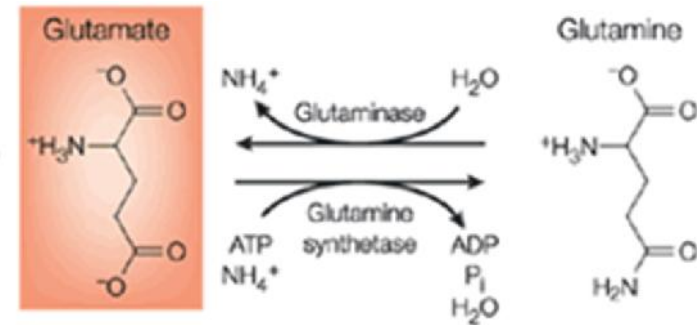


Glutamine/glutamate analysis results

Network analysis of untargeted lipidomics

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05/31/2017



# Metabolites of Glutamate Metabolism Are Associated with Incident Type 2 Diabetes

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# Background

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- Glutamine (Gln) enhances cardiac recovery in mice and human.
- Plasma Glutamate (Glu) levels are **positively** associated with, and Gln levels or glutamine-to-glutamate ratio (Gln:Glu) are **inversely** associated with BMI, blood pressure, and insulin resistance.
- **Higher** circulating Gln:Glu levels are also associated with a **reduced** type 2 diabetes (T2D) risk.
- However, evidence that relates these metabolites directly and, moreover, relates changes in these metabolites, to T2D risk is limited.

Cheng S, et al. *Circulation*. 2012.  
Khogali SE, et al. *JMCC*. 1998.  
Khogali SE, et al. *Nutrition*. 2002.

