

Combined analysis of neuroimaging and metabolomics data for Parkinson's disease

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Overview of analyses

- 1) Cohort overview
- 2) Neuroimaging data analysis (FDG and F-DOPA PET)
- 3) Metabolomics analysis (cross-sectional & longitudinal)
- 4) Machine learning analyses:
 - a) FDOPA PET
 - b) FDG PET
 - c) FDOPA PET + metabolomics
 - d) FDG PET + metabolomics
 - e) ROC curves

Cohort overview

- **60 PD patients** and **15 healthy age- and gender-matched controls** (University Hospitals Cologne, Giessen and Marburg; Prof. C. Eggers)
- **Medication:** PD patients had been 12 hours off levodopa and 72 hours off dopamine agonists

	PD patients	Controls	P-value
N (female/male)	60 (19/41)	15 (8/7)	.14
Age	65.7 ± 9.0	65.1 ± 8.4	.831
UPDRS III	25.1 ± 9.7	2.1 ± 2.6	.000
H&Y stage	2.3 ± 0.4	-	-
BMI	26.8 ± 4.7	24.6 ± 4.1	.101

Analyses overview

- **Metabolomics**

- Gas chromatography coupled to mass spectrometry (GC-MS)
- Determination of metabolomic profiles for blood plasma samples:
 - Baseline: entire cohort (60 patients and 15 controls)
 - Follow-up exam after 1 year: 18 patients

- **Neuroimaging**

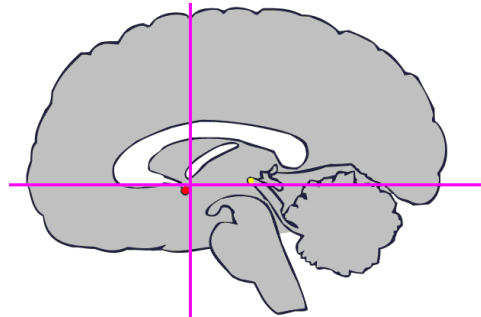
Positron emission tomography (PET):

- 3,4-dihydroxy-6-¹⁸F-fluoro-L-phenyl-alanine (**FDOPA**)
44 patients and 14 controls
→ How does dopamine metabolism change?
- 2-[fluorine-18]fluoro-2-deoxy-D-glucose (**FDG**)
51 patients and 15 controls
→ How does glucose metabolism change?

PET imaging data pre-processing

- All pre-processing steps performed in SPM12 (Matlab)
- Co-registration of each subject's averaged FDG and FDOPA images

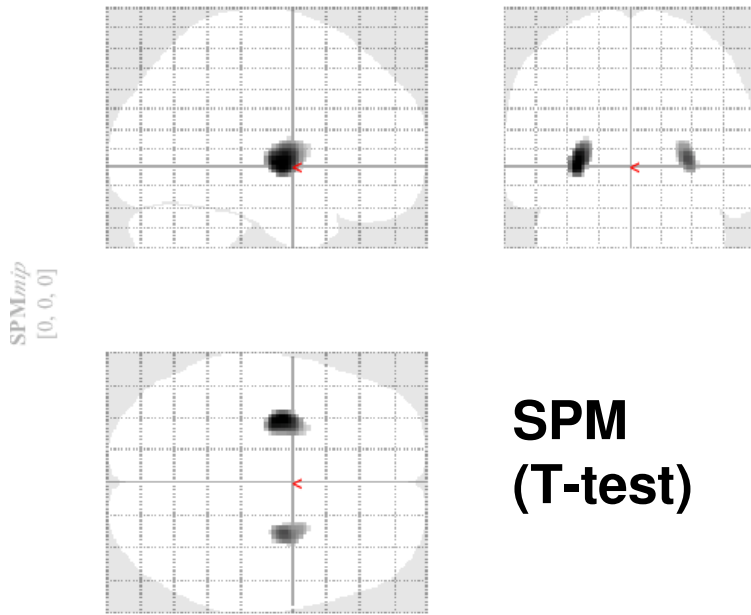
- Centering on the anterior commissure and horizontal alignment



