progetti @

Network analysis integrating miRNA expression profiling with MRI biomarkers and clinical data for prostate cancer early detection:

a proof-of-concept study

FEDERICA CONTE, PHD

Institute of Systems Analysis and Computer Science (IASI) "A. Ruberti"

CNR - Roma

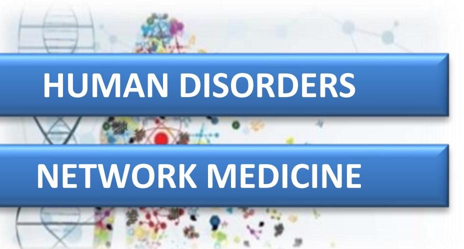
RESEARCH ACTIVITY

Systems Biology & Modeling Analysis

- K-RAS TUMORS
- mRNA HALF-LIVES
- INSULIN SIGNALING
- CANCER RADIOTHERAPY
- NEURODEGENERATIVE DISEASES

Bioinformatics & Network Analysis

- VITICULTURE



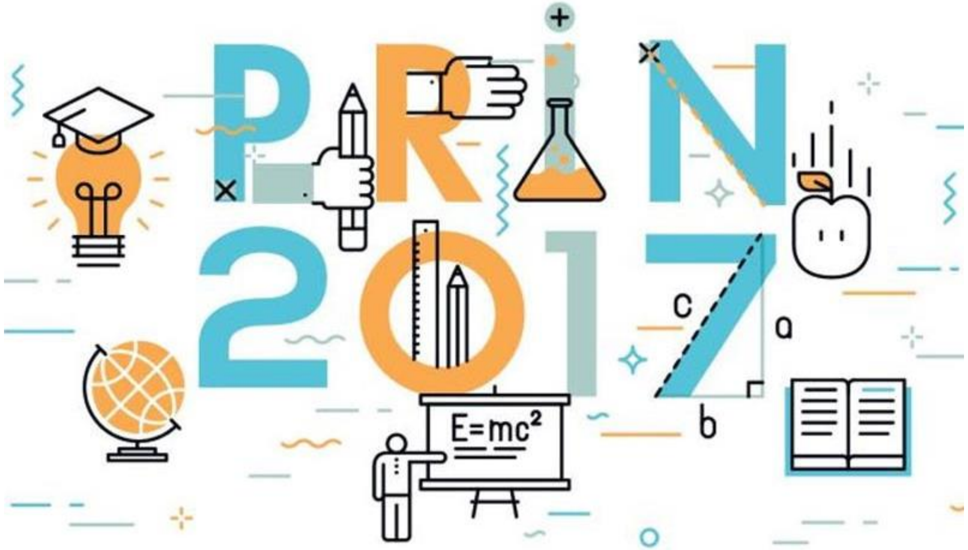
- HUMAN DISORDERS
- NETWORK MEDICINE

COLLABORATIONS

UNIVERSITA' DEGLI STUDI DI MILANO BICOCCA
IBFM
UNIVERSITAS CATHOLICA SACRI CORDIS JESU MEDIOLANI
SAPIENZA UNIVERSITÀ DI ROMA
MODIAG Modeling for Diagnostic Genomics and NeuroScience

IGA ISTITUTO DI GENOMICA APPLICATA
UNIVERSITÀ DEGLI STUDI DI VERONA
UNIVERSITY OF TRIESTE
SAPIENZA UNIVERSITÀ DI ROMA
Bambino Gesù OSPEDALE PEDIATRICO
UMBERTO I POLICLINICO DI ROMA
ISTITUTO DI RICERCA SDN IRCCS

MiUr



MIUR.AOODGRIC.REGISTRO_PRIN2017.0003798.29-03-2018

Ministero dell'Istruzione dell'Università e della Ricerca

Dipartimento per la formazione superiore e per la Ricerca

Direzione Generale per il Coordinamento, la promozione e la valorizzazione della Ricerca

PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2017

Prot. 20178L3P38

UNIVERSITÀ DEGLI STUDI DI VERONA

IGA
ISTITUTO DI GENOMICA APPLICATA

2. Research project title

Regulation of gene expression in grapevine: analysis of genetic and epigenetic determinants

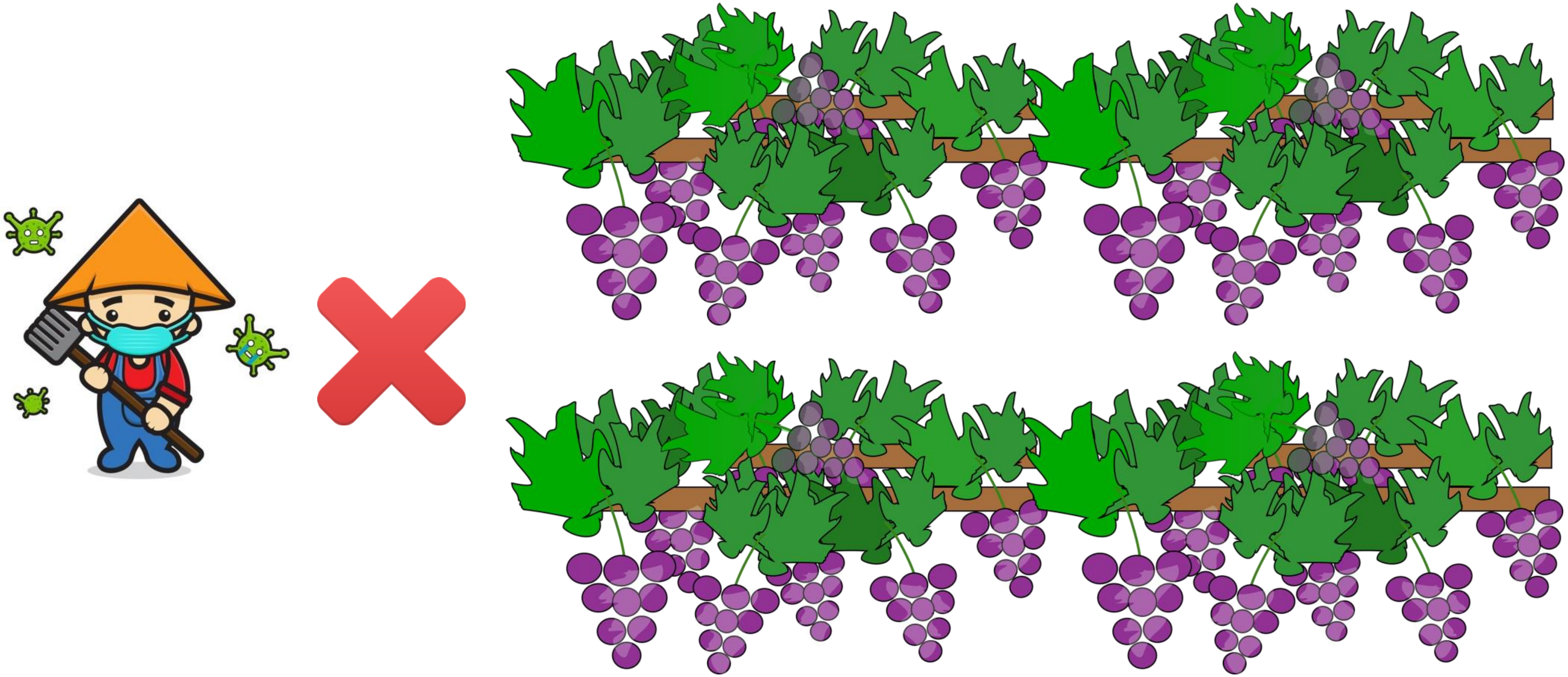
Personnel of the research unit: Paola Paci

Research proposal

The aim of this project is to analyse in depth the grapevine genome (~500 Mbp) to search for putative regulatory regions and cis-regulatory variants with a range of wet lab and bioinformatic approaches that will use biochemical, evolutionary and genetic data to look at both genetic and epigenetic factors involved in gene regulation.

Objective of our research unit

The task of our research unit is the in silico identification of regulatory regions in the grapevine genome and the integration, through bioinformatics approaches, of different types of data (RNASeq for gene expression, ChIPSeq for histone modifications, ATACSeq for chromatin accessibility, BSSeq for DNA methylation, DAPSeq for transcription factor binding site identification). Up to now, our research activity focused on the **development of a pipeline for analyzing RNA-seq data**. This will not only provide basic information on the levels and patterns of gene expression, but will also allow us to evaluate the correlation between the transcriptional activity of genes and epigenetic information when it becomes available during the duration of the project.



DELAYED

1. Network-based approaches

Why we need networks

- **Networks are everywhere…**

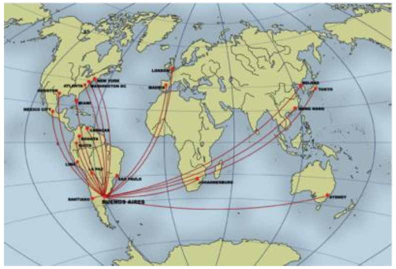
- Molecular networks
- Cell-cell communication
- Nervous systems
- Social networks

- **Networks are powerful tools…**

- Reduce complexity
- More efficient than tables
- Great for data integration
- Intuitive visualization


Social network


Computer network: internet

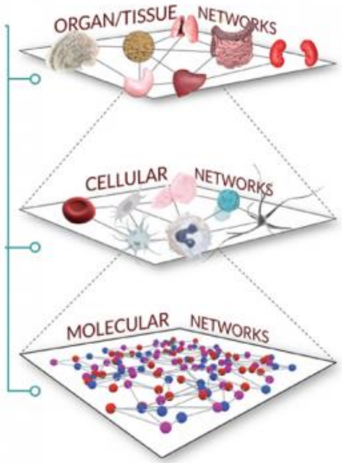

Network of airports


Networks of underground lines: London tube map

Why we need networks

- **Networks are everywhere...**
especially in biology!

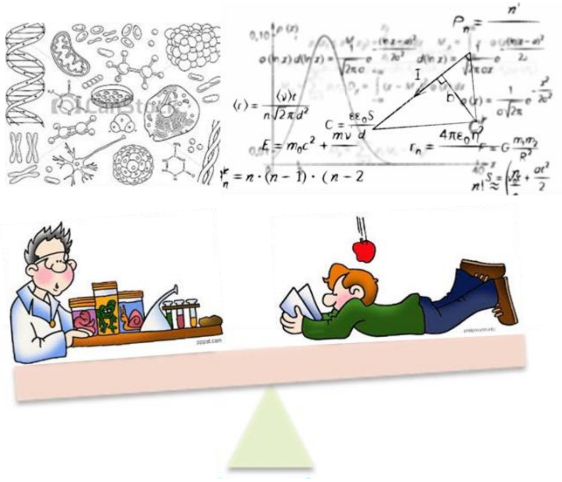
- Molecular networks
- Cell-cell communication
- Nervous systems
- Social networks



Goldman, et al., Frontiers in Physiology 2015

- **Networks are powerful tools...**
especially in biology!

- Reduce complexity
- More efficient than tables
- Great for data integration
- Intuitive visualization
- Common language



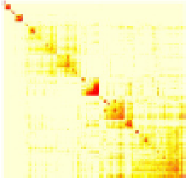
2. Weighted Gene Co-expression Network Analysis (WGCNA)

Input Data

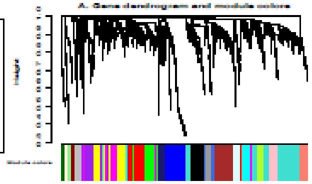
- **Gene expression data:**
  - Microarray
  - RNA-Seq
- **Clinical/phenotypical traits** from the same samples (optional):
  - Weight, race, stage, level of glucose, ecc.

WGCNA workflow

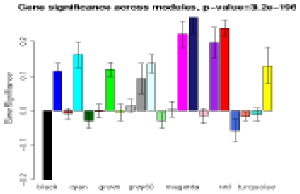
Construct a gene co-expression network
Rationale: make use of interaction patterns among genes
Tools: correlation as a measure of co-expression



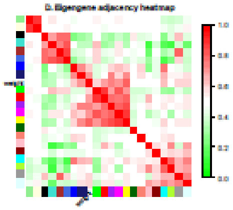
Identify modules
Rationale: module (pathway) based analysis
Tools: hierarchical clustering, Dynamic Tree Cut



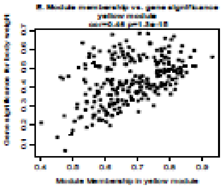
Relate modules to external information
Array Information: clinical data, SNPs, proteomics
Gene Information: ontology, functional enrichement
Rationale: find biologically interesting modules



Study module relationships
Rationale: biological data reduction, systems-level view
Tools: Eigengene Networks



Find the key drivers in *interesting* modules
Rationale: experimental validation, biomarkers
Tools: intramodular connectivity, causality testing

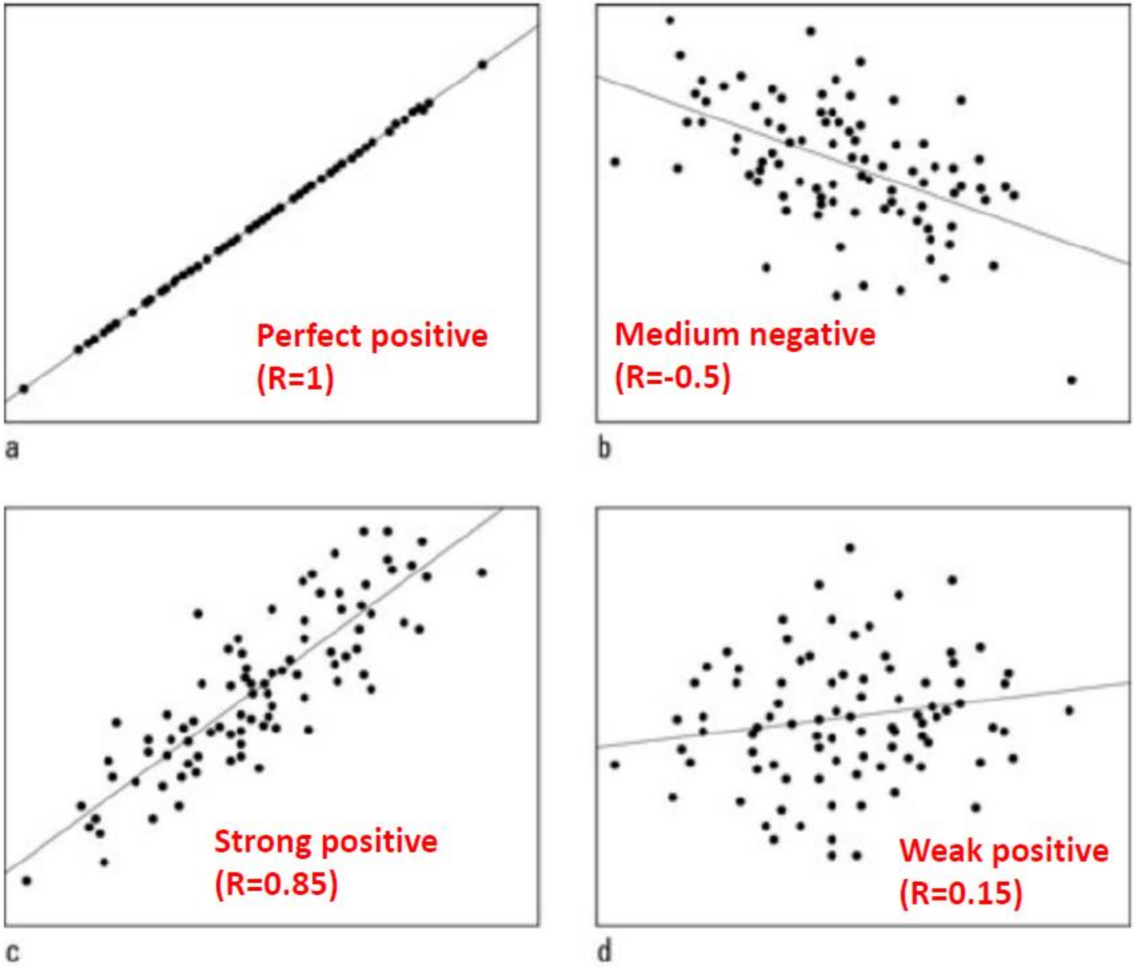


Gene co-expression network

- **Co-expression networks**: undirected, weighted gene networks where nodes correspond to genes and edges between them are determined by the pairwise correlations between their expression profiles.
- **Correlation**: statistical measure for the extent to which two variables fluctuate together
  - Positive correlation: variables increase/decrease together
  - Negative correlation: variables increase/decrease in opposing direction

Correlation examples

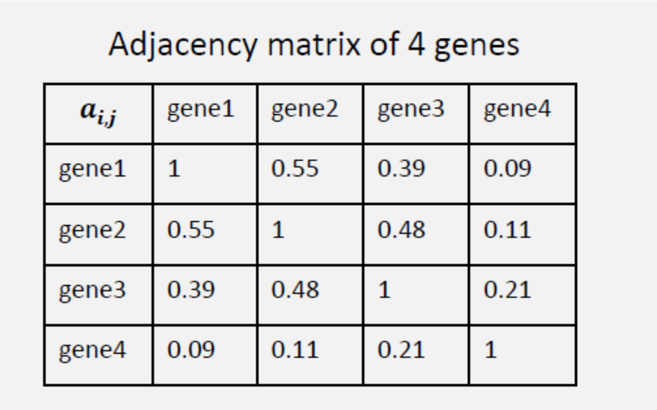
Pearson correlation coefficient R



Adiacency matrix

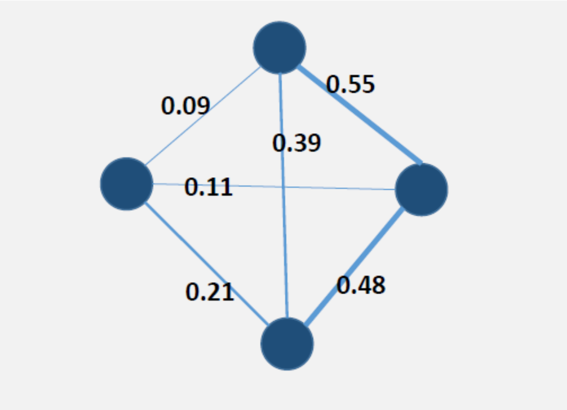
- The correlation between each pair of genes (i, j) is raised to a power β (soft thresholding):

$$a_{i,j} = |corr(i,j)|^{\beta}$$



Adjacency matrix of 4 genes

$a_{i,j}$	gene1	gene2	gene3	gene4
gene1	1	0.55	0.39	0.09
gene2	0.55	1	0.48	0.11
gene3	0.39	0.48	1	0.21
gene4	0.09	0.11	0.21	1



- This amplifies disparity between strong and weak correlations and leads to a fully connected network with genes as nodes and $a_{i,j}$ as edge weights.