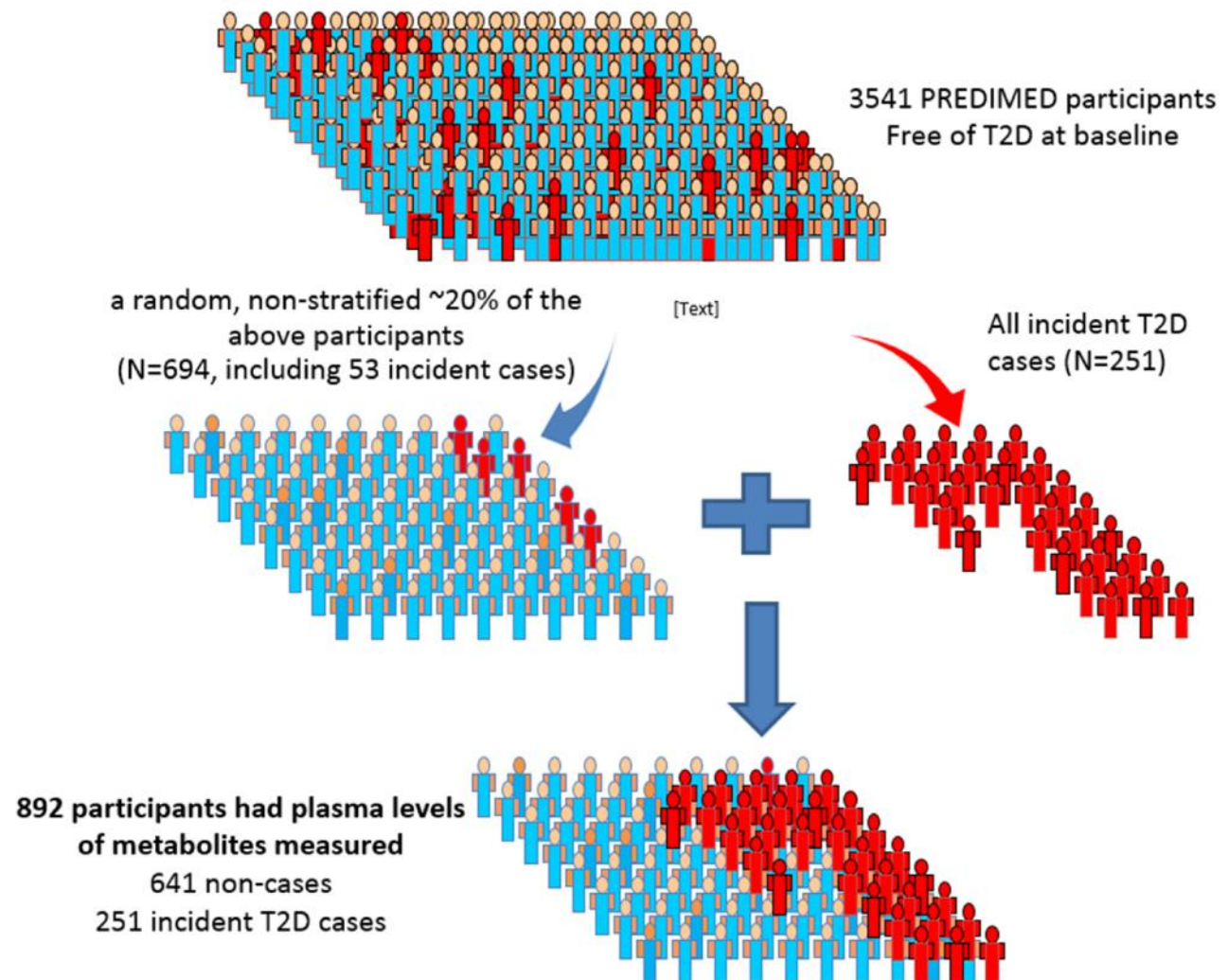
# Research questions

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- 1) Are baseline levels of these metabolites (Gln, Glu, and their ratio) or their year-1 changes associated with the risk of T2D?
- 2) Do the MedDiet interventions change metabolite levels after 1 year?
- 3) Whether the cardioprotective effects of the Mediterranean diet interventions are modified by these metabolites?

# Study design

- Case-cohort design



# Study design

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- Case-cohort design
- Metabolomics platform
  - Liquid chromatography tandem mass spectrometry (LC-MS/MS) techniques by Broad Institute
  - Rank-based inverse normal transformations
- Weighted Cox PH models
  - metabolites as both continuous variables and in quartile categories

# Baseline Characteristics

**Table 1. Baseline participant characteristics in the random subcohort and of the cases**

	Subcohort <sup>a</sup>	Cases
n	694	251
Age (years)	66.5 (5.7)	66.4 (5.7)
Sex (% women),	62.8	55.0
Intervention group, %		
MedDiet+EVOO	30.7	29.9
MedDiet+nuts	37.2	33.9
Control	32.1	36.3
Hypertension, %	90.8	96.0
Dyslipidemia, %	85.0	79.7
Smoking, %		
Never	61.0	52.6
Former	22.6	22.3
Current	16.4	25.1
Waist circumference, cm	99.5 (10.7)	103.4 (10.0)
Body mass index, kg/m <sup>2</sup>	29.9 (3.6)	30.8 (3.3)
Physical activity, METs/d	238 (238)	249 (232)
Education, %		
Elementary or lower	75.4	76.5
Secondary or higher	24.6	23.5
Total energy intake, kcal/d	2277 (566)	2327 (622)
Score for adherence to Mediterranean diet <sup>b</sup>	8.6 (1.9)	8.5 (1.8)
Fasting Glucose, mg/dl	99.7 (15.2)	117.2 (17.6)

Abbreviation: EVOO, Extra-virgin olive oil; CHD, coronary heart disease; MET, metabolic equivalent. Values are mean (SD) or percentage.<sup>a</sup> 37 cases are included in the randomly selected subcohort.

<sup>b</sup>This score is based on the 14-item dietary screener.<sup>33</sup>



# Risk of incident T2D

Table 2. Incident diabetes by Baseline Plasma Amino Acid Concentrations in the PREDIMED Trial, 2003–2010: Observed Event Rates and Hazard Ratios (251 cases, 694 participants in sub-cohort) <sup>a</sup>

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend	P-interaction <sup>d</sup>
<b>Univariable models</b>						
Glutamine	1.00 (ref)	1.24 (0.83, 1.85)	0.93 (0.61, 1.43)	0.84 (0.54, 1.3)	0.25	0.25
Glutamate	1.00 (ref)	2.39 (1.43, 3.99)	4.13 (2.54, 6.72)	2.43 (1.44, 4.1)	0.0001	0.66
Glutamine to glutamate ratio	1.00 (ref)	1.36 (0.93, 2)	0.78 (0.52, 1.19)	0.39 (0.23, 0.64)	0.00002	0.84
<b>Multivariable models <sup>b</sup> (main model)</b>						
Glutamine	1.00 (ref)	1.14 (0.68, 1.93)	0.8 (0.47, 1.36)	0.64 (0.36, 1.12)	0.04	0.39
Glutamate	1.00 (ref)	2.99 (1.15, 7.74)	3.83 (1.64, 8.94)	2.03 (0.72, 5.73)	0.0002	0.64
Glutamine to glutamate ratio	1.00 (ref)	1.18 (0.72, 1.93)	0.69 (0.4, 1.2)	0.31 (0.16, 0.57)	0.0001	0.75
<b>Multivariable models + baseline fasting glucose <sup>c</sup> (sensitivity analysis)</b>						
Glutamine	1.00 (ref)	0.82 (0.39, 1.71)	0.47 (0.19, 1.18)	0.38 (0.15, 0.96)	0.02	0.92
Glutamate	1.00 (ref)	2.24 (1.22, 4.11)	4.34 (2.42, 7.78)	2.78 (1.43, 5.41)	0.14	0.36
Glutamine to glutamate ratio	1.00 (ref)	1.17 (0.49, 2.82)	0.55 (0.2, 1.49)	0.34 (0.12, 0.94)	0.03	0.36

