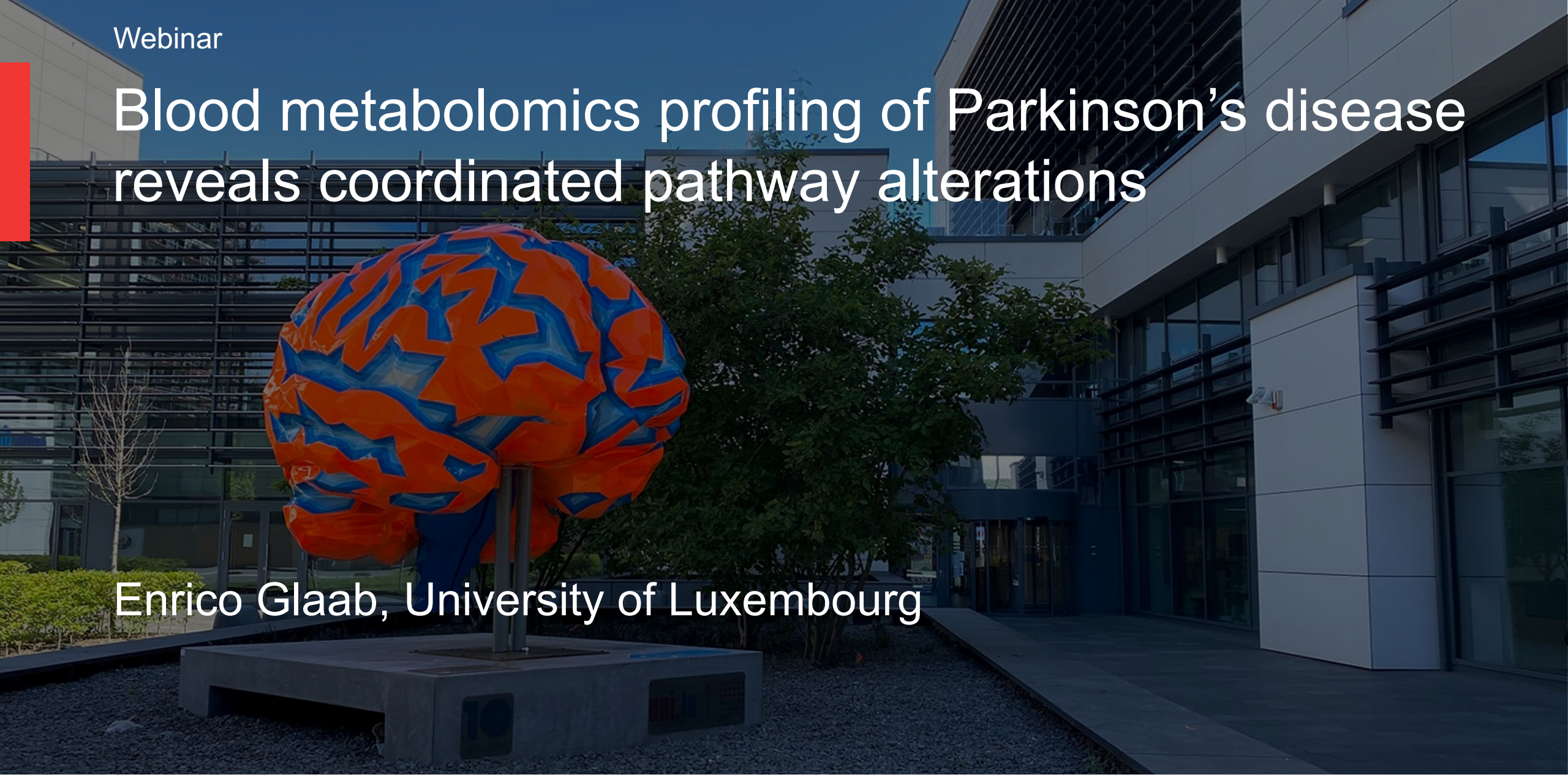
Webinar

Blood metabolomics profiling of Parkinson's disease reveals coordinated pathway alterations



Enrico Glaab, University of Luxembourg

Outline

- Background on Parkinson‘s disease (PD), the cohort, and the metabolomics study design
- Differential analysis of metabolites for *de novo* patients, treated patients, and controls
- Metabolomics changes at pathway and network level
- Machine learning analysis of the metabolite data

Background: Parkinson‘s disease

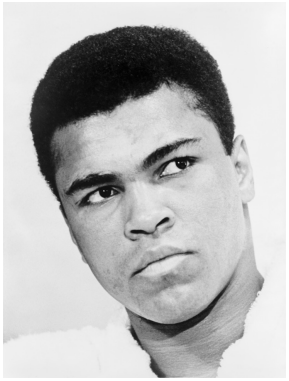
- a currently incurable brain disorder
- affects mainly people over 60 years of age, less commonly younger people

Symptoms:

- Motor impairments:
  Resting tremor, rigidity, slowness of movement, postural instability

- Non-motor impairments:
  Sleep disorders, constipation, sensory impairments, among others

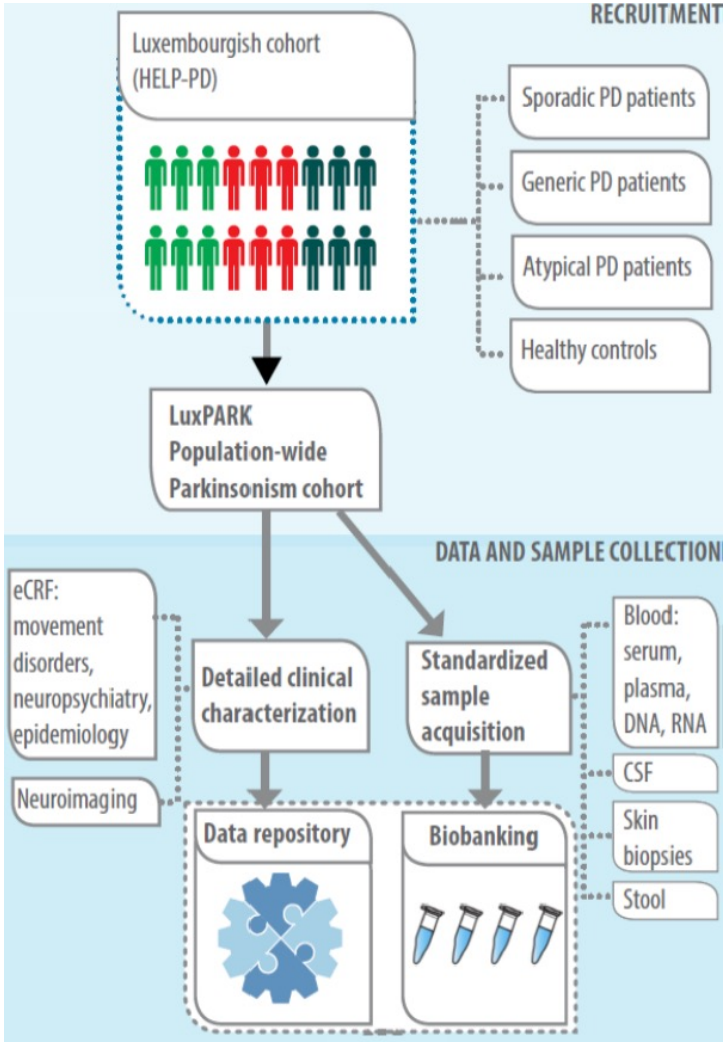
Illustration: Parkinson‘s patient
(Source: W. R. Gowers, 1886)



