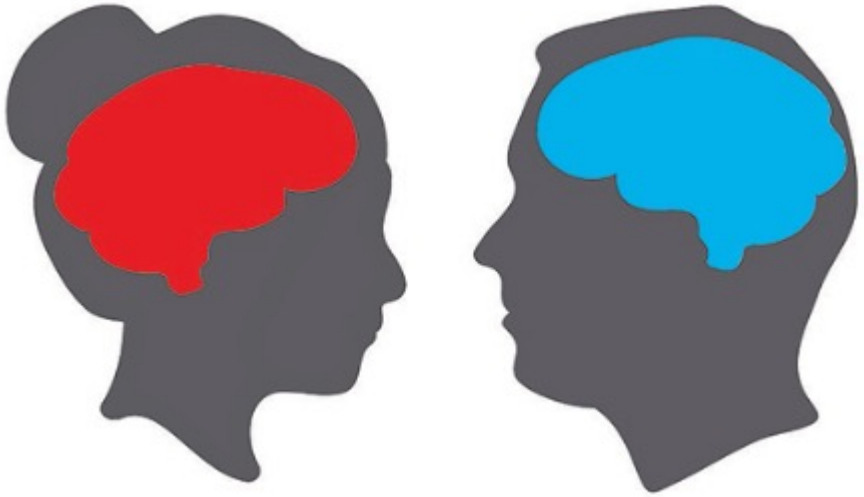
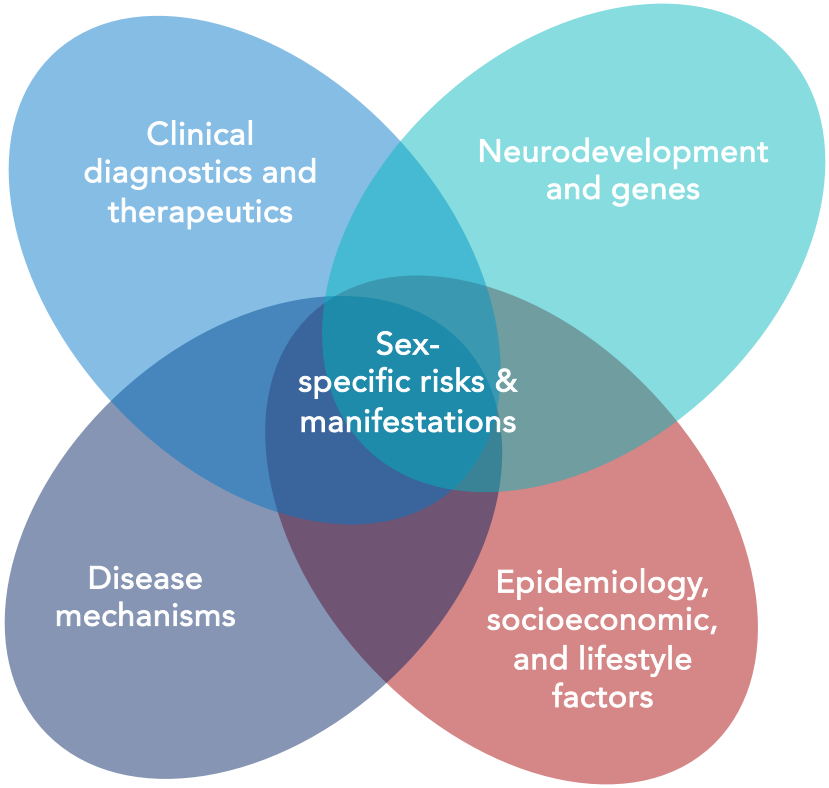


Sex differences in omics data for neurodegenerative diseases

Enrico Glaab, Luxembourg Centre for Systems Biomedicine

Why study sex differences in complex diseases?

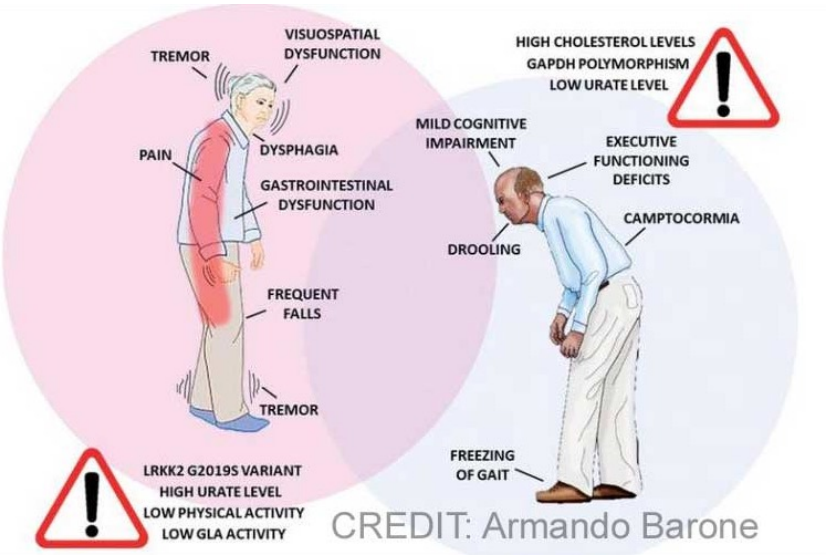


→ Sex differences can affect disease risk, manifestations, progression & therapy

Sex differences in Alzheimer‘s disease (AD)

- Higher incidence rate among females in the older age groups, also when adjusting for differential survival (Gao et al., 1998)
- Females have more global AD pathology (Barnes et al., 2005), while males suffer a more aggressive disease progression and earlier mortality (Dubal et al., 2020)
- Molecular mechanisms and mediators of these sex differences are unknown

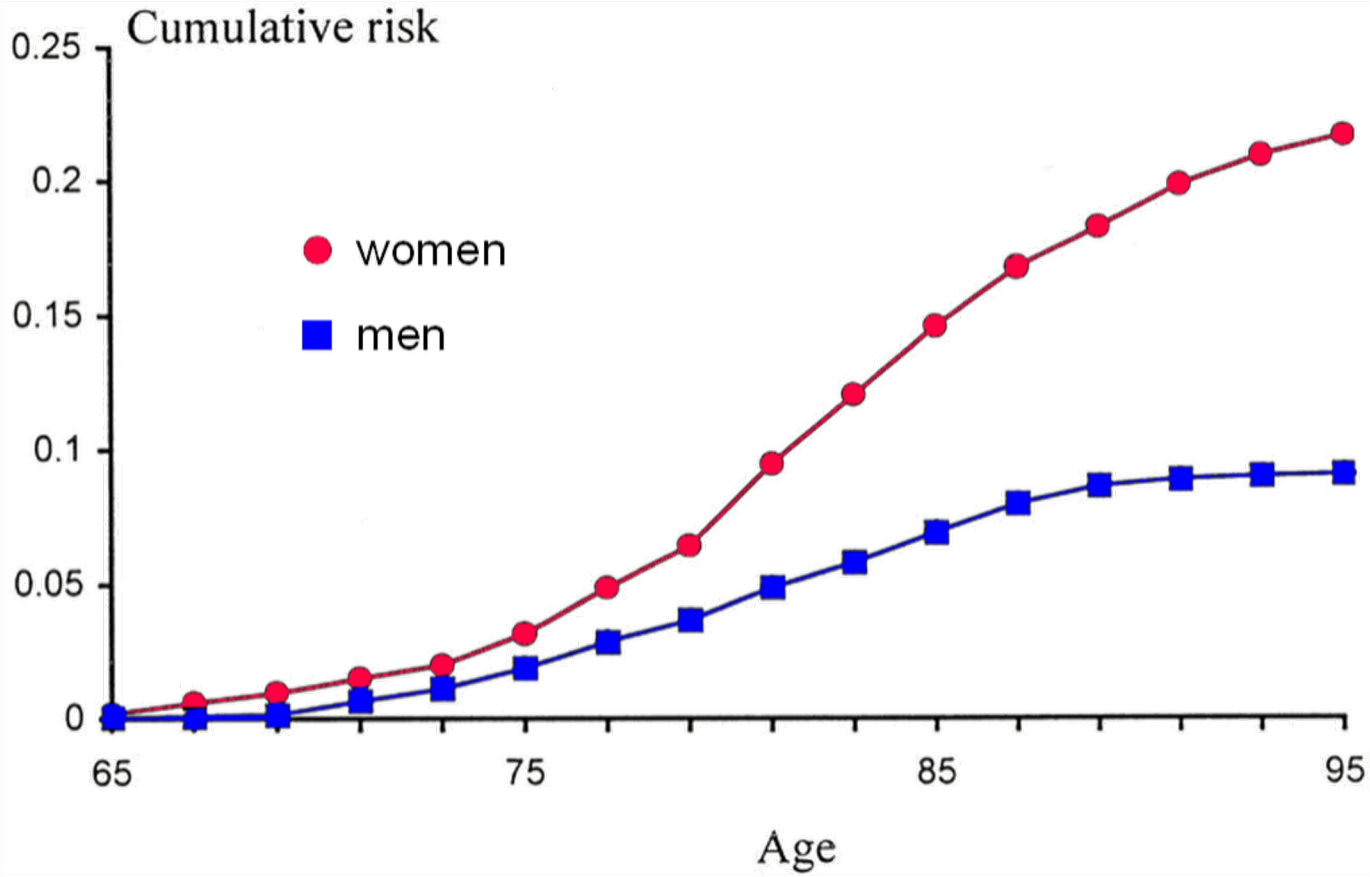
Sex differences in Parkinson‘s disease (PD)