<sup>a</sup> Inverse normal transformation was applied to raw values.

<sup>b</sup> Stratified by recruitment center and intervention group (MedDiet+EVOO, MedDiet+nuts, low fat), and adjusted for age (years), sex (male, female), body mass index (kg/m<sup>2</sup>), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia and hypertension.

<sup>c</sup> glucose levels were available in 537 participants.

<sup>d</sup> p for interaction with 1 degree of freedom: amino acid level × intervention arm (intervention vs. control)



# Correlation with fasting glucose

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Table 3 correlation of glucose with amino acids

	glutamate	glutamine	Glutamine to glutamate ratio
<b>Correlation coefficient</b>	0.10	-0.07	-0.13
<b>p value</b>	0.02	0.10	0.002

# Risk of incident T2D

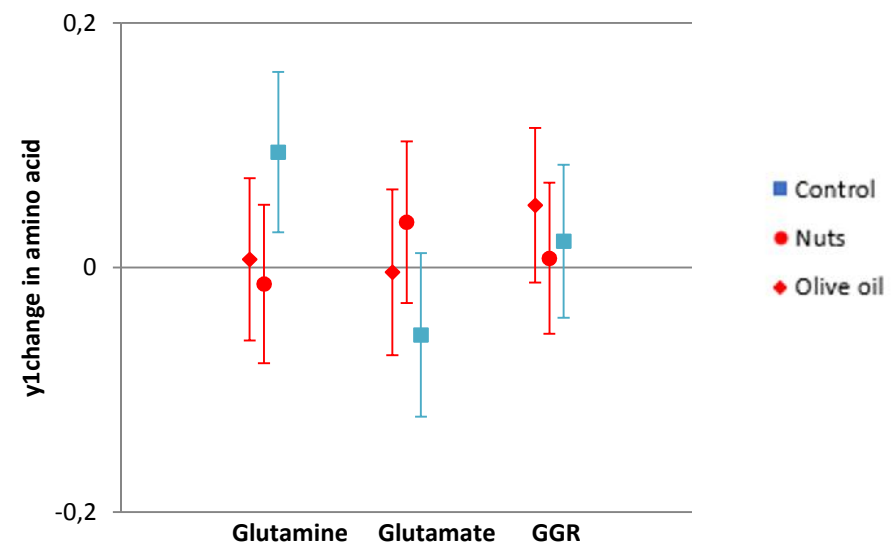
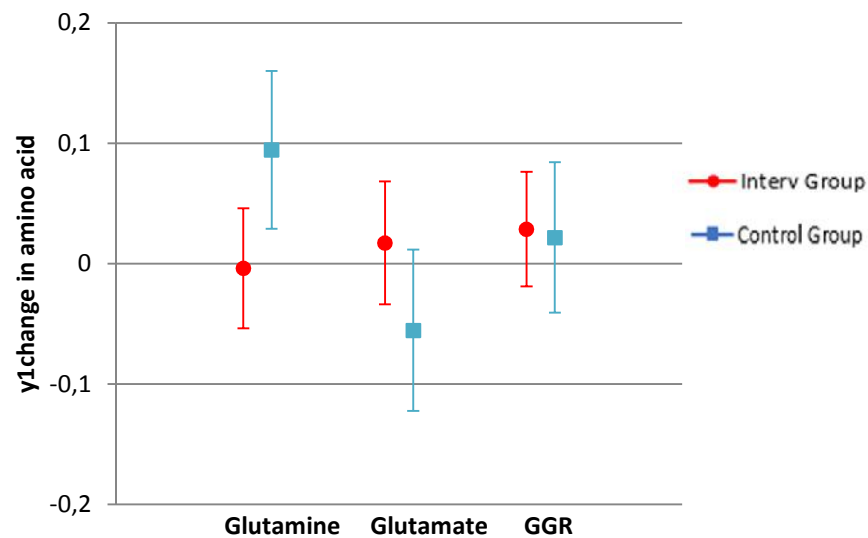
Table 4. Incident Type 2 diabetes by Changes in Plasma Amino Acid Concentrations in the PREDIMED Trial, 2003–2010: Observed Event Rates and Hazard Ratios (251 cases, 694 participants in sub-cohort) <sup>a</sup>