Example

Power term $\boldsymbol{\beta} = \mathbf{4}$

Correlation

$$cor(i,j) = 0.8$$

$$cor(k,l) = 0.2$$

$$\frac{0.8}{0.2} = 4\ fold\ of\ difference$$

Adjacencies

$$|0.8|^4 = 0.4096$$

$$|0.2|^4 = 0.0016$$

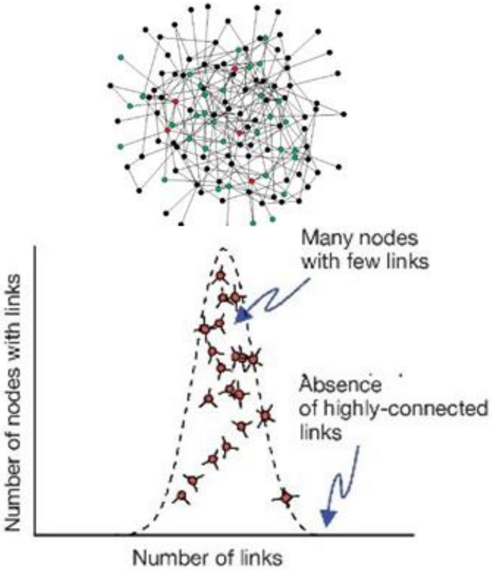
$$\frac{0.4096}{0.0016} = 256\ fold\ of\ difference$$

Selection criterion of power term

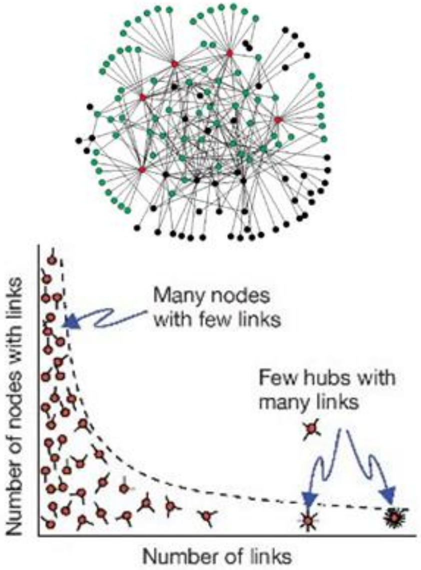
Pick the lowest possible β leading to an approximately scale-free network topology, that is the typical structure of most of biological networks:

- few nodes with many connections (hub nodes)
- many nodes with few connections (peripheral nodes)
- degree distribution follows a power law, i.e. the probability for a node of having k links is $k^{-\gamma}$

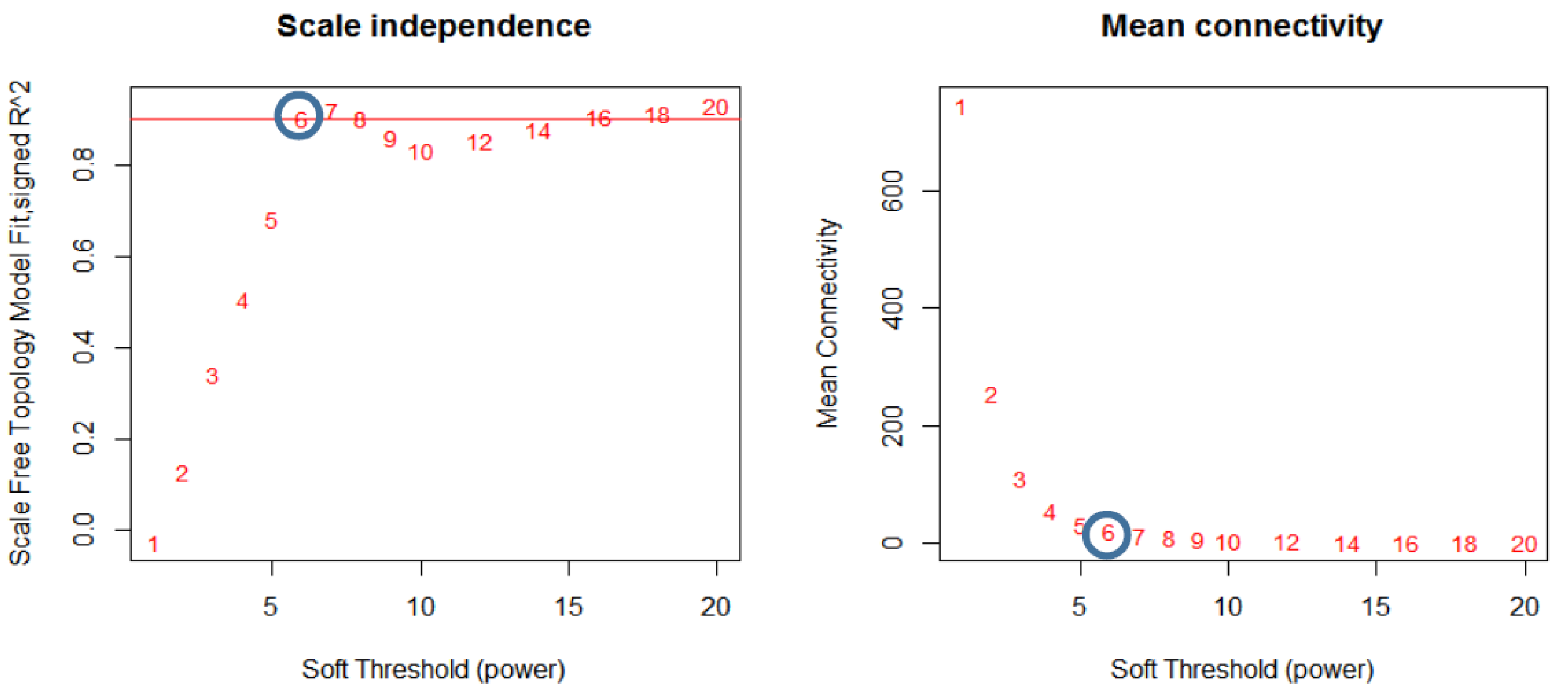
Random network



Scale-free network

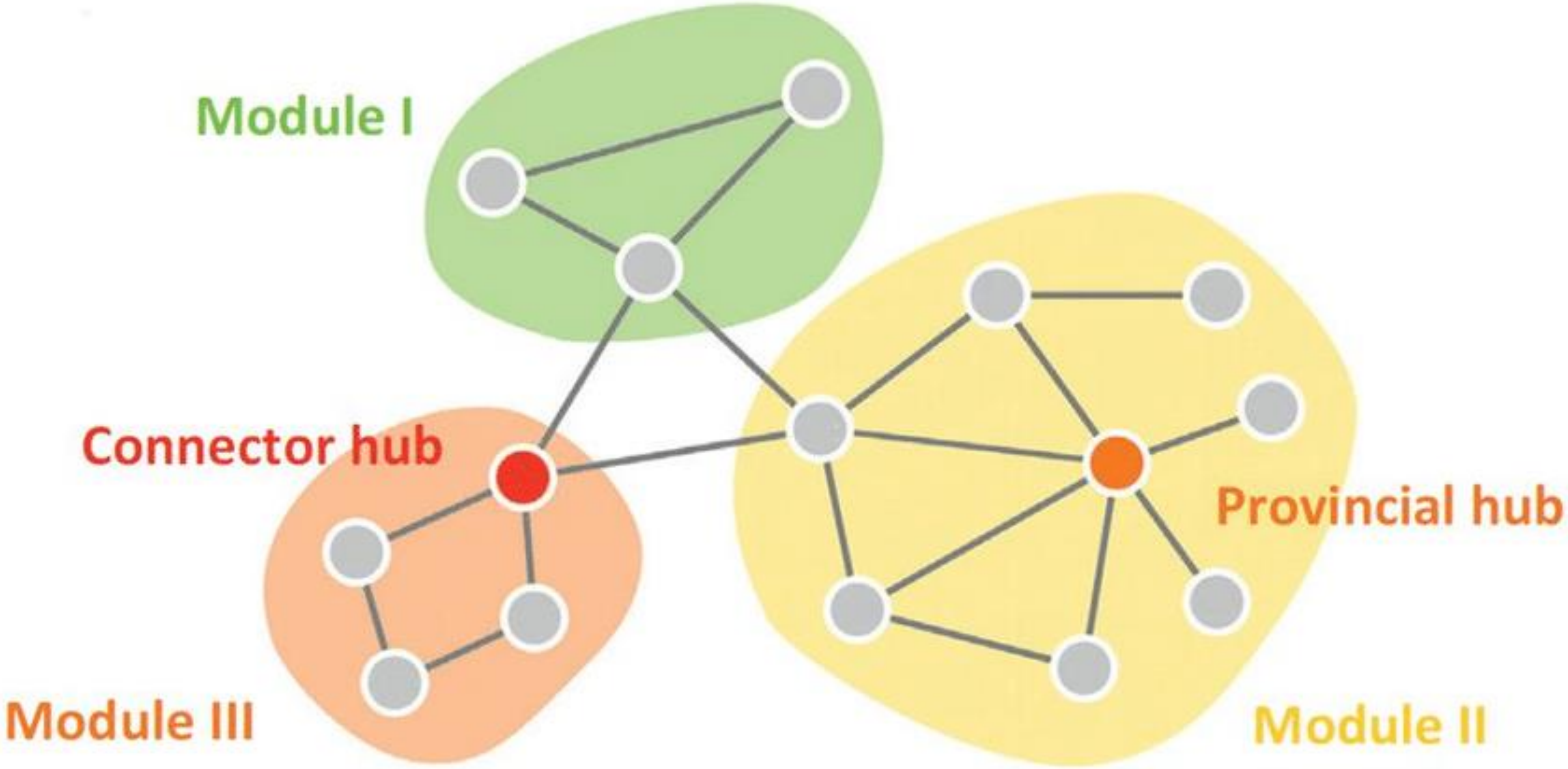


Pick a power term: Visual Aid in WGCNA



- Left plot: Choose power 6. Lowest possible power term where topology approximately fits a scale free network (on or above red horizontal line).
- Right plot: mean connectivity drops as power goes up. Must not drop too low.

Modules detection



Topological Overlap Measure (TOM)

- TOM is a pairwise similarity measure between network nodes (genes) defined as:

$$TOM_{ij} = \frac{\sum_u a_{iu} a_{uj} + a_{ij}}{min(k_i, k_j) + 1 - a_{ij}}$$

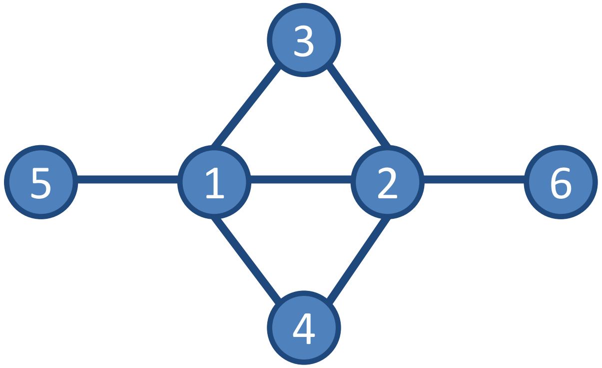
$$DistTOM_{ij} = 1 - TOM_{ij}$$

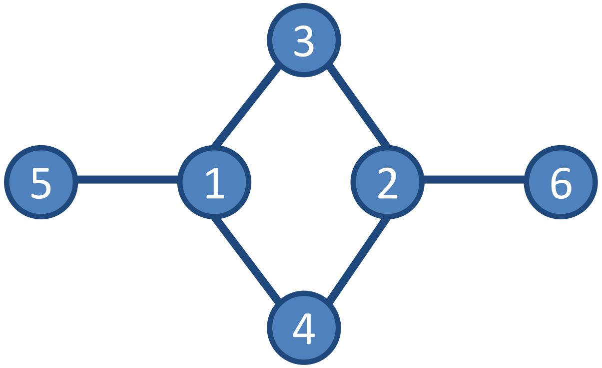
- TOM(i,j) is high if genes i,j have many shared neighbors (overlap of their network neighbors is large)
- High TOM(i,j) implies that genes have similar expression patterns

Topological Overlap Measure (TOM)

$$TOM_{ij} = \frac{\sum_u a_{iu} a_{uj} + a_{ij}}{min(k_i, k_j) + 1 - a_{ij}}$$

$$TOM_{ij} = \frac{\#common\ neighbors + link\ between\ them}{\min(\#\ link\ i, \#\ link\ j) + 1 - link\ between\ them}$$

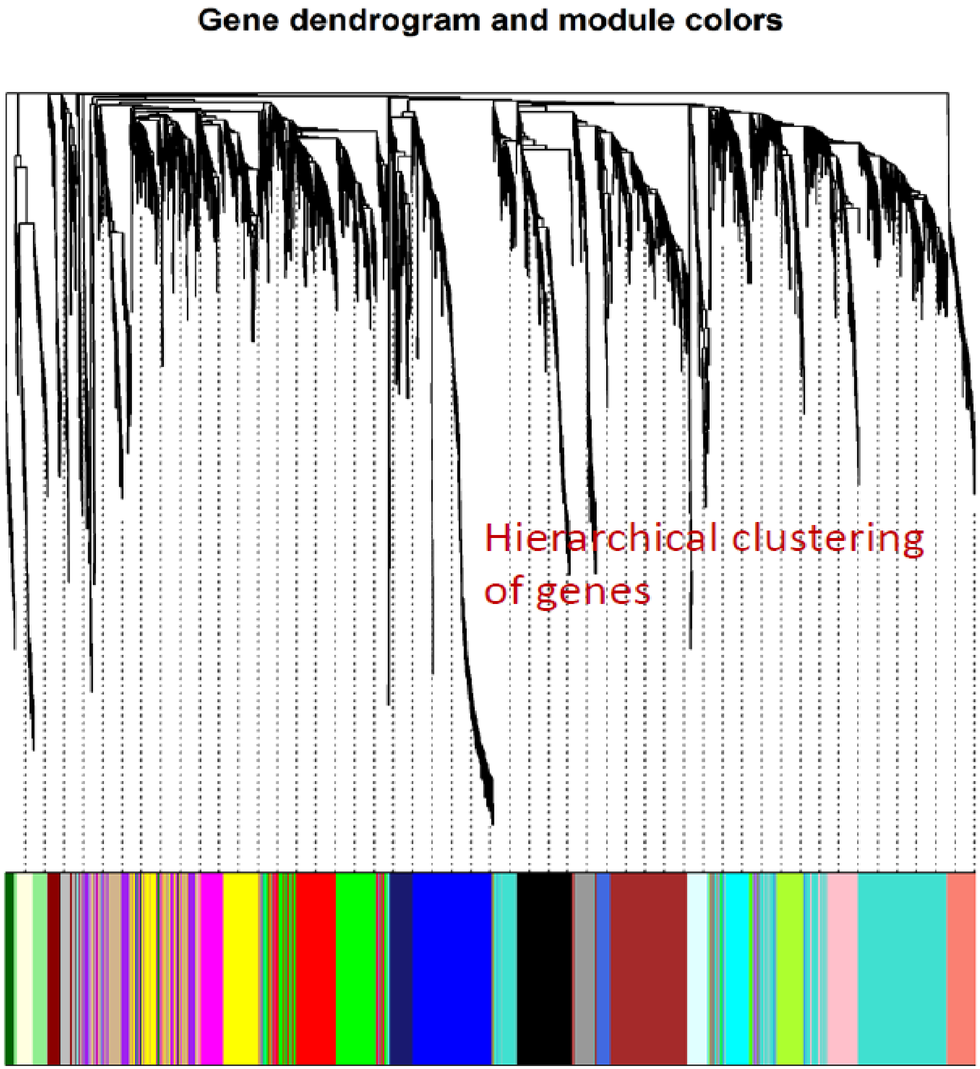




$$TOM_{1,2} = \frac{2+1}{\min(4,4)+1-1} = \frac{3}{4} = 0.75$$

$$TOM_{1,2} = \frac{2+0}{\min(3,3)+1-0} = \frac{2}{4} = 0.5$$

Hierarchical clustering



Hierarchical clustering of genes

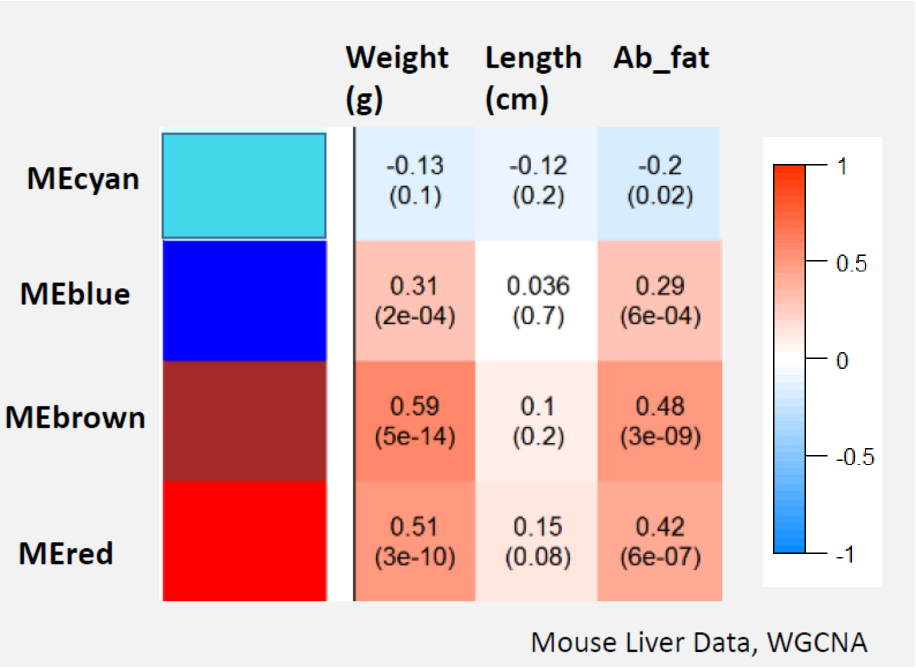
Module eigengene (ME)