Heterogeneous disease course and manifestations:
Patients Michael J. Fox and Muhammed Ali

The Luxembourg Parkinson Study (LuxPARK)

- a nationwide, monocentric, observational, longitudinal study
- covers ~800 patients with typical or atypical parkinsonism and ~800 controls
- Patients: annual follow-up, controls: after 4 years
- **Clinical data**: Motor symptoms (MDS-UPDRS), Non-motor symptoms (Cognition, Sleep, Quality of Life, Environment, among others)
- **Biosampling**: Blood, DNA, RNA, Urine, Saliva, Nasal Washes, Stool, CSF, Skin & Colon biopsies



LuxPARK Metabolomics Study - Overview

Goals: - Characterize blood metabolomics changes in PD vs. controls & PD sub-groups

- Interpret cellular pathway/network alterations & build prediction models for clinical outcomes

Data: LC-MS metabolomics (Metabolon) using 1500 LuxPARK blood plasma samples, covering 1409 metabolites

Baseline analysis
(1300 samples)

- PD (593, including 56 *de novo* PD) and Controls (592)
- PD with dementia (46)
- Atypical forms of parkinsonism (PSP: 46, MSA-P: 12, CBS: 11)

Longitudinal analysis
(200 samples)

- Annual follow-up visits 1 & 2 for 100 PD patients in the baseline analysis
- Balanced sexes (50/50)

Study limitations

- **Dietary effects:** No possibility to ensure that all subjects receive the same diet or participate in the fasting state → need to consider dietary influences for data interpretation

- **Batch effects:** Samples were allocated across multiple measurement batches, only an approximate balancing of sample group representations possible → increased data variance

- **Treatment effects:** Most patients received dopaminergic treatment, which can confound the measurements → adjust for confounding effects & compare against *de novo* patients

Algorithmic sample matching & selection

Goal: Select baseline biospecimens from PD patients (593) & controls (592), such that:

- Sexes are matched and balanced
- Age distributions are matched
- BMI distributions are matched
- Use of arterial medication is balanced
- Minimized occurrence of other comorbidities (non-PD related medications)

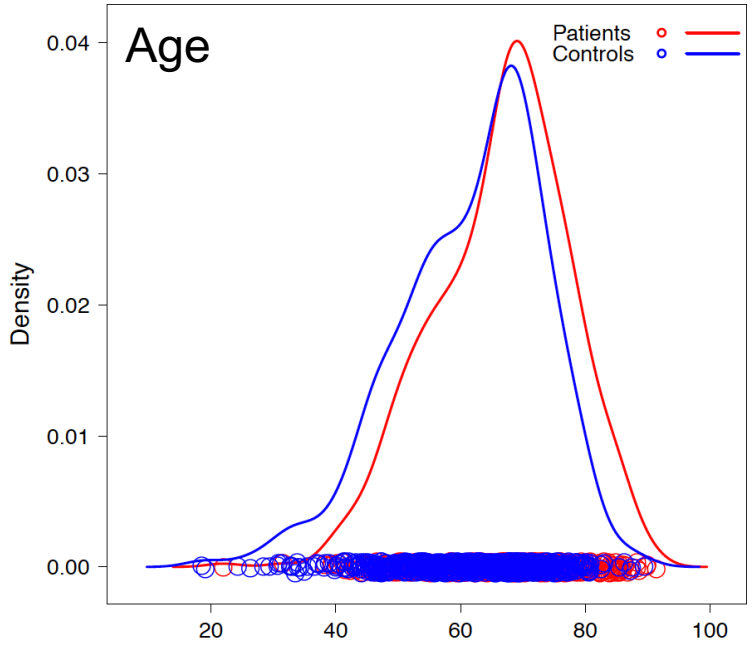
Benefits:

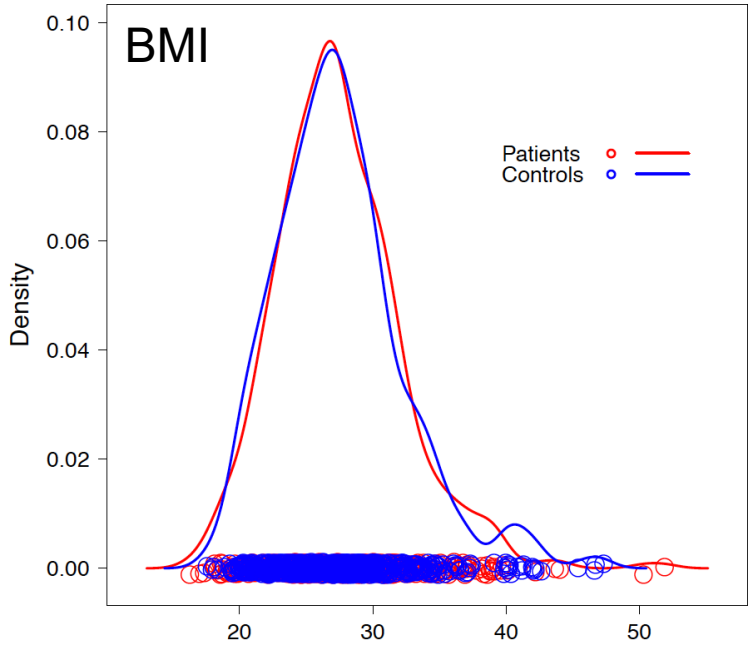
→ Reduces influence of confounding factors

→ Removes unwanted sources of variance

Matched sample selection

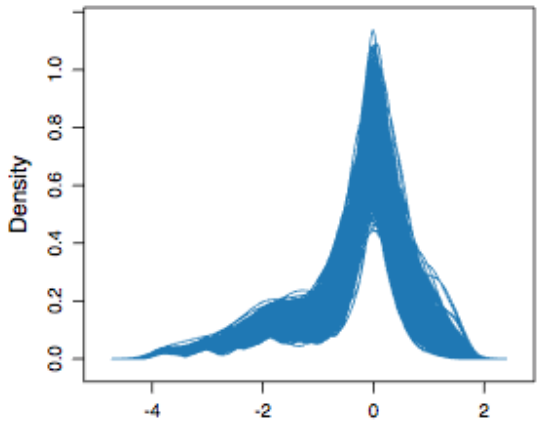
- Sex representation statistics: PD (207 females, 386 males), controls (207 females, 385 males)
- Arterial medication: PD (~38.0%), controls (~37.4%)
- Medication for other symptoms: PD (~50.4%), controls (~45.2%)



