Leids Universitair
Medisch Centrum

Multi-omics analysis powered by massive data integration

RE(ACT) congress
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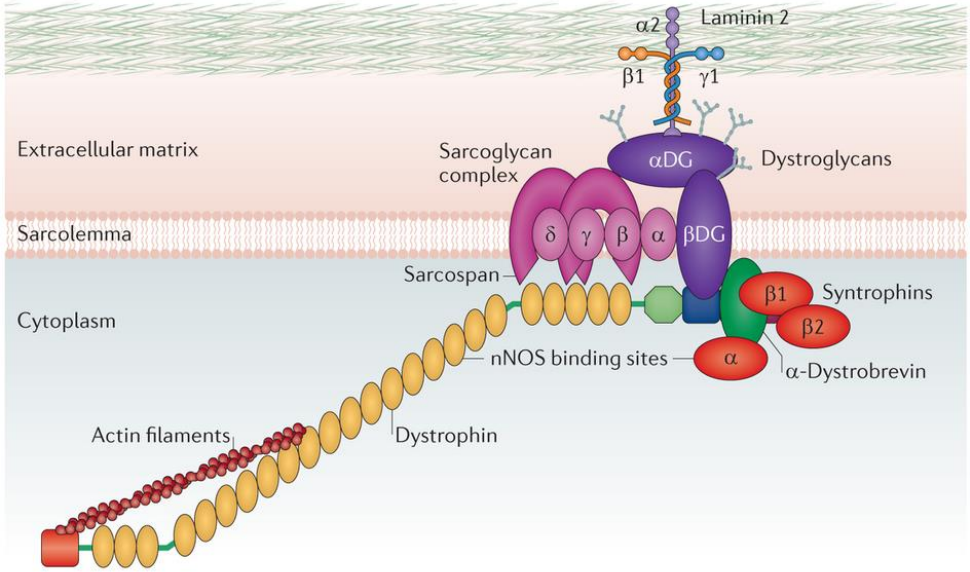
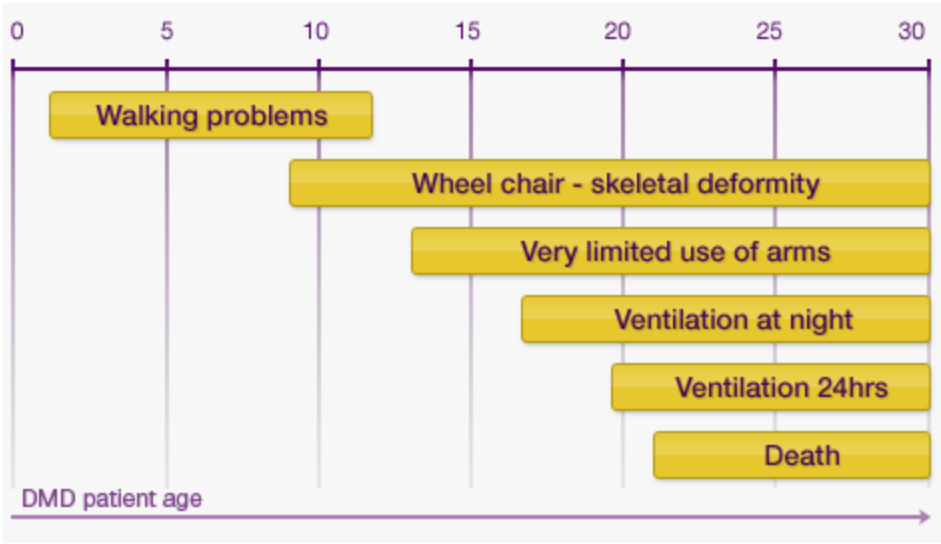
LEIDEN UNIVERSITY MEDICAL CENTER

Duchenne muscular dystrophy (DMD)

- X-linked
  - Mutations in dystrophin - in DMD patients dystrophin is virtually absent; Becker muscular dystrophy patients have 10% to 40% of the normal amount
  - Muscle atrophy, inflammation, fibrosis, fat deposits
- Incidence 1:5000, costs: $80.000 - $120.000 p.p/p.y



Nature Reviews | Genetics



https://kin450-neurophysiology.wikispaces.com/Duchenne+Muscular+Dystrophy

Role of muscle biopsies in clinical trials

- Muscle biopsies are often requested in interventional clinical trials to show proof of concept that the drug is working e.g. dystrophin restoration after gene therapy, but muscle biopsies are invasive for the patient

Can bio-signature detection in blood offer an alternative?

- Identification of blood biomarkers able to monitor disease progression and response to therapy would enable:
  - Better marketing authorization of medicinal products for DMD and muscular dystrophies in general
    - The analysis of blood should support drug developers to show the drug is working (or not working) without taking muscle biopsies

Aim

Gain insights into the effect of dystrophin deficiency on a molecular level

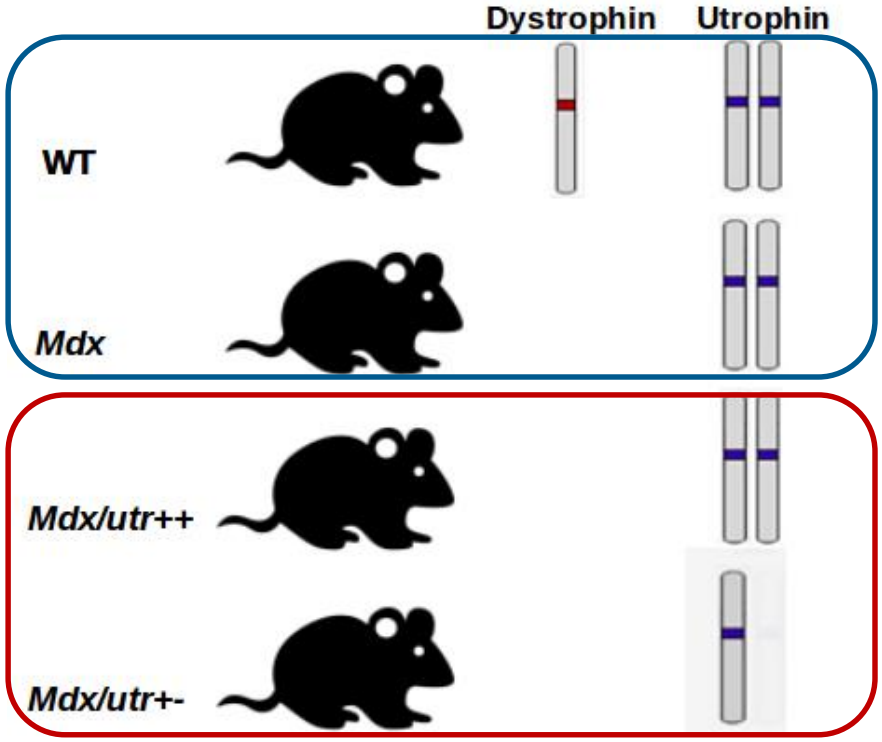
identification of novel biomarkers for disease severity and progression

Research question

Identify groups of genes that remain active and drive the pathology

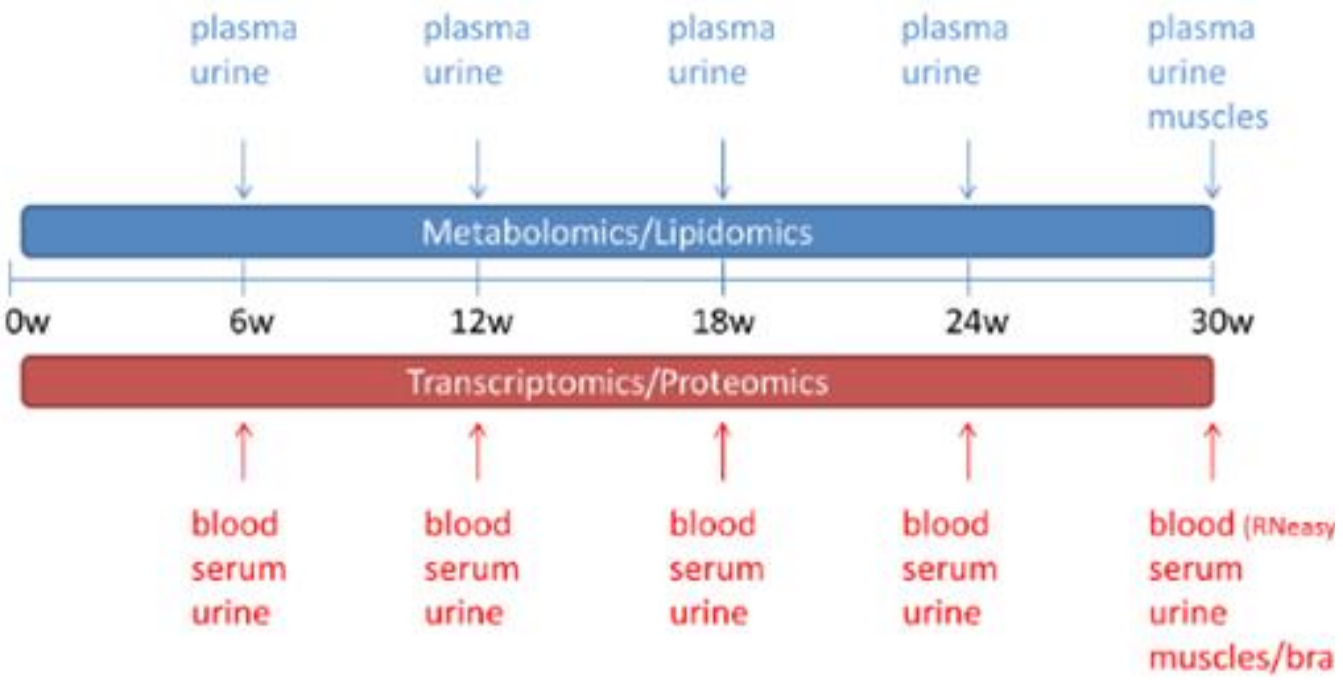
Dystrophin-deficient *mdx* mice

- Bred at the LUMC
- DMD mouse model
  - Nonsense mutation *dystrophin*
- Capable of producing functional analog *Utrophin*
  - Less severe symptoms