- **What it is:**
  - a 1-dimensional data vector, summarizing the expression data of the genes forming a module
- **How it is computed:**
  - 1st principal component of the expression data of the genes forming a module
- **What it is used for:**
  - modules can be correlated with one another
  - modules can be clustered
  - modules can be correlated with external traits

Correlate modules to external traits

- Compute correlations between each module eigengene and each trait variable
- Identify modules highly correlated to traits of interest
- Identify traits highly correlated to multiple modules

Heatmap of module–trait relationships



	Weight (g)	Length (cm)	Ab_fat
MEcyan	-0.13 (0.1)	-0.12 (0.2)	-0.2 (0.02)
MEblue	0.31 (2e-04)	0.036 (0.7)	0.29 (6e-04)
MEbrown	0.59 (5e-14)	0.1 (0.2)	0.48 (3e-09)
MEred	0.51 (3e-10)	0.15 (0.08)	0.42 (6e-07)

Mouse Liver Data, WGCNA

Driver genes identification

- **Aim:**
  - Identify key genes that may influence the expression or function of other genes **OR**
  - may be causal factors for a trait of interest
- **Caveat:**
  - WGCNA cannot show whether gene-gene or gene-trait relationships are causal **BUT**
  - can help find candidate genes for further study
- **Strategy:**
  - Identify those genes within a module that are most representative or highly connected within the module (highest **module membership**) **AND**
  - most strongly correlated with a trait of interest (highest **gene significance**)

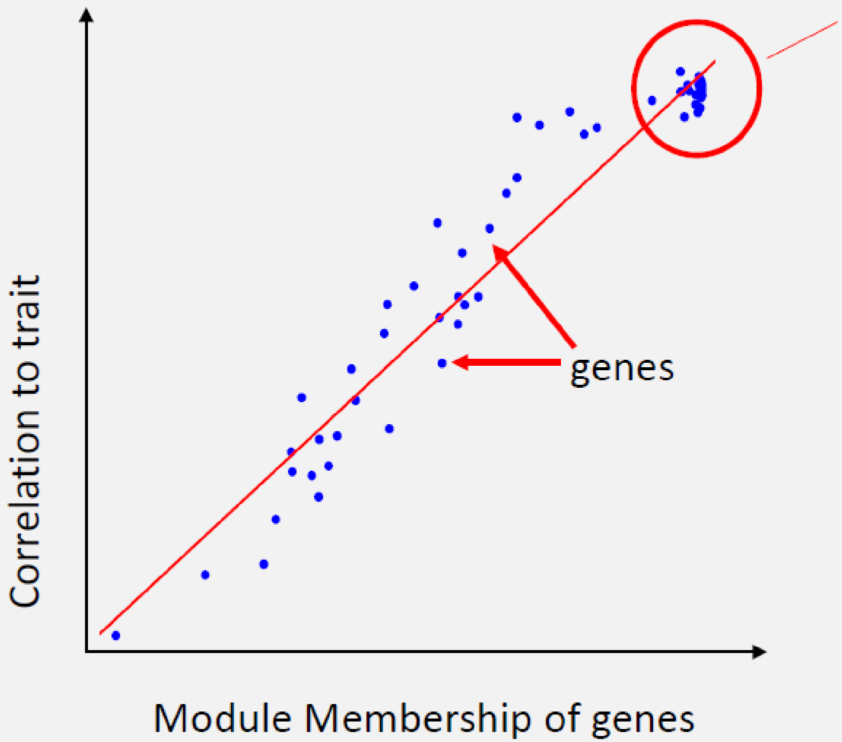
Module membership (MM)

- MM of a gene is the correlation between its expression profile and the module eigengene of a given module
- Genes with high MM are good representatives of the overall expression profile in the module
- Genes with high MM tend to be “hub” in the module
  - If MM → 0 the gene is not part of the module.
  - if MM → 1 or -1, the gene is highly connected to the genes of that module. The sign of MM encodes whether the gene has a positive or a negative relationship with the module eigengene.

Gene significance (GS)

- GS of a gene is the correlation between its expression profile and external sample traits
- Genes with higher GS are the more biologically significant genes
  - If GS → 0 that the gene is not significant with regard to the biological question of interest
  - if GS → 1 or -1, the gene is significant with regard to the biological question of interest. The sign of GS encodes whether the gene has a positive or a negative relationship with the trait of interest.

Potential driver genes



“driver” genes:
most correlated with
both module and trait

(picked by visual
inspection)

3. WGCNA application for prostate cancer early detection

Prostate cancer

- Prostate cancer (PCa) is the carcinoma of prostate gland that may spread to other parts of the body particularly bones and lymph nodes.
- PCa is the fourth most common malignancy worldwide and the second cause of cancer-related death in men.
- PCa can be clinically significant (csPCa) or insignificant (ciPCa):
  - csPCa is often aggressive and potentially metastatic, requiring early detection and possibly multi-modal therapy;
  - ciPCa often never progress and can be safely treated with active surveillance strategies.

Diagnostic Parameters

- **Prostate-Specific Antigen (PSA) Blood Test:** to look for raised levels of PSA in the blood that may be a sign of cancer in its early stages
- **Digital Rectal Examination:** to rule out prostate enlargement caused by benign prostatic hyperplasia
- **Magnetic Resonance Imaging (MRI):** to access the extension into the bladder and lymph nodes for staging the cancer and to evaluate bone metastasis.
- **Biopsy:** to aid in the diagnosis and help to determine the Gleason score

Gleason score is based upon microscopic appearance and ranges from 2 to 10, with higher numbers indicating higher risks, aggressiveness and mortality (≥7 means csPCa)

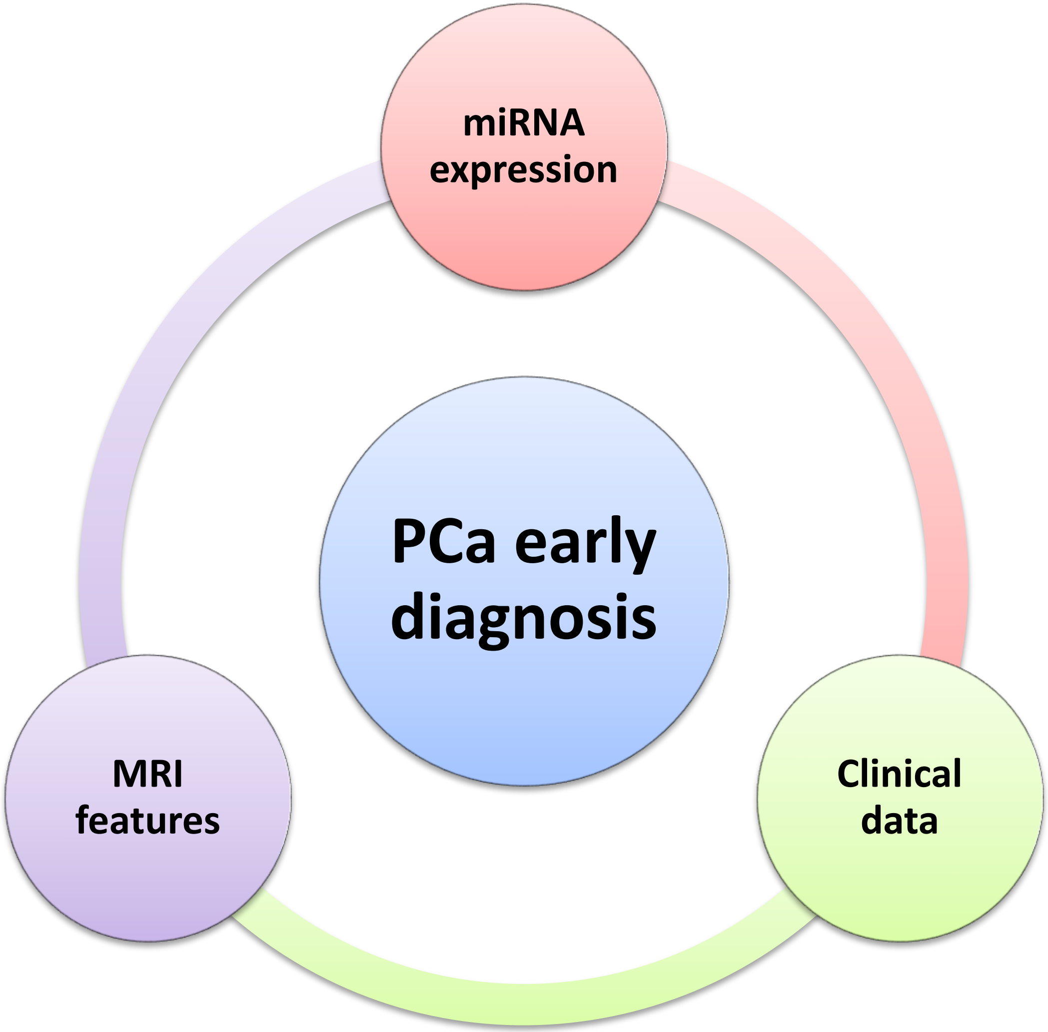
Magnetic Resonance Imaging (MRI)

- Prostate MRI is recommended by the European association of Urology guidelines as triage test to identify men with csPCa in view of its **high accuracy**
- Nonetheless, MRI still **misses around 11%** csPCa
- Guidelines suggest the use of complementary non-invasive tests (**blood- or urine-based biomarkers**) to implement the accuracy of MRI

microRNAs as promising biomarkers

- MicroRNAs (miRNAs) are small, non-coding RNA molecules of 21–23 nucleotides
- They are important post-transcriptional regulators of gene expression by translation inhibition or degradation of their target mRNAs
- They represent promising biomarkers for the early detection of PCa, given their stability in tissue and biological fluids

WGCNA for a non-invasive diagnosis



miRNA expression

PCa early diagnosis

MRI features

Clinical data

Sample collection

Equally sized cohort:

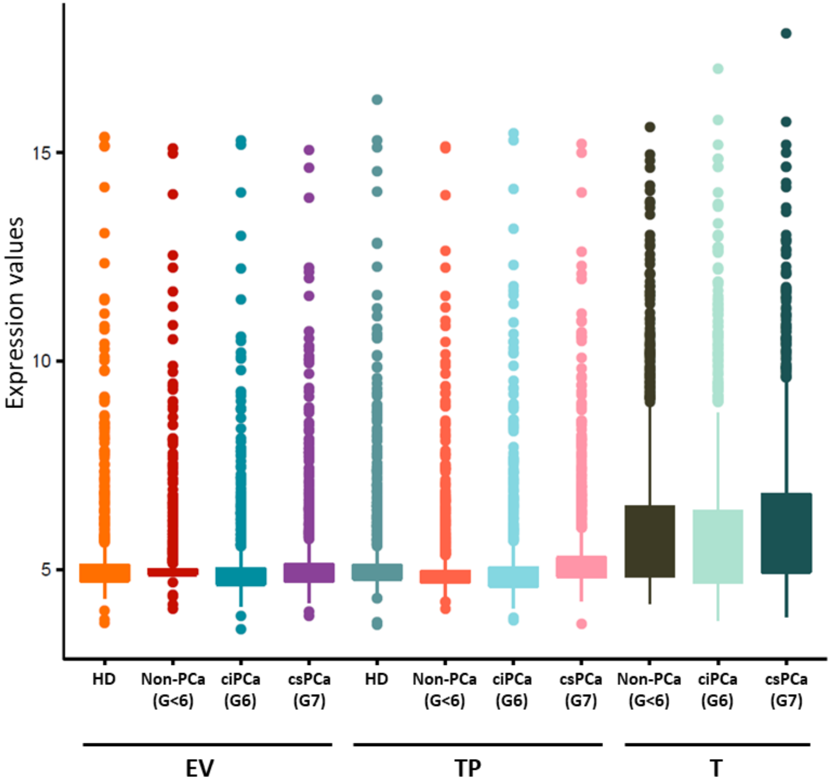
- 5 patiens with csPCa (Gleason 7)
- 5 patiens with ciPCa (Gleason 6)
- 5 patiens with Non-PCa (Gleason < 6)

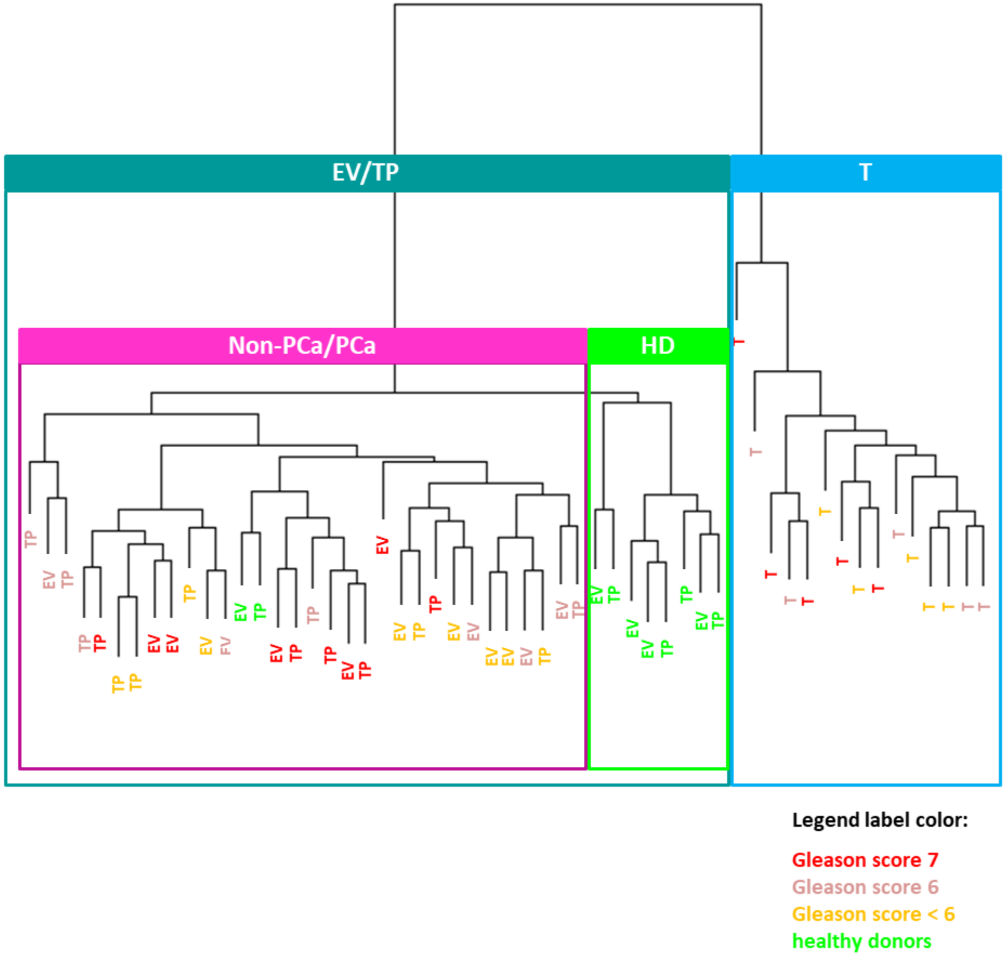
miRNA from total plasma (TP), extracellular vesicles (EV) and prostate tissue (T)
\+
mpMRI features
\+
Clinical data

- 5 healthy donors (HD)