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
<b>Univariable models</b>					
Glutamine change	1.00 (ref)	1.33 (0.80, 2.20)	1.33 (0.80, 2.20)	1.33 (0.80, 2.20)	0.75
Glutamate change	1.00 (ref)	0.81 (0.52, 1.29)	0.81 (0.52, 1.29)	0.81 (0.52, 1.29)	0.14
Glutamine to glutamate ratio change	1.00 (ref)	4.88 (2.51, 9.47)	4.88 (2.51, 9.47)	4.88 (2.51, 9.47)	0.009
<b>Multivariable models <sup>b</sup> (main model)</b>					
Glutamine change	1.00 (ref)	1.12 (0.59, 2.13)	1.12 (0.59, 2.13)	1.12 (0.59, 2.13)	0.64
Glutamate change	1.00 (ref)	1.00 (0.49, 2.04)	1.00 (0.49, 2.04)	1.00 (0.49, 2.04)	0.84
Glutamine to glutamate ratio change	1.00 (ref)	3.03 (1.37, 6.70)	3.03 (1.37, 6.70)	3.03 (1.37, 6.70)	0.41

<sup>a</sup> Inverse normal transformation was applied to raw values.

<sup>b</sup> Stratified by recruitment center and intervention group (MedDiet+EVOO, MedDiet+nuts, low fat), and adjusted for age (years), sex (male, female), body mass index (kg/m<sup>2</sup>), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, and baseline level of respective amino acid.

# Dietary Effects on Metabolites Change



There were no statistically significant differences in mean first-year changes of metabolites between intervention and control groups, after adjustment for age (years), sex (male, female), body mass index (kg/m<sup>2</sup>), smoking (never, current, former), leisure-time physical activity (metabolic equivalent tasks in minutes/day), dyslipidemia, hypertension, and baseline level of respective amino acid.

# Conclusion

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- Our results suggest that participants with higher Glu levels may have a higher risk, and those with a higher Gln:Glu might have a lower risk of T2D.
- The intervention diets may not change these metabolite levels after 1 year.

# Untargeted Lipids Signed Network Analysis in PREDIMED preliminary results



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# Weighted correlation network analysis

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- Weighted correlation network analysis (WGCNA) is a widely used data mining method based on *pairwise correlations between variables.*
- WGCNA can be used for
  - finding clusters (modules) of highly correlated metabolites,
  - summarizing such clusters using the module eigengene or an intramodular hub gene/metabolite,
  - relating modules to one another and to external clinical traits.

[https://en.wikipedia.org/wiki/Weighted\\_correlation\\_network\\_analysis](https://en.wikipedia.org/wiki/Weighted_correlation_network_analysis)

<https://labs.genetics.ucla.edu/horvath/CoexpressionNetwork/Rpackages/WGCNA/>

# Data outline

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## **CVD project**

- 6359 metabolites (201 named lipids and 6158 unnamed lipids)
- Samples were from 984 participants at baseline
- Metabolite levels were natural log-transformed before any further analysis

## **T2D project**

- 3374 metabolites (301 named lipids and 3073 unnamed lipids)
- Samples were from 1017 participants at baseline
- Metabolite levels were natural log-transformed before any further analysis