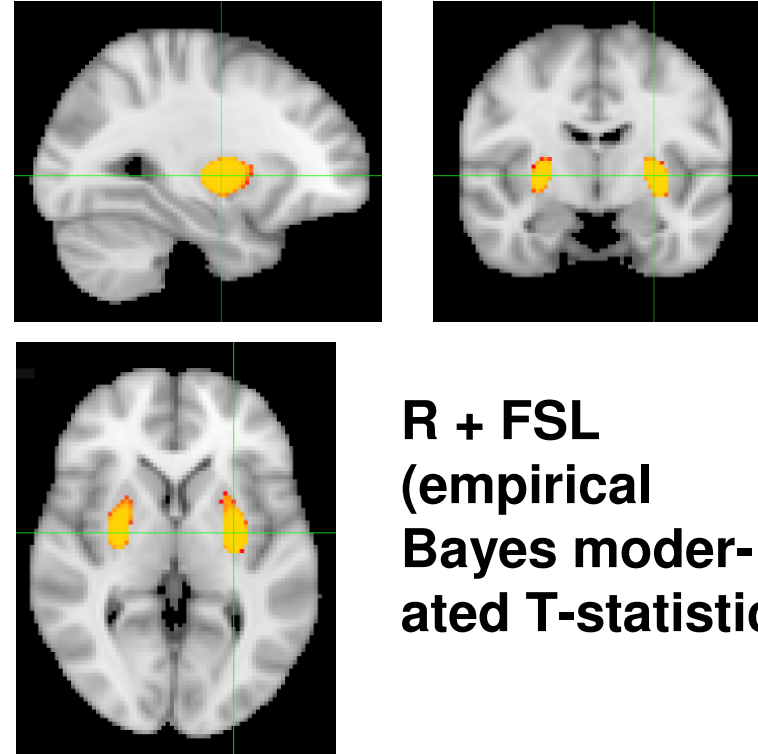
- Spatial normalization to Montreal Neurological Institute standard space (MNI152) performed using tracer-specific templates
- Spatial smoothing (Gaussian kernel, 5 mm FWHM)

FDOPA PET analyses after global mean normalization

FDOPA PET: Significant changes in putamen/striatum (FDR < 0.05)



SPMresults: /PD_imaging/FDopa_controls
Height threshold T = 5.112656 {p<0.05 (FWE)}
Extent threshold k = 0 voxels

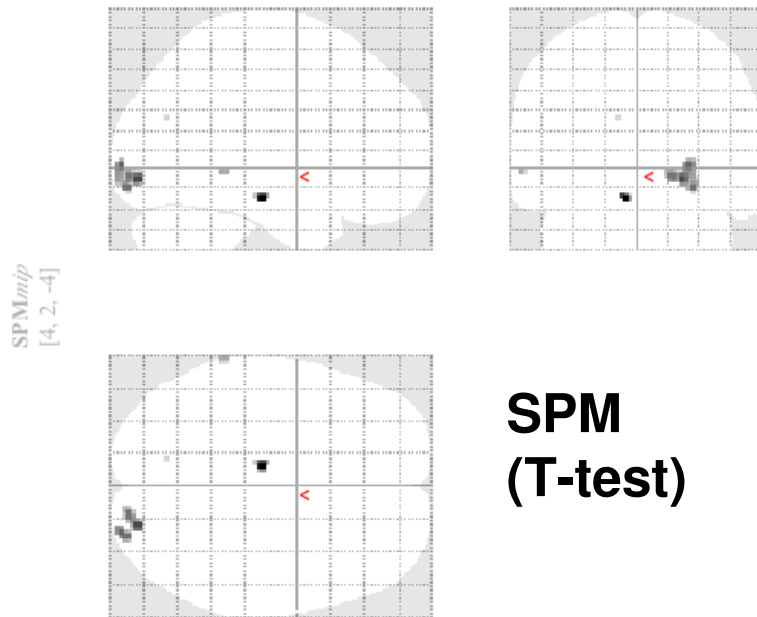


Best FDR < 1E-3
(only 3 digits behind comma reported)