Experimental design

- *mdx* mice:
  - Samples at 6, 12, 18, 24 and 30 weeks of age
  - Sacrifice at week 30
  - 4 groups, 5 - 6 mouse per group
  - C57BL or hybrid

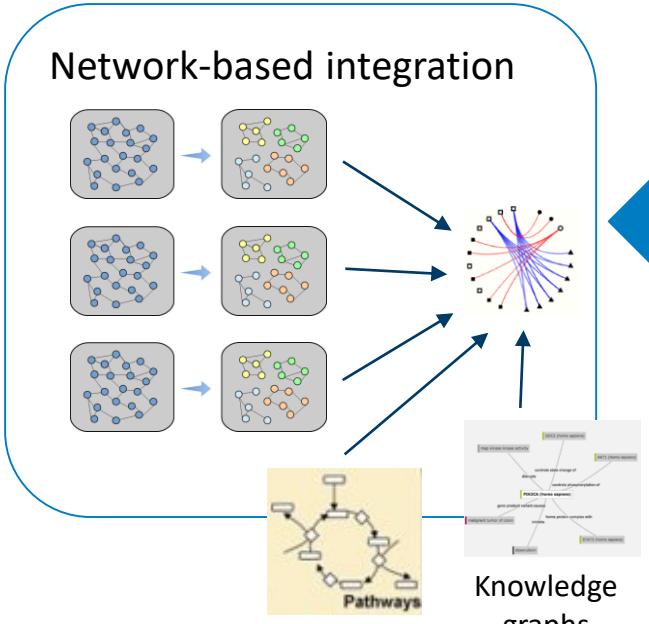


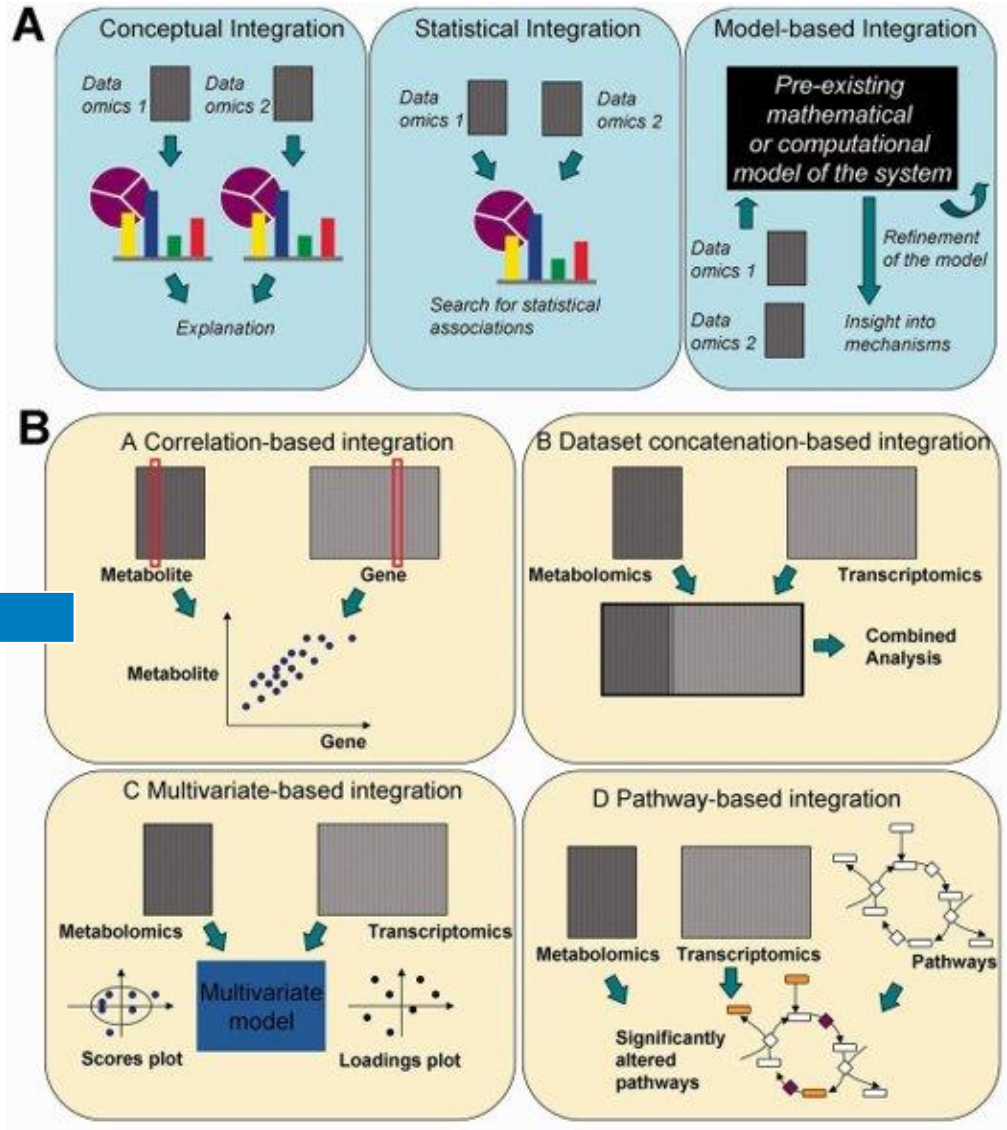
Multi-omics data integration, networks and knowledge discovery

Problems:

- Different sizes of data sets
- Multiple testing burden

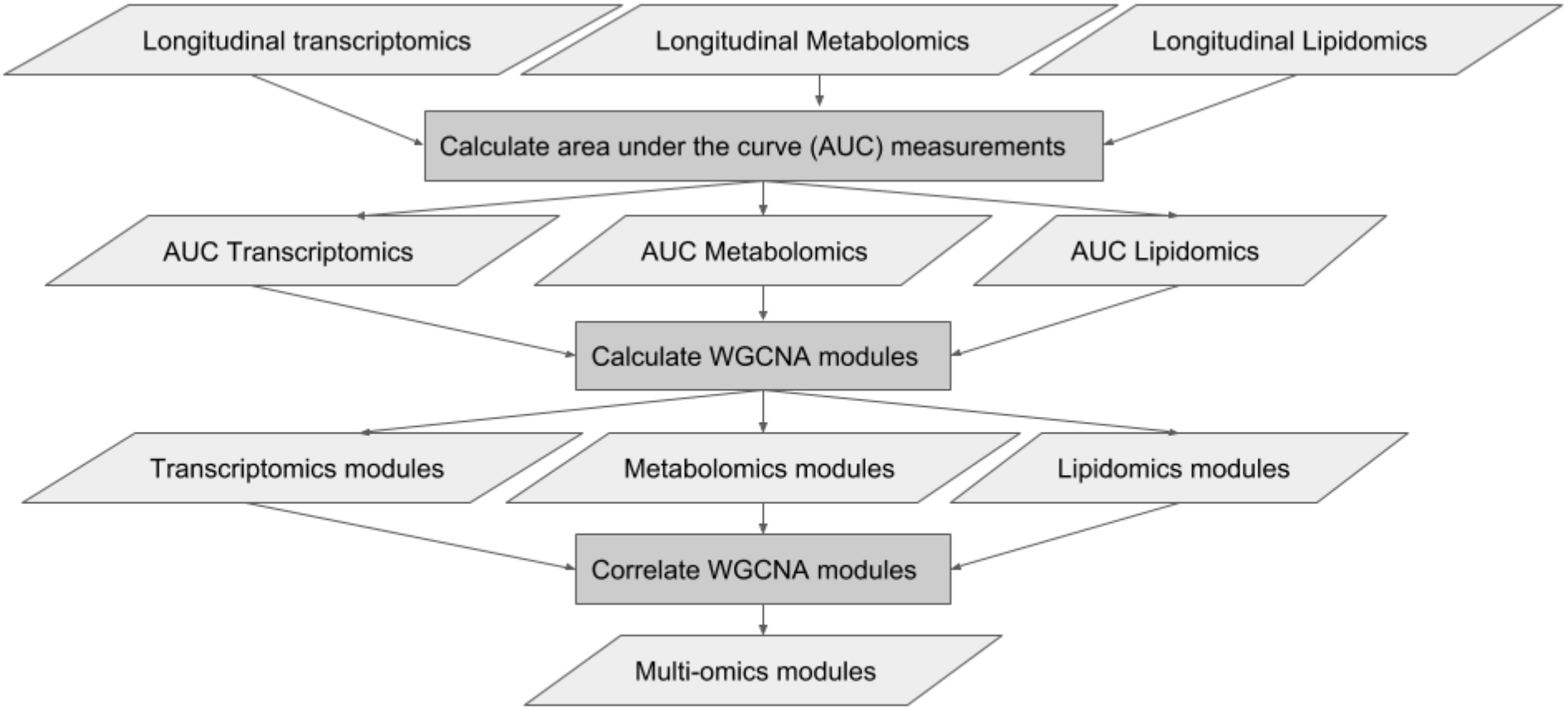
Solution:





Rachel Cavill et al. Brief Bioinform, Dec 2015;bib.bbv090

Approach *mdx* study

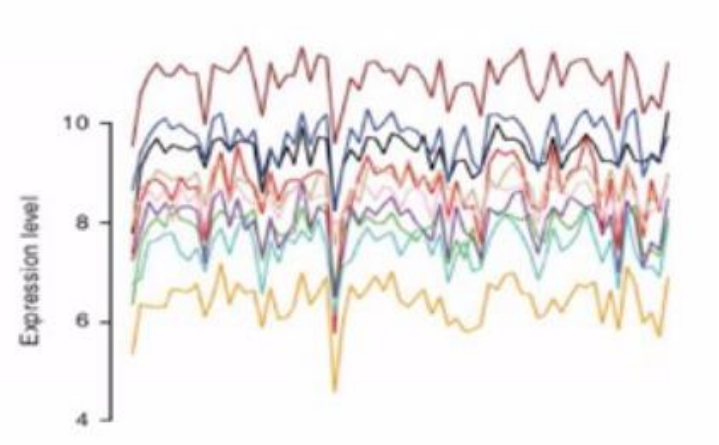


AUC=area under the curve
WGCNA=weighted gene co-expression analysis

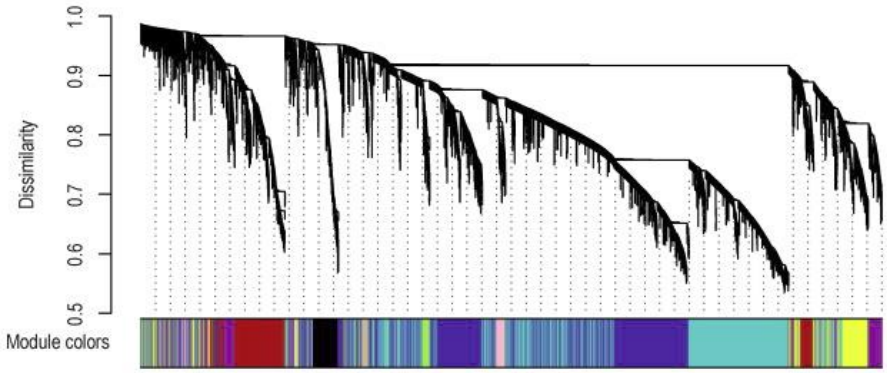
=data =process

Weighted correlation network analysis (WGCNA)