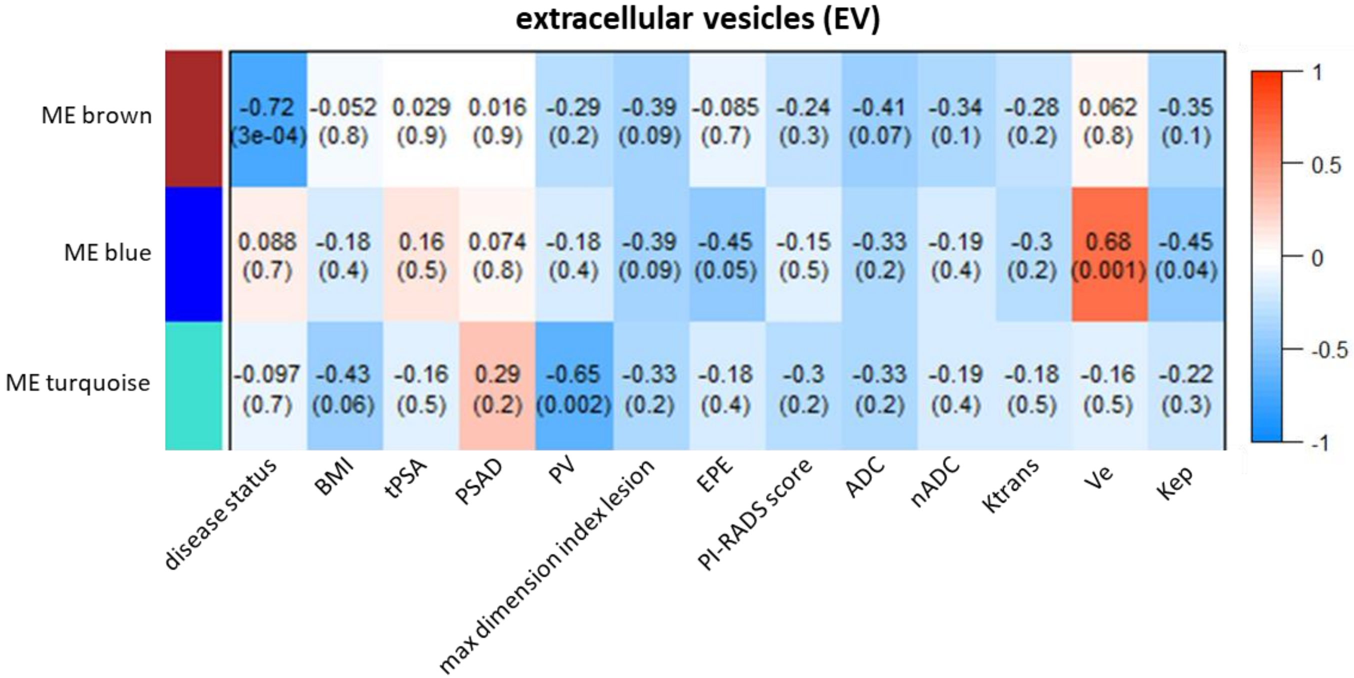
miRNA from TP and EV

miRNA expression data



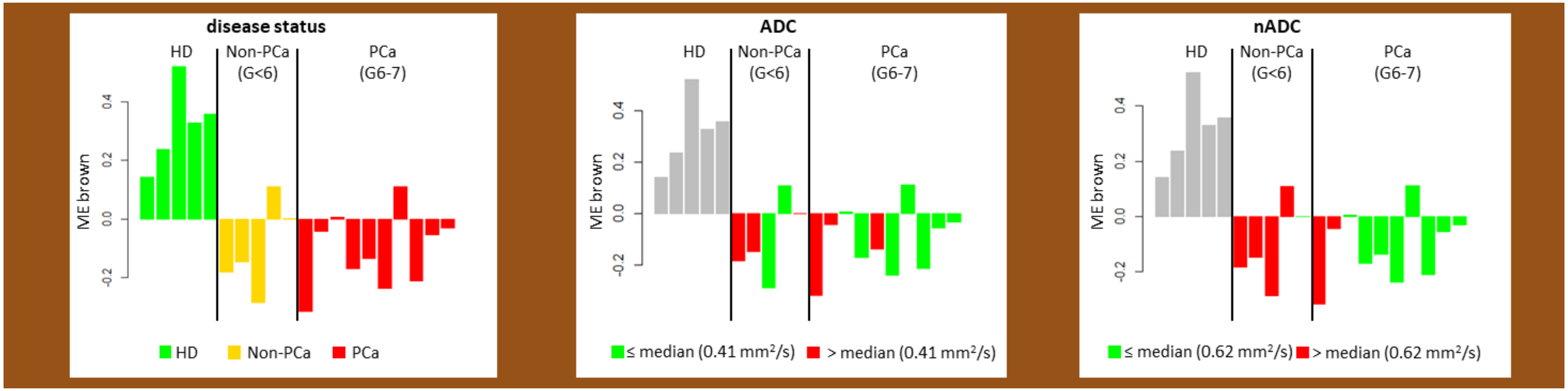


WGCNA on all cohort of patients

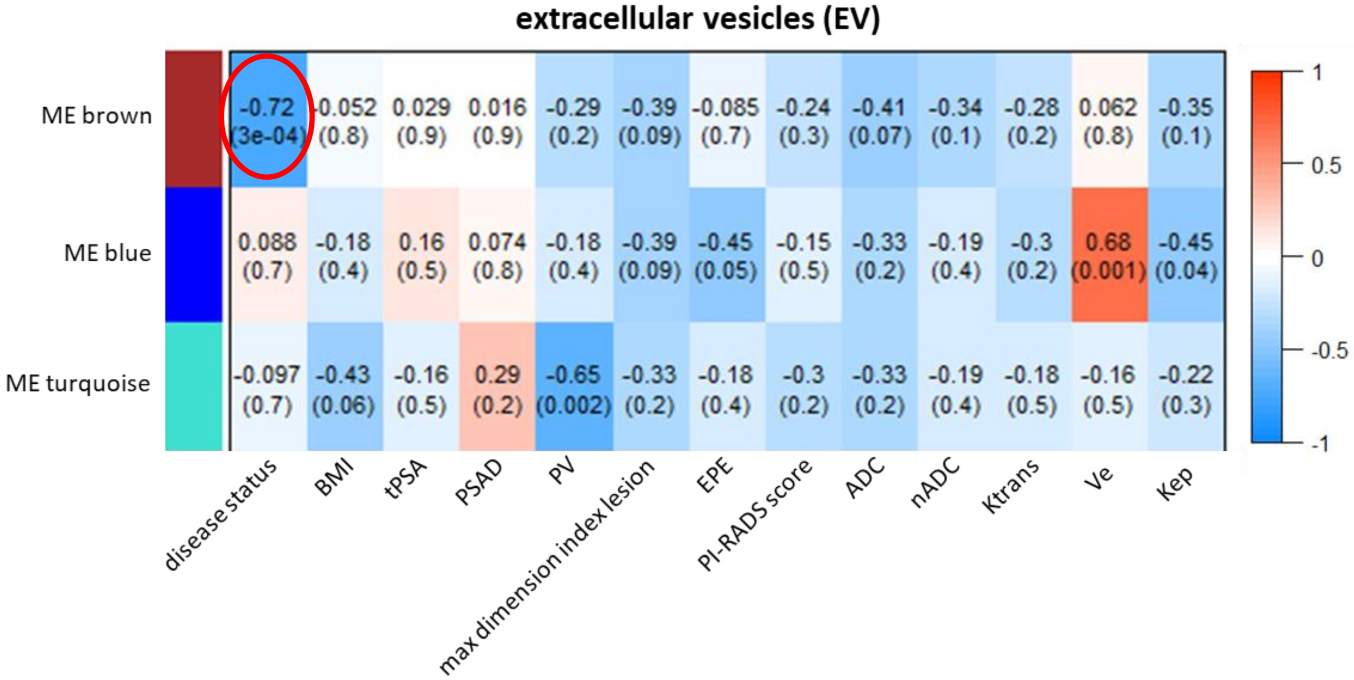


extracellular vesicles (EV)

	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME brown	-0.72 (3e-04)	-0.052 (0.8)	0.029 (0.9)	0.016 (0.9)	-0.29 (0.2)	-0.39 (0.09)	-0.085 (0.7)	-0.24 (0.3)	-0.41 (0.07)	-0.34 (0.1)	-0.28 (0.2)	0.062 (0.8)	-0.35 (0.1)
ME blue	0.088 (0.7)	-0.18 (0.4)	0.16 (0.5)	0.074 (0.8)	-0.18 (0.4)	-0.39 (0.09)	-0.45 (0.05)	-0.15 (0.5)	-0.33 (0.2)	-0.19 (0.4)	-0.3 (0.2)	0.68 (0.001)	-0.45 (0.04)
ME turquoise	-0.097 (0.7)	-0.43 (0.06)	-0.16 (0.5)	0.29 (0.2)	-0.65 (0.002)	-0.33 (0.2)	-0.18 (0.4)	-0.3 (0.2)	-0.33 (0.2)	-0.19 (0.4)	-0.18 (0.5)	-0.16 (0.5)	-0.22 (0.3)

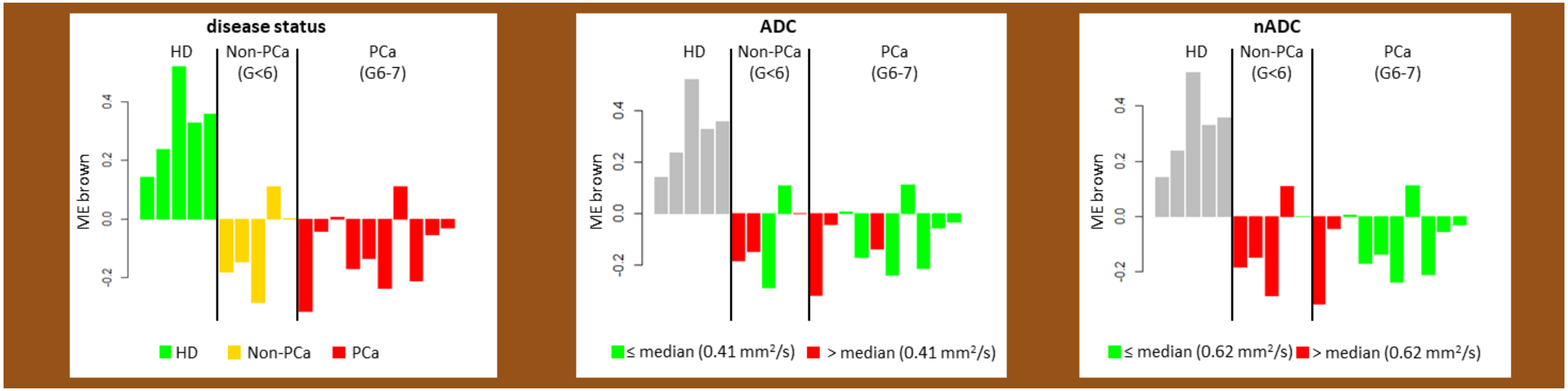


WGCNA on all cohort of patients

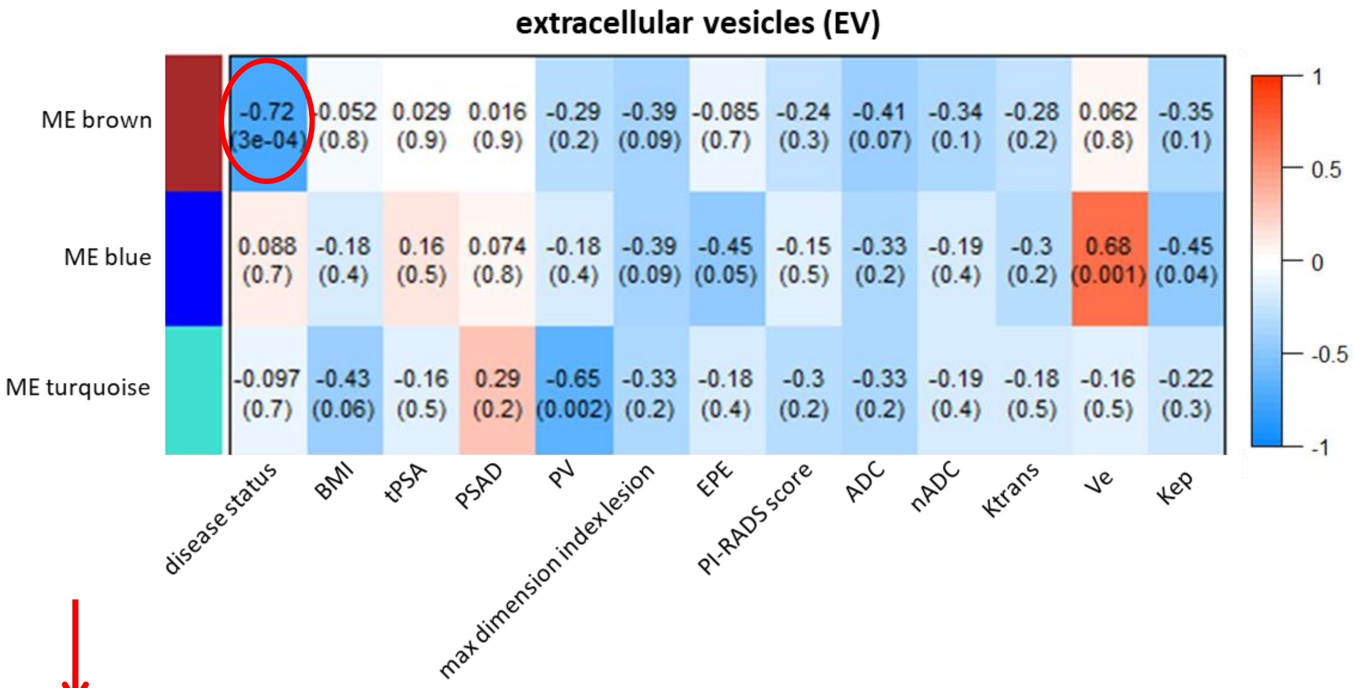


extracellular vesicles (EV)

	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME brown	-0.72 (3e-04)	-0.052 (0.8)	0.029 (0.9)	0.016 (0.9)	-0.29 (0.2)	-0.39 (0.09)	-0.085 (0.7)	-0.24 (0.3)	-0.41 (0.07)	-0.34 (0.1)	-0.28 (0.2)	0.062 (0.8)	-0.35 (0.1)
ME blue	0.088 (0.7)	-0.18 (0.4)	0.16 (0.5)	0.074 (0.8)	-0.18 (0.4)	-0.39 (0.09)	-0.45 (0.05)	-0.15 (0.5)	-0.33 (0.2)	-0.19 (0.4)	-0.3 (0.2)	0.68 (0.001)	-0.45 (0.04)
ME turquoise	-0.097 (0.7)	-0.43 (0.06)	-0.16 (0.5)	0.29 (0.2)	-0.65 (0.002)	-0.33 (0.2)	-0.18 (0.4)	-0.3 (0.2)	-0.33 (0.2)	-0.19 (0.4)	-0.18 (0.5)	-0.16 (0.5)	-0.22 (0.3)

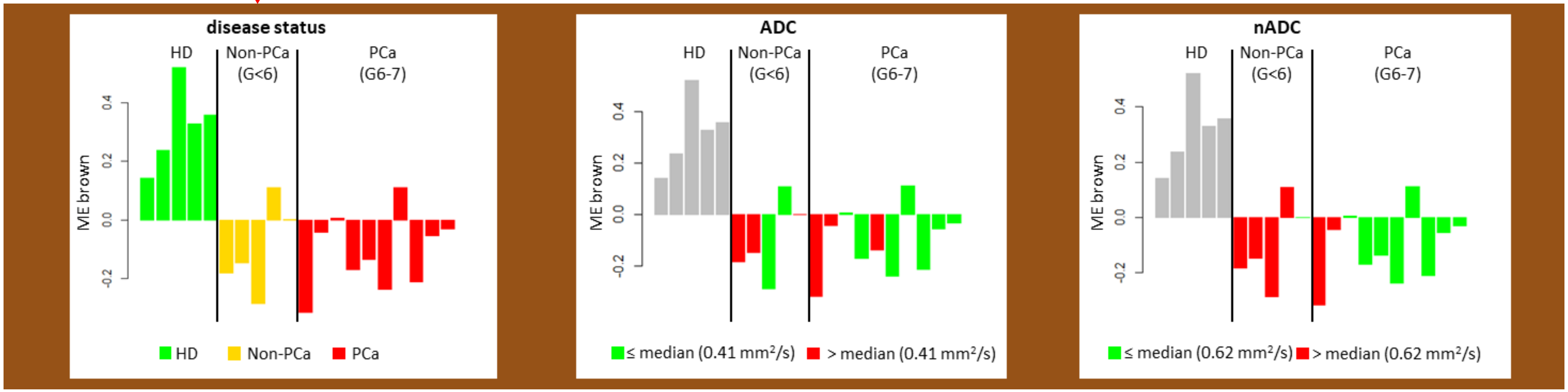


WGCNA on all cohort of patients

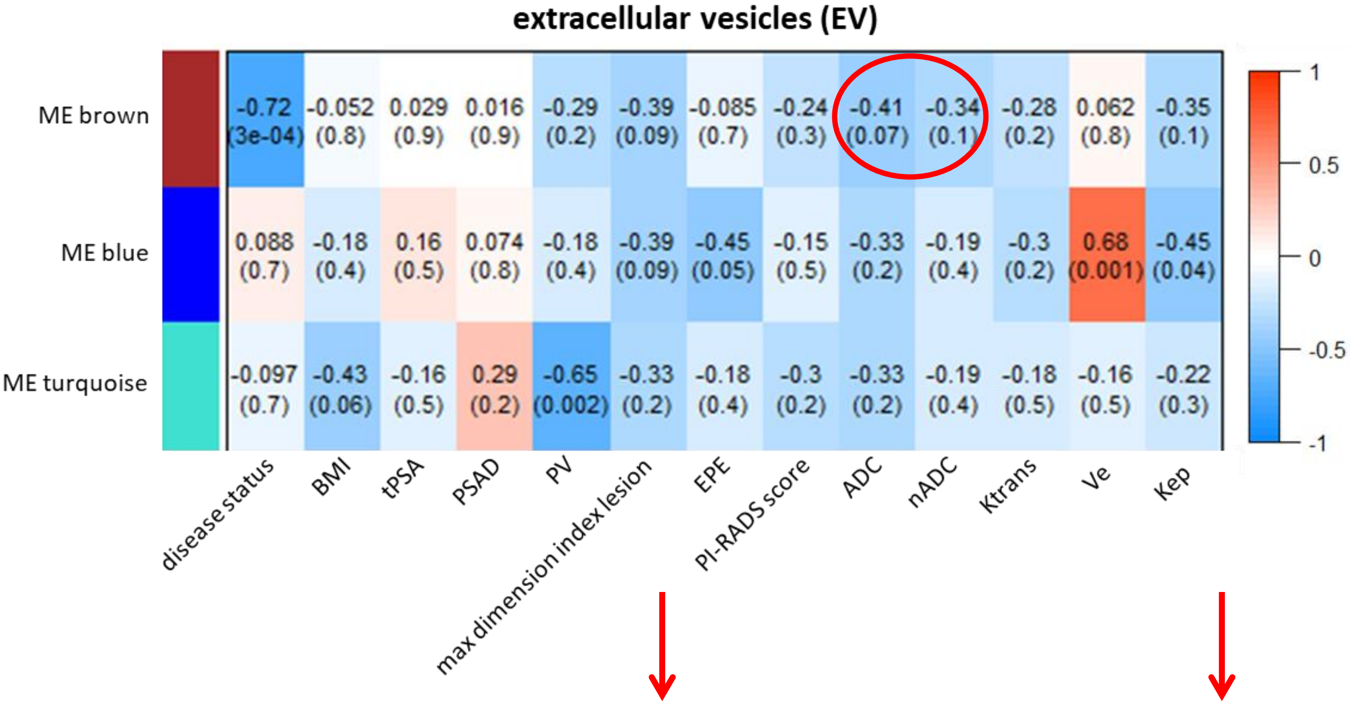


extracellular vesicles (EV)