- Higher incidence rate among males for idiopathic PD in most studies (Mayeux et al., 1995; Baldereschi et al., 2000, Wooten et al., 2004)
- Women present more often with tremor, associated with milder motor deterioration and striatal degeneration (Haaxma et al., 2007)
- Molecular mechanisms and mediators of these sex differences are unknown

Age-dependence of sex differences in AD

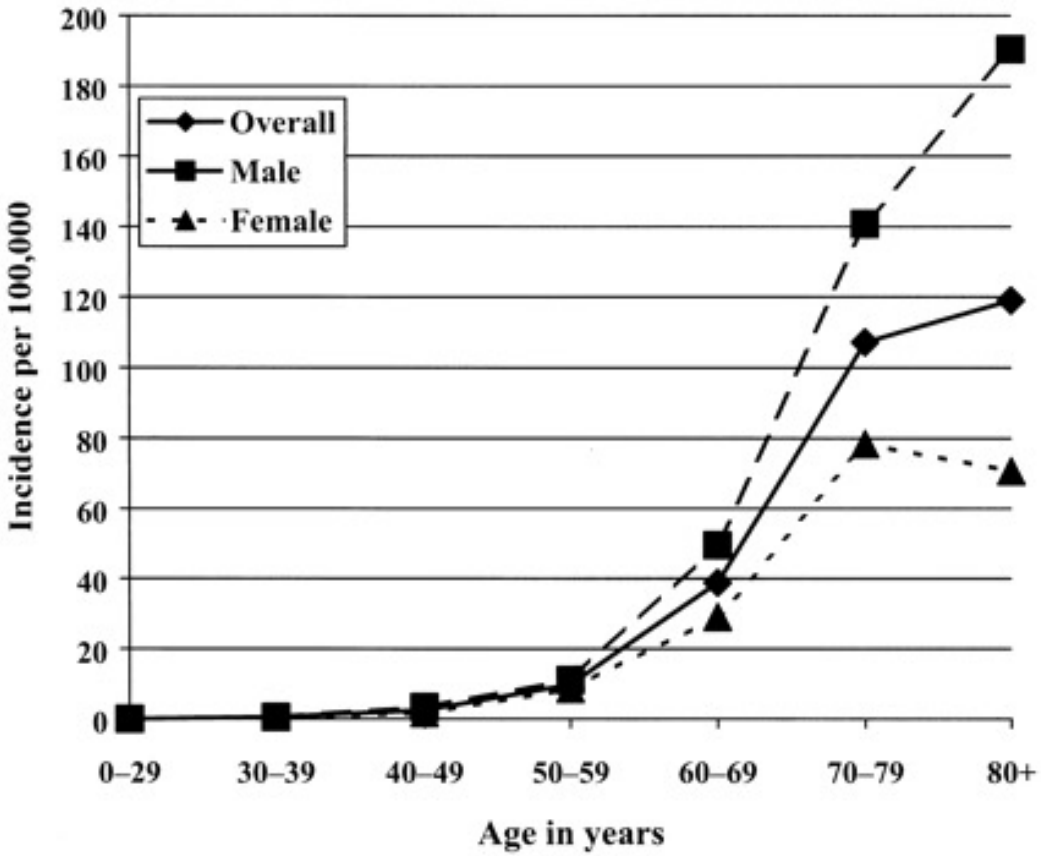
Sex-specific cumulative risk for a 65-year-old to develop AD by 95 years of age



Adapted from Andersen K et al. Neurology 1999;53:1992-1992

Age-dependence of sex differences in PD

Incidence of Parkinson's disease by age and gender



Source: Kaiser Permanente, 1994–1995

Previous hypotheses

Potential generic causes for sex differences in AD/PD:

- Life-style / occupation related causes (e.g., exposure to toxicants, head trauma)
- Differences in hormone levels (e.g., hormones with neuroprotective functions)
- Genetic differences (sex chromosomes and genetic variations)
- Differences in mitochondrial (dys)function (proteins vs. lipids as fuel sources; Demarest & McCarthy, 2015)
- Differences in neuroinflammatory processes and microglia signaling (more active microglia in some brain regions in males; Lenz & McCarthy, 2015)

Limitations of previous hypotheses

Sex disparities are not generic:

- Observed clinical sex differences are disease-specific (e.g., PD vs. AD)
- Sex disparities differ in sporadic vs. familial disease forms (e.g., increased female prevalence in LRRK2-PD; Cilia et al., 2014, Alcalay et al., 2013)
- Sex differences show different patterns within the same disease across different clinical, neuropathological and molecular readouts

→ Investigate contributing roles of disease / subtype / symptom-specific molecular factors

Method: Testing sex differences in omics data

- Directly test for statistical interactions between sex and disease status

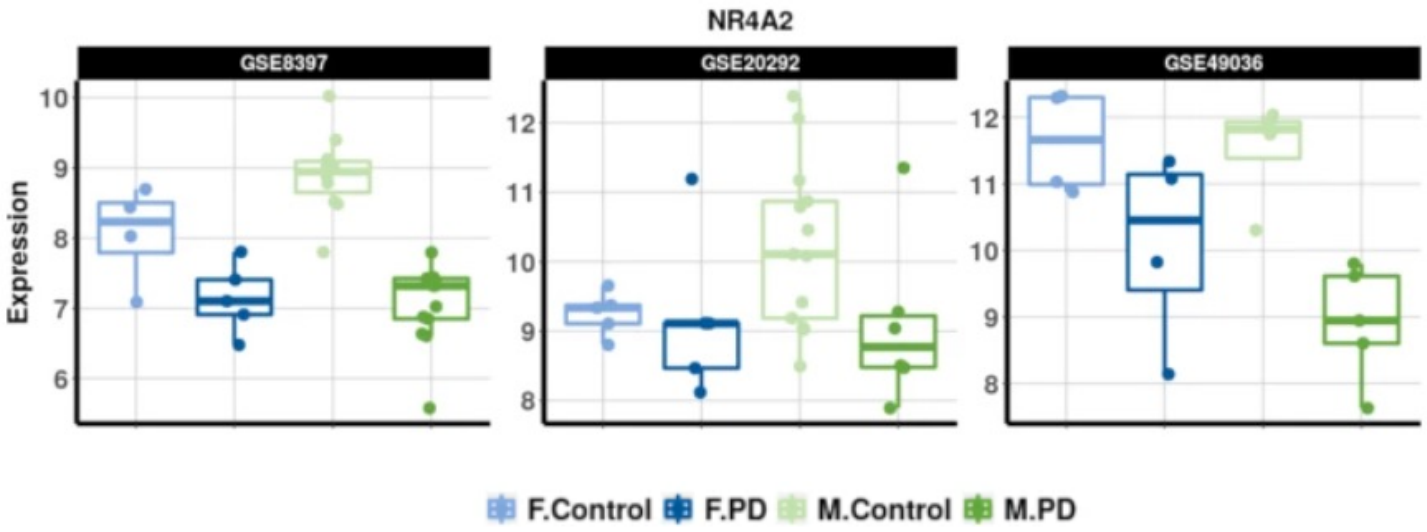
  → a purely sex-stratified analysis is prone to issues with significance thresholds

- Categorize sex differences in disease associations:

  - Sex-specific: Significant in one sex (FDR < 0.05) and not close to significance in the other (p > 0.5)
  - Sex-dimorphic: Significant in both sexes (FDR < 0.05) and with opposite direction of the effect (different signs of log fold change + min. absolute difference)
  - Sex-modulated: Significant in both sexes (FDR < 0.05) with shared direction but differing significantly in the magnitude of the effect

PD transcriptomics: Gene-level analysis

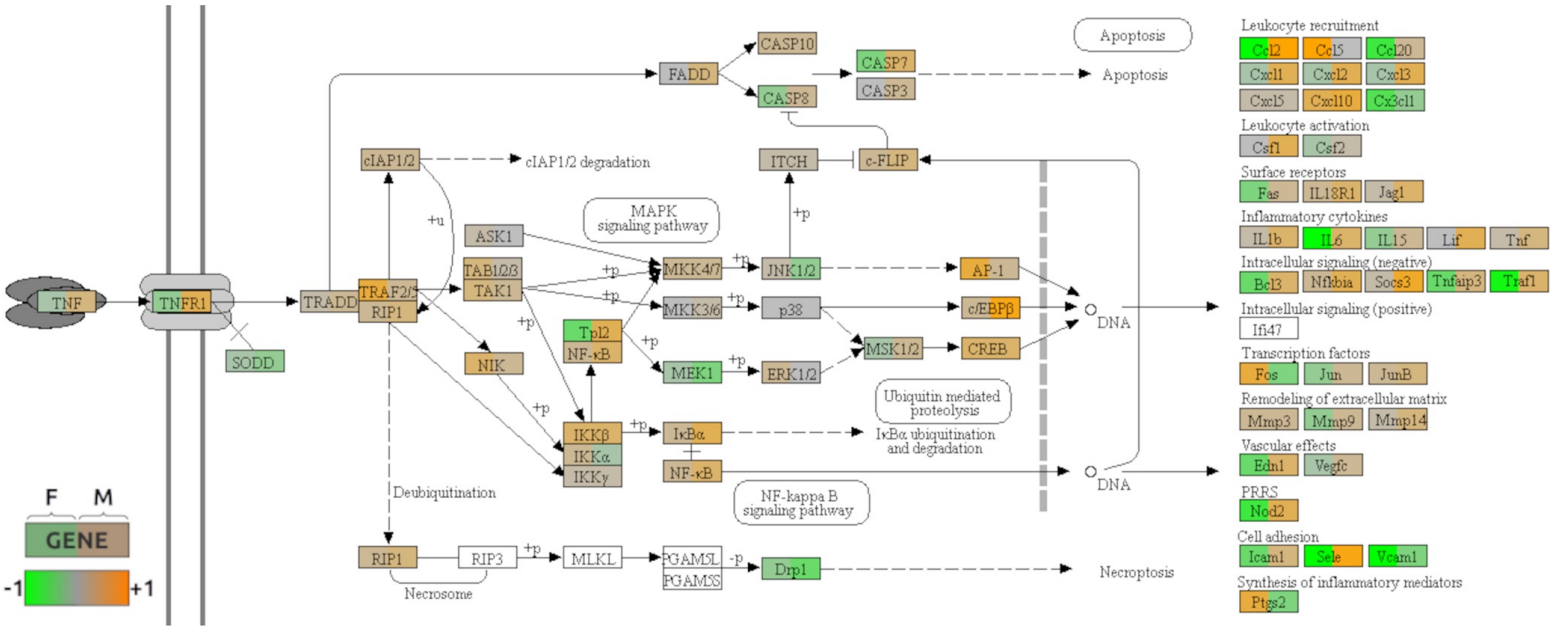
- Several sex-dependent differentially expressed genes (DEGs) identified
- Many top significant genes have well-known PD associations
- Example: NR4A2 → dopamine metabolism regulator, mutations in familial PD