1. Calculate co-expression values



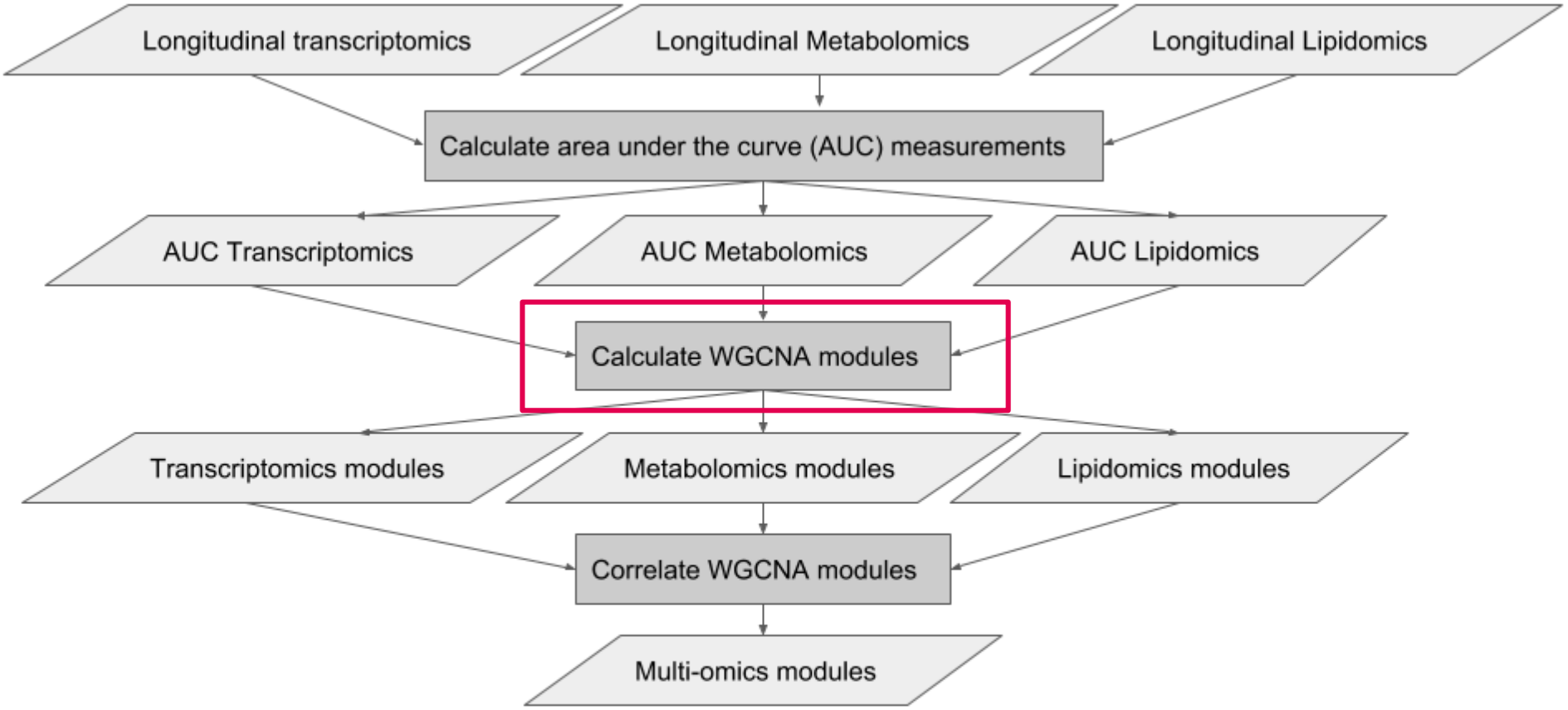
2. Cluster co-expression values



3. Relate modules (lists of genes, metabolites or lipids) to genotype, phenotype, or clinical parameters

Langfelder and Horvath, 2008

Approach



AUC=area under the curve
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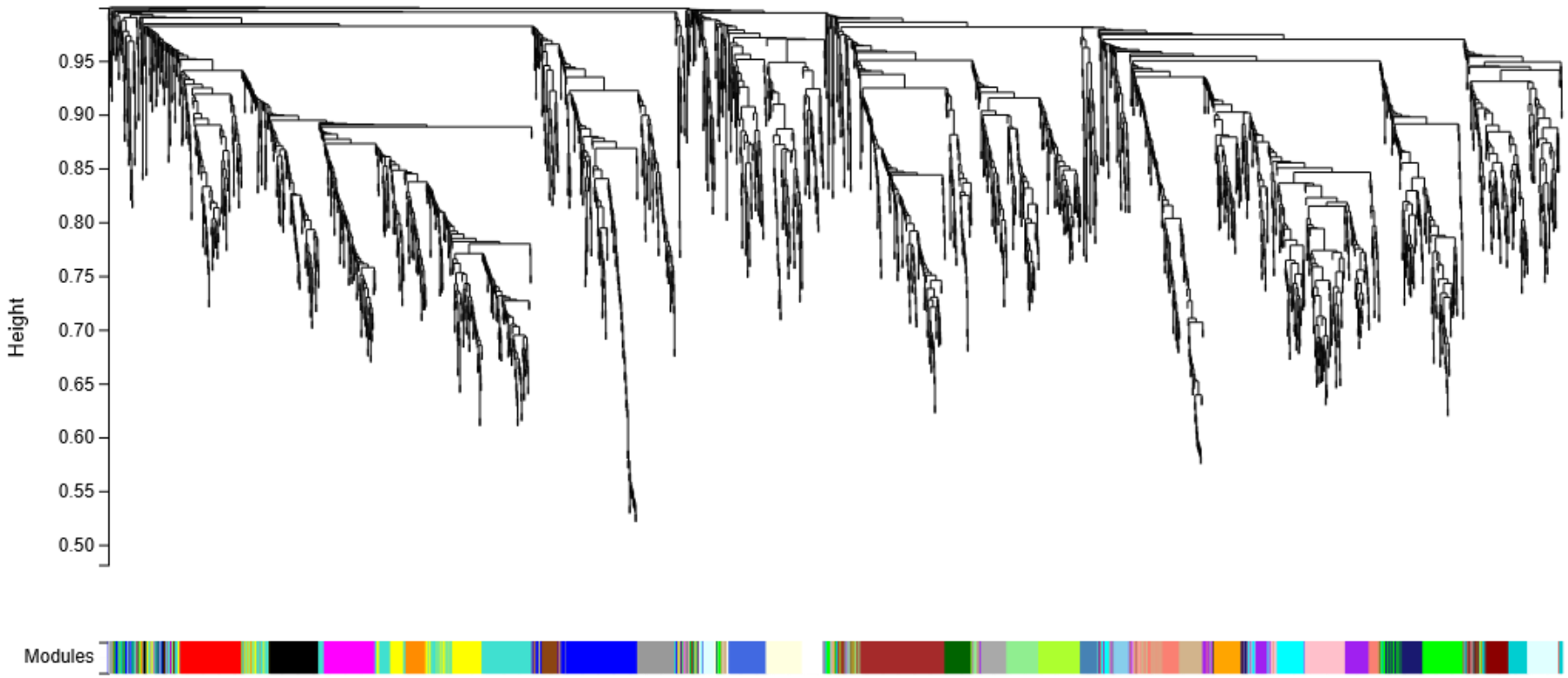
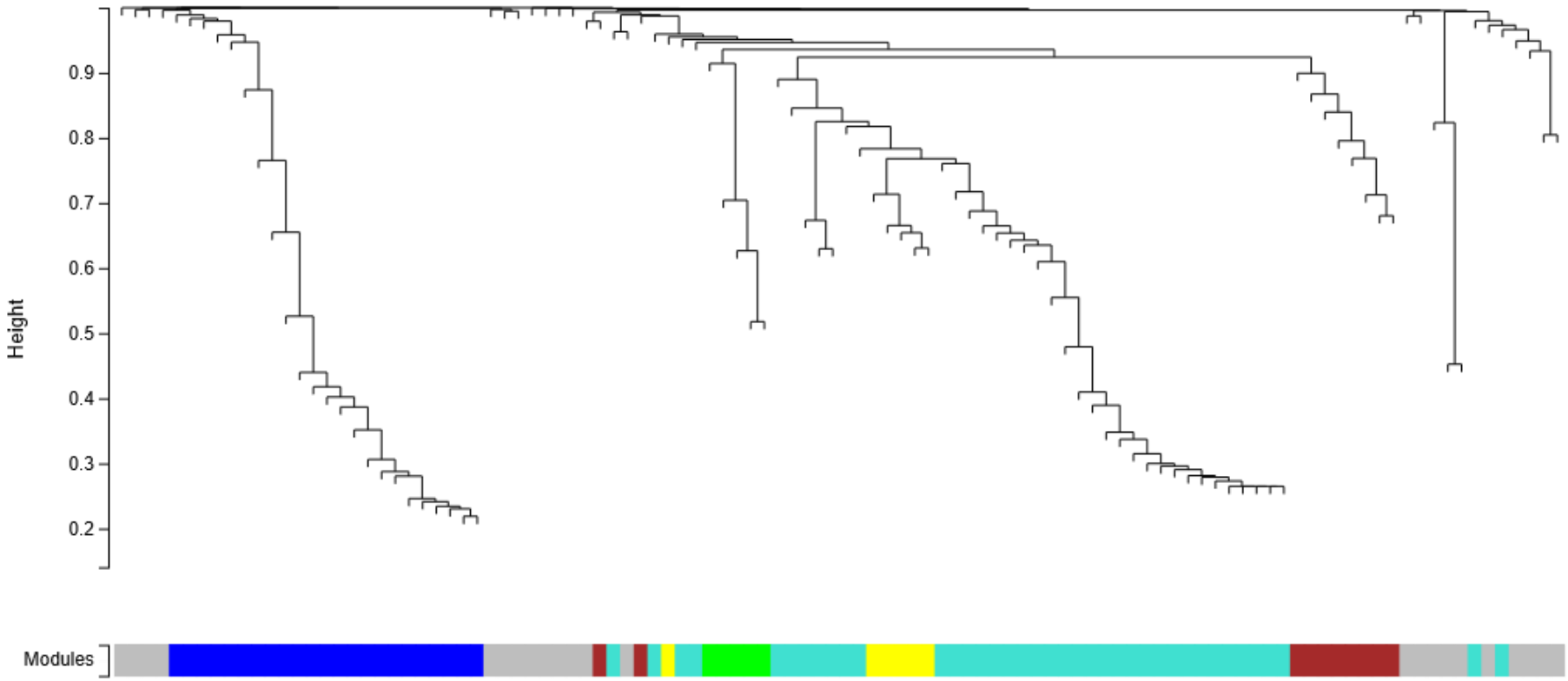
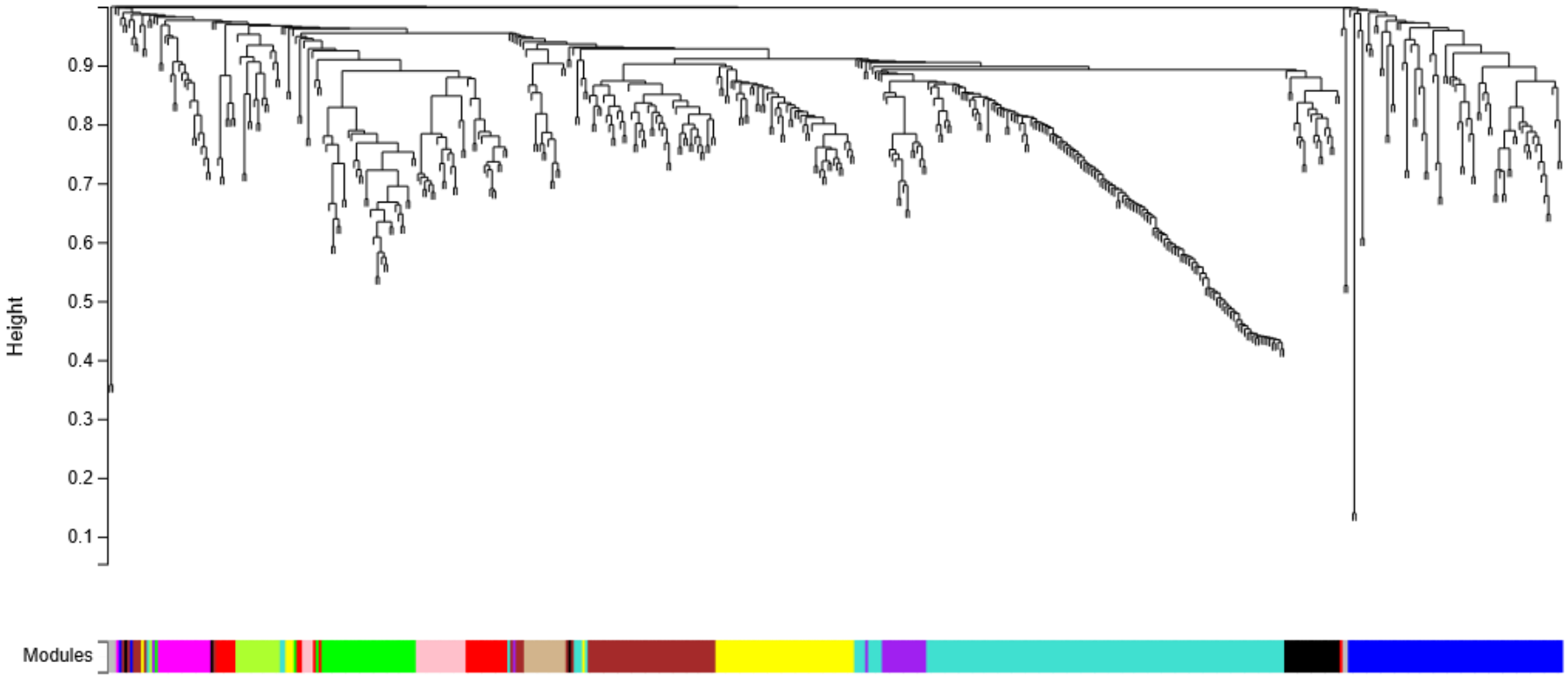
Transcriptomics modules



Metabolomics modules



Lipidomics modules

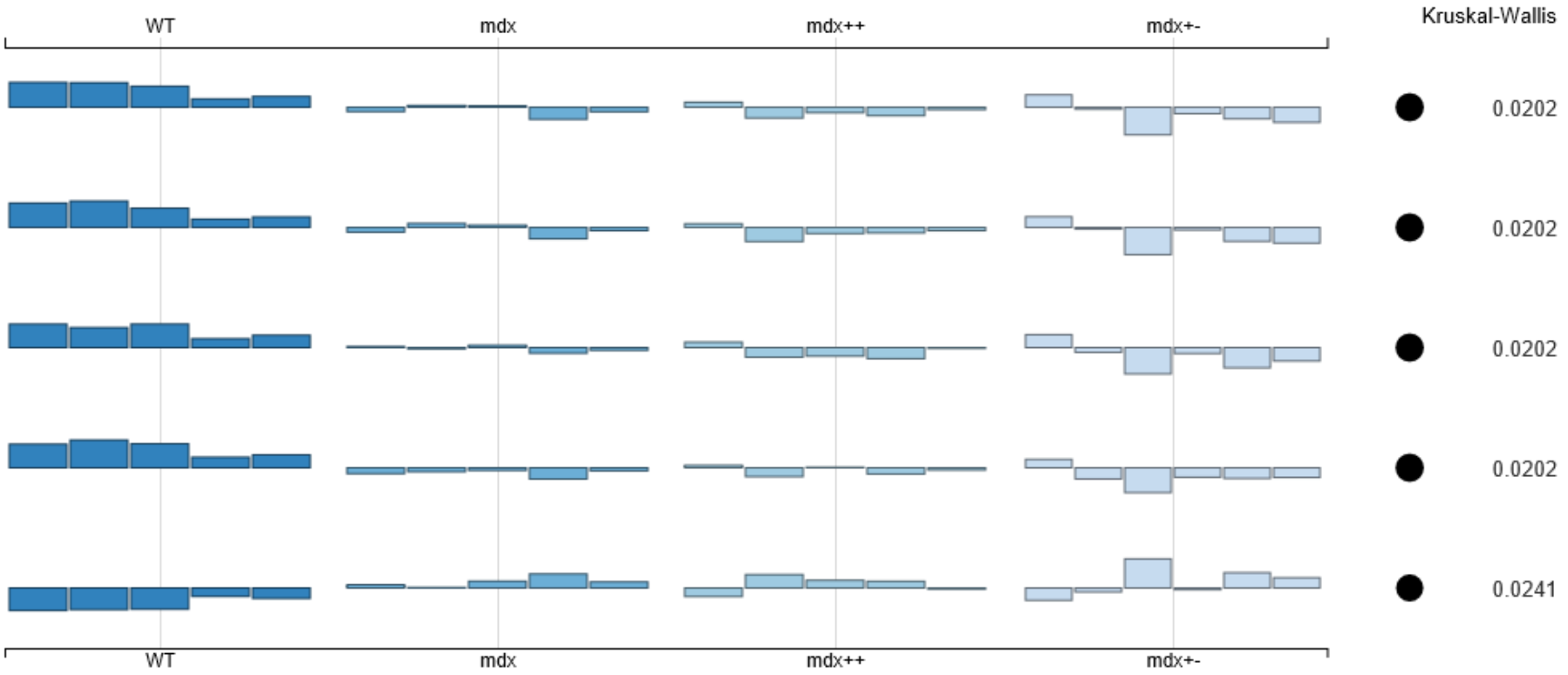


Calculate significance

- Does a module have any relation to the disease?
- Calculate module eigengenes
  - The first principal component of the standardized expression profiles
- Module eigengenes not always normally distributed
  - Calculate significance by comparing the group means of the module eigengene values using the parametric Kruskal-Wallis test
  - Significance ($p < 0.05$) indicates a general difference in expression of the module's molecules between one or more group pairs (e.g. wt vs mdx)