Best FDR < 1.18E-5

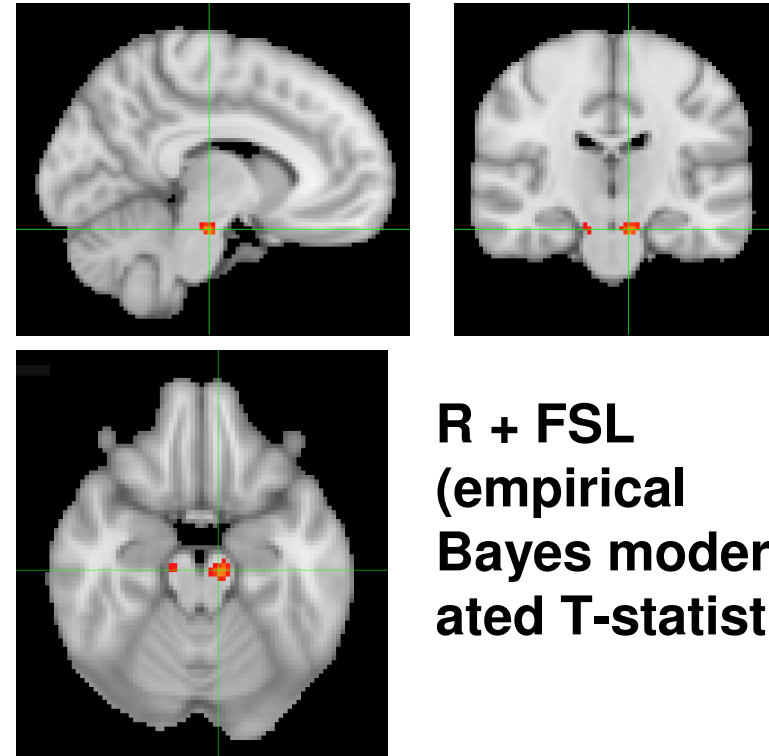
FDG PET analyses after global mean normalization

FDG PET: Significant changes in lower midbrain (FDR < 0.05)



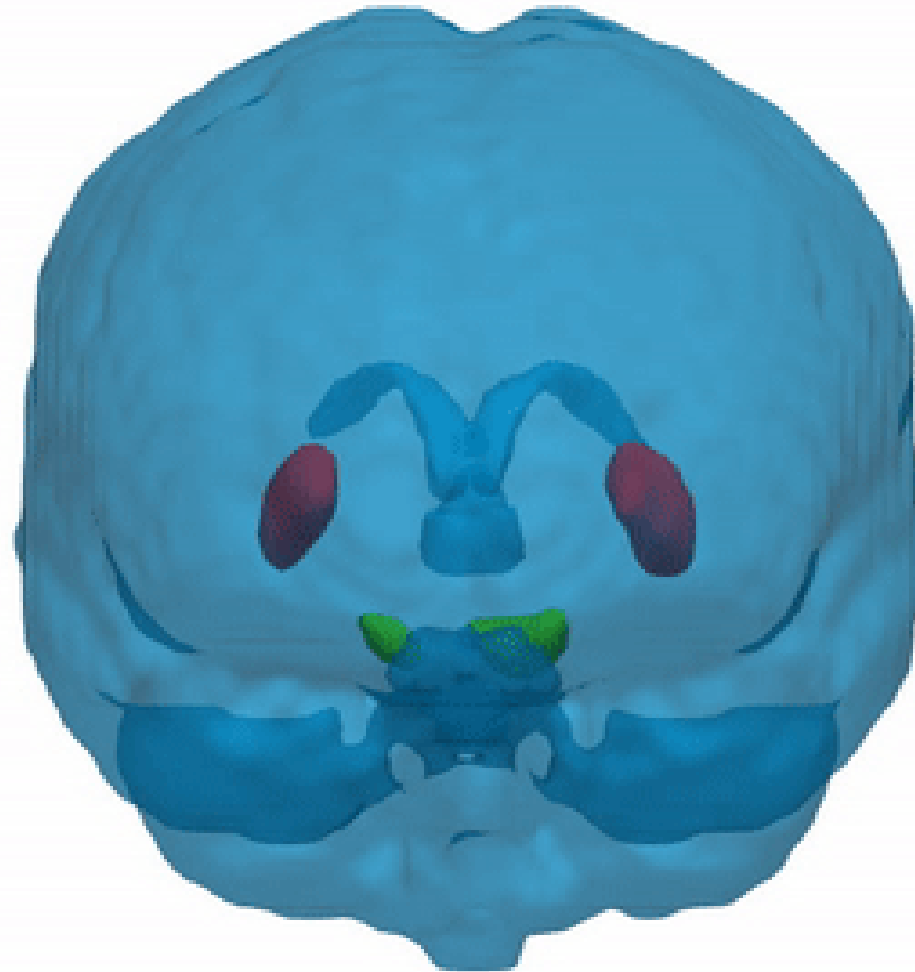
SPMresults: ./FDG_controls/warped
Height threshold T = 5.312996 {p<0.05 (FWE)}
Extent threshold k = 0 voxels