→ significantly stronger changes in males

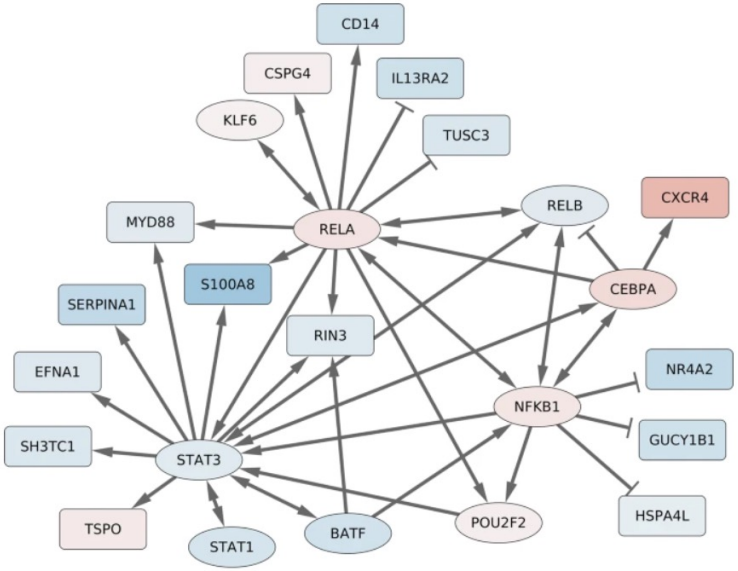
PD transcriptomics: Pathway analysis

- Enrichment of sex-dependent DEGs in pathways (KEGG, GO, Reactome)
- Main changes in mitochondrial and inflammatory response pathways
- Example: TNF signaling pathway (KEGG)

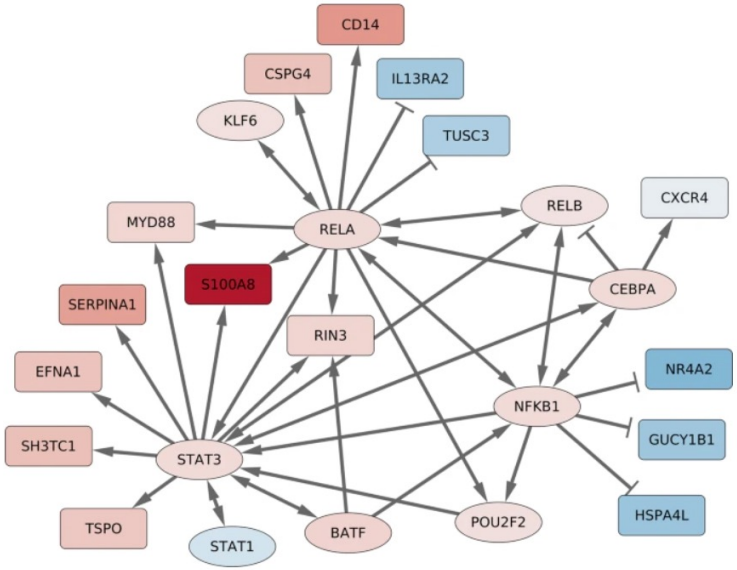


PD transcriptomics: Regulatory network analysis

- Transcription factors (TFs) with sex-dependent changes & sex differences in their downstream target genes were identified
- Multiple of these TFs are members of the statin or NFκB family (e.g., STAT3)



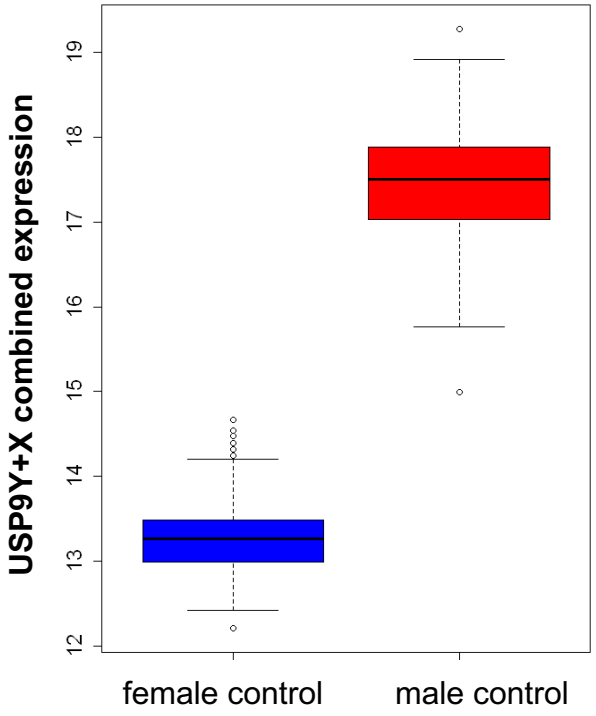
STAT3 network: logFC in males

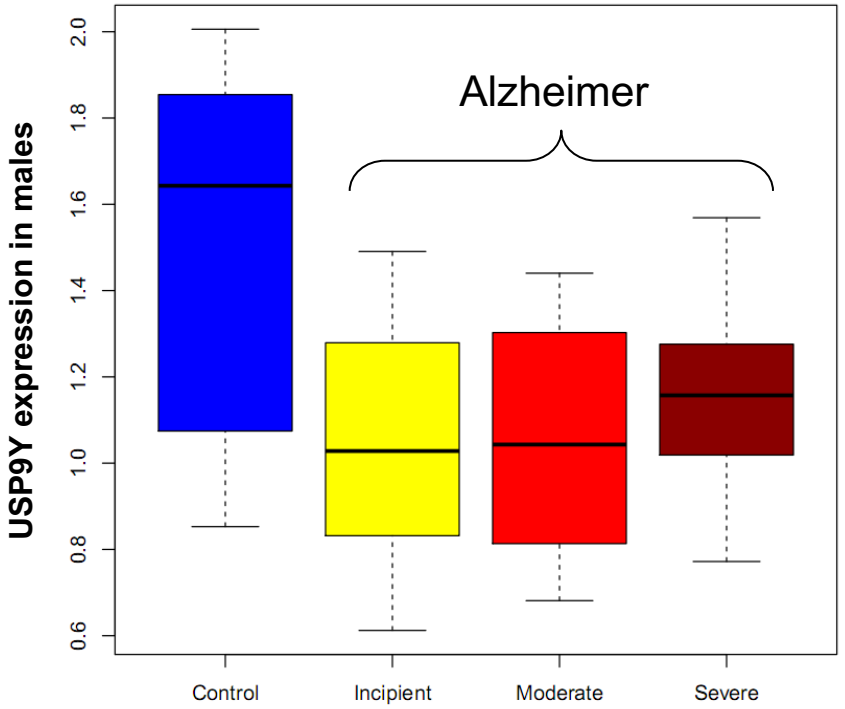


STAT3 network: logFC in females

Alzheimer‘s disease (AD) transcriptomics analysis

- AD vs. control meta-analysis → similar workflow as for PD meta-analysis
- Focus on the hippocampus as the main affected brain area
- Key gene: USP9X/Y (ubiquitin-specific peptidase 9) shows strong generic sex differences (no dosage compensation) & male-specific decrease in AD