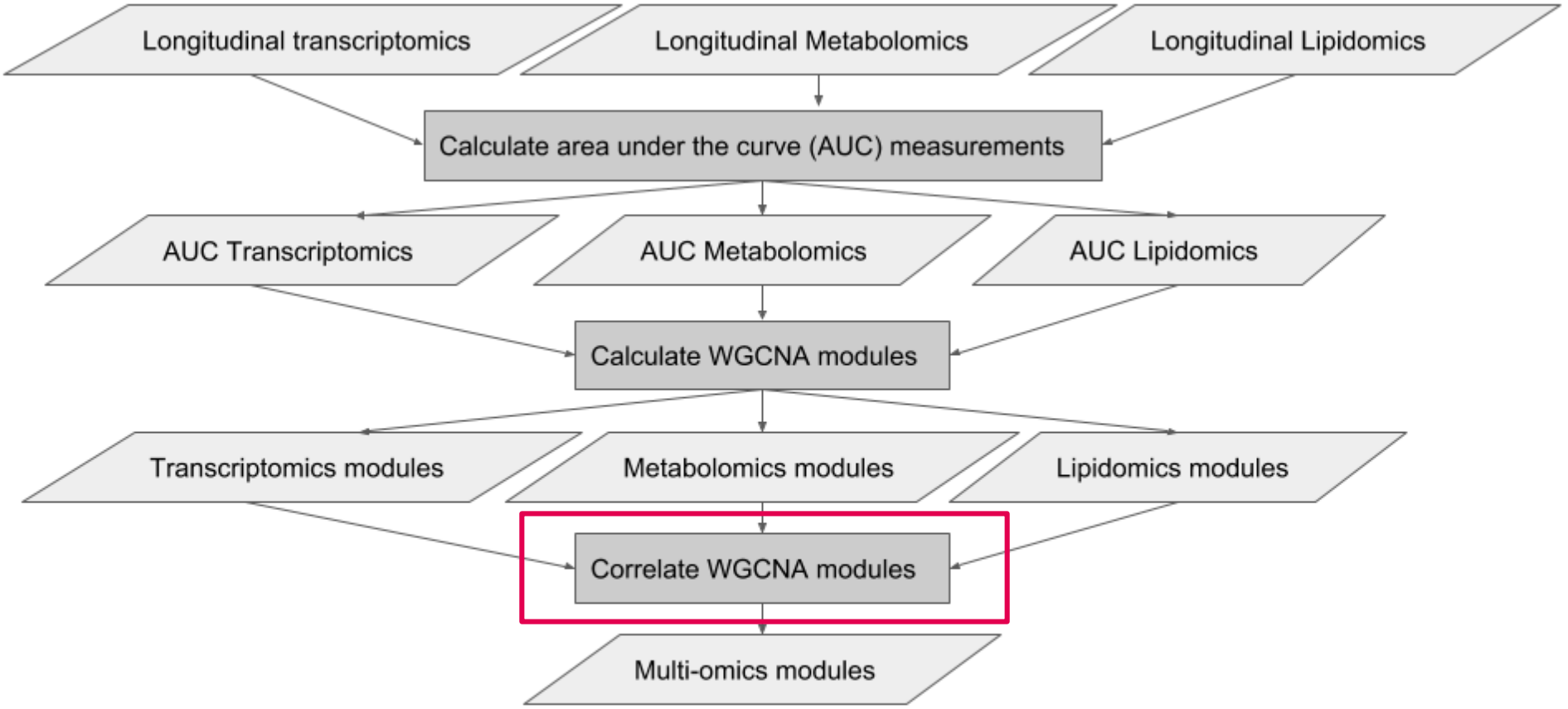
All metabolomics modules significant, all but one lipidomics module, no transcriptomics modules