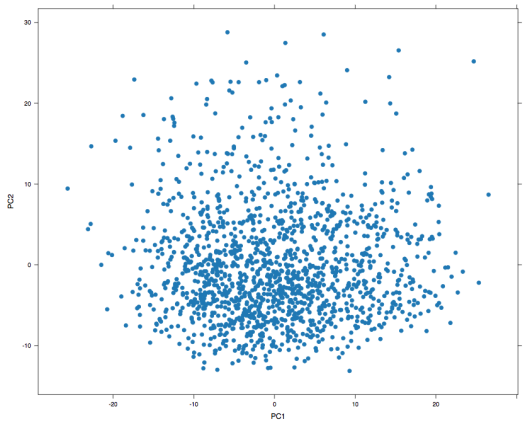


LC-MS Metabolomics data – Quality controls

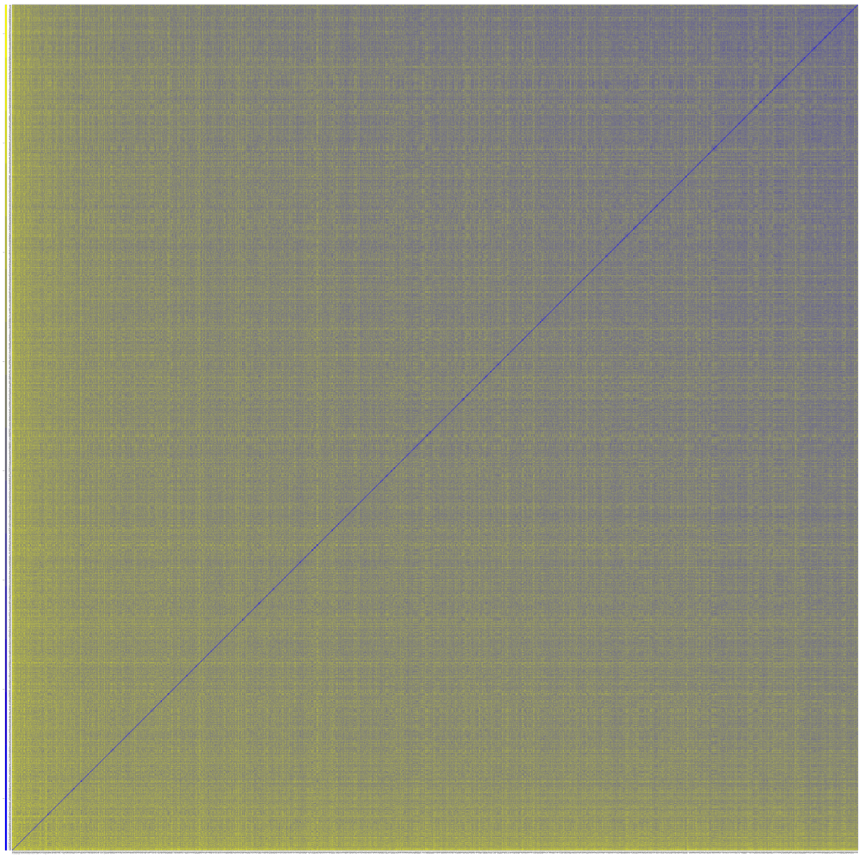
Signal intensity distributions



PCA Plot / Outlier detection



Distances between samples / Outlier detection



Differential statistical analysis

Goal: Identify PD-associated metabolite changes, independent from medication, age and sex effects

Method:

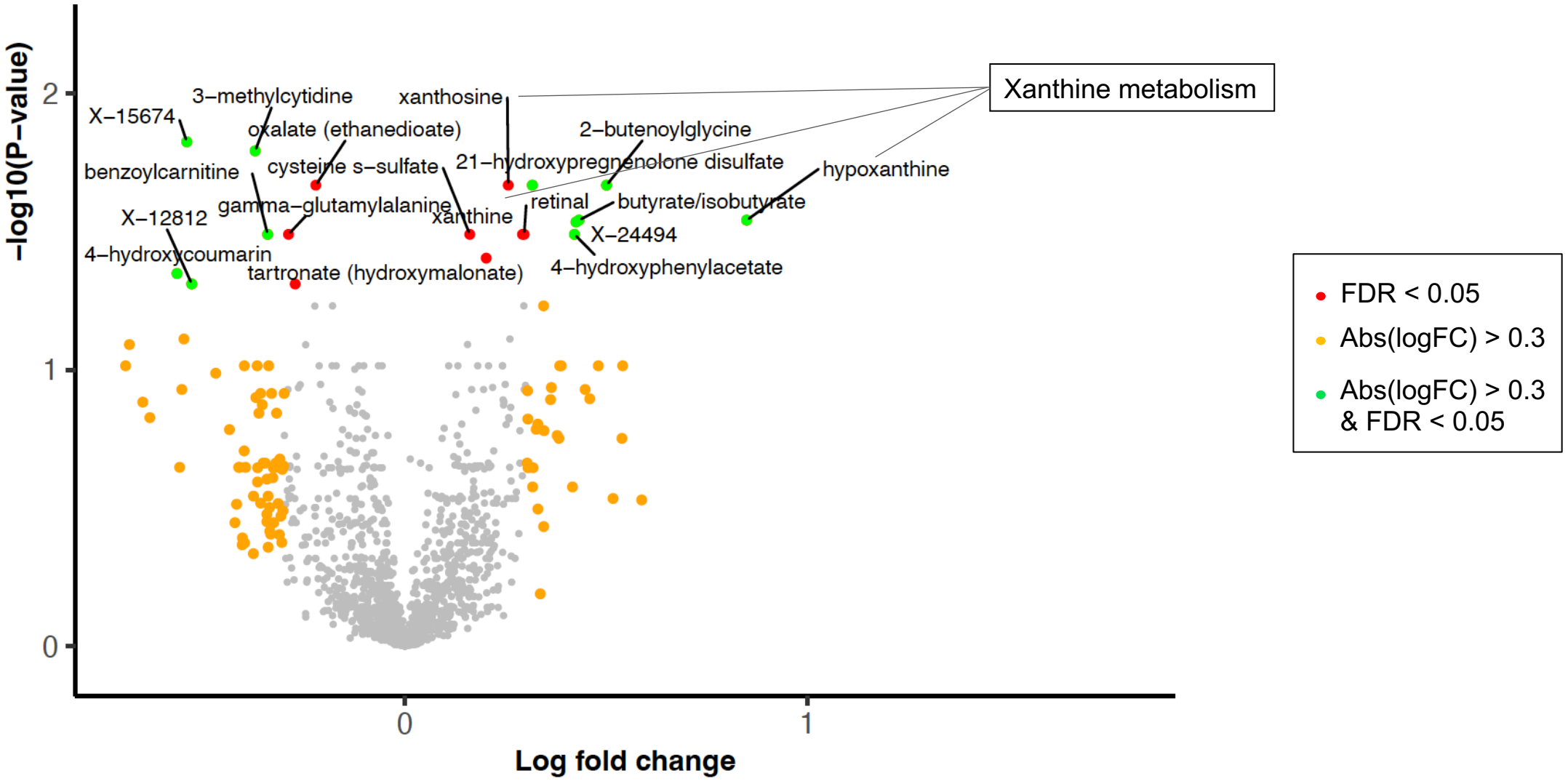
- Differential abundance analysis: PD vs. Controls