**Best FDR = 0.026
(FWE < 1E-3)**



Best FDR = 0.009

FDOPA PET – Visualization of significant clusters

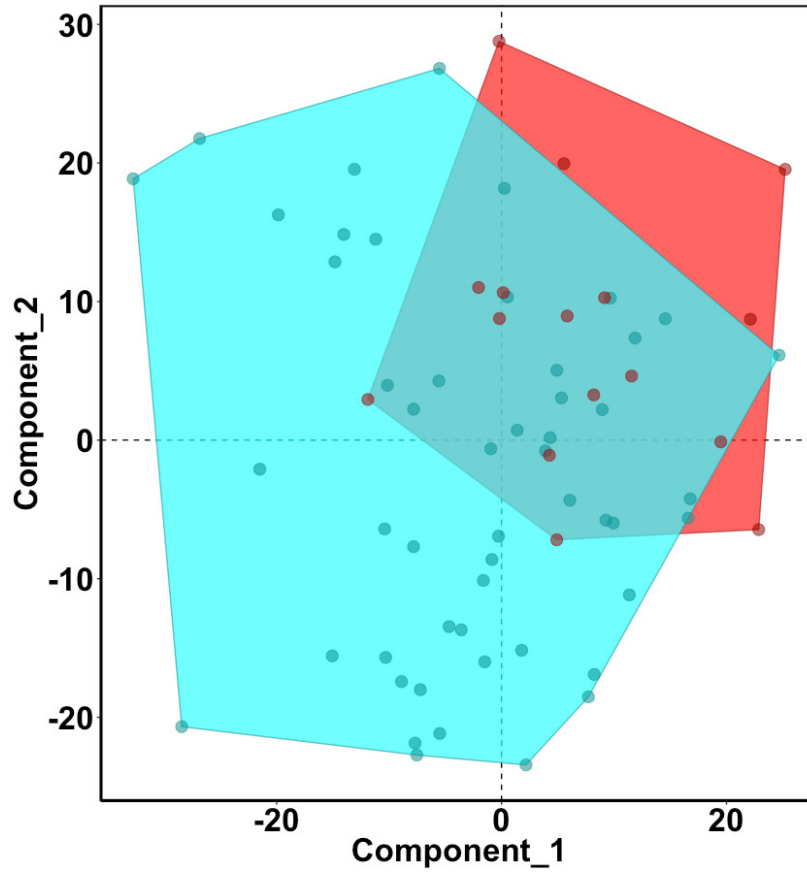


Most significant voxel clusters (eBayes, FDR < 0.05):