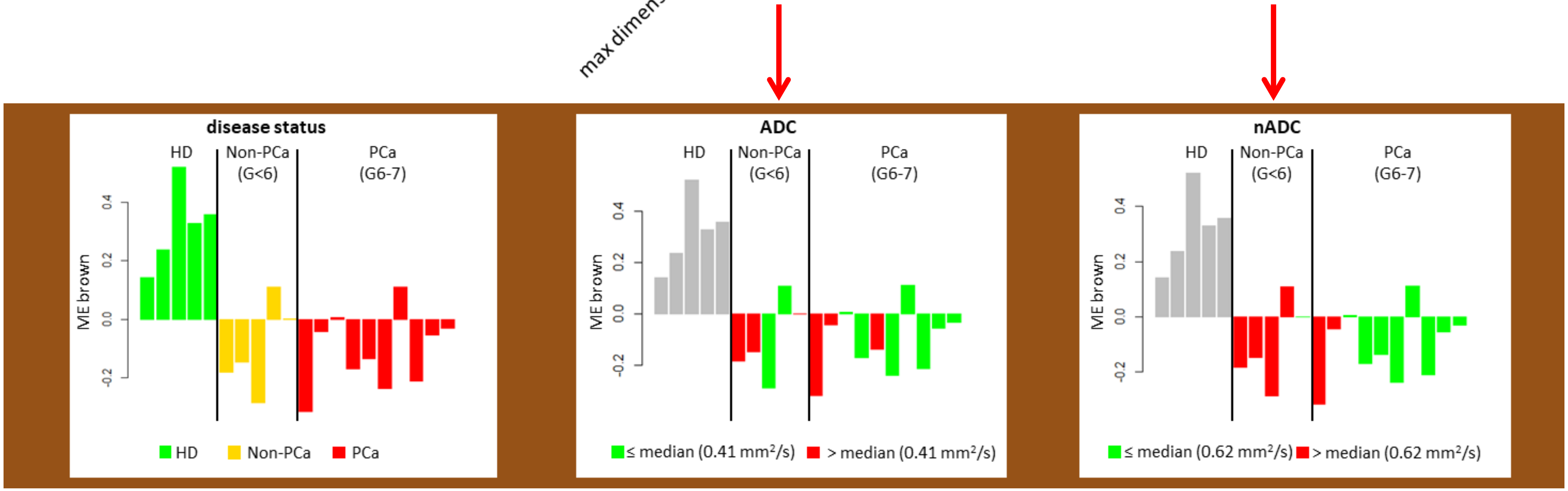
	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME brown	-0.72 (3e-04)	-0.052 (0.8)	0.029 (0.9)	0.016 (0.9)	-0.29 (0.2)	-0.39 (0.09)	-0.085 (0.7)	-0.24 (0.3)	-0.41 (0.07)	-0.34 (0.1)	-0.28 (0.2)	0.062 (0.8)	-0.35 (0.1)
ME blue	0.088 (0.7)	-0.18 (0.4)	0.16 (0.5)	0.074 (0.8)	-0.18 (0.4)	-0.39 (0.09)	-0.45 (0.05)	-0.15 (0.5)	-0.33 (0.2)	-0.19 (0.4)	-0.3 (0.2)	0.68 (0.001)	-0.45 (0.04)
ME turquoise	-0.097 (0.7)	-0.43 (0.06)	-0.16 (0.5)	0.29 (0.2)	-0.65 (0.002)	-0.33 (0.2)	-0.18 (0.4)	-0.3 (0.2)	-0.33 (0.2)	-0.19 (0.4)	-0.18 (0.5)	-0.16 (0.5)	-0.22 (0.3)



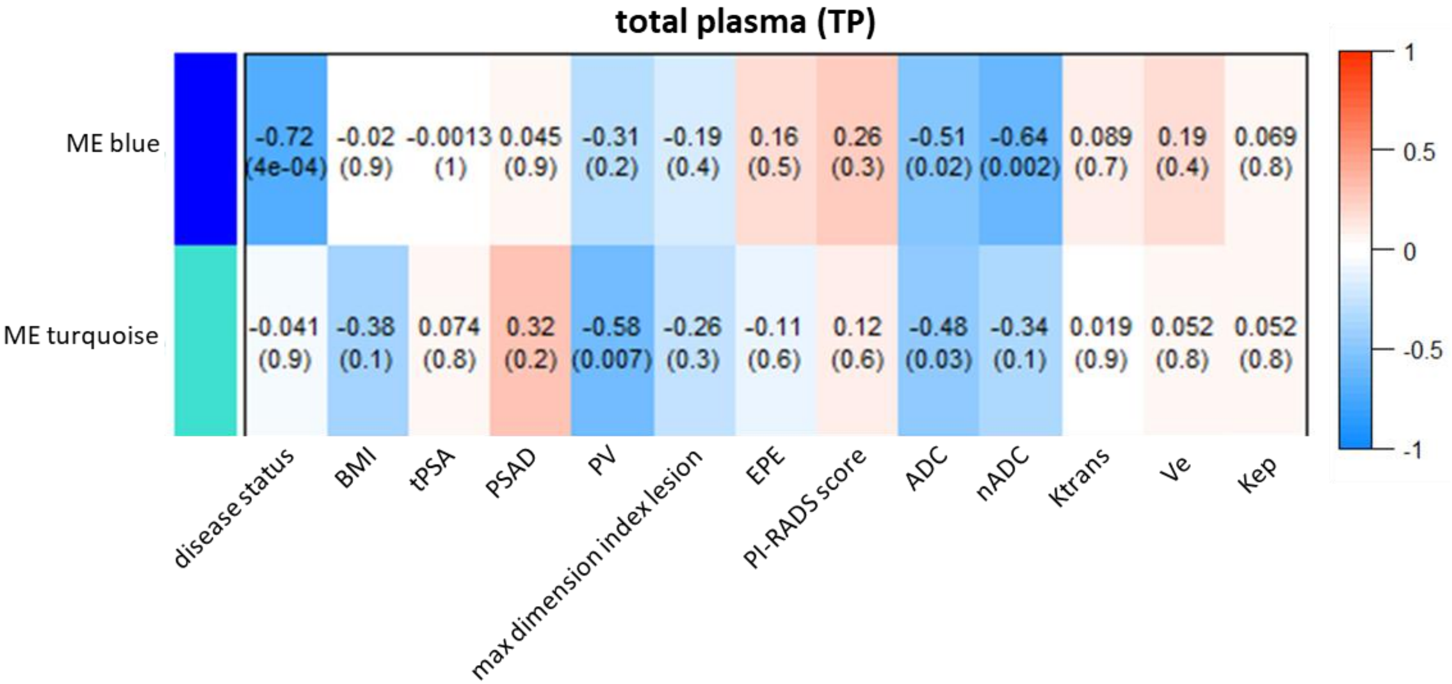
WGCNA on all cohort of patients


extracellular vesicles (EV)

	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME brown	-0.72 (3e-04)	-0.052 (0.8)	0.029 (0.9)	0.016 (0.9)	-0.29 (0.2)	-0.39 (0.09)	-0.085 (0.7)	-0.24 (0.3)	-0.41 (0.07)	-0.34 (0.1)	-0.28 (0.2)	0.062 (0.8)	-0.35 (0.1)
ME blue	0.088 (0.7)	-0.18 (0.4)	0.16 (0.5)	0.074 (0.8)	-0.18 (0.4)	-0.39 (0.09)	-0.45 (0.05)	-0.15 (0.5)	-0.33 (0.2)	-0.19 (0.4)	-0.3 (0.2)	0.68 (0.001)	-0.45 (0.04)
ME turquoise	-0.097 (0.7)	-0.43 (0.06)	-0.16 (0.5)	0.29 (0.2)	-0.65 (0.002)	-0.33 (0.2)	-0.18 (0.4)	-0.3 (0.2)	-0.33 (0.2)	-0.19 (0.4)	-0.18 (0.5)	-0.16 (0.5)	-0.22 (0.3)

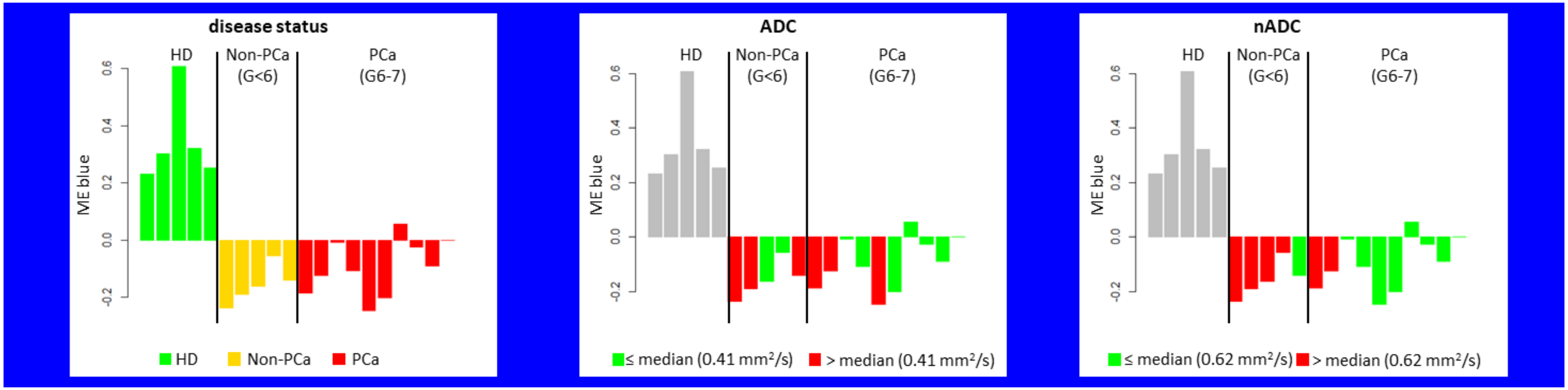


WGCNA on all cohort of patients

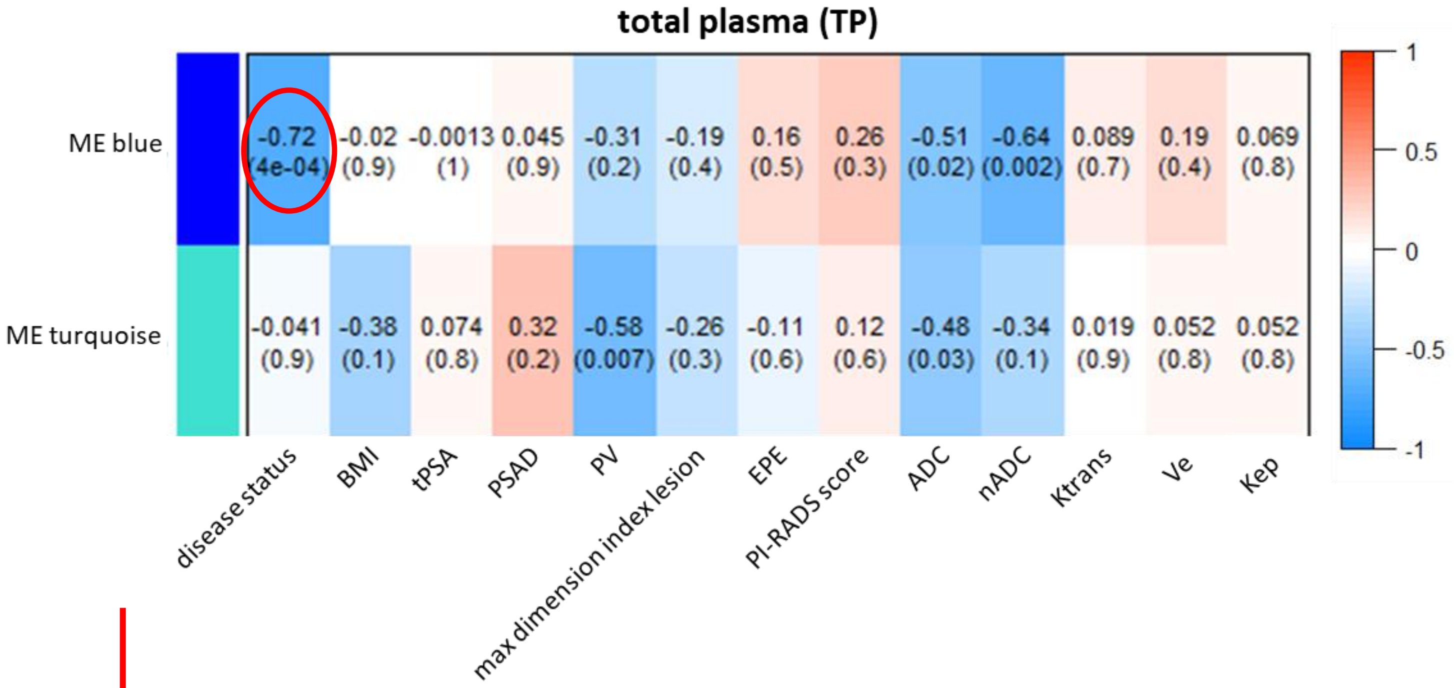


total plasma (TP)

	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME blue	-0.72 (4e-04)	-0.02 (0.9)	-0.0013 (1)	0.045 (0.9)	-0.31 (0.2)	-0.19 (0.4)	0.16 (0.5)	0.26 (0.3)	-0.51 (0.02)	-0.64 (0.002)	0.089 (0.7)	0.19 (0.4)	0.069 (0.8)
ME turquoise	-0.041 (0.9)	-0.38 (0.1)	0.074 (0.8)	0.32 (0.2)	-0.58 (0.007)	-0.26 (0.3)	-0.11 (0.6)	0.12 (0.6)	-0.48 (0.03)	-0.34 (0.1)	0.019 (0.9)	0.052 (0.8)	0.052 (0.8)