  Statistical test: empirical Bayes moderated t-statistic

- Confounder adjustment: age, sex, treatment effects (L-Dopa treatment captured using 3-O-Methyldopa metabolite) + compare with *de novo* PD vs. controls results

- Check for other confounders: Principal variance component analysis (PCVA) does not suggest the presence of additional confounders with major effects in our clinical data

De novo PD vs. control - Volcano plot

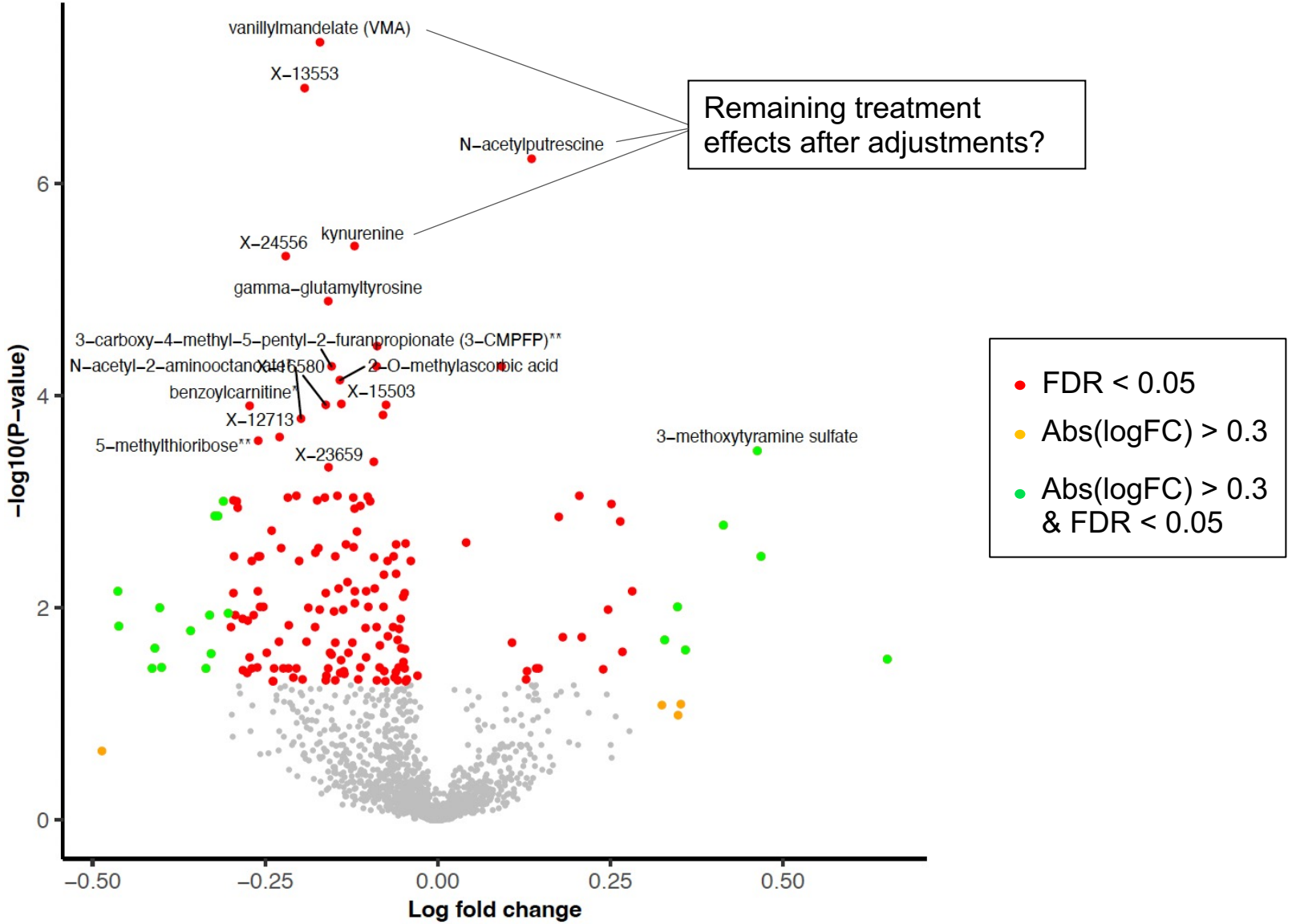


Top-ranked metabolites - *de novo* PD vs. control

Metabolite	logFC	P-value	adj. P-value
oxazepam (sedative, likely medication effect)	0.12	1.83E-07	2.63E-04
inosine	1.79	4.44E-06	3.19E-03
X-15674	-0.54	3.12E-05	1.49E-02
3-methylcytidine	-0.37	4.49E-05	1.61E-02
2-butenoylglycine	0.50	8.18E-05	2.14E-02
xanthosine	0.26	1.17E-04	2.14E-02
oxalate (ethanedioate)	-0.22	1.19E-04	2.14E-02
21-hydroxypregnenolone disulfate	0.32	1.19E-04	2.14E-02
butyrate/isobutyrate	0.43	1.86E-04	2.87E-02
hypoxanthine	0.85	2.00E-04	2.87E-02
X-24494	0.43	2.23E-04	2.91E-02
gamma-glutamylalanine	-0.29	3.23E-04	3.23E-02
4-hydroxyphenylacetate	0.42	3.59E-04	3.23E-02
benzoylcarnitine	-0.34	3.64E-04	3.23E-02
retinal	0.30	3.73E-04	3.23E-02
cysteine s-sulfate	0.16	3.79E-04	3.23E-02
xanthine	0.29	3.82E-04	3.23E-02
2-hydroxy-4-(methylthio)butanoic acid	0.20	4.93E-04	3.94E-02
4-hydroxycoumarin (anticoagulant medication)	-0.57	5.92E-04	4.48E-02
tartronate (hydroxymalonate)	-0.27	7.12E-04	4.89E-02