Example: metabolomics eigengene plots



mdx+/+ = mdx/utrn+/+
mdx+/- = mdx/utrn+/-

Approach

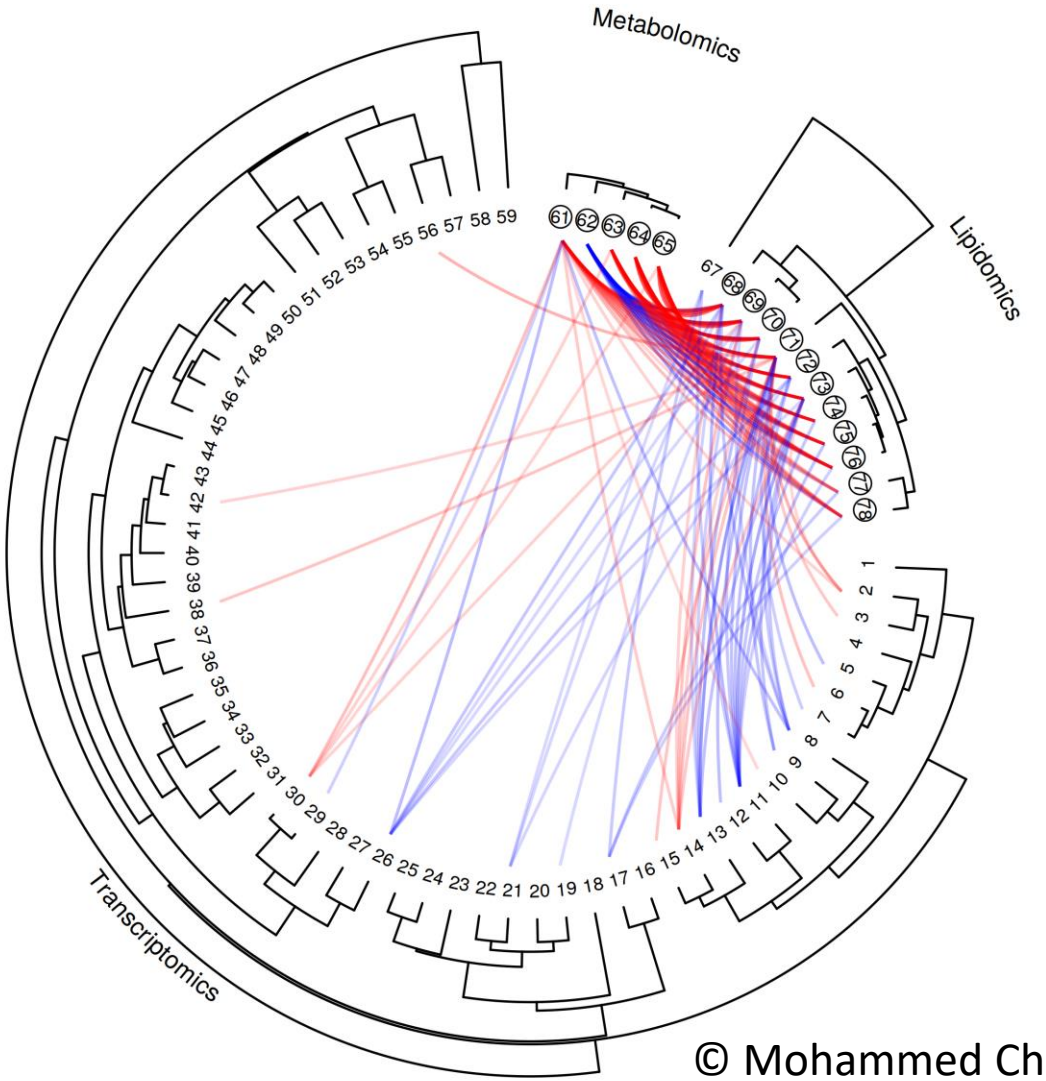


AUC=area under the curve
WGCNA=weighted gene co-expression analysis

=data =process

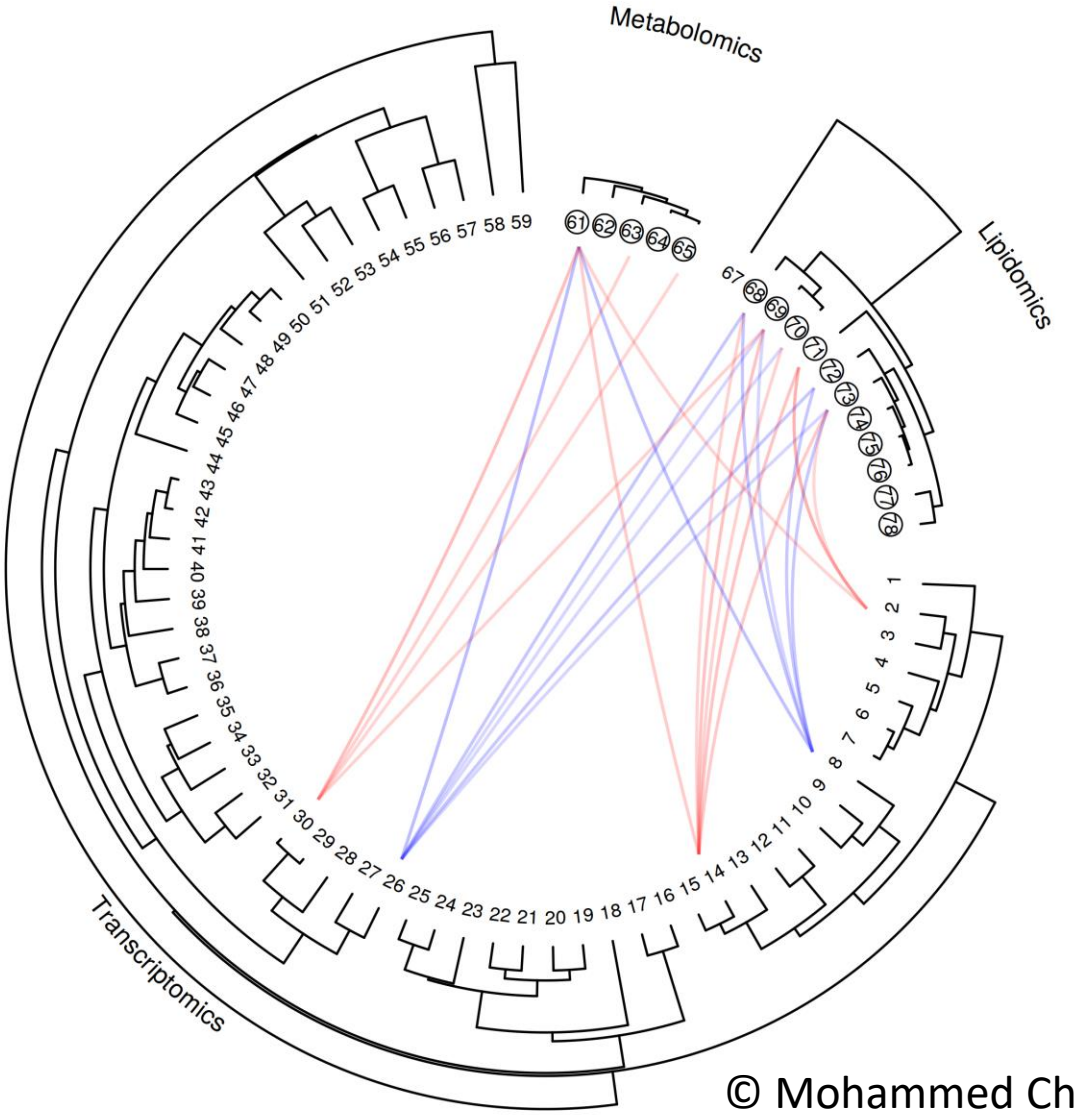
Cross-correlation between module eigengenes reveals multi-omic correlations

Module eigengene: the first principal component of the standardized expression profiles



Encircled numbers: significant
Red line: positive correlation
Blue line: negative correlation

Cross-correlation filtered for three-way omics

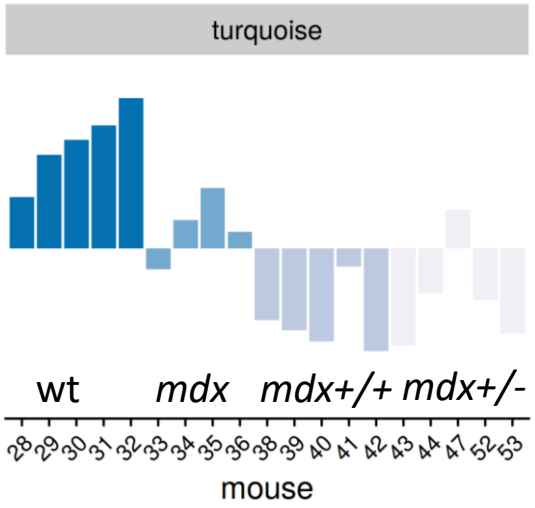


Red line: positive correlation
Blue line: negative correlation

Cross-correlation filtered for three-way omics

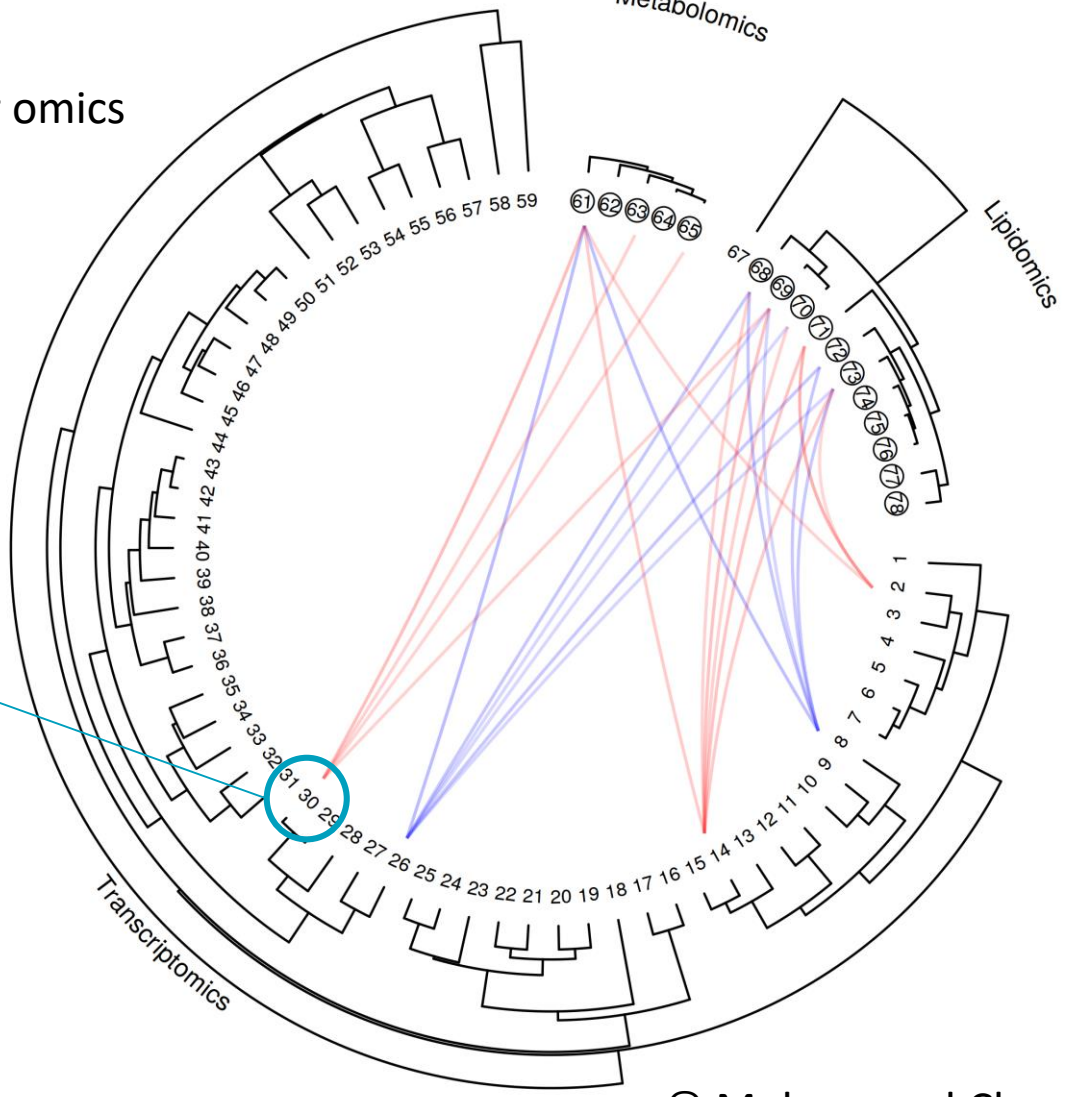
Turquoise module

- Highly connected
- Significant correlations to other omics
  - 0.55 (p=0.01)
  - 0.48 (p=0.04)
  - 0.47 (p=0.04)
  - 0.48 (p=0.04)