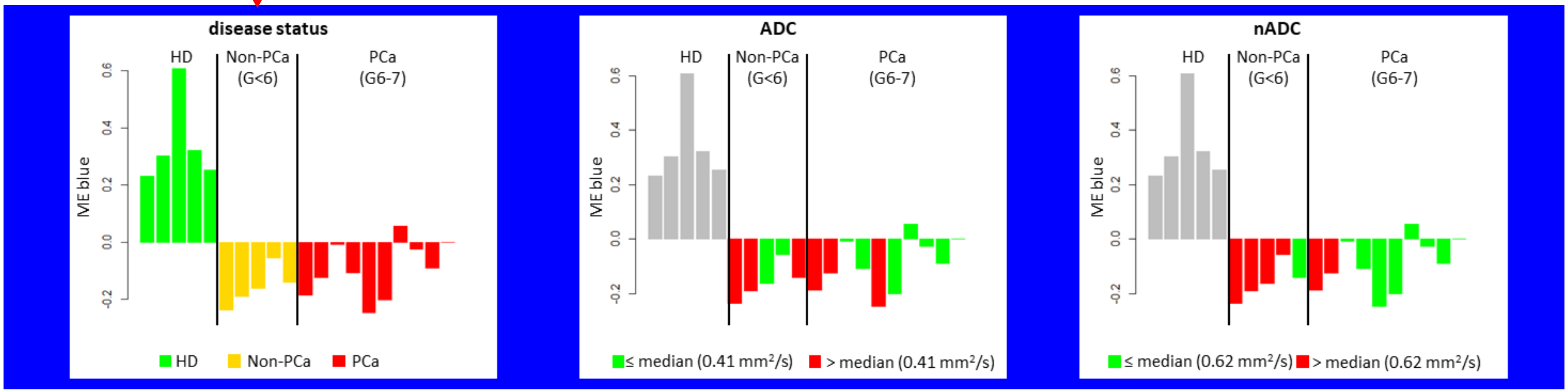


WGCNA on all cohort of patients

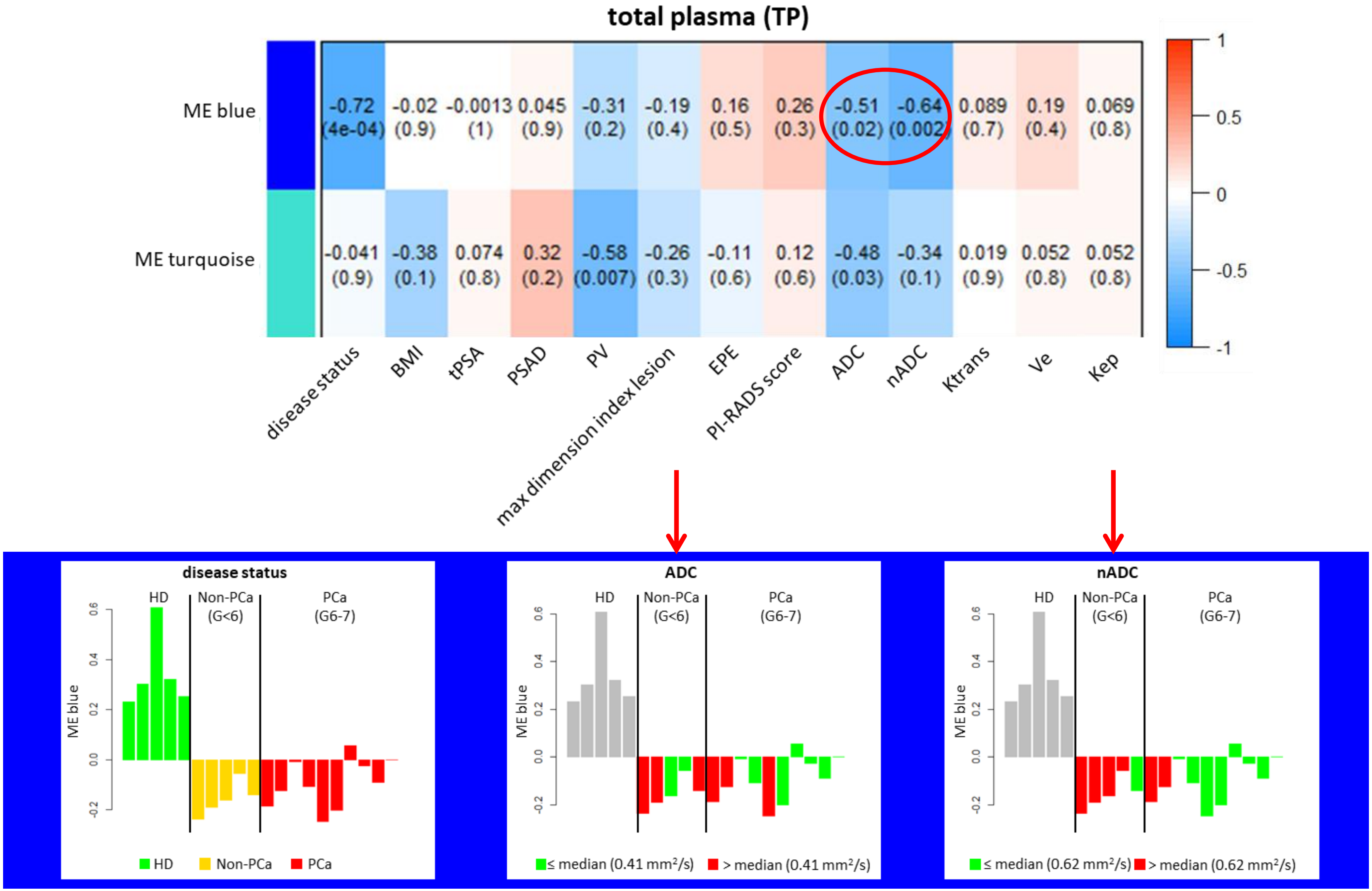


total plasma (TP)

	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME blue	-0.72 (4e-04)	-0.02 (0.9)	-0.0013 (1)	0.045 (0.9)	-0.31 (0.2)	-0.19 (0.4)	0.16 (0.5)	0.26 (0.3)	-0.51 (0.02)	-0.64 (0.002)	0.089 (0.7)	0.19 (0.4)	0.069 (0.8)
ME turquoise	-0.041 (0.9)	-0.38 (0.1)	0.074 (0.8)	0.32 (0.2)	-0.58 (0.007)	-0.26 (0.3)	-0.11 (0.6)	0.12 (0.6)	-0.48 (0.03)	-0.34 (0.1)	0.019 (0.9)	0.052 (0.8)	0.052 (0.8)



WGCNA on all cohort of patients

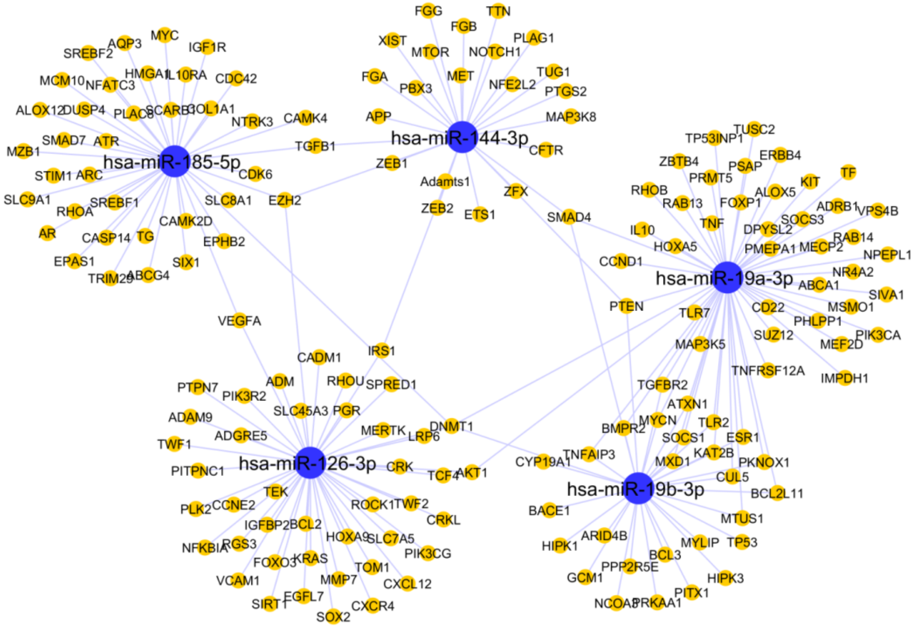


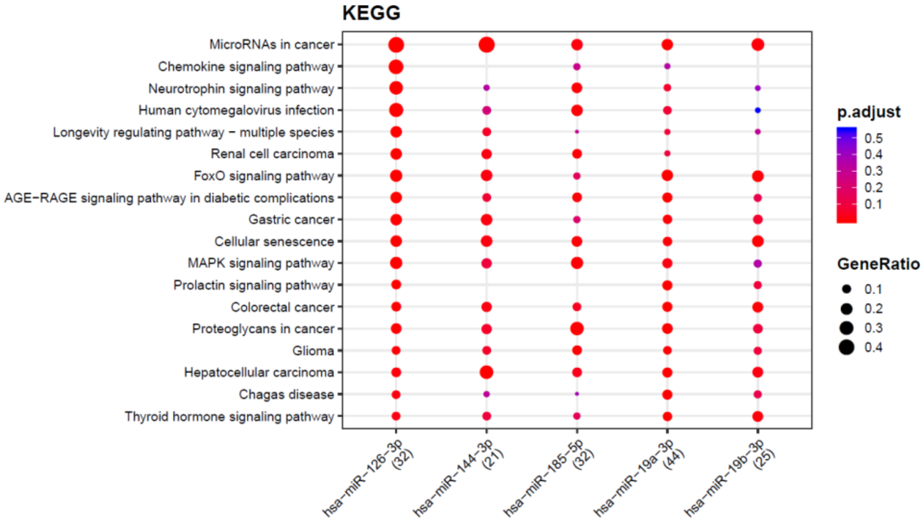
total plasma (TP)

	disease status	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep
ME blue	-0.72 (4e-04)	-0.02 (0.9)	-0.0013 (1)	0.045 (0.9)	-0.31 (0.2)	-0.19 (0.4)	0.16 (0.5)	0.26 (0.3)	-0.51 (0.02)	-0.64 (0.002)	0.089 (0.7)	0.19 (0.4)	0.069 (0.8)
ME turquoise	-0.041 (0.9)	-0.38 (0.1)	0.074 (0.8)	0.32 (0.2)	-0.58 (0.007)	-0.26 (0.3)	-0.11 (0.6)	0.12 (0.6)	-0.48 (0.03)	-0.34 (0.1)	0.019 (0.9)	0.052 (0.8)	0.052 (0.8)

WGCNA on all cohort of patients

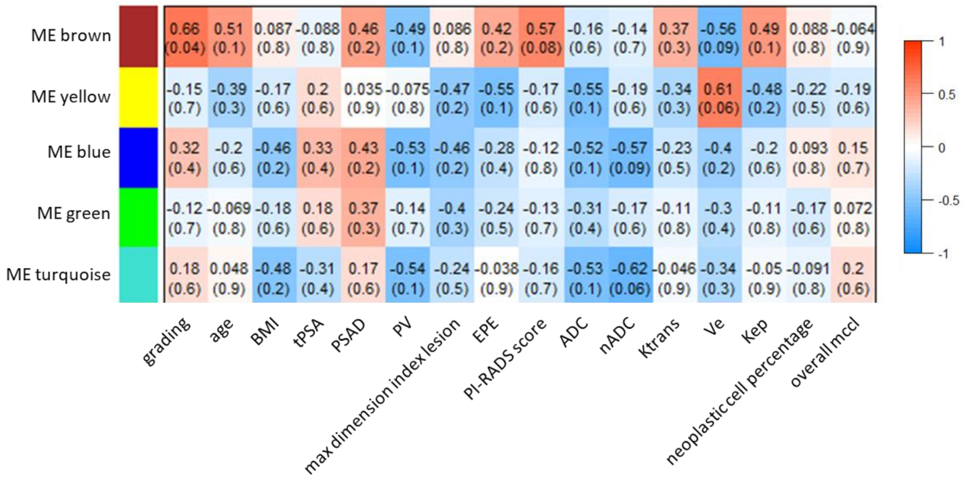
MIENTURNET
MIcroRNAENrichmentTURnedNETwork





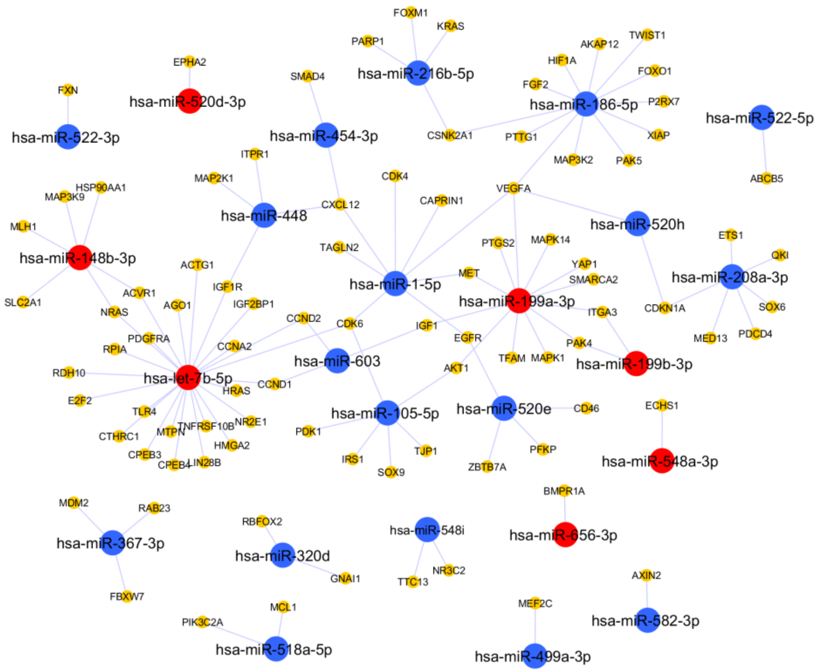
WGCNA on PCa (G6-7) patients

extracellular vesicles (EV)



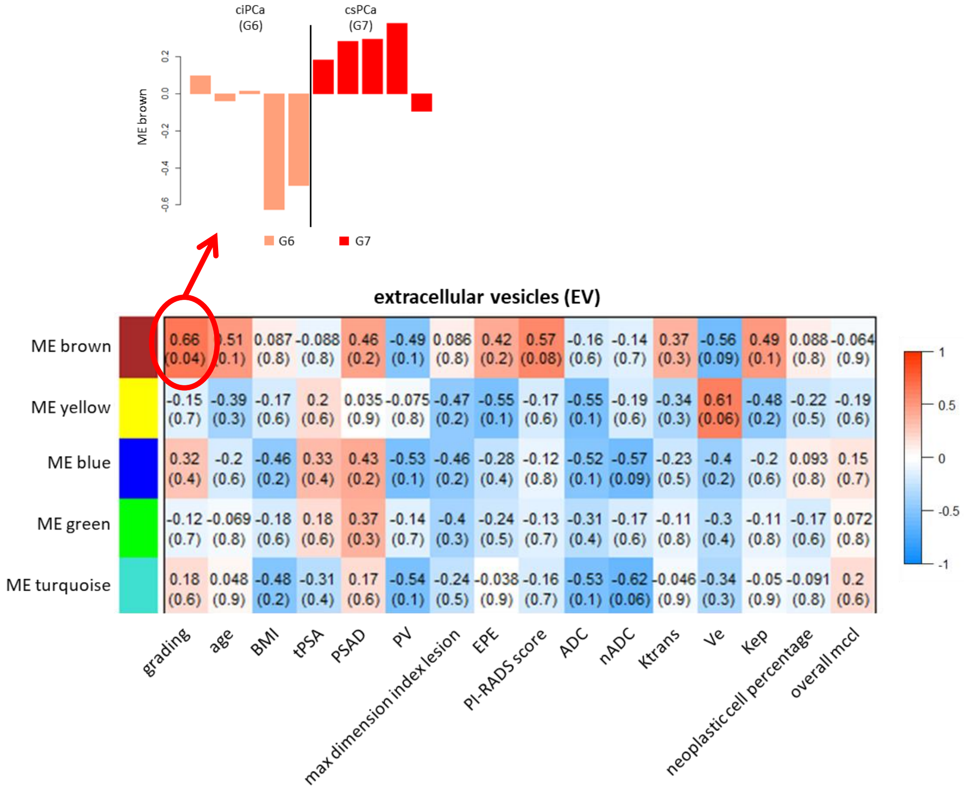
	grading	age	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep	neoplastic cell percentage	overall mccl
ME brown	0.66 (0.04)	0.51 (0.1)	0.087 (0.8)	-0.088 (0.8)	0.46 (0.2)	-0.49 (0.1)	0.086 (0.8)	0.42 (0.2)	0.57 (0.08)	-0.16 (0.6)	-0.14 (0.7)	0.37 (0.3)	-0.56 (0.09)	0.49 (0.1)	0.088 (0.8)	-0.064 (0.9)
ME yellow	-0.15 (0.7)	-0.39 (0.3)	-0.17 (0.6)	0.2 (0.6)	0.035 (0.9)	-0.075 (0.8)	-0.47 (0.2)	-0.55 (0.1)	-0.17 (0.6)	-0.55 (0.1)	-0.19 (0.6)	-0.34 (0.3)	0.61 (0.06)	-0.48 (0.2)	-0.22 (0.5)	-0.19 (0.6)
ME blue	0.32 (0.4)	-0.2 (0.6)	-0.46 (0.2)	0.33 (0.4)	0.43 (0.2)	-0.53 (0.1)	-0.46 (0.2)	-0.28 (0.4)	-0.12 (0.8)	-0.52 (0.1)	-0.57 (0.09)	-0.23 (0.5)	-0.4 (0.2)	-0.2 (0.6)	0.093 (0.8)	0.15 (0.7)
ME green	-0.12 (0.7)	-0.069 (0.8)	-0.18 (0.6)	0.18 (0.6)	0.37 (0.3)	-0.14 (0.7)	-0.4 (0.3)	-0.24 (0.5)	-0.13 (0.7)	-0.31 (0.4)	-0.17 (0.6)	-0.11 (0.8)	-0.3 (0.4)	-0.11 (0.8)	-0.17 (0.6)	0.072 (0.8)
ME turquoise	0.18 (0.6)	0.048 (0.9)	-0.48 (0.2)	-0.31 (0.4)	0.17 (0.6)	-0.54 (0.1)	-0.24 (0.5)	-0.038 (0.9)	-0.16 (0.7)	-0.53 (0.1)	-0.62 (0.06)	-0.046 (0.9)	-0.34 (0.3)	-0.05 (0.9)	-0.091 (0.8)	0.2 (0.6)