Xanthine metabolism

Treated PD vs. control, confounder adjustment – Volcano plot

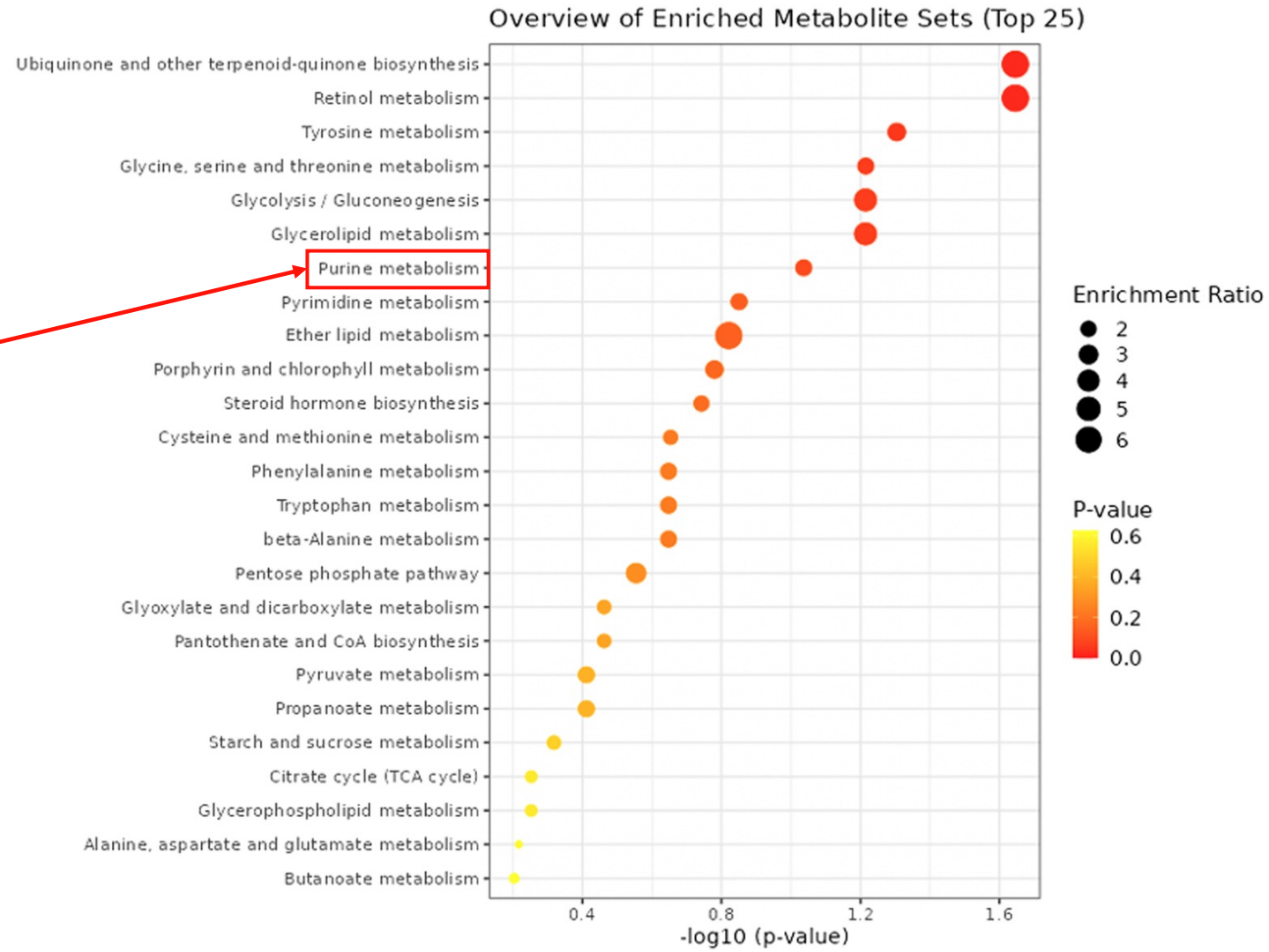


Shared significant metabolites – *de novo* PD & all PD analysis

	***De novo* PD vs. Control**			**All PD vs. Control (adjusted)**		
Metabolite	**logFC**	**P-Value**	**adj. P-Value**	**logFC**	**P-Value**	**adj. P-Value**
inosine	1.79	4.44E-06	3.19E-03	0.63	3.57E-03	3.71E-02
hypoxanthine	0.85	2.00E-04	2.87E-02	0.45	2.26E-04	5.92E-03
gamma-glutamylalanine	-0.29	0.000323	0.032301	-0.13	4.71E-03	4.44E-02
benzoylcarnitine	-0.34	3.64E-04	3.23E-02	-0.25	9.15E-06	7.76E-04
retinal	0.30	3.73E-04	3.23E-02	0.13	3.30E-03	3.48E-02
4-hydroxycoumarin	-0.57	5.92E-04	4.48E-02	-0.30	1.10E-03	1.62E-02
tartronate (hydroxymalonate)	-0.27	7.12E-04	4.89E-02	-0.18	3.45E-04	7.65E-03
X-12812	-0.53	7.14E-04	4.89E-02	-0.28	1.28E-03	1.80E-02