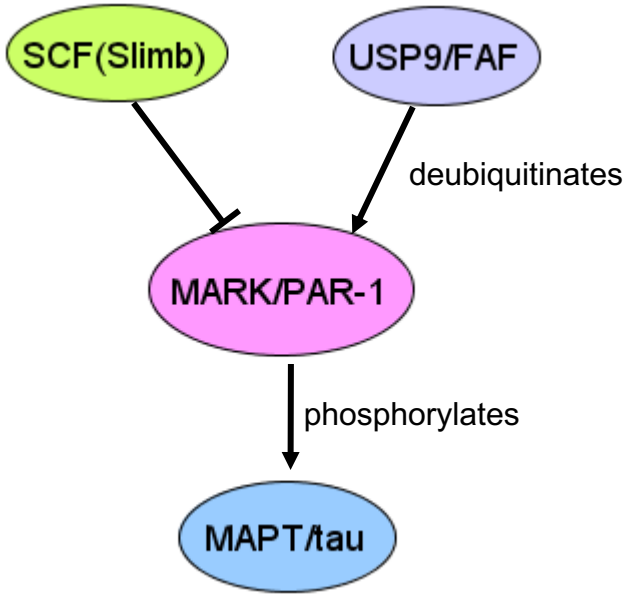




USP9 prior knowledge

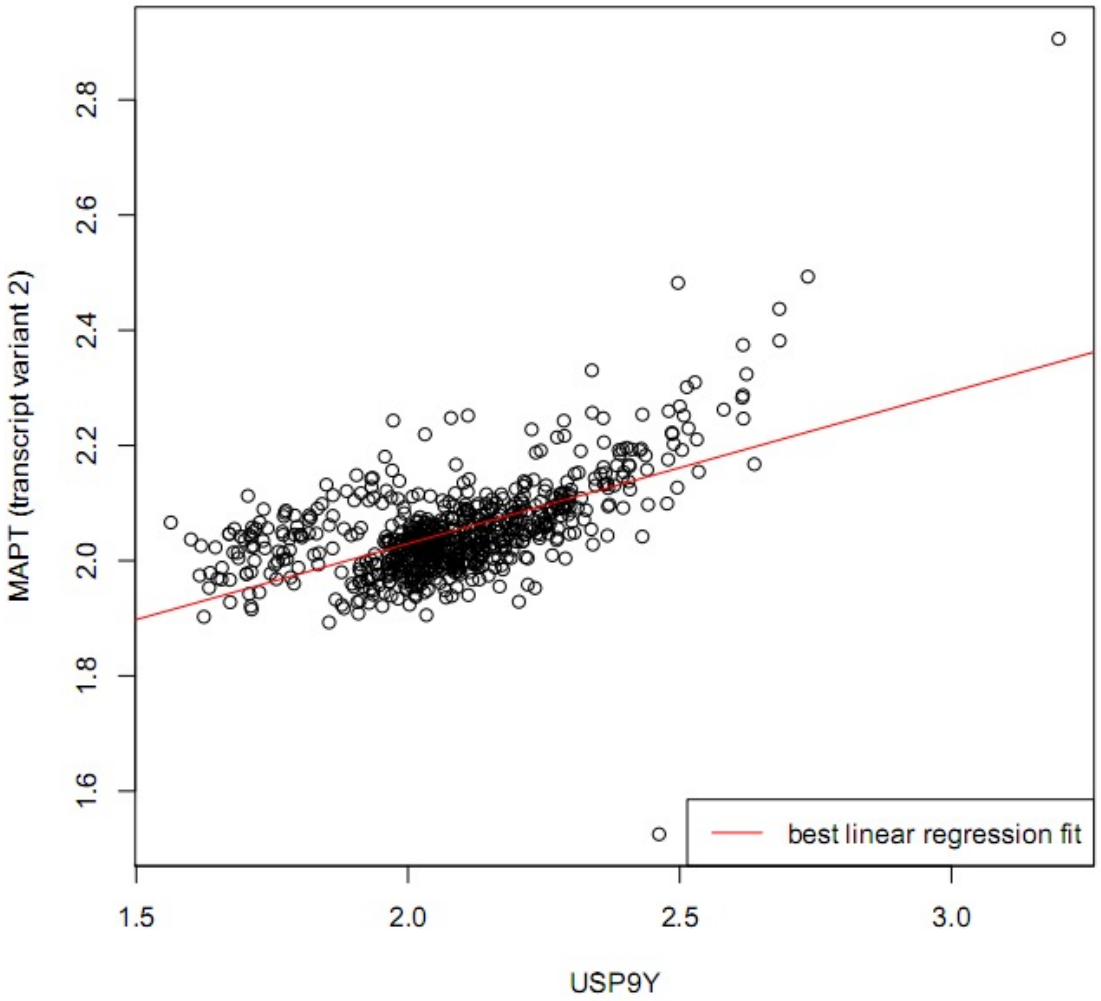
- USP9 deubiquitinates other target proteins, preventing their degradation
- In a drosophila model, USP9/FAF deubiquitinates a kinase that phosphorylates the microtubule-associated protein tau (MAPT, Lee et al., 2011)
- USP9 is highly expressed in the CNS and its targets are implicated in neuro-developmental signaling pathways, including Notch, Wnt, and TGF-ß

Role of USP9 in MAPT regulation (Drosophila model)



(source: S. Lee et al., Nature Communications, 2011)

Correlation between USP9 and MAPT

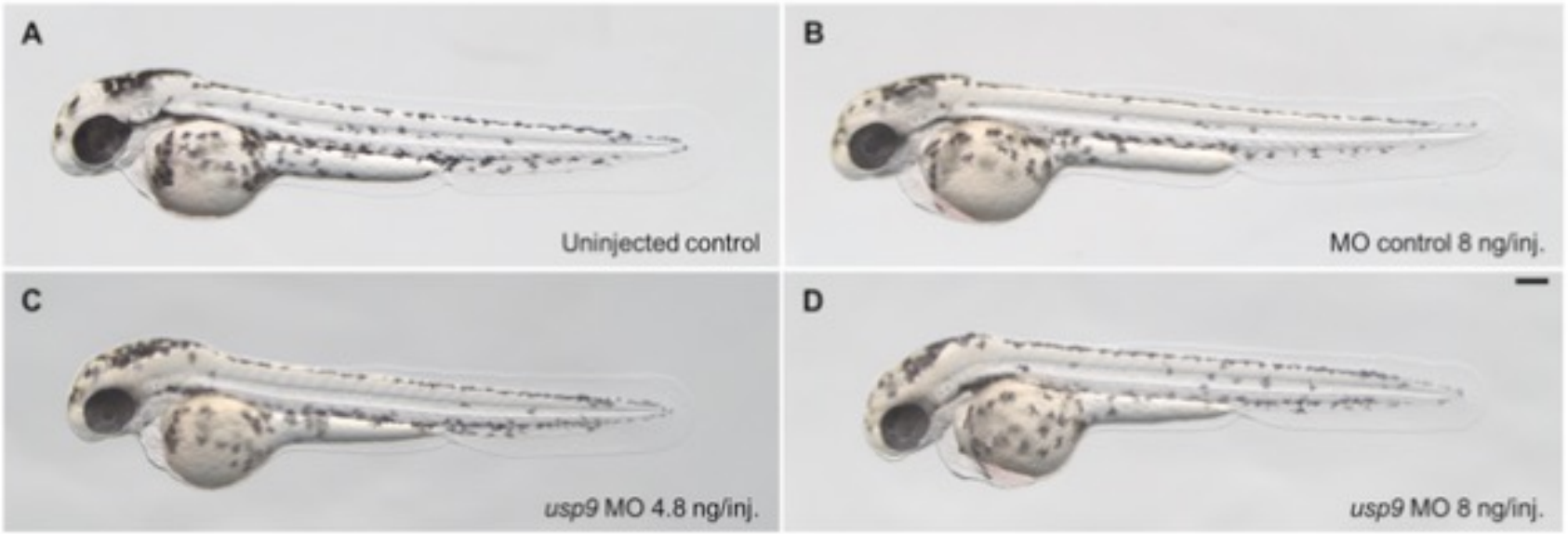


Pearson correlation: 0.602

Significance: $p < 2.2e\text{-}16$

Correlation plot of gene expression levels in human post-mortem brain samples for USP9 and MAPT (dataset by Zhang et al., 2013)

Analysis in zebrafish embryo model (USP9 Knockdown)



A: Zebrafish prior to injection of morpholino oligos (MO) for *usp9* knockdown

B: 8 ng control injection

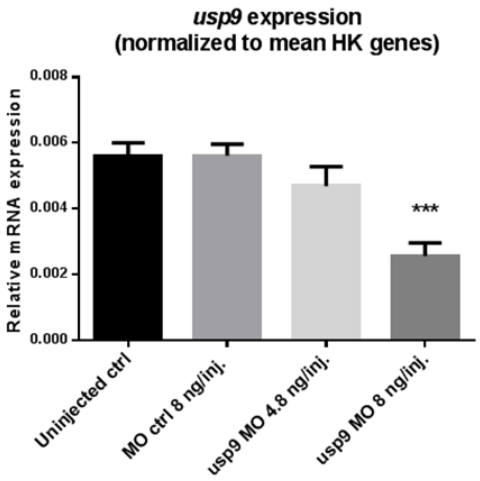
C: 4.8 ng MO injection

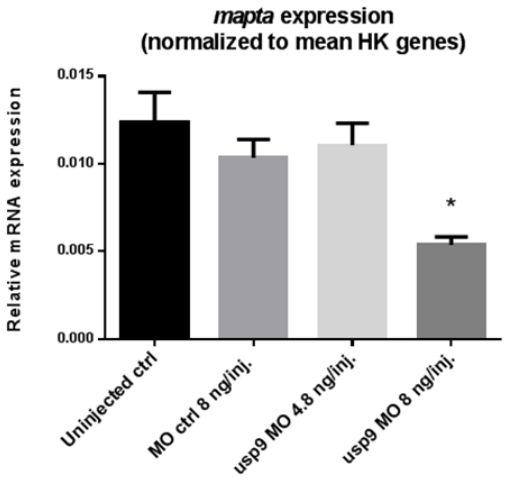
D: 8 ng MO injection

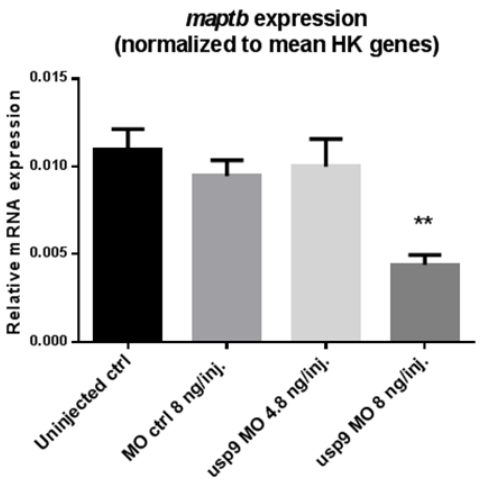
Analysis of usp9/tau associations in zebrafish embryo model