MIENTURNET
MIcroRNAENrichmentTURnedNETwork



miRNA driver for:
● grading

WGCNA on PCa (G6-7) patients

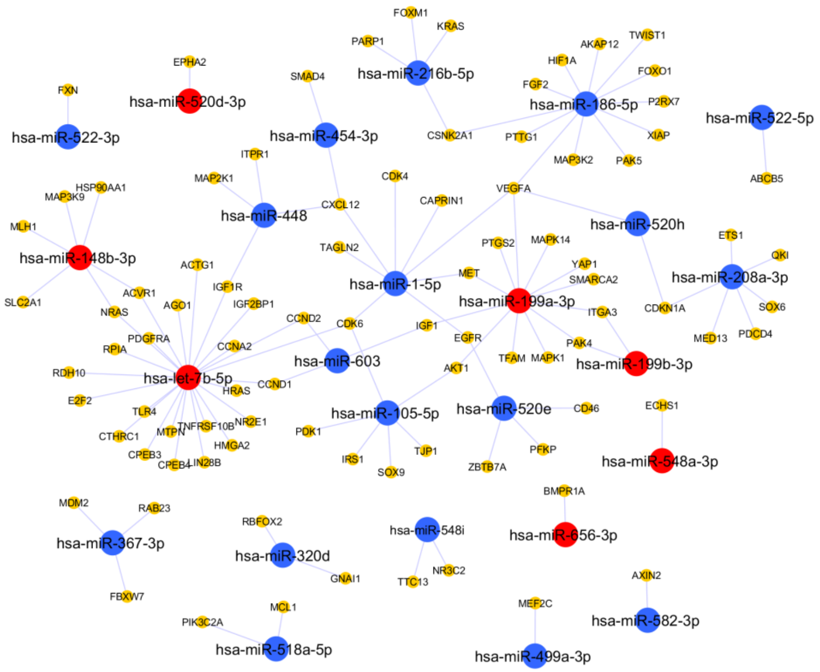


extracellular vesicles (EV)

	grading	age	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep	neoplastic cell percentage	overall mccl
ME brown	0.66 (0.04)	.51 (0.1)	0.087 (0.8)	-0.088 (0.8)	0.46 (0.2)	-0.49 (0.1)	0.086 (0.8)	0.42 (0.2)	0.57 (0.08)	-0.16 (0.6)	-0.14 (0.7)	0.37 (0.3)	-0.56 (0.09)	0.49 (0.1)	0.088 (0.8)	-0.064 (0.9)
ME yellow	-0.15 (0.7)	-0.39 (0.3)	-0.17 (0.6)	0.2 (0.6)	0.035 (0.9)	-0.075 (0.8)	-0.47 (0.2)	-0.55 (0.1)	-0.17 (0.6)	-0.55 (0.1)	-0.19 (0.6)	-0.34 (0.3)	0.61 (0.06)	-0.48 (0.2)	-0.22 (0.5)	-0.19 (0.6)
ME blue	0.32 (0.4)	-0.2 (0.6)	-0.46 (0.2)	0.33 (0.4)	0.43 (0.2)	-0.53 (0.1)	-0.46 (0.2)	-0.28 (0.4)	-0.12 (0.8)	-0.52 (0.1)	-0.57 (0.09)	-0.23 (0.5)	-0.4 (0.2)	-0.2 (0.6)	0.093 (0.8)	0.15 (0.7)
ME green	-0.12 (0.7)	-0.069 (0.8)	-0.18 (0.6)	0.18 (0.6)	0.37 (0.3)	-0.14 (0.7)	-0.4 (0.3)	-0.24 (0.5)	-0.13 (0.7)	-0.31 (0.4)	-0.17 (0.6)	-0.11 (0.8)	-0.3 (0.4)	-0.11 (0.8)	-0.17 (0.6)	0.072 (0.8)
ME turquoise	0.18 (0.6)	0.048 (0.9)	-0.48 (0.2)	-0.31 (0.4)	0.17 (0.6)	-0.54 (0.1)	-0.24 (0.5)	-0.038 (0.9)	-0.16 (0.7)	-0.53 (0.1)	-0.62 (0.06)	-0.046 (0.9)	-0.34 (0.3)	-0.05 (0.9)	-0.091 (0.8)	0.2 (0.6)

MIENTURNET

MIcroRNAENrichmentTURnedNETwork

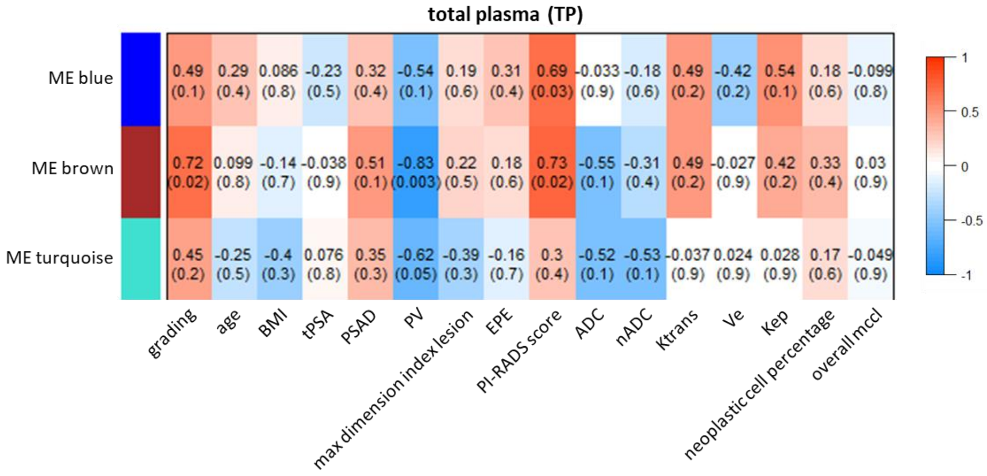


miRNA driver for:

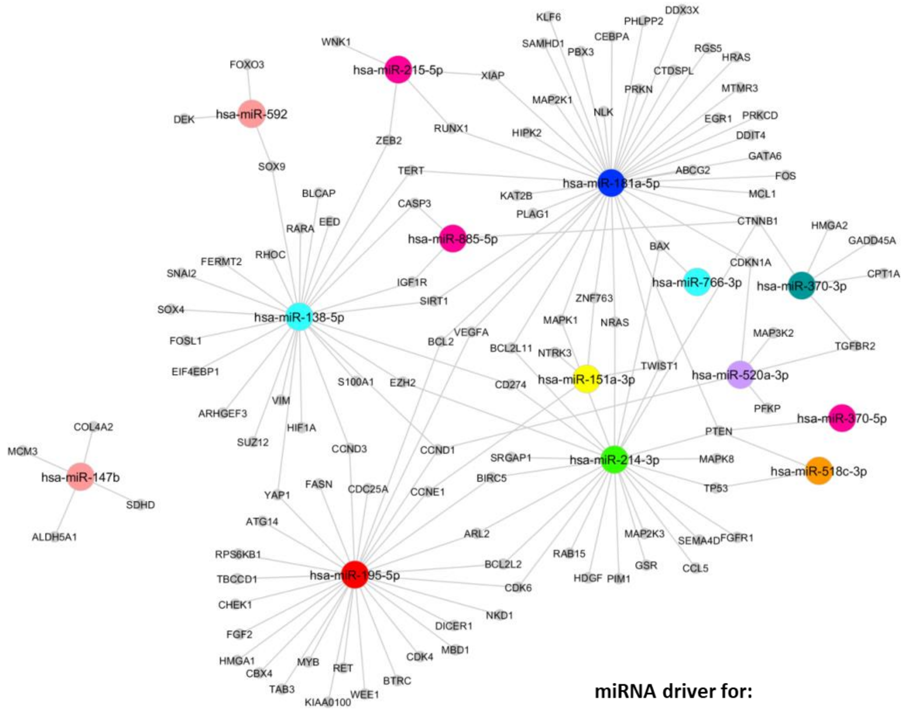
● grading

WGCNA on PCa (G6-7) patients

MIENTURNET
MIcroRNAENrichmentTURnedNETwork



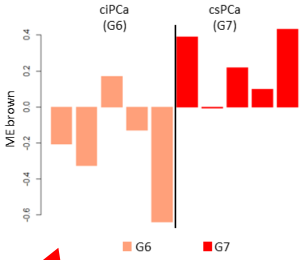
	grading	age	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep	neoplastic cell percentage	overall mccl
ME blue	0.49 (0.1)	0.29 (0.4)	0.086 (0.8)	-0.23 (0.5)	0.32 (0.4)	-0.54 (0.1)	0.19 (0.6)	0.31 (0.4)	0.69 (0.03)	-0.033 (0.9)	-0.18 (0.6)	0.49 (0.2)	-0.42 (0.2)	0.54 (0.1)	0.18 (0.6)	-0.099 (0.8)
ME brown	0.72 (0.02)	0.099 (0.8)	-0.14 (0.7)	-0.038 (0.9)	0.51 (0.1)	-0.83 (0.003)	0.22 (0.5)	0.18 (0.6)	0.73 (0.02)	-0.55 (0.1)	-0.31 (0.4)	0.49 (0.2)	-0.027 (0.9)	0.42 (0.2)	0.33 (0.4)	0.03 (0.9)
ME turquoise	0.45 (0.2)	-0.25 (0.5)	-0.4 (0.3)	0.076 (0.8)	0.35 (0.3)	-0.62 (0.05)	-0.39 (0.3)	-0.16 (0.7)	0.3 (0.4)	-0.52 (0.1)	-0.53 (0.1)	-0.037 (0.9)	0.024 (0.9)	0.028 (0.9)	0.17 (0.6)	-0.049 (0.9)

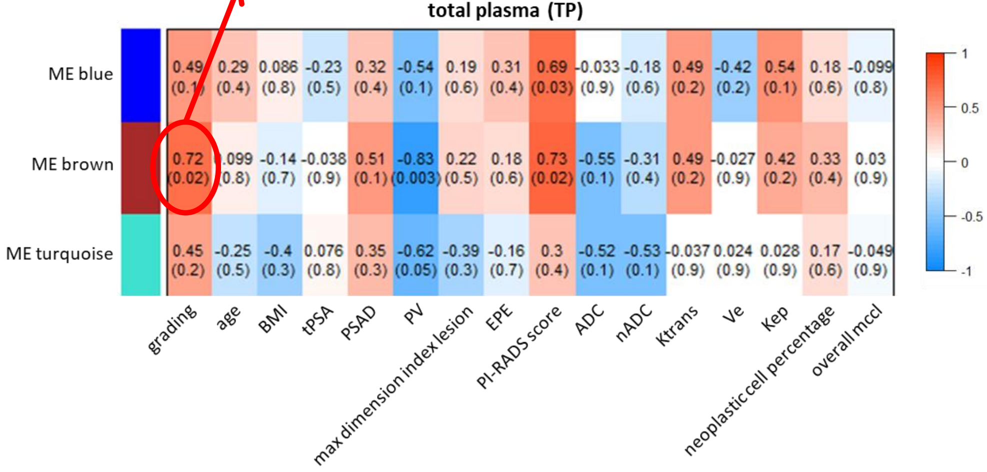


miRNA driver for:

- ● grading
- ● PV
- ● PSAD
- ● grading, ADC
- ● PI-RADS, PSAD, Kep
- ● PI-RADS, Kep, Ktrans
- ● PSAD, PV, ADC
- ● PI-RADS, PSAD, PV, Kep, Ktrans
- ● PI-RADS, PSAD, PV, ADC, Kep
- ● grading, PI-RADS, ADC, Kep, Ktrans

WGCNA on PCa (G6-7) patients

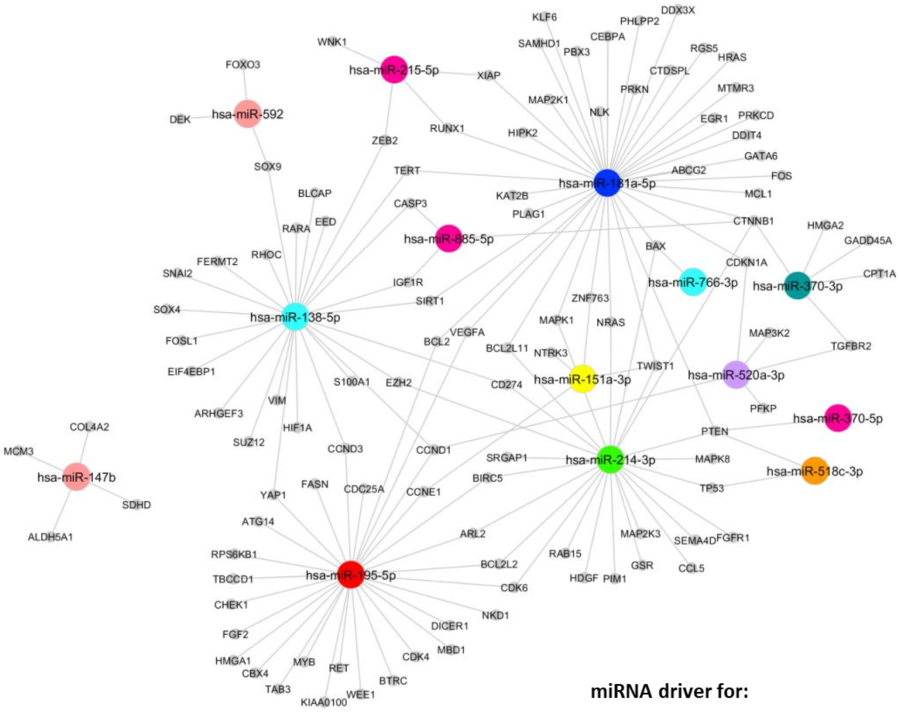




total plasma (TP)

	grading	age	BMI	tPSA	PSAD	PV	max dimension index lesion	EPE	PI-RADS score	ADC	nADC	Ktrans	Ve	Kep	neoplastic cell percentage	overall mccl
ME blue	0.49 (0.1)	0.29 (0.4)	0.086 (0.8)	-0.23 (0.5)	0.32 (0.4)	-0.54 (0.1)	0.19 (0.6)	0.31 (0.4)	0.69 (0.03)	-0.033 (0.9)	-0.18 (0.6)	0.49 (0.2)	-0.42 (0.2)	0.54 (0.1)	0.18 (0.6)	-0.099 (0.8)
ME brown	0.72 (0.02)	0.099 (0.8)	-0.14 (0.7)	-0.038 (0.9)	0.51 (0.1)	-0.83 (0.003)	0.22 (0.5)	0.18 (0.6)	0.73 (0.02)	-0.55 (0.1)	-0.31 (0.4)	0.49 (0.2)	-0.027 (0.9)	0.42 (0.2)	0.33 (0.4)	0.03 (0.9)
ME turquoise	0.45 (0.2)	-0.25 (0.5)	-0.4 (0.3)	0.076 (0.8)	0.35 (0.3)	-0.62 (0.05)	-0.39 (0.3)	-0.16 (0.7)	0.3 (0.4)	-0.52 (0.1)	-0.53 (0.1)	-0.037 (0.9)	0.024 (0.9)	0.028 (0.9)	0.17 (0.6)	-0.049 (0.9)

MIENTURNET
MIcroRNAENrichmentTURnedNETwork



miRNA driver for:

- ● grading
- ● PV
- ● PSAD
- ● grading, ADC
- ● PI-RADS, PSAD, Kep
- ● PI-RADS, Kep, Ktrans
- ● PSAD, PV, ADC
- ● PI-RADS, PSAD, PV, Kep, Ktrans
- ● PI-RADS, PSAD, PV, ADC, Kep
- ● grading, PI-RADS, ADC, Kep, Ktrans

Conclusions

- Our study enabled the identification of several miRNAs with good diagnostic potential when used as a single biomarker or in combination with other imaging/clinical parameters.
- After an experimental validation, they could be suitable for non-invasive diagnosis of PCa using blood as a source of cancer-derived transcripts.
- The clinical implications of this approach would be a dramatic reduction in the number of unnecessary biopsies and a more accurate patient stratification, thus reducing overdiagnosis, overtreatment, and overall related-costs.