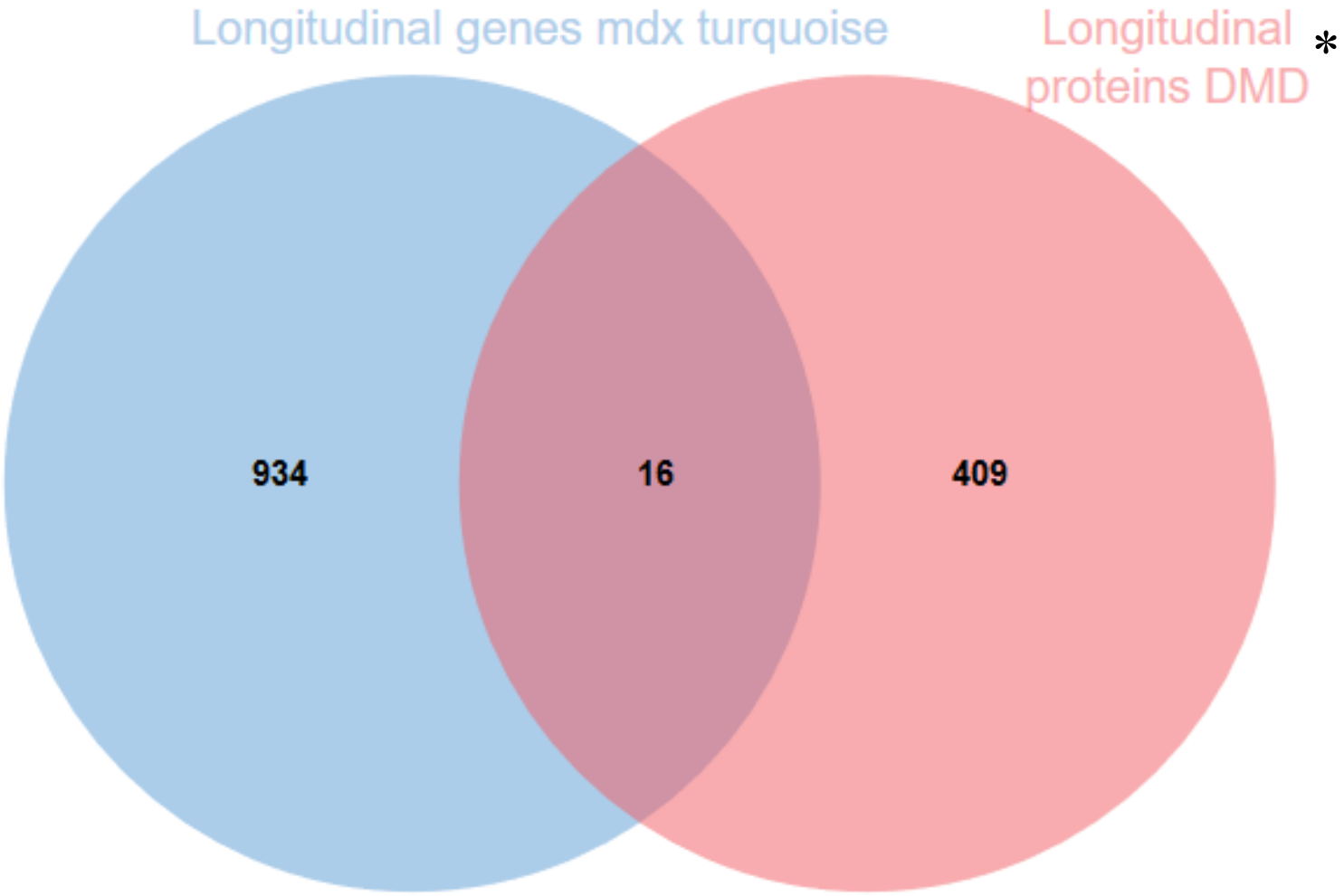


Red line: positive correlation
Blue line: negative correlation

mdx+/+ = mdx/utrn+/+
mdx+/- = mdx/utrn+/-



Overlap with longtitudinal proteomics in DMD patients



*Proteins changed over time in serum from DMD patients from Spitali et al. 2018. *Under review*.

Physiologic functional annotation with Euretos

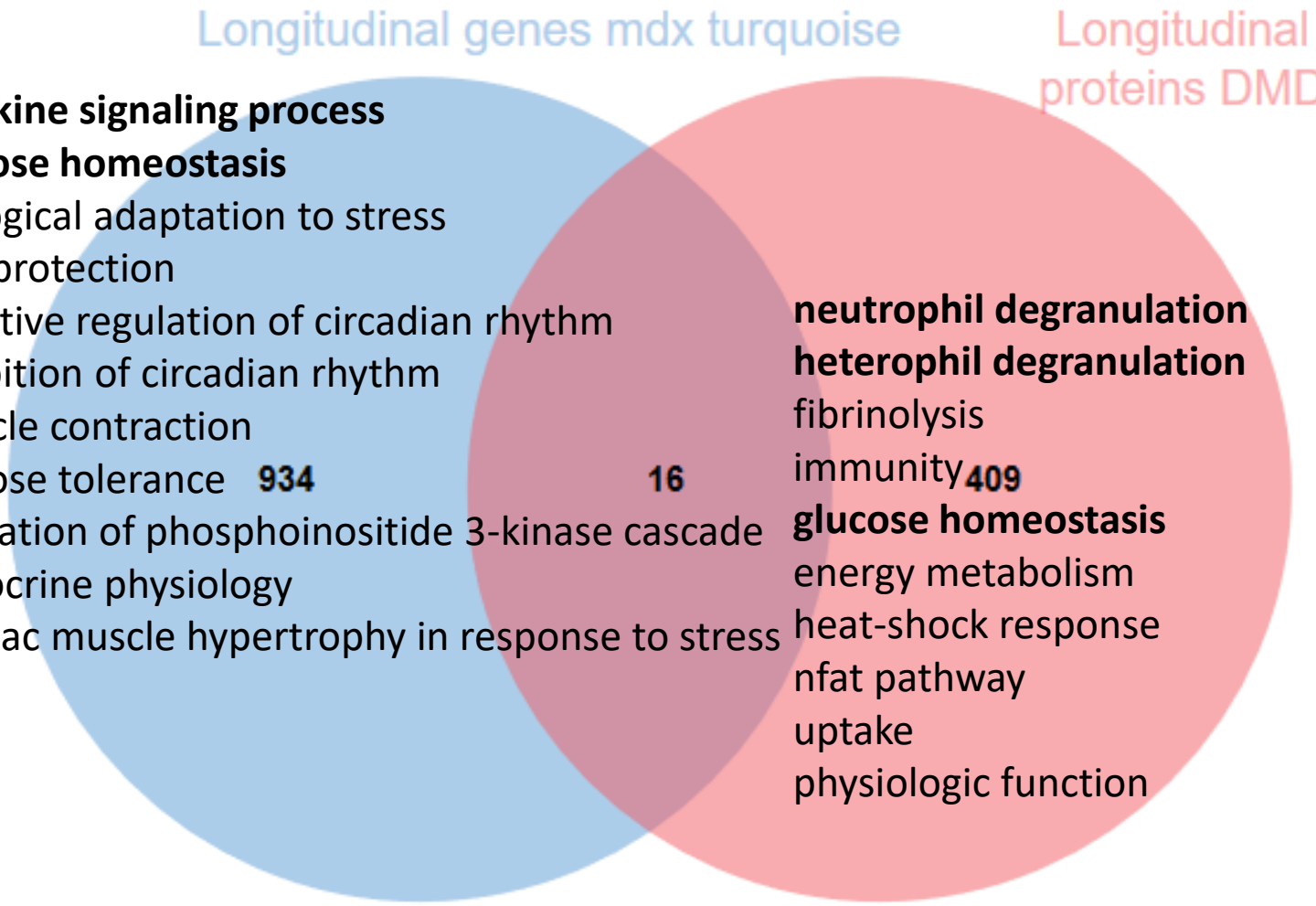
Data Sources: 200+ http://www.euretos.com/files/EuretosSources2018.pdf