- Zebrafish have 2 MAPT-paralogs, *mapta* & *maptb*, which resemble the two main human tau isoforms (3R- and 4R-tau) → Analyze *usp9* knockdown effect on *mapta* & *maptb*

- The morpholino knockdown of *usp9* results in a concentration-dependent decrease of *mapta* and *maptb* gene expression

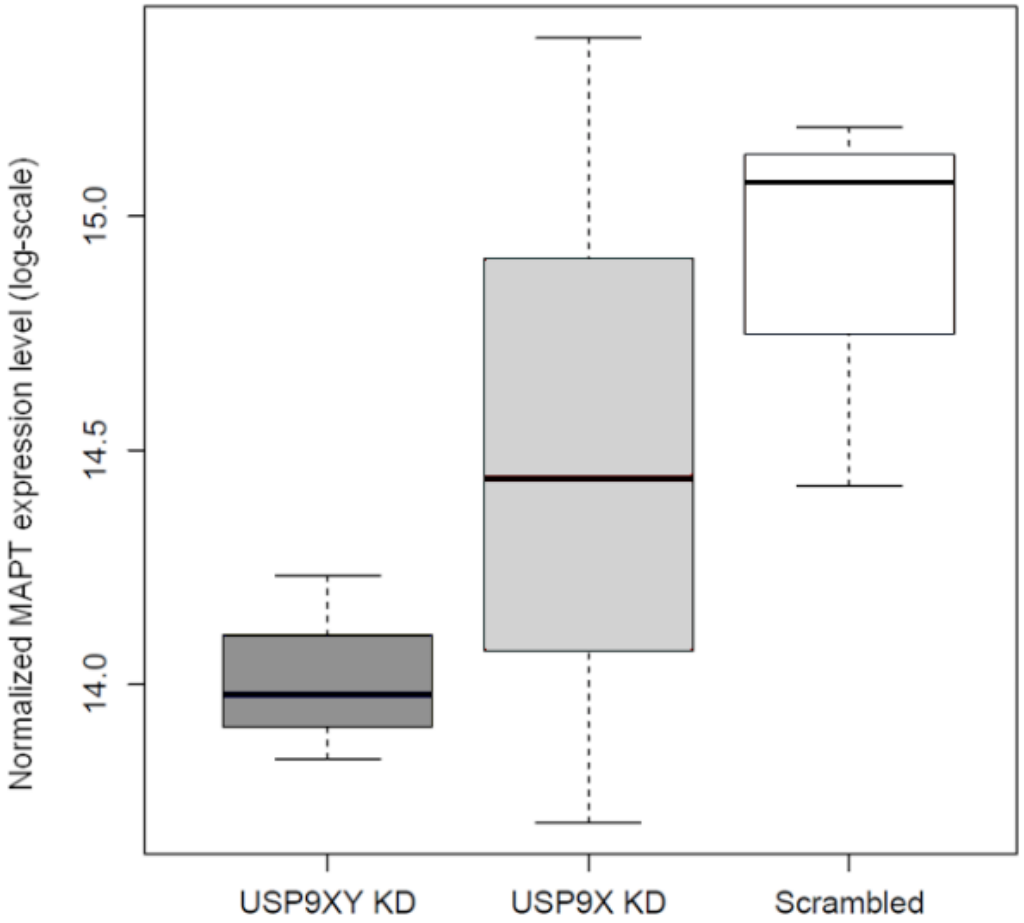






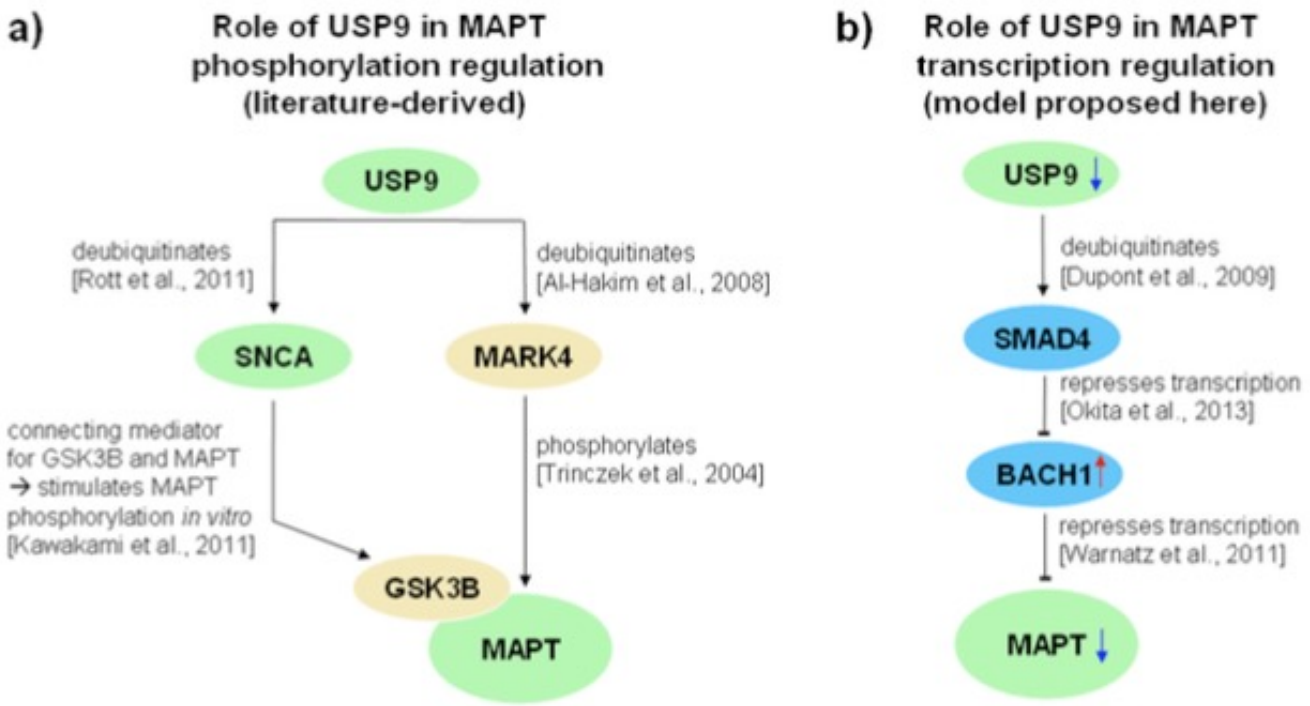
Analysis of USP9/tau associations in DU145 cell culture model

- Knockdown of USP9X and USP9X/Y results in significantly reduced MAPT expression levels
- The effect is strongest for the combined knockdown of USP9X and USP9Y



Model for the role of USP9 in MAPT regulation

Gene regulatory and protein-protein interaction links between USP9 and MAPT:



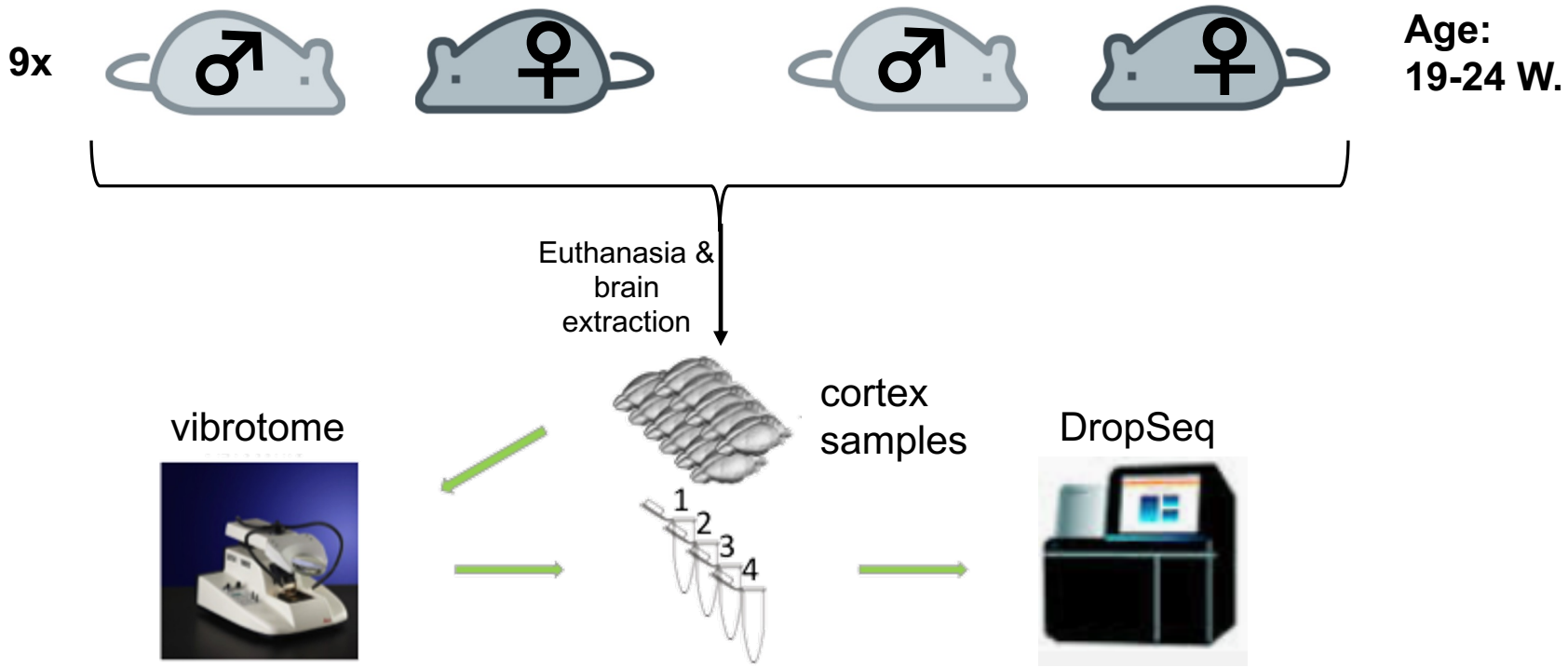
a) USP9 can modulate MAPT phosphorylation via SNCA and MARK4