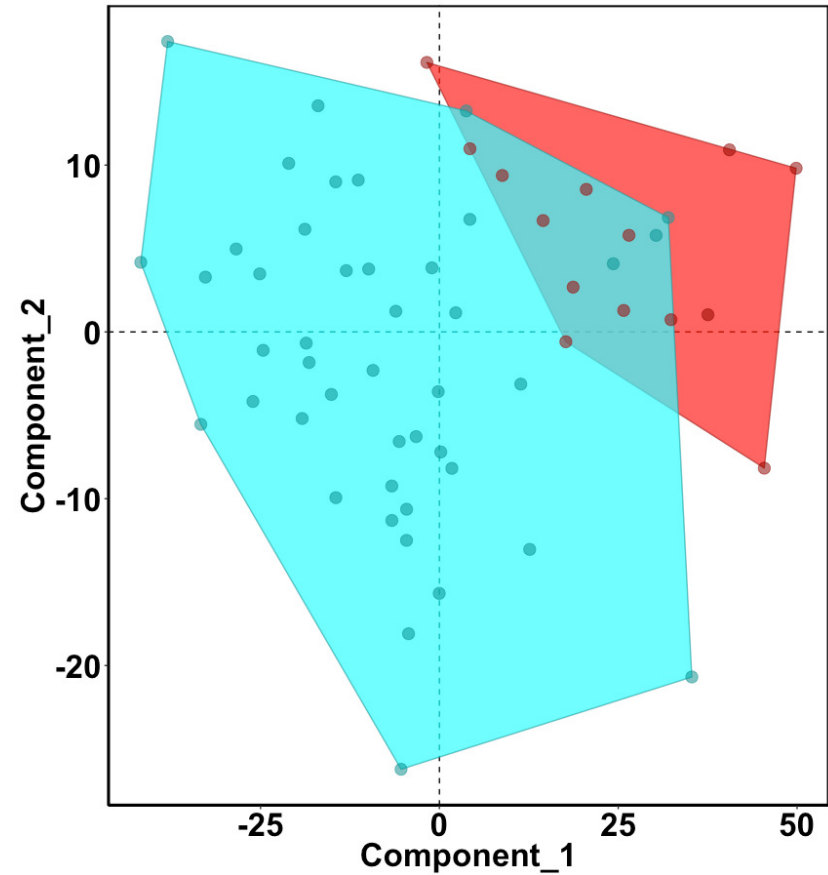
- FDOPA
- FDG

Partial Least Squares Discriminant Analysis

FDG PET



FDOPA PET

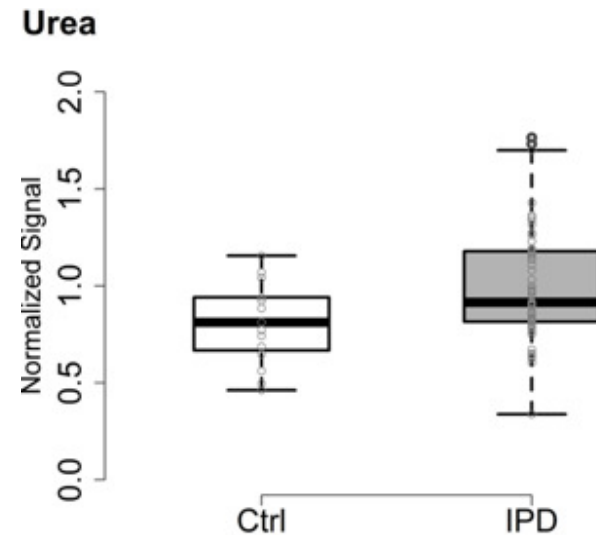


control PD

Metabolomics analyses (Baseline)

- 1 unknown metabolite (RI 1446) with higher abundance in PD (FDR < 0.05)
- Urea = top-ranked known metabolite → marker of oxidative stress (but FDR > 0.05, see box plot)

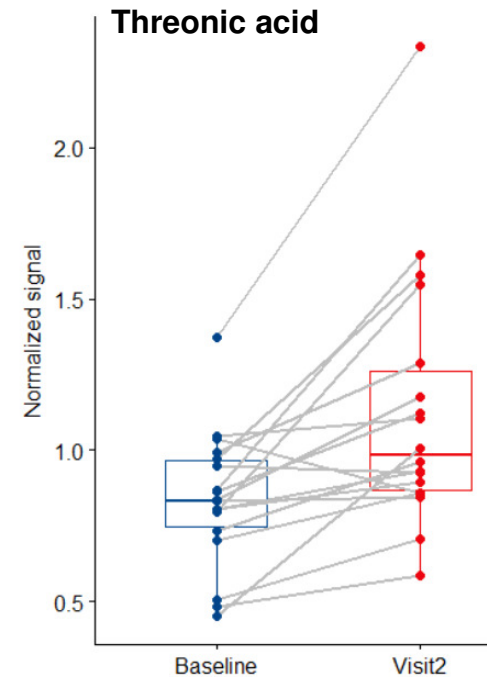
Metabolite	Fold-change	P-value	FDR
RI 1446 (unknown)	1.270	0.001	0.039
Urea	1.262	0.005	0.140
RI 1050 (unknown)	1.324	0.006	0.140
Hexadecanoic acid	1.256	0.030	0.371
Dodecanoic acid	1.403	0.033	0.371



Metabolomics analyses (Longitudinal: Visit 2 vs. Visit 1)

- Threonic and glycolic acid are top-ranked, but FDR > 0.05
- Most top-ranked metabolites tend to have higher abundance in PD