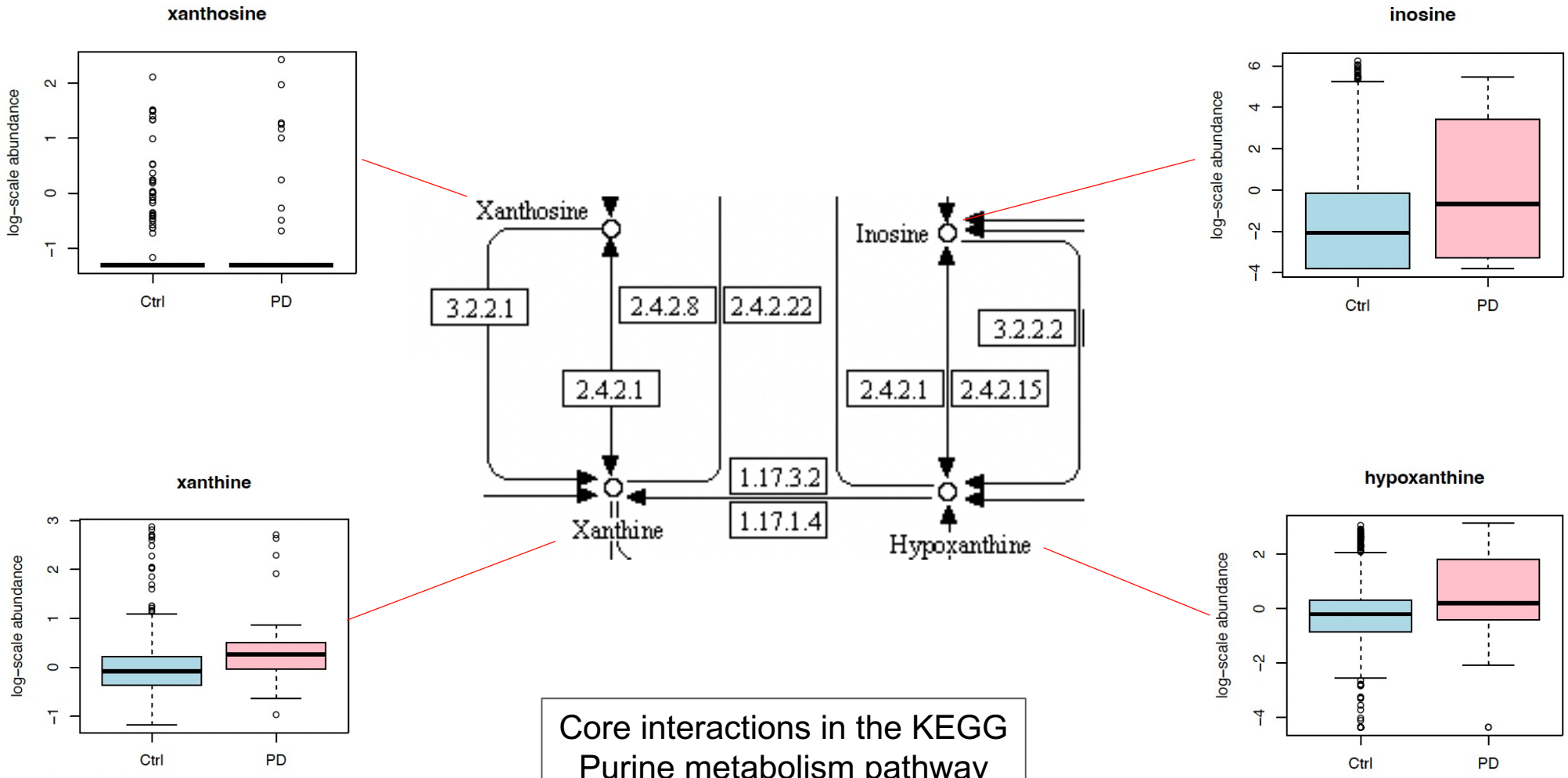
Blue = xanthine metabolites

Pathway enrichment analysis: *de novo* PD vs. control (KEGG)



Enriched in xanthines

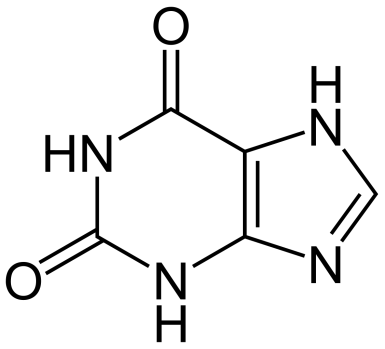
Purine metabolism in *de novo* PD vs. controls



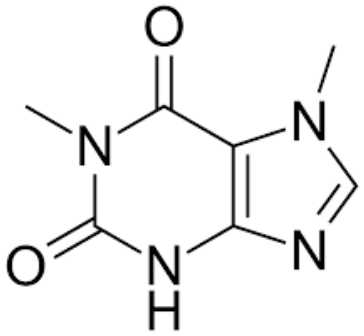
Core interactions in the KEGG Purine metabolism pathway

Prior links between xanthines and PD (1)

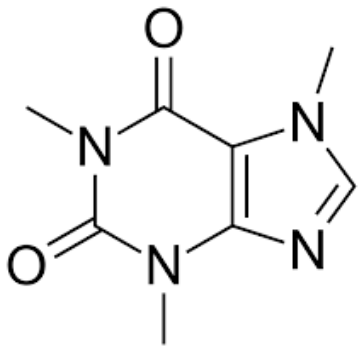
- **Drug candidates**: Xanthine derivatives show benefits in PD patients and mouse models (Xu et al., 2010)
- **Scaffold for drugs**: Xanthine is a sub-structure of various drugs, including Istradefylline (an A2A receptor antagonist)
- **Mechanisms**: - **A2A Receptor Antagonism**: enhances dopaminergic signaling
  - **MAO-B Inhibition**: reduces dopamine breakdown



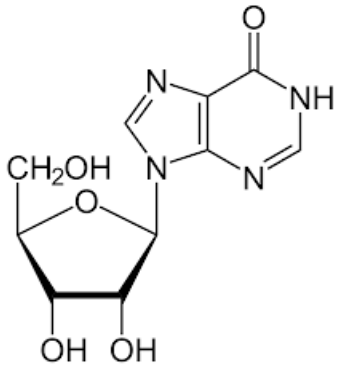
xanthine



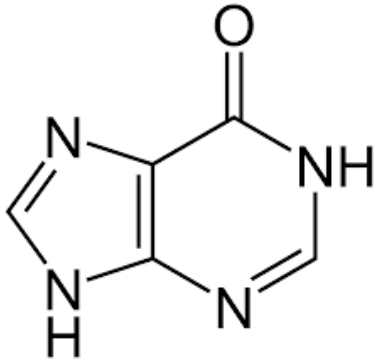
paraxanthine



caffeine



inosine



hypoxanthine

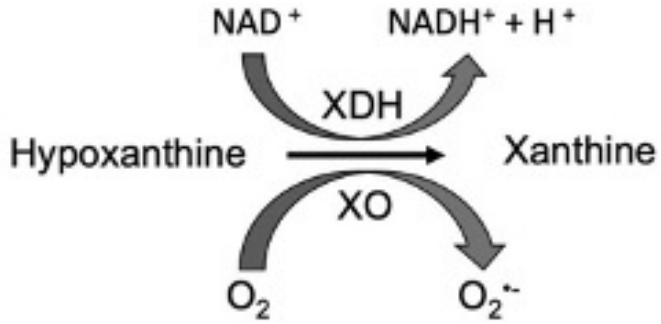
Prior links between xanthines and PD (2)

- **Reactive oxygen species (ROS) generation by xanthine dehydrogenase (XDH)**:

  - XDH converts hypoxanthine to xanthine, producing ROS (O_2^- and H_2O_2)

  - ROS induce oxidative stress, damaging mitochondria and neurons in PD

  - increased levels of XDH observed in PD patients (Çokal et al., 2017)