4. WGCNA application in grapevine

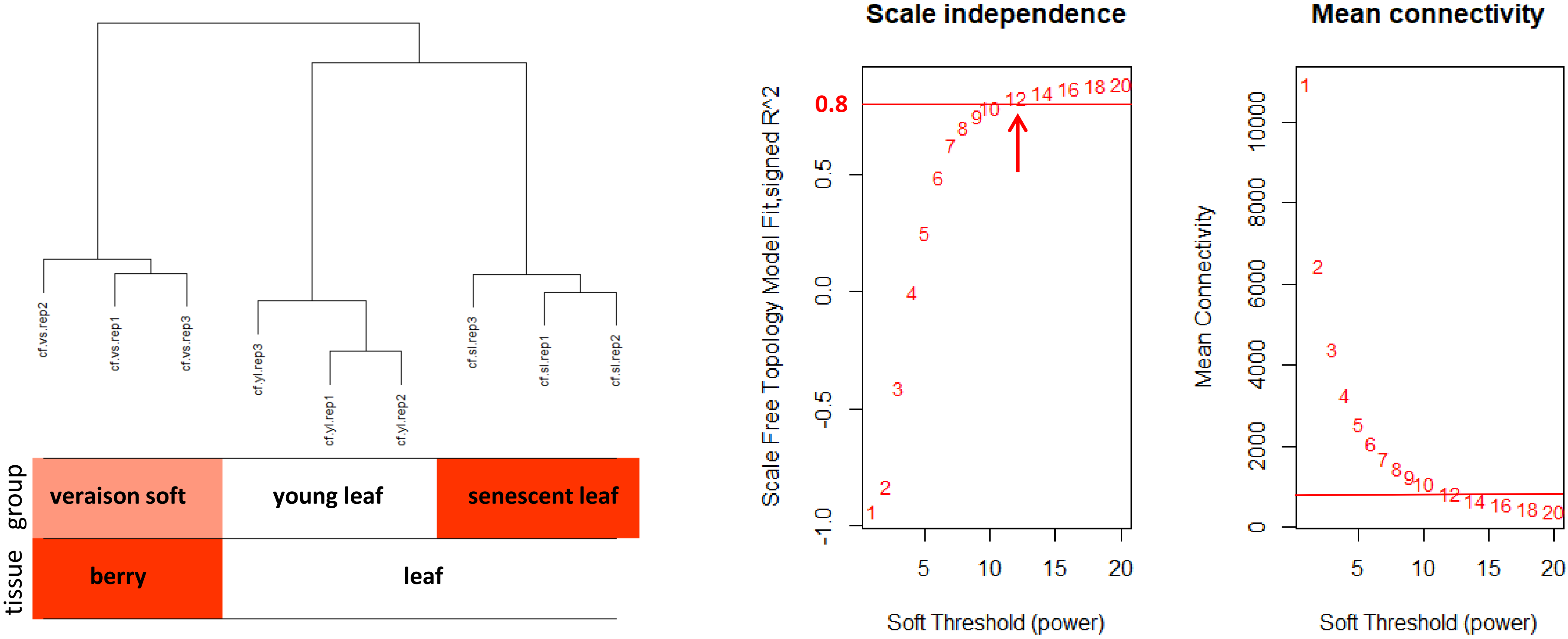
Working in progress

	young leaf			senescent leaf			veraison soft			young leaf			senescent leaf			veraison soft		
	cf.yl.rep1	**cf.yl.rep2**	**cf.yl.rep3**	**cf.sl.rep1**	**cf.sl.rep2**	**cf.sl.rep3**	**cf.vs.rep1**	**cf.vs.rep2**	**cf.vs.rep3**	**rk.yl.rep1**	**rk.yl.rep2**	**rk.yl.rep3**	**rk.sl.rep1**	**rk.sl.rep2**	**rk.sl.rep3**	**rk.vs.rep1**	**rk.vs.rep2**	**rk.vs.rep3**
VIT_201s0011g00010	67.64305	84.40926	46.56563	100.756	109.8212	72.31631	167.3562	172.0807	123.0081	105.5222	98.65743	144.002	151.6068	123.1155	134.4458	110.2557	117.2131	120.8792
VIT_201s0011g00030	23.97475	28.96396	72.70773	1.538259	21.96424	10.19845	74.58265	66.92026	57.24139	7.914168	25.36905	39.65272	22.15792	19.52867	12.15329	31.89215	43.66763	56.16609
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

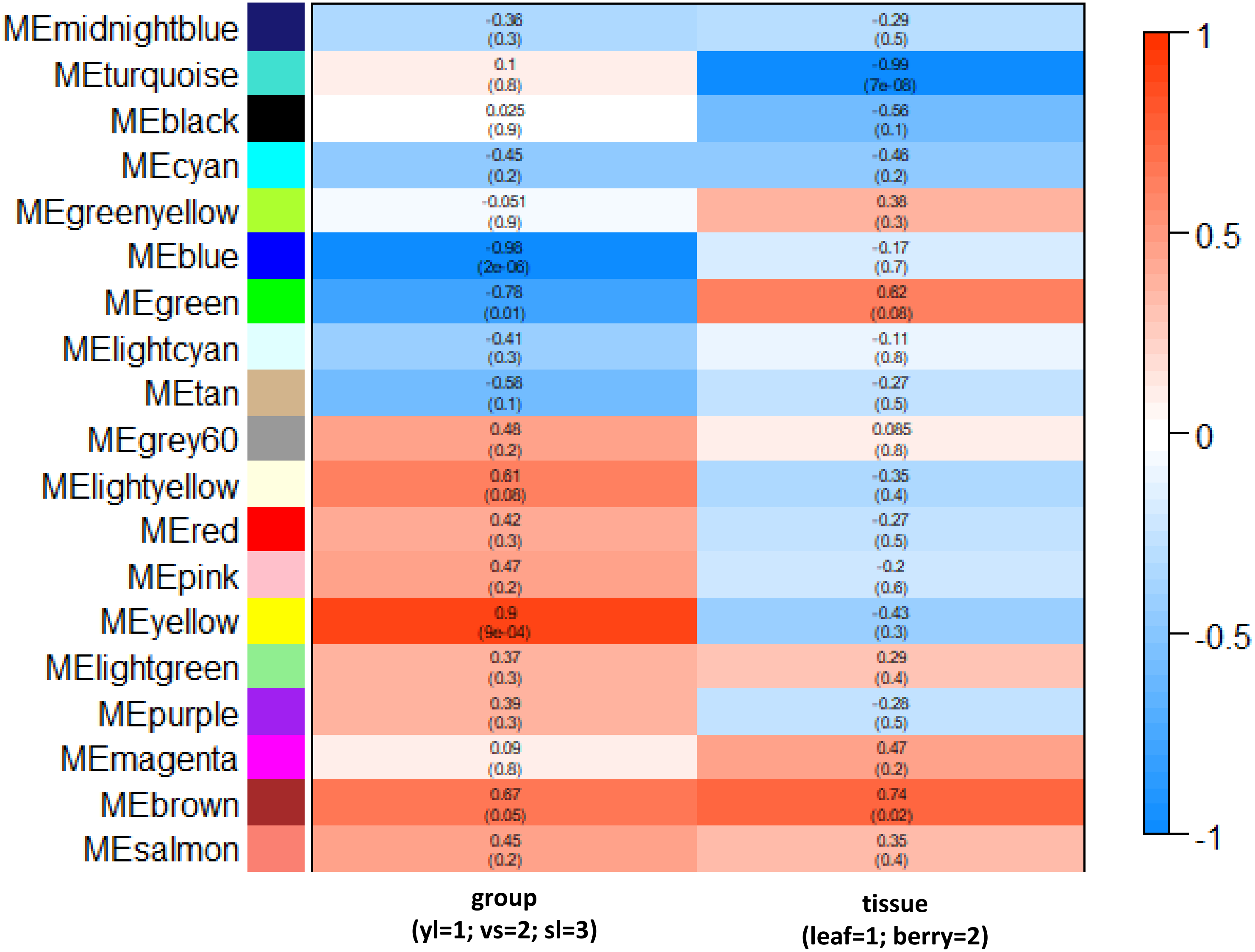
cabernet

rkatsiteli

WGCNA on cabernet

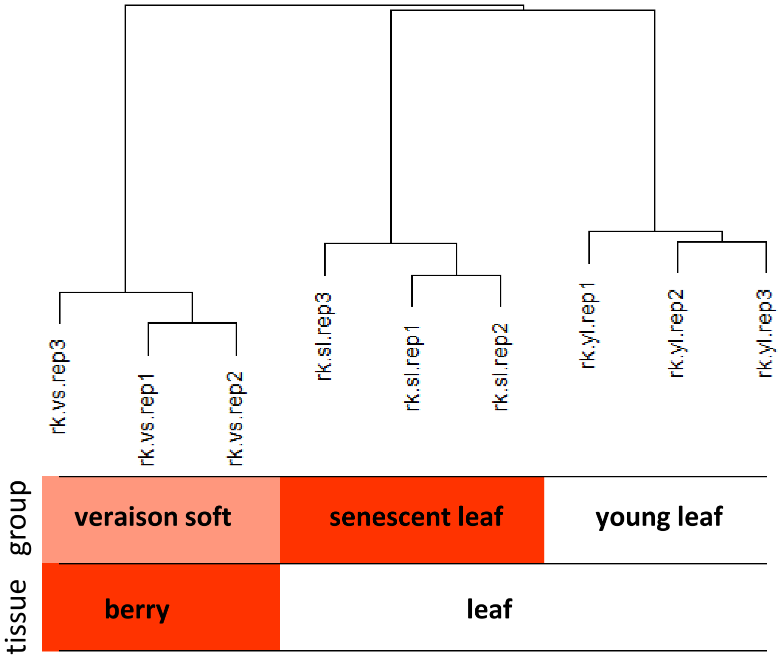


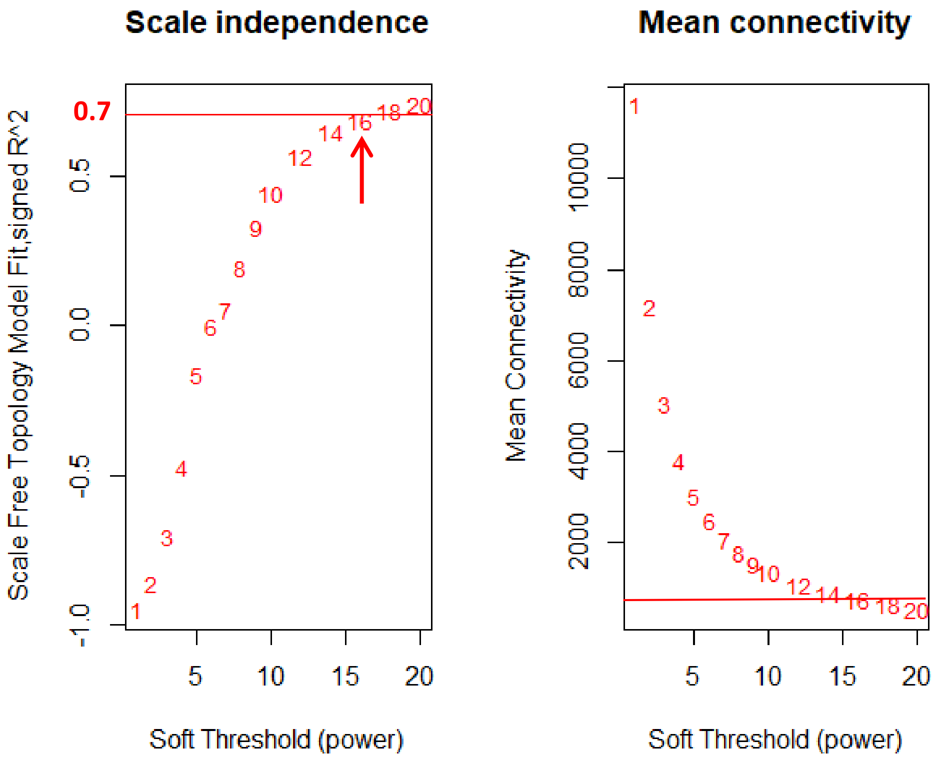
WGCNA on cabernet



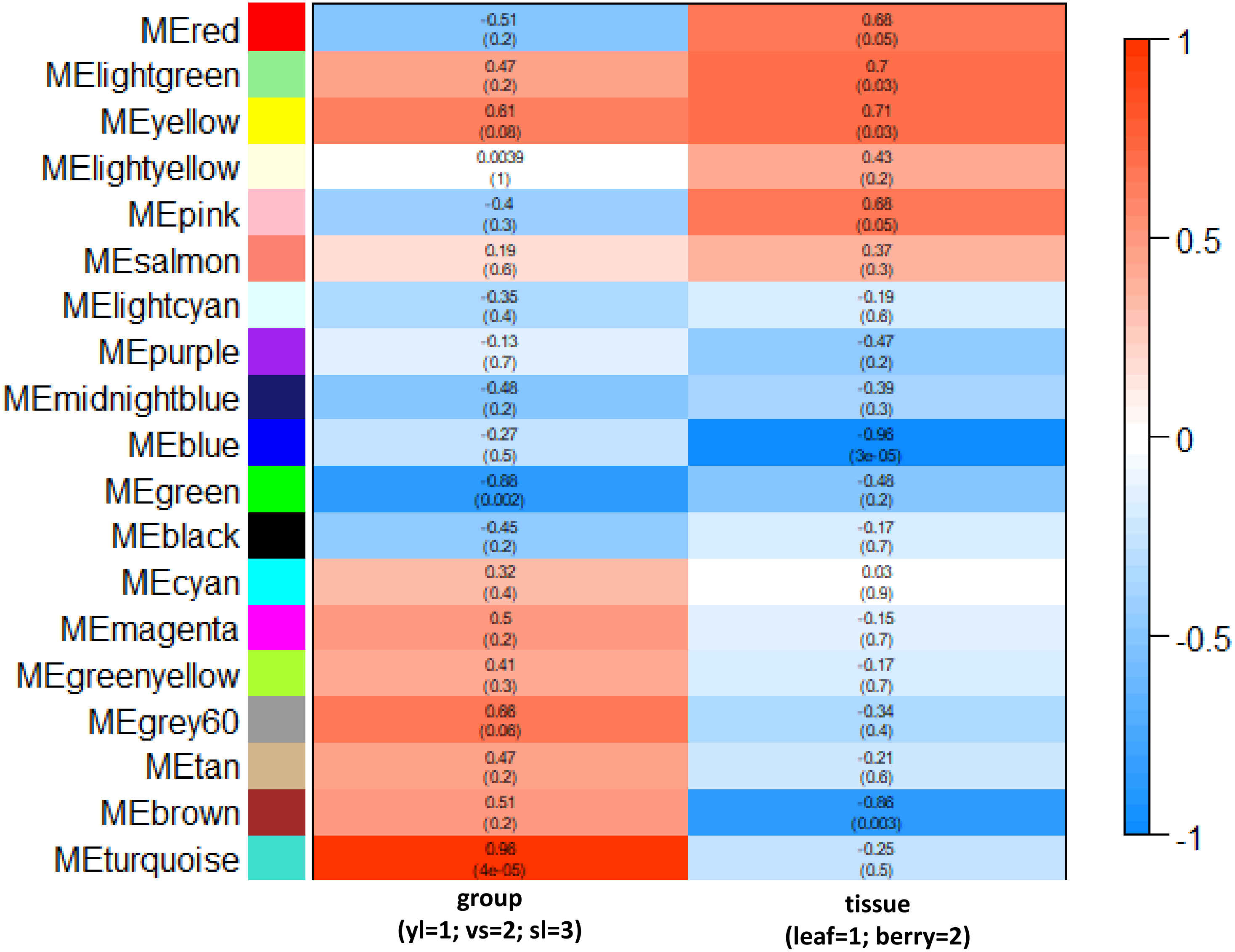
	group (yl=1; vs=2; sl=3)	tissue (leaf=1; berry=2)
MEmidnightblue	-0.36 (0.3)	-0.29 (0.5)
MEturquoise	0.1 (0.8)	-0.99 (7e-08)
MEblack	0.025 (0.9)	-0.56 (0.1)
MEcyan	-0.45 (0.2)	-0.46 (0.2)
MEgreenyellow	-0.051 (0.9)	0.38 (0.3)
MEblue	-0.98 (2e-06)	-0.17 (0.7)
MEgreen	-0.78 (0.01)	0.62 (0.08)
MElightcyan	-0.41 (0.3)	-0.11 (0.8)
MEtan	-0.58 (0.1)	-0.27 (0.5)
MEgrey60	0.48 (0.2)	0.085 (0.8)
MElightyellow	0.61 (0.08)	-0.35 (0.4)
MEred	0.42 (0.3)	-0.27 (0.5)
MEpink	0.47 (0.2)	-0.2 (0.6)
MEyellow	0.9 (9e-04)	-0.43 (0.3)
MElightgreen	0.37 (0.3)	0.29 (0.4)
MEpurple	0.39 (0.3)	-0.28 (0.5)
MEmagenta	0.09 (0.8)	0.47 (0.2)
MEbrown	0.67 (0.05)	0.74 (0.02)
MEsalmon	0.45 (0.2)	0.35 (0.4)

WGCNA on rkatsiteli





WGCNA on rkatsiteli



	group (yl=1; vs=2; sl=3)	tissue (leaf=1; berry=2)
MEred	-0.51 (0.2)	0.68 (0.05)
MElightgreen	0.47 (0.2)	0.7 (0.03)
MEyellow	0.61 (0.08)	0.71 (0.03)
MElightyellow	0.0039 (1)	0.43 (0.2)
MEpink	-0.4 (0.3)	0.68 (0.05)
MEsalmon	0.19 (0.6)	0.37 (0.3)
MElightcyan	-0.35 (0.4)	-0.19 (0.6)
MEpurple	-0.13 (0.7)	-0.47 (0.2)
MEmidnightblue	-0.48 (0.2)	-0.39 (0.3)
MEblue	-0.27 (0.5)	-0.96 (3e-05)
MEgreen	-0.88 (0.002)	-0.48 (0.2)
MEblack	-0.45 (0.2)	-0.17 (0.7)
MEcyan	0.32 (0.4)	0.03 (0.9)
MEmagenta	0.5 (0.2)	-0.15 (0.7)
MEgreenyellow	0.41 (0.3)	-0.17 (0.7)
MEgrey60	0.66 (0.08)	-0.34 (0.4)
MEtan	0.47 (0.2)	-0.21 (0.6)
MEbrown	0.51 (0.2)	-0.86 (0.003)
MEturquoise	0.98 (4e-05)	-0.25 (0.5)


THANK
YOU