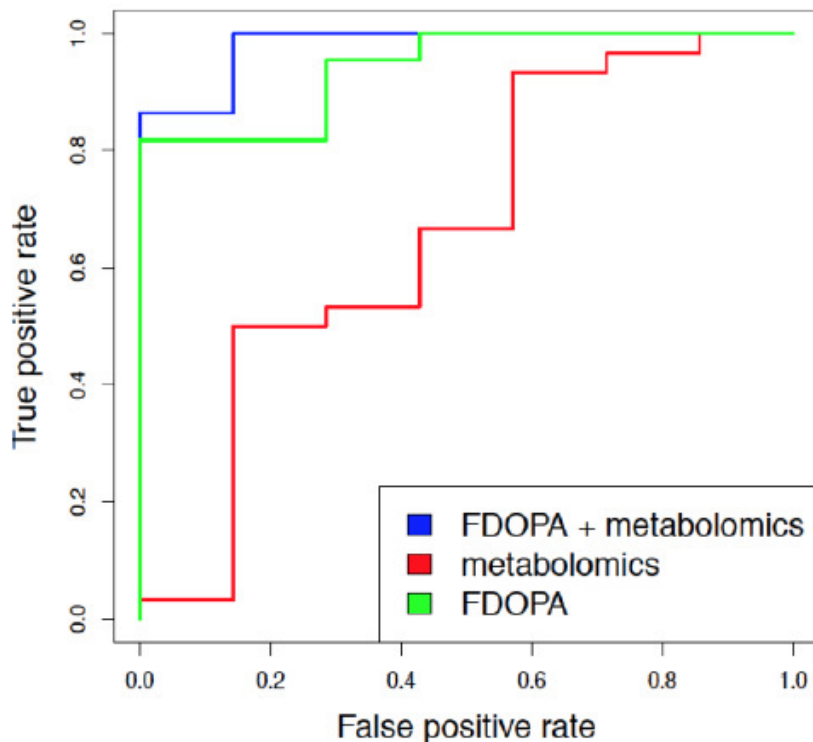
Metabolite	FC	P	FDR
Threonic acid	1.353	0.001	0.059
Glycolic acid	1.258	0.002	0.059
Iminodiacetic acid	1.154	0.007	0.140
Glycerol	0.642	0.008	0.140
Succinic acid	1.161	0.029	0.317
Mannose	1.148	0.030	0.317
Glyceric acid	1.229	0.031	0.317
Citric acid	1.144	0.046	0.375
RI 1708	0.876	0.048	0.375



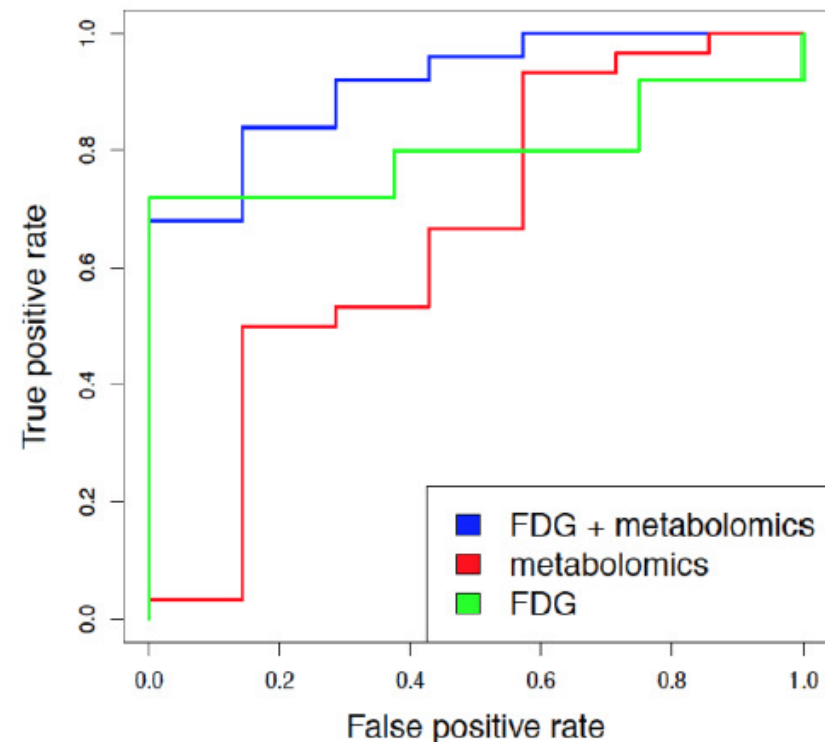
Machine Learning (SVM) – ROC Curve Analyses

Combine attributes from **FDOPA** and **FDG** PET data with **metabolomics data** for the same samples to create integrated machine learning models

ROC curves (FDOPA)



ROC curves (FDG)