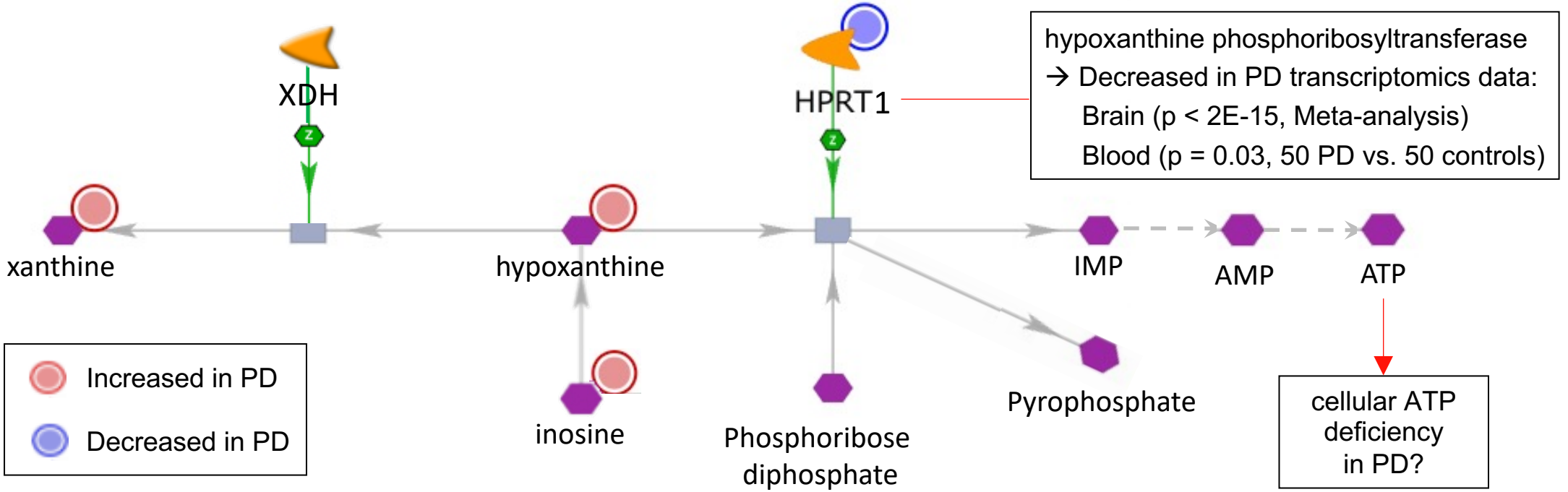


- **Oxidative stress in PD**

  - contributes to loss of dopaminergic neurons in the midbrain

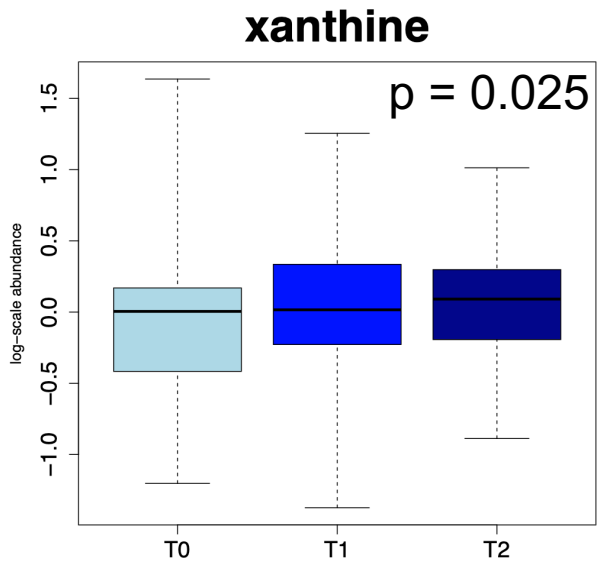
  - decreased gamma-glutamylalanine in PD may affect glutathione metabolism, reducing antioxidant defense

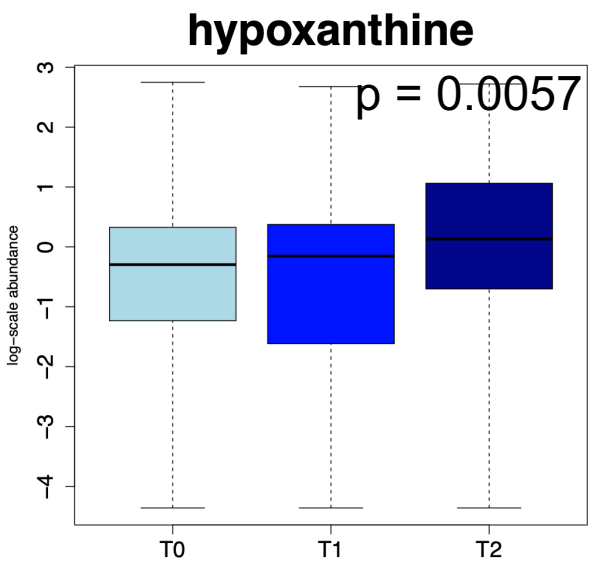
Joint metabolomics & transcriptomics network analysis

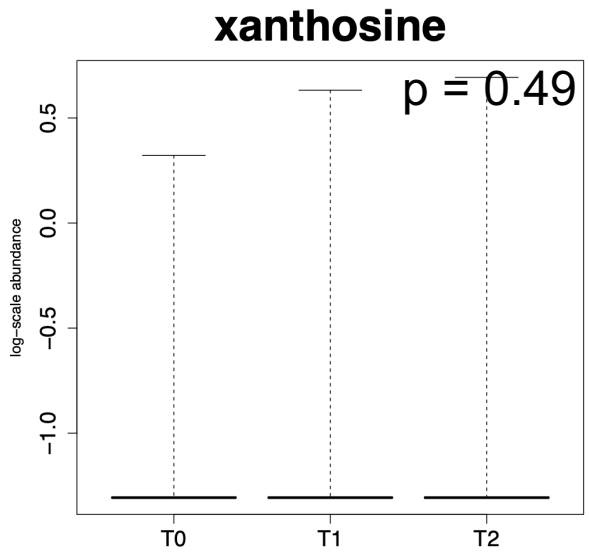


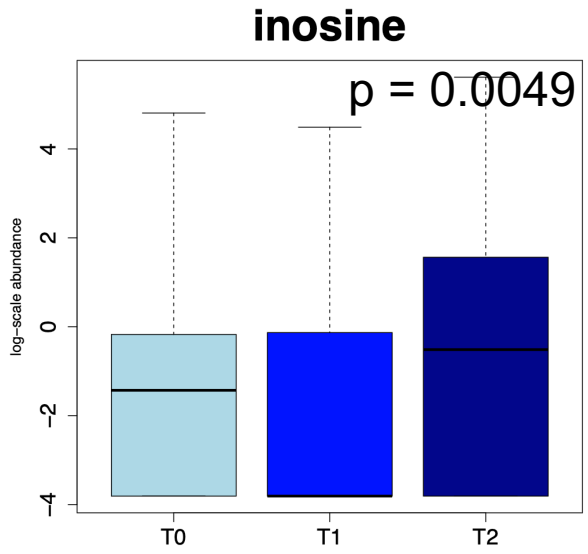
- A genetic **HPRT1** deficiency, Lesch-Nyhan's syndrome, involves loss of dopamine in the basal ganglia and hyperkinetic movements
- **HPRT1** over-expression inhibits DA neuron loss in 6-OHDA mice by activating Wnt/ß-catenin signaling (Jiang et al., Aging, 2020)
- Open-label, single arm trial (26 PD patients): **inosine** + **XDH** inhibitor increased blood hypoxanthine and ATP, and decreased UPDRS III score (Watanabe et al., 2020)