Phenotype <–> Pathways <–> Proteomics <–>Metabolomics<–> Genetics

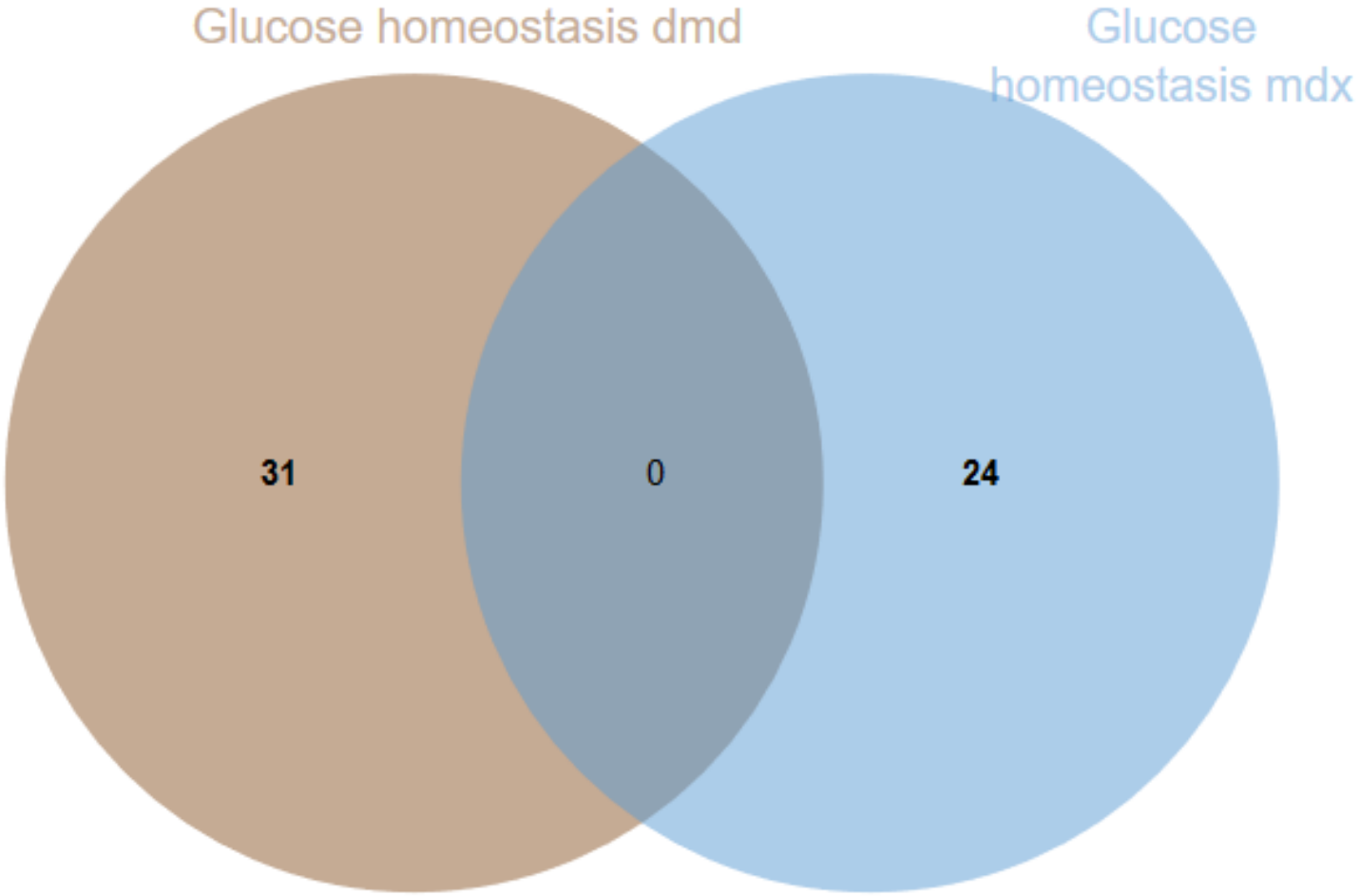
EURETOS

Knowledge Platform

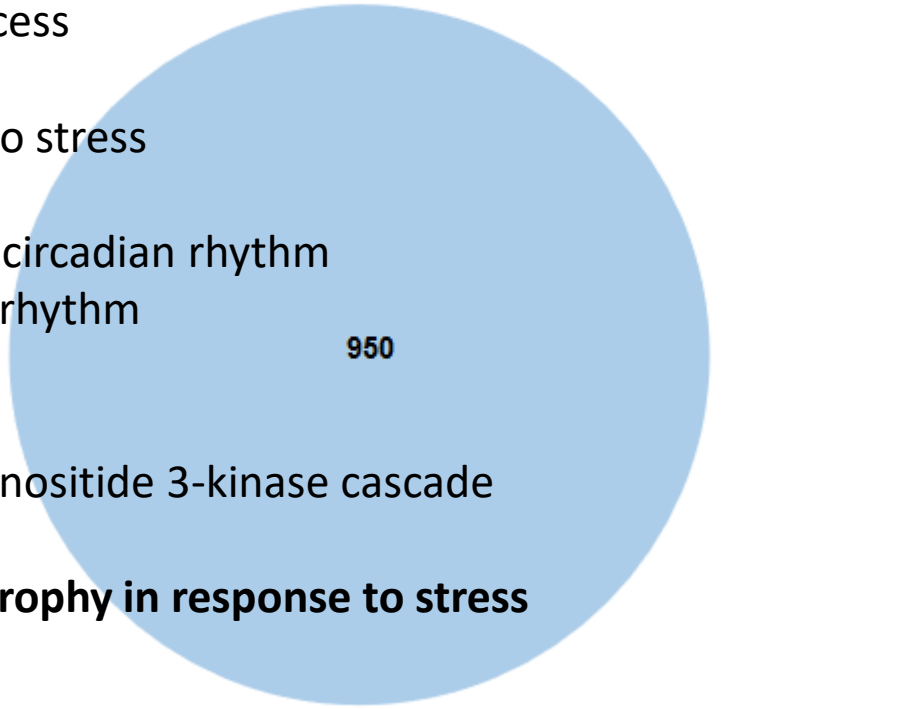
Euretos physiologic function annotation reveals annotation overlap



Functional annotation overlap does not have to mean gene overlap



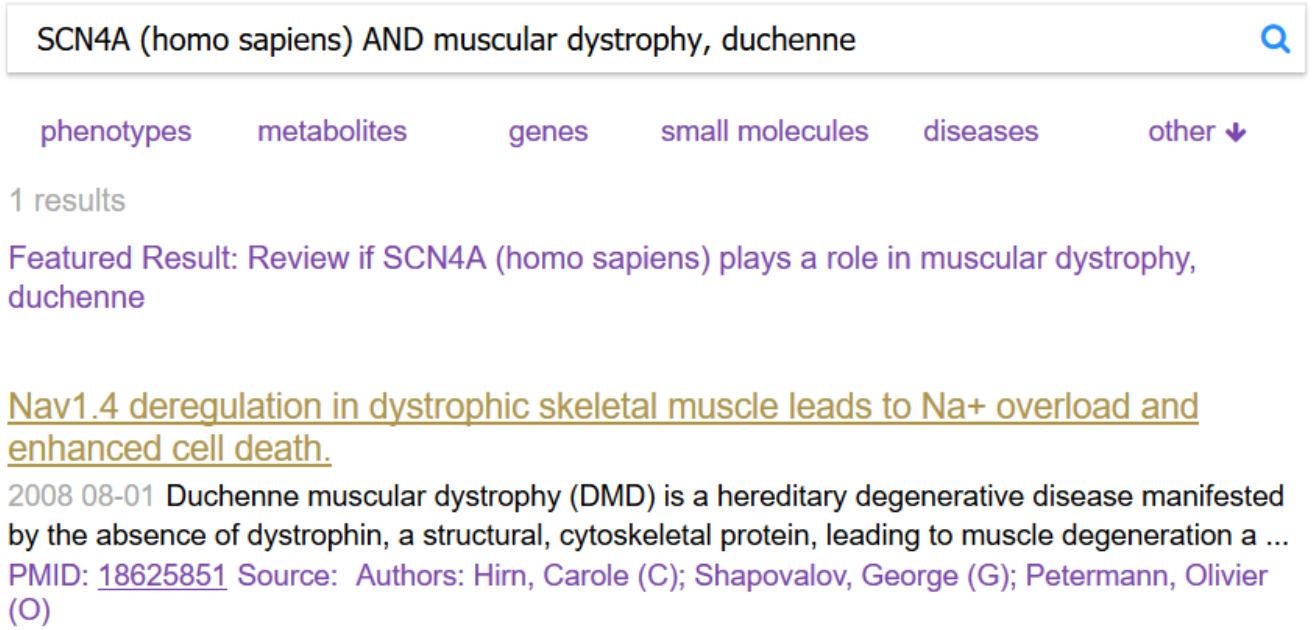
Euretos physiologic function annotation reveals muscle-related processes



cytokine signaling process
glucose homeostasis
biological adaptation to stress
cytoprotection
negative regulation of circadian rhythm
inhibition of circadian rhythm
muscle contraction
glucose tolerance
activation of phosphoinositide 3-kinase cascade
endocrine physiology
cardiac muscle hypertrophy in response to stress

Digging deeper with gene-disease analysis: SCN4A from muscle contraction process

EURETOS



Inspired by: Hettne KM. PLoS One. 2016 Feb. doi:10.1371/journal.pone.0149621.

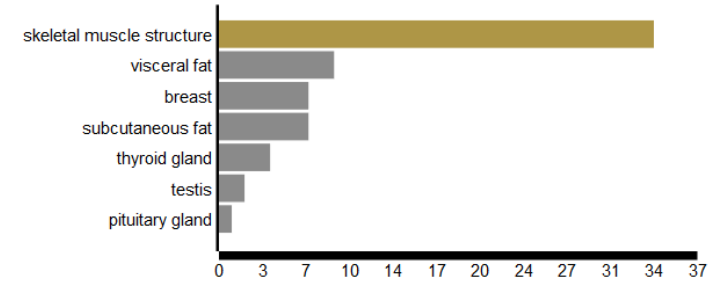
SCN4A is expressed in healthy muscle

Role of SCN4A (homo sapiens) in muscular dystrophy, duchenne

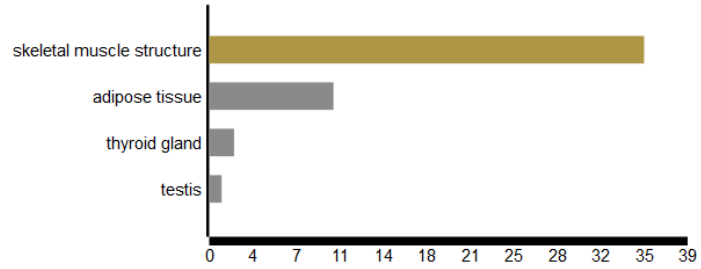
SCN4A (homo sapiens) has the following RNA and protein expression patterns in healthy tissues. The top 3 tissues associated with muscular dystrophy, duchenne are highlighted. See the information panel above for more details.

RNA Expression (FPKM)

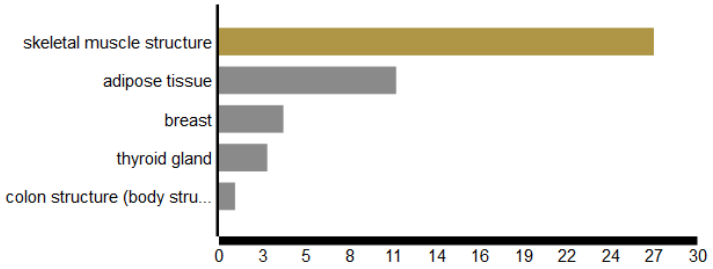
GTEx



Human Protein Atlas



Illumina Body map



Fantom 5

Summary

- Disease-related biomolecular modules were found in mouse blood metabolomics and lipidomics data
- Cross-correlation between module eigengenes revealed multi-omic correlations
- Significantly correlated turquoise transcriptomics module was related to cytokine production, glucose homeostasis and muscle contraction

- *In conclusion:*
  - Multi-omics network analysis reveals a muscular dystrophy-related signature in mouse blood

Data management

- When published: data in public repositories, scripts on GitHub
- Now:
  - Scripts on LUMC GitLab
  - Metadata and result data files on FAIR Data Point
  - WGCNA web tool by Mohammed Charrout creates report with parameters used on GitHub
    - https://github.com/mochar/wgcna

Next steps

- Drug repurposing
  - In progress, integrative pipeline to match targets with drugs in place
- Uptake in the RD-Connect platform
  - Multi-omics analysis pipeline under construction
  - Will incorporate lessons learned from the past years multi-omics analyses:
    - **Huntington's Disease** (transcriptomics, metabolomics, lipidomics; mouse [1] and human [2])
    - **Duchenne muscular dystrophy** (proteomics, transcriptomics, metabolomics, lipidomics; mouse [3] and human [4])
    - **SCA3** (transcriptomics, metabolomics, lipidomics; mouse [5])
    - **Beta-thalassemia** (transcriptomics, proteomics, human [6])

[1] Mina E, et al. Orphanet J Rare Dis. 2016 and *in preparation*.
[2] Mastrokolias A, et al. Metabolomics. 2016. PMID: 27524956
[3] Hettne K, et al. *in preparation*.
[4] Spitali P, et al. J Cell Mol Med. 2018 and *under review*.
[5] Toonen L, et al. *under review.*
[6] Katsantoni E, et al. *in preparation*.

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Thank you

BioSemantics/Bioinformatics (LUMC)

Mohammed Charrout, Eleni Mina, Marco Roos, Mark Thompson, Kees Burger, Rajaram Kaliyaperumal, Barend Mons, Annika Jacobsen

Peter A.C. 't Hoen (since Feb 2018 Radboud UMC)

DMD exon skip group (LUMC)

Pietro Spitali, Annemieke Aartsma-Rus

Medical Statistics (LUMC)

Roula Tsonaka

Profilomic/MedDay

Alexandre Seyer