlling the survival of neurons.

GO term	Network association score (XD-score)	Significance of overlap q-value (Fisher's exact test)	Overlap size
Dopamine metabolic process (GO:0042417)	1.61	0.38	4
Synaptic vesicle endocytosis (GO:0048488)	1.47	0.46	3
Positive regulation of synaptic transmission (GO:0050806)	1.47	0.46	3
Synaptic transmission, dopaminergic (GO:0001963)	1.46	0.60	3
Regulation of long-term neuronal synaptic plasticity (GO:0048169)	1.38	0.19	6
Positive regulation of endocytosis (GO:0045807)	1.36	0.33	4
Phosphatidylinositol metabolic process (GO:0046488)	1.27	0.16	6
Synaptic transmission (GO:0007268)	0.29	0.00067	38

Tab. 1

4 Network analysis

Method: Significantly altered genes in PD vs. controls and during adult brain aging were mapped onto a genome-scale protein-protein interaction network together with public pathway annotations. After determining the cellular pathways most strongly associated with the PD- and aging-altered genes as described in the pathway analysis section, **representative sub-networks were extracted from the interactome** by determining, for each pathway, the **smallest connected network component of pathway members and significant genes**. To display these networks of shared gene alterations affecting specific pathways, a force-directed graph layout algorithm [5] was applied to every sub-network scored as significant.

Result: The extracted sub-networks from the interactome show that the **significant PD- and aging-associated genes are densely clustered together** in distinct network regions and **tightly interlinked with the significant cellular processes** identified in the pathway analysis by numerous direct interactions. The generated sub-network visualizations enable a detailed investigation of how the top-significant pathways are associated with genes altered in PD or during brain aging at the level of individual molecular interactions. For example, the central network region for the sub-network extracted for the **dopamine metabolic process** in Fig. 4 reveals that two interaction partners of the process member and familial PD-associated alpha-synuclein protein (***SNCA***), the microtubule-associated protein tau (***MAPT***) and the SNCA-degrading serine protease ***KLK6***, are significantly altered during adult aging.

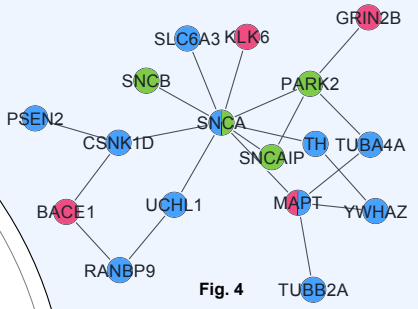


Fig. 4

- significantly altered during brain aging
- significantly altered in PD vs. controls
- involved in dopamine metabolic process

5 Conclusions

Transcriptome changes associated with natural aging of the adult brain and Parkinson's disease are significantly correlated. In particular, the following main observations are made:

- **120 genes are found significantly altered in both PD and aging** with highly correlated changes, including genes with known links to PD and aging.
- For the most significant shared gene, **NR4A2 (NURR1)**, a downstream analysis provides a **mechanistic explanation of how its negative correlation with aging may contribute to an age-related increased risk for PD** via declining dopamine levels and reduced mitochondrial activity.
- **PD and aging share significant cellular pathway alterations**. Apart from observed changes in processes known to be involved in PD and aging, we find previously unreported joint pathway alterations (e.g. affecting synaptic vesicle endocytosis and phosphatidylinositol metabolism).
- Genes undergoing significant changes in PD and during brain aging form **dense clusters in a molecular interaction network**. These clusters are **tightly interconnected with the identified significant shared pathway alterations**. Visualizations of the corresponding sub-networks reveal new insights on how these most affected pathways are interlinked with PD- or aging-related genes at the level of single interactions.

References

[1] Glaab, E., Baudot, A., Krasnogor, N., Schneider, R., Valencia, A. (2012) EnrichNet: network-based gene set enrichment analysis, *Bioinformatics*, 28(18), i451
[2] Glaab, E., Schneider, R. (2015) Comparative pathway and network analysis of brain transcriptome changes during adult aging and in Parkinson's disease, *Neurobiology of Disease*, 74, 1
[3] Marot, G. et al. (2009) Moderated effect size and P-value combinations for microarray meta-analyses, *Bioinformatics*, 25(20), 2692
[4] Kang, H. J. et a. (2011) Spatio-temporal transcriptome of the human brain, *Nature*, 478(7370), 483
[5] Fruchterman, T. M. J., Reingold, E. M. (1991) Graph drawing by force-directed placement, *Software: Practice and Experience*, 21(11), 1129
[6] Jones, A. R. et al., (2009) The Allen brain atlas: 5 years and beyond, *Nature Reviews Neuroscience*, 10(11), 821

This work was supported by the Luxembourg National Research Fund through grants C13/BM/5782168 and NCER-PD (I1R-BIC-PFN-15NCER)