Metabolite changes over time in PD – Xanthine metabolism









Significant increase over time in 3 out of 4 xanthines (Spearman correlation test)

Machine learning: *de novo* PD vs. control classification

Metabolite	Linear AUC (Train)	Linear AUC (Test)	Radial AUC (Train)	Radial AUC (Test)	Avg. AUC
Xanthine	0.68	0.71	0.63	0.72	0.68
2-ketocaprylate	0.58	0.74	0.54	0.78	0.66
Glutarylcarnitine (C5-DC)	0.64	0.68	0.62	0.66	0.65
4-hydroxyphenylpyruvate	0.57	0.68	0.61	0.66	0.63
N-palmitoyl-sphingadienine (d18:2/16:0)	0.57	0.58	0.70	0.65	0.63
3-hydroxyoleoylcarnitine	0.57	0.62	0.64	0.68	0.62
3-hydroxybutyroylglycine	0.61	0.62	0.66	0.60	0.62
Cortisol	0.53	0.70	0.56	0.69	0.62
Hypotaurine	0.60	0.66	0.59	0.63	0.62
Palmitoleoylcarnitine (C16:1)	0.59	0.65	0.57	0.66	0.62

Ranking of the top 10 known metabolite features for supervised sample classification of *de novo* PD vs. control samples, by the sum-of-ranks of the Area Under the ROC Curve (AUC) for linear and radial Support Vector Machine (SVM) classifiers.

Summary

- **Metabolite-level analysis**

  → statistically significant alterations in *de novo* PD vs. controls,
  particularly in xanthines (e.g., inosine, hypoxanthine, xanthine, xanthosine)

- **Pathway- & network-level analysis**

  → coordinated PD-associated changes in purine / xanthine metabolism

  → integrated analysis with transcriptomics data suggests *HPRT1* as a key regulator

- **Machine learning analysis**

  → identified candidate metabolites for *de novo* PD vs. control discrimination,
  xanthine is top-ranked predictor

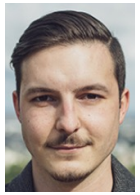
Thank you!

Quentin Klopfenstein
(Machine learning)

Elisa Gomez
(Pathway/network analysis)

Muhammad Ali
(Statistics, network analysis)

Varsha Murthy
(Animal model analyses)



Cyril Brzenczek
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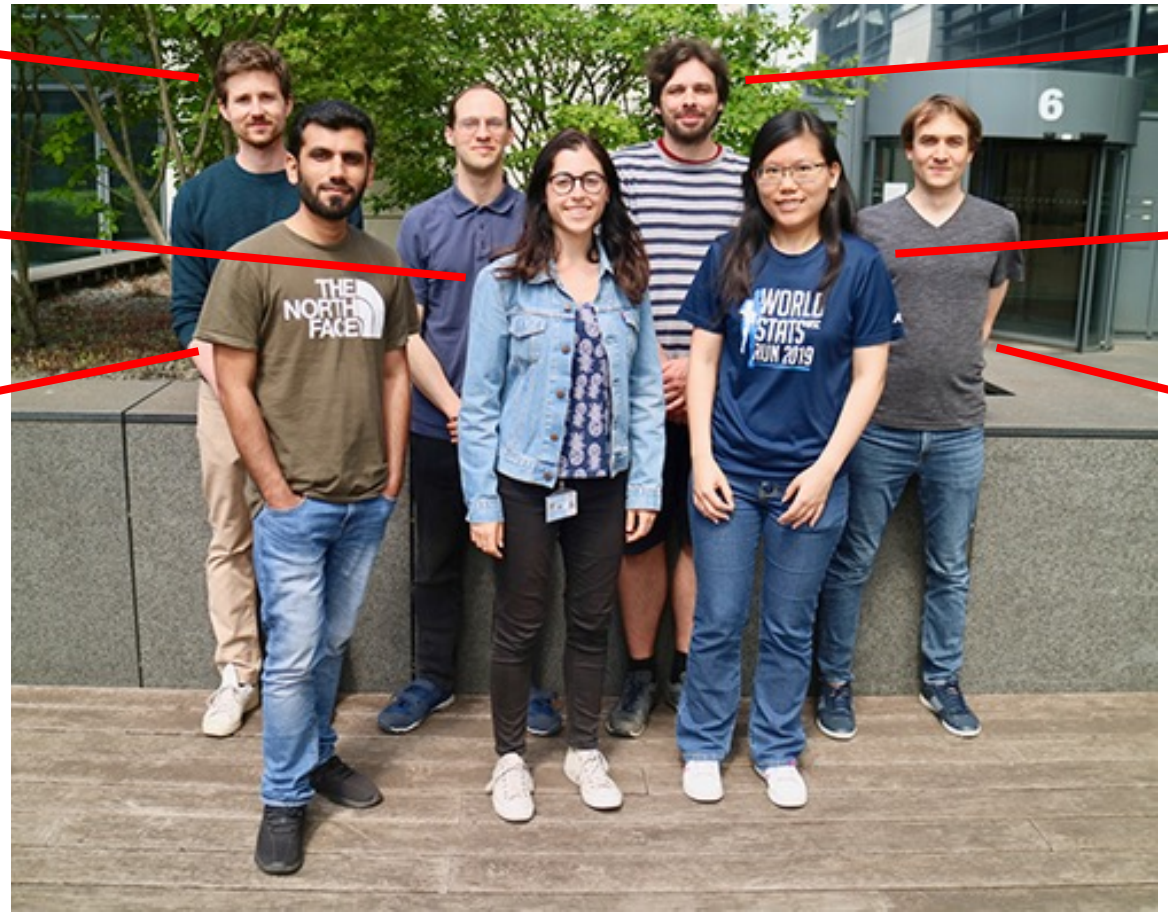

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Rebecca Ting Jiin Loo
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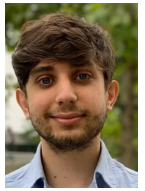
Armin Rauschenberger
(Statistics, Machine learning)



Francesco Nasta
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Sophie Lebars
(Network analysis and modeling)

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