Summary

- **PET analyses:** Significant changes in both FDOPA (putamen/striatum) and FDG (midbrain) analysis
- **Metabolomics analyses:** Few significant changes; top-ranked metabolites tend to have associations with oxidative stress and mitochondrial dysfunction
- **Integrated machine learning:** Combination of standardized PET + metabolomics features tends to provide higher predictive performance than PET or metabolomics only

References

1. E. Glaab, *Using prior knowledge from cellular pathways and molecular networks for diagnostic specimen classification*, Briefings in Bioinformatics (2015), 17(3), pp. 440
2. E. Glaab, R. Schneider, *Comparative pathway and network analysis of brain transcriptome changes during adult aging and in Parkinson's disease*, Neurobiology of Disease (2015), 74, 1-13
3. N. Vlassis, E. Glaab, *GenePEN: analysis of network activity alterations in complex diseases via the pairwise elastic net*, Statistical Applications in Genetics and Molecular Biology (2015), 14(2), 221
4. S. Köglberger, M. L. Cordero-Maldonado, P. Antony, J. I. Forster, P. Garcia, M. Buttini, A. Crawford, E. Glaab, *Gender-specific expression of ubiquitin-specific peptidase 9 modulates tau expression and phosphorylation: possible implications for tauopathies*, Molecular Neurobiology (2016), in press (doi: 10.1007/s12035-016-0299-z)
5. L. Grandbarbe, S. Gabel, E. Koncina, G. Dorban, T. Heurtaux, C. Birck, E. Glaab, A. Michelucci, P. Heuschling, *Inflammation promotes a conversion of astrocytes into neural progenitor cells via NF- κ B activation*, Molecular Neurobiology (2016), Vol. 53, No. 8, 5041-5055
6. S. Kleiderman, J. Sá, A. Teixeira, C. Brito, S. Gutbier, L. Evje, M. Hadera, E. Glaab, M. Henry, S. Agapios, P. Alves, U. Sonnewald, M. Leist, *Functional and phenotypic differences of pure populations of stem cell-derived astrocytes and neuronal precursor cells*, Glia (2016), Vol. 64, No. 5, 695-715
7. E. Glaab, R. Schneider, *RepExplore: Addressing technical replicate variance in proteomics and metabolomics data analysis*, Bioinformatics (2015), 31(13), pp. 2235
8. E. Glaab, *Building a virtual ligand screening pipeline using free software: a survey*, Briefings in Bioinformatics (2015), 17(2), pp. 352
9. E. Glaab, A. Baudot, N. Krasnogor, R. Schneider, A. Valencia. *EnrichNet: network-based gene set enrichment analysis*, Bioinformatics, 28(18):i451-i457, 2012
10. E. Glaab, R. Schneider, *PathVar: analysis of gene and protein expression variance in cellular pathways using microarray data*, Bioinformatics, 28(3):446-447, 2012
11. E. Glaab, J. Bacardit, J. M. Garibaldi, N. Krasnogor, *Using rule-based machine learning for candidate disease gene prioritization and sample classification of cancer gene expression data*, PLoS ONE, 7(7):e39932, 2012
12. E. Glaab, A. Baudot, N. Krasnogor, A. Valencia. *TopoGSA: network topological gene set analysis*, Bioinformatics, 26(9):1271-1272, 2010
13. E. Glaab, A. Baudot, N. Krasnogor, A. Valencia. *Extending pathways and processes using molecular interaction networks to analyse cancer genome data*, BMC Bioinformatics, 11(1):597, 2010
14. E. Glaab, J. M. Garibaldi and N. Krasnogor. *ArrayMining: a modular web-application for microarray analysis combining ensemble and consensus methods with cross-study normalization*, BMC Bioinformatics, 10:358, 2009
15. E. Glaab, J. M. Garibaldi, N. Krasnogor. *Learning pathway-based decision rules to classify microarray cancer samples*, German Conference on Bioinformatics 2010, Lecture Notes in Informatics (LNI), 173, 123-134
16. E. Glaab, J. M. Garibaldi and N. Krasnogor. *VRMLGen: An R-package for 3D Data Visualization on the Web*, Journal of Statistical Software, 36(8), 1-18, 2010
17. C. Jaeger, E. Glaab, A. Michelucci, T. M. Binz, S. Koeglsberger, P. Garcia, J. P. Trezzi, J. Ghelfi, R. Balling, M. Buttini, *The Mouse Brain Metabolome: Region-Specific Signatures and Response to Excitotoxic Neuronal Injury*, American Journal of Pathology (2015), Vol. 185, No. 6, pp. 1699