b) USP9 can modulate MAPT gene expression via SMAD4 and BACH1

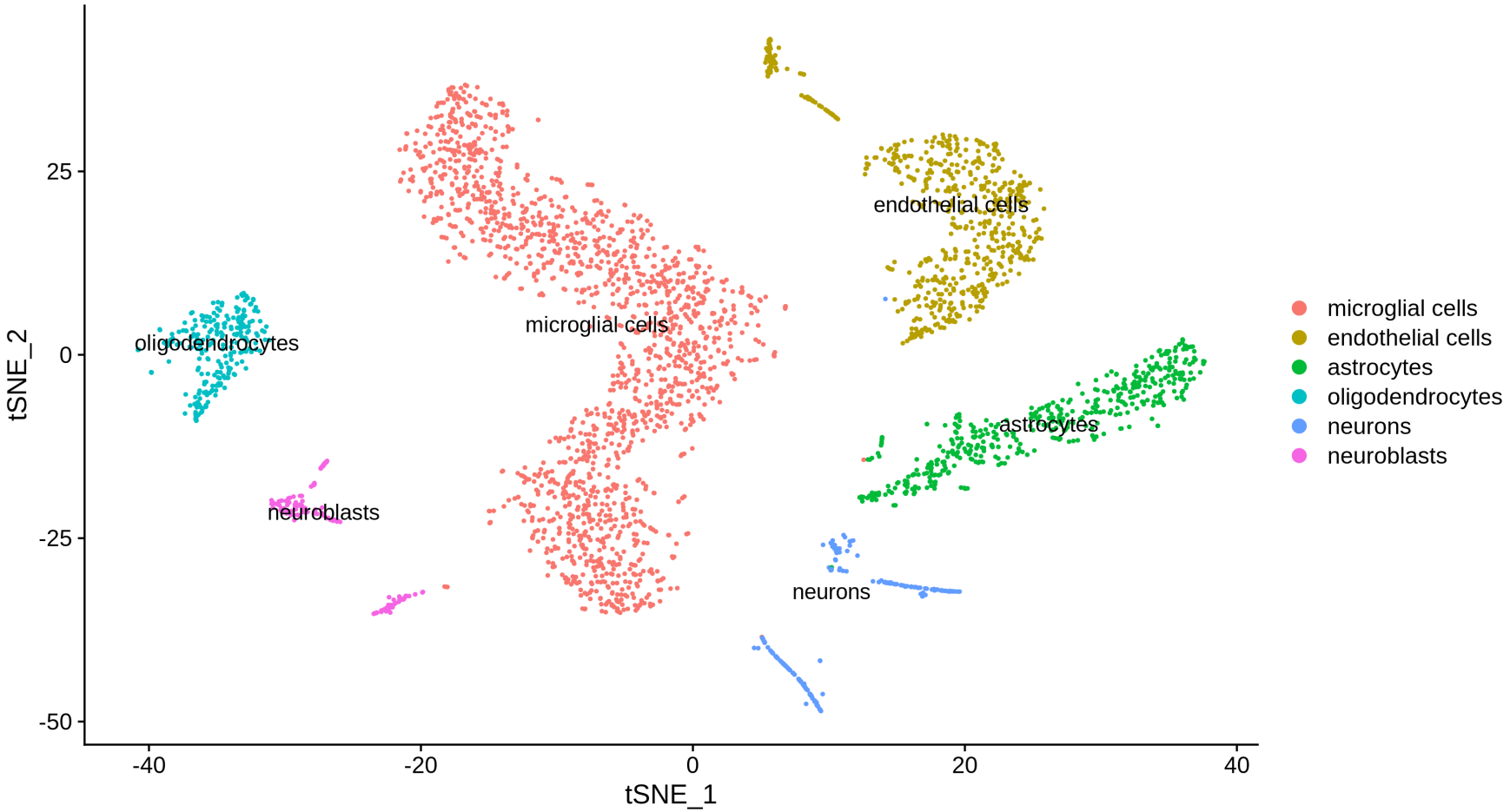
Sex differences in single cells in a model for early AD

AD mouse model: Tg2576 (overexpresses mutant APP: K670/671L)

Study set-up:

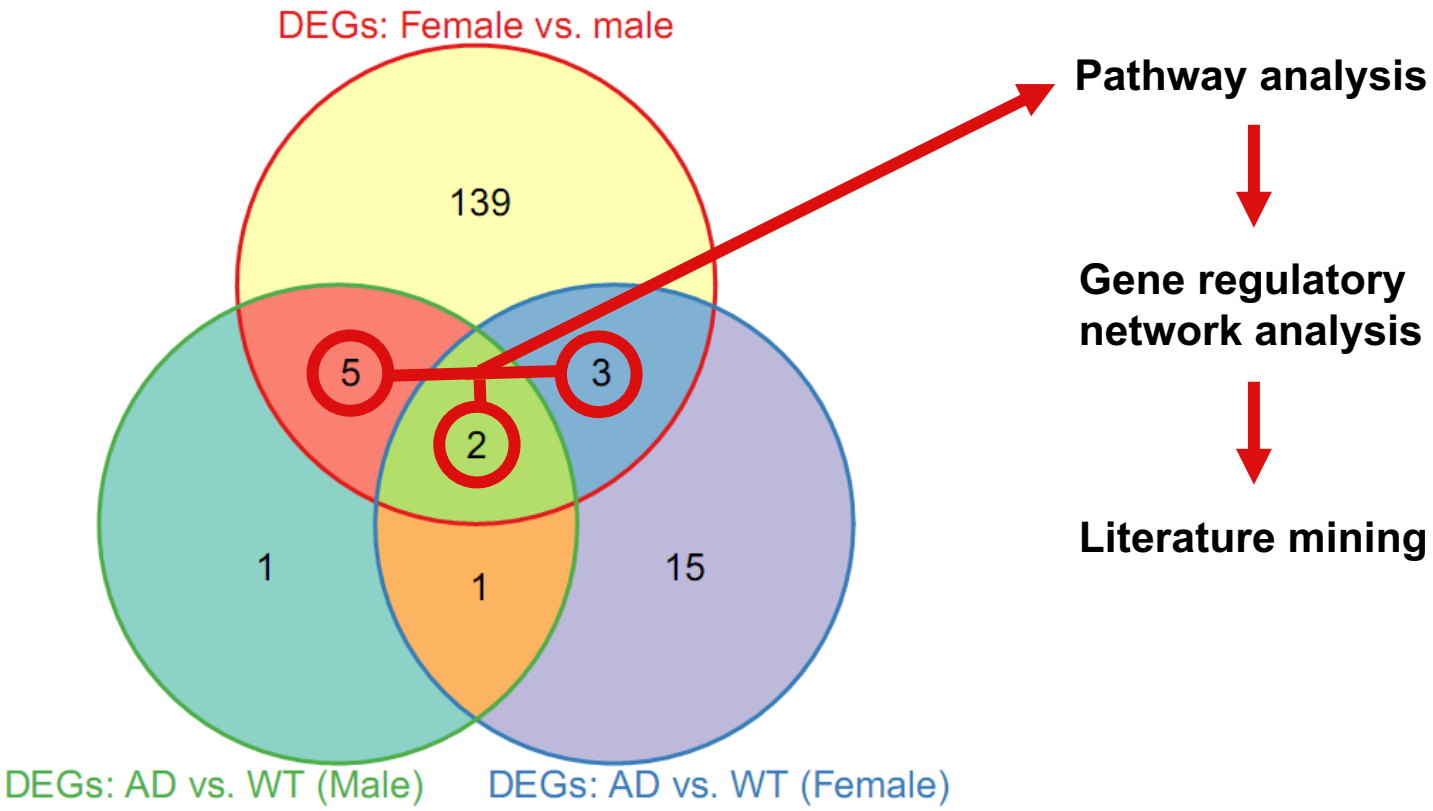


Clustering of cells and cell type annotation



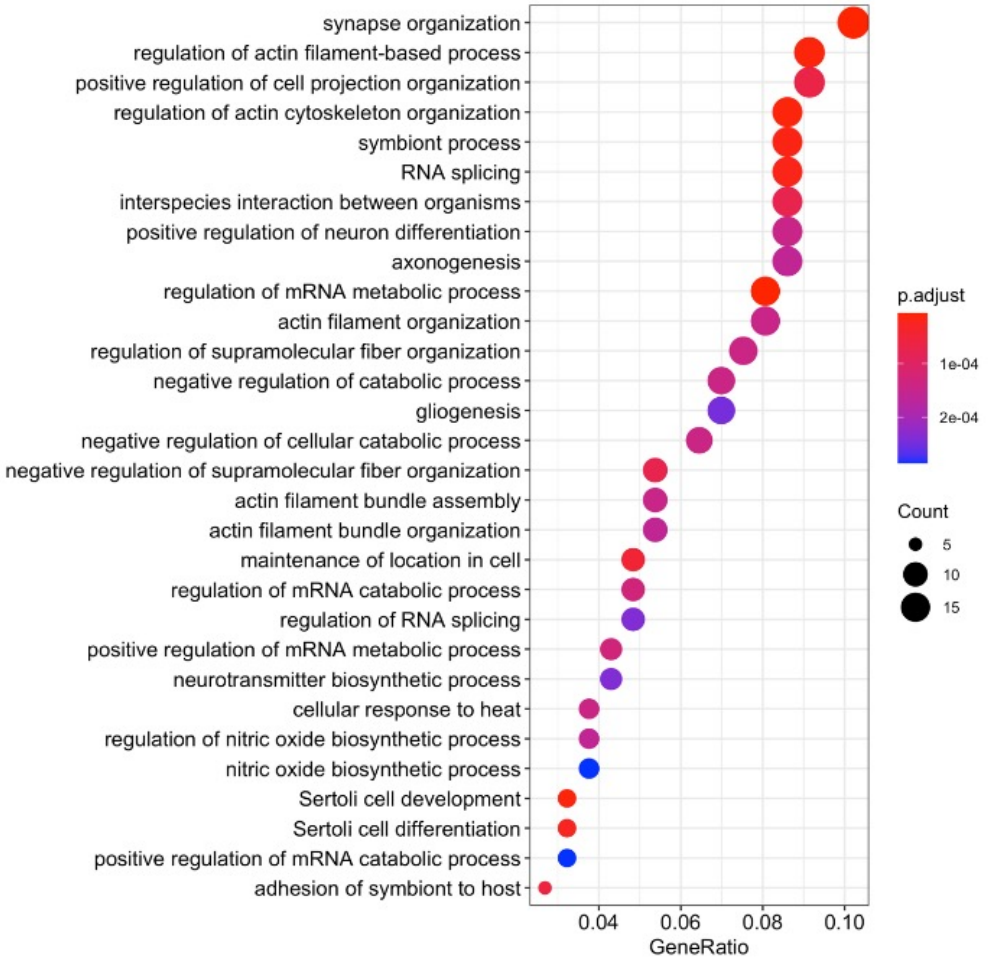
Analysis workflow

Compute sex-specific and sex-dimorphic DEGs between TG and WT for all cell types:

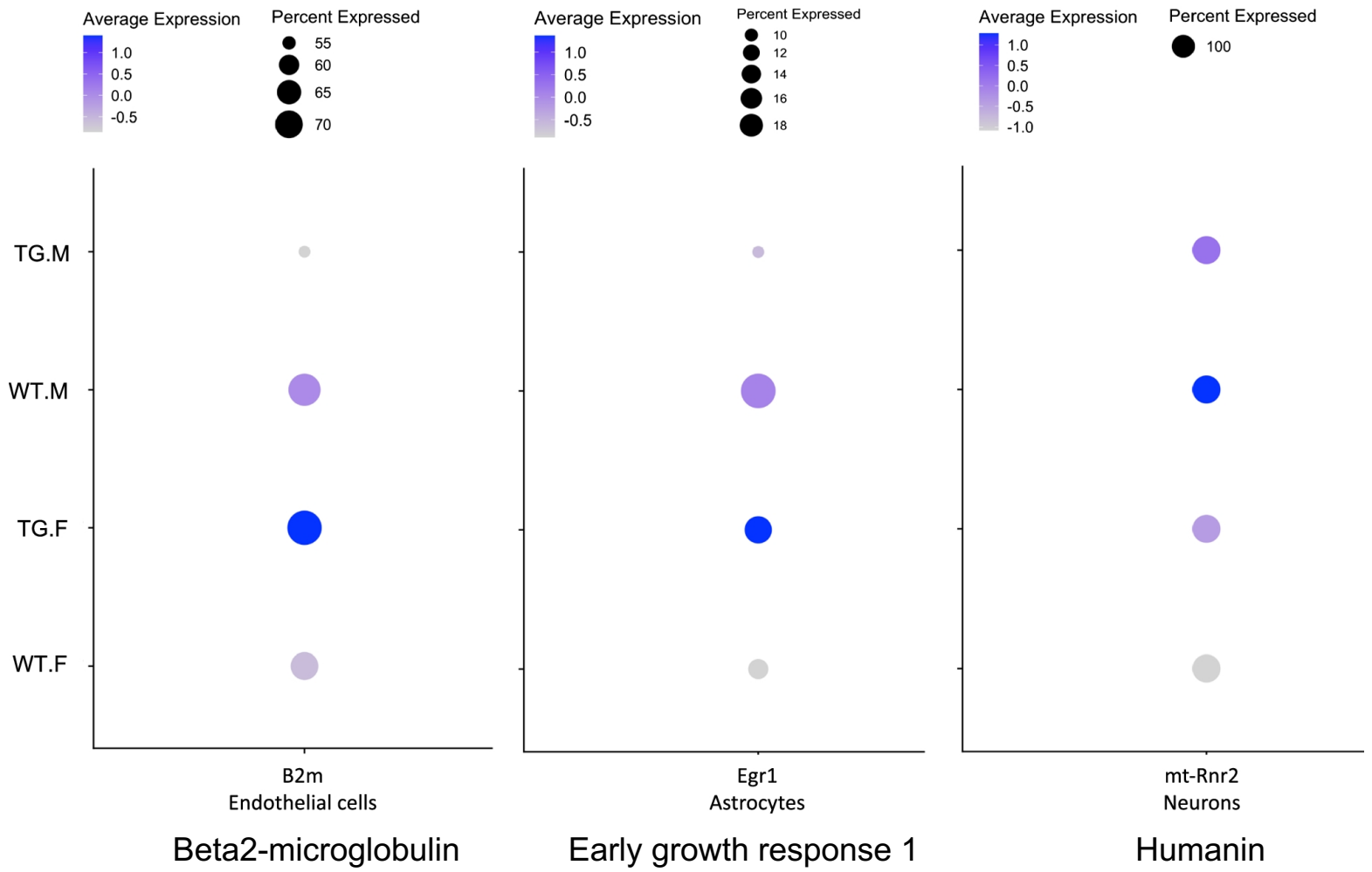


Sex-specific pathway alterations

Enrichment of sex-specific changes in processes related to synapse organization, neuron differentation and RNA splicing:



Sex-dimorphic changes



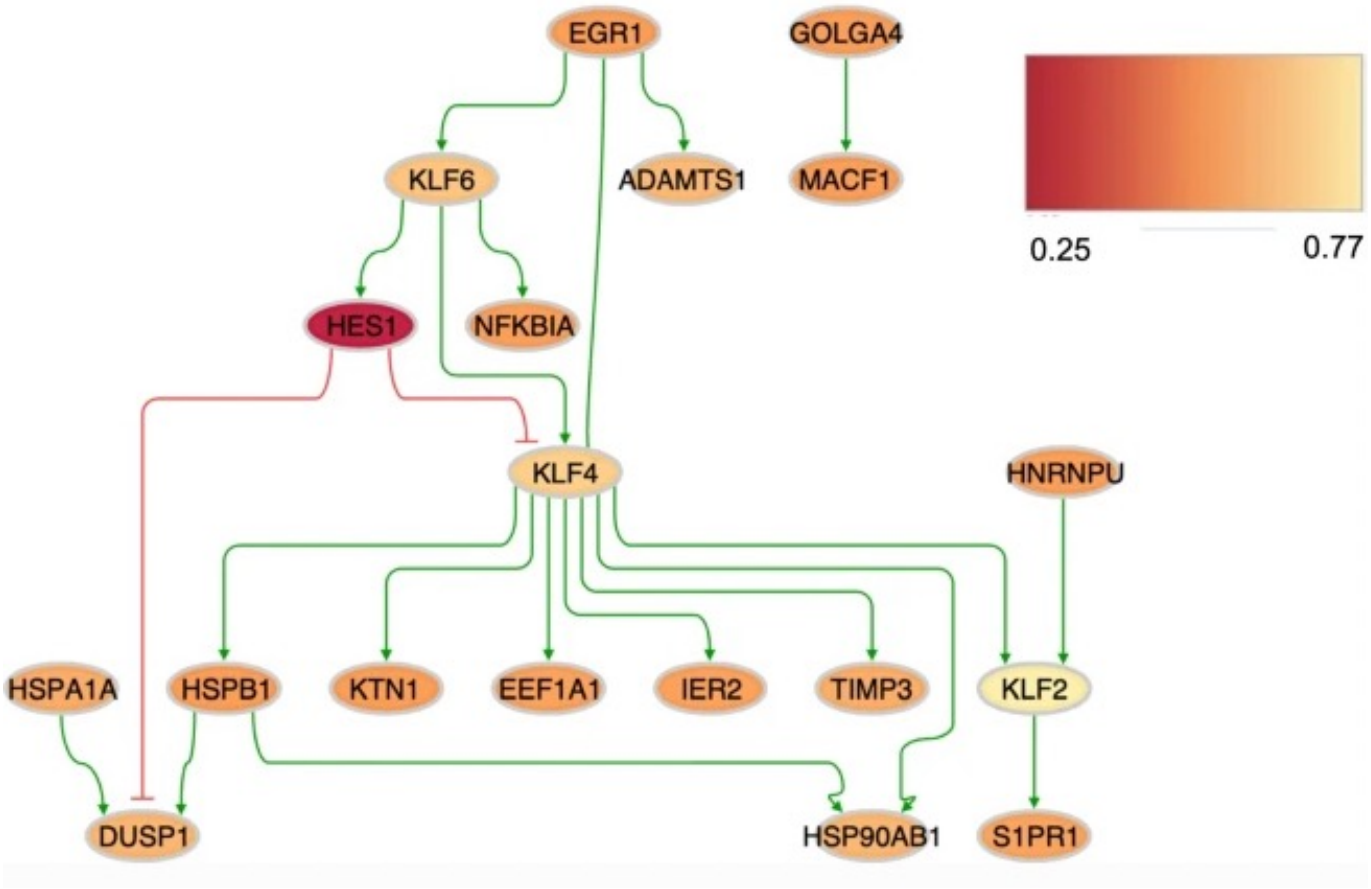
Beta2-microglobulin

Early growth response 1

Humanin

Gene regulatory network (GRN) analysis

Example: Male-specific sub-network alteration in endothelial cells



Log fold change in Tg2576 vs. WT

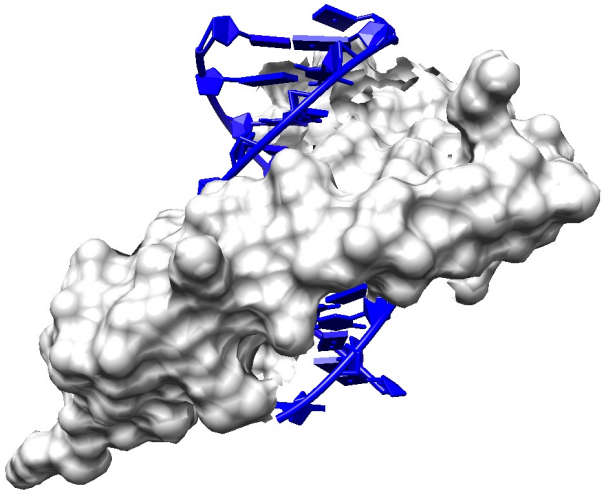
EGR1 (Early Growth Response 1) in Alzheimer‘s disease

- **Functions**: transcription factor associated with response to growth factors, DNA damage and ischemia

- **AD associations**:

  - Silencing Egr1 in the 3xTg-AD model lowers Abeta pathology and improves cognition (Qin et al., 2017)

  - Expression of EGR1 and acetylcholine-esterase (AchE) correlate in humans and in the 3xTg model; and EGR1 upregulates AchE *in vitro* (Hu et al., 2019)



EGR1 crystal structure (gray) bound to DNA (blue) (PDB: 4X9J)

Summary

- Significant disease-associated sex differences in AD and PD for individual genes, pathways and sub-networks

- PD: Main changes in mitochondrial and inflammatory pathways; key transcription factors associated with dopamine metabolism (NR4A2) and NF-κB and STAT3 signaling pathways

- AD: Tg2576 model shows changes in synapse organization, neuron differentiation, and RNA splicing. The regulatory gene *Egr1* upregulates acetylcholine-esterase, and its inhibition is protective in the 3xTG model.

Acknowledgements

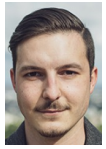
Thank you!

Quentin Klopfenstein
(Machine learning)

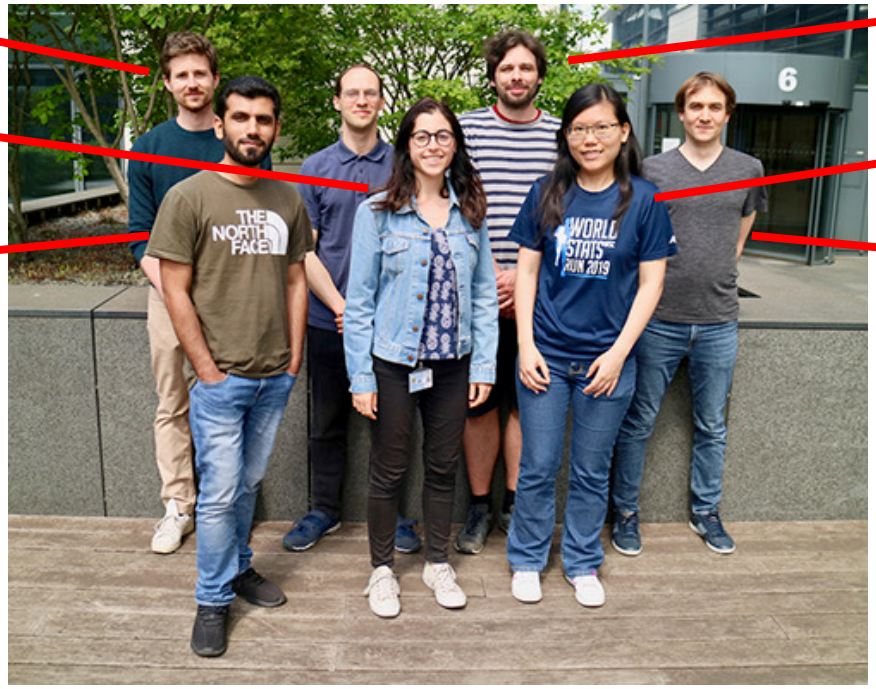
Elisa Gomez
(Pathway/network analysis)

Muhammad Ali
(Statistics, network analysis)

Varsha Murthy
(Animal model analyses)



Cyril Brzenczek
(Machine learning, MRI analysis)



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Francesco Nasta
(Machine learning, Image analysis)

Sophie Lebars
(Network analysis and modeling)

References

1. A. Rauschenberger, Z. Landoulsi, M. A. van de Wiel, E. Glaab. *Penalized regression with multiple sources of prior effects*, Bioinformatics (2022), 39(12), doi: 10.1007/s12035-022-02985-2.
2. M. Ali, O. Uriarte Huarte, T. Heurtaux, P. Garcia, B. Pardo Rodriguez, K. Grzyb, R. Halder, A. Skupin, M. Buttini, E. Glaab. *Single-Cell Transcriptional Profiling and Gene Regulatory Network Modeling in Tg2576 Mice Reveal Gender-Dependent Molecular Features Preceding Alzheimer-Like Pathologies*, Mol Neurobiol (2022), doi:10.1007/s12035-022-02985-2.
3. A. Rauschenberger, E. Glaab. *Predicting Dichotomised Outcomes from High-Dimensional Data in Biomedicine*, Journal of Applied Statistics, (2023), doi: 10.1080/02664763.2023.2233057.
4. L. C. Tranchevent, R. Halder, E. Glaab. *Systems level analysis of sex-dependent gene expression changes in Parkinson's disease*, NPJ Parkinson's Disease, (2022), 9, 8.
5. A. Rauschenberger, E. Glaab, *Predicting correlated outcomes from molecular data*, Bioinformatics (2021), 37(21), 3889–3895
6. R. Diaz-Uriarte, E. Gómez de Lope, R. Giugno, H. Fröhlich, P. V. Nazarov, I. A. Nepomuceno-Chamorro, A. Rauschenberger, E. Glaab, *Ten Quick Tips for Biomarker Discovery and Validation Analyses Using Machine Learning*, PLoS Computational Biology (2022), doi:10.1371/journal.pcbi.1010357
7. E. Glaab, J.P. Trezzi, A. Greuel, C. Jäger, Z. Hodak, A. Drzezga, L. Timmermann, M. Tittgemeyer, N. J. Diederich, C. Eggers, Integrative analysis of blood metabolomics and PET brain neuroimaging data for Parkinson's disease, Neurobiology of Disease (2019), Vol. 124, No. 1, pp. 555
8. S. Köglsberger, 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 (2017), 54(10), pp. 7979
9. 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
10. E. Glaab, *Using prior knowledge from cellular pathways and molecular networks for diagnostic specimen classification*, Briefings in Bioinformatics (2015), 17(3), pp. 440
11. 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
12. E. Glaab, R. Schneider, *RepExplore: Addressing technical replicate variance in proteomics and metabolomics data analysis*, Bioinformatics (2015), 31(13), pp. 2235
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, A. Baudot, N. Krasnogor, R. Schneider, A. Valencia. EnrichNet: network-based gene set enrichment analysis, Bioinformatics, 28(18):i451-i457, 2012
15. E. Glaab, A. Rauschenberger, R. Banzi, C. Gerardi, P. Garcia, J. Demotes-Mainard, and the PERMIT Group, Biomarker discovery studies for patient stratification using machine learning analysis of omics data: a scoping review, BMC Open (2021), 11, e053674
16. D. M. Hendrickx, P. Garcia, A. Ashrafi, A. Sciortino, K. J. Schmit, H. Kollmus, N. Nicot, T. Kaoma, L. Vallar, M. Buttini, E. Glaab, A new synuclein-transgenic mouse model for early Parkinson's reveals molecular features of preclinical disease, Molecular Neurobiology (2020), 58, 576-602