# Data pre-management

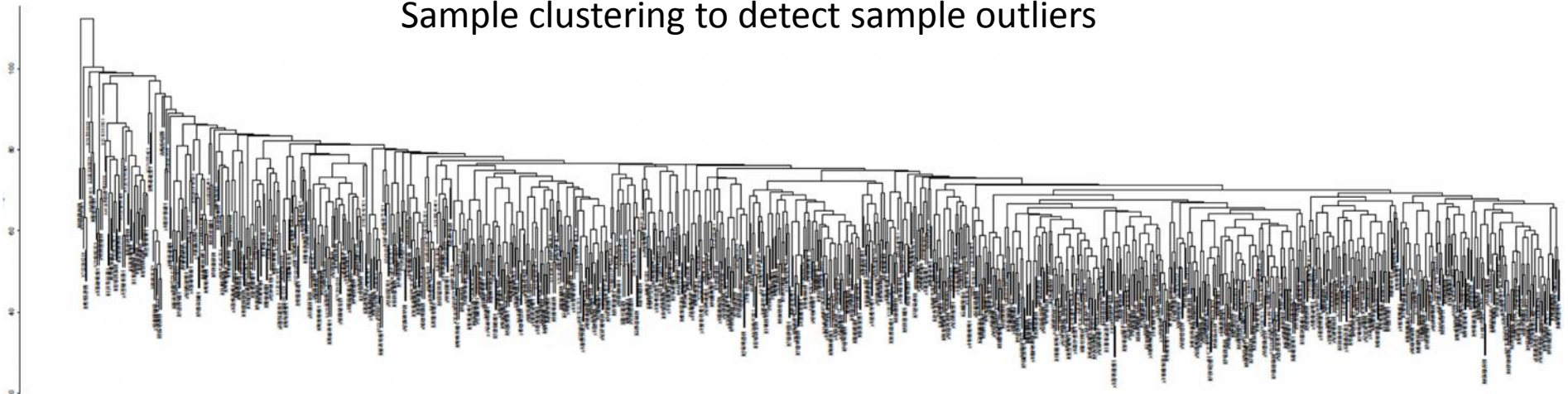
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Using WGCNA package for R:

<https://labs.genetics.ucla.edu/horvath/CoexpressionNetwork/Rpackages/WGCNA/>

Detected and deleted metabolite outliers due to >50% missing samples or zero variance, and **5633 metabolites were left for further analysis in CVD project and 3327 metabolites in T2D project**

Sample clustering to detect sample outliers



X-axis: each sample (individual); Y-axis: measure of similarity between samples based on metabolites;  
I did not exclude any sample based on this figure->**984 samples for further analysis**

# Data pre-management

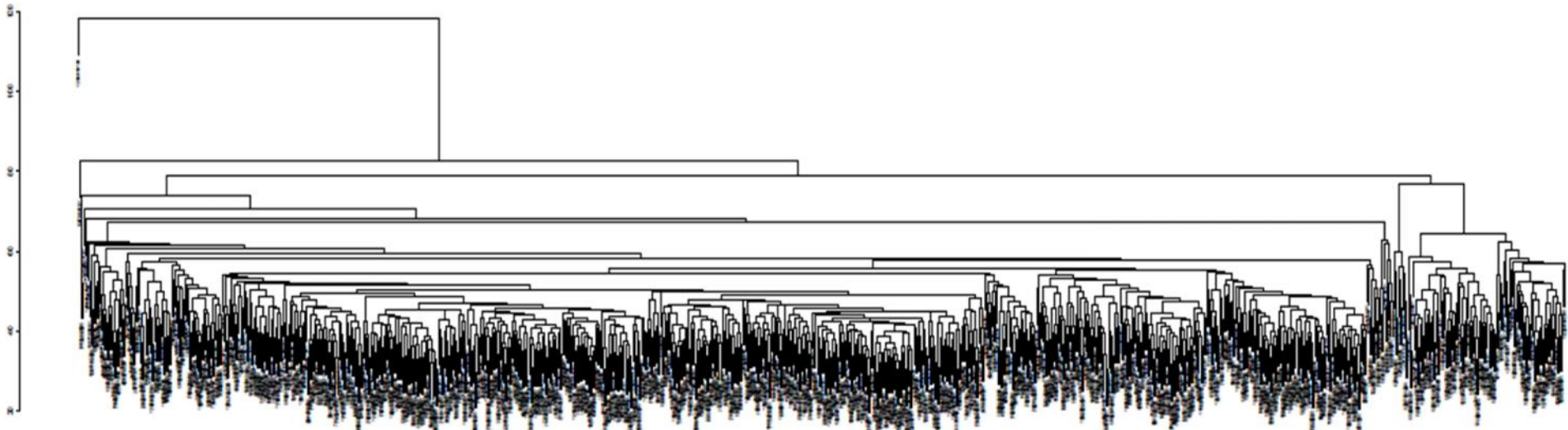
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Using WGCNA package for R:

<https://labs.genetics.ucla.edu/horvath/CoexpressionNetwork/Rpackages/WGCNA/>

Detected and deleted metabolite outliers due to >50% missing samples or zero variance, and **5633 metabolites were left for further analysis in CVD project and 3327 metabolites in T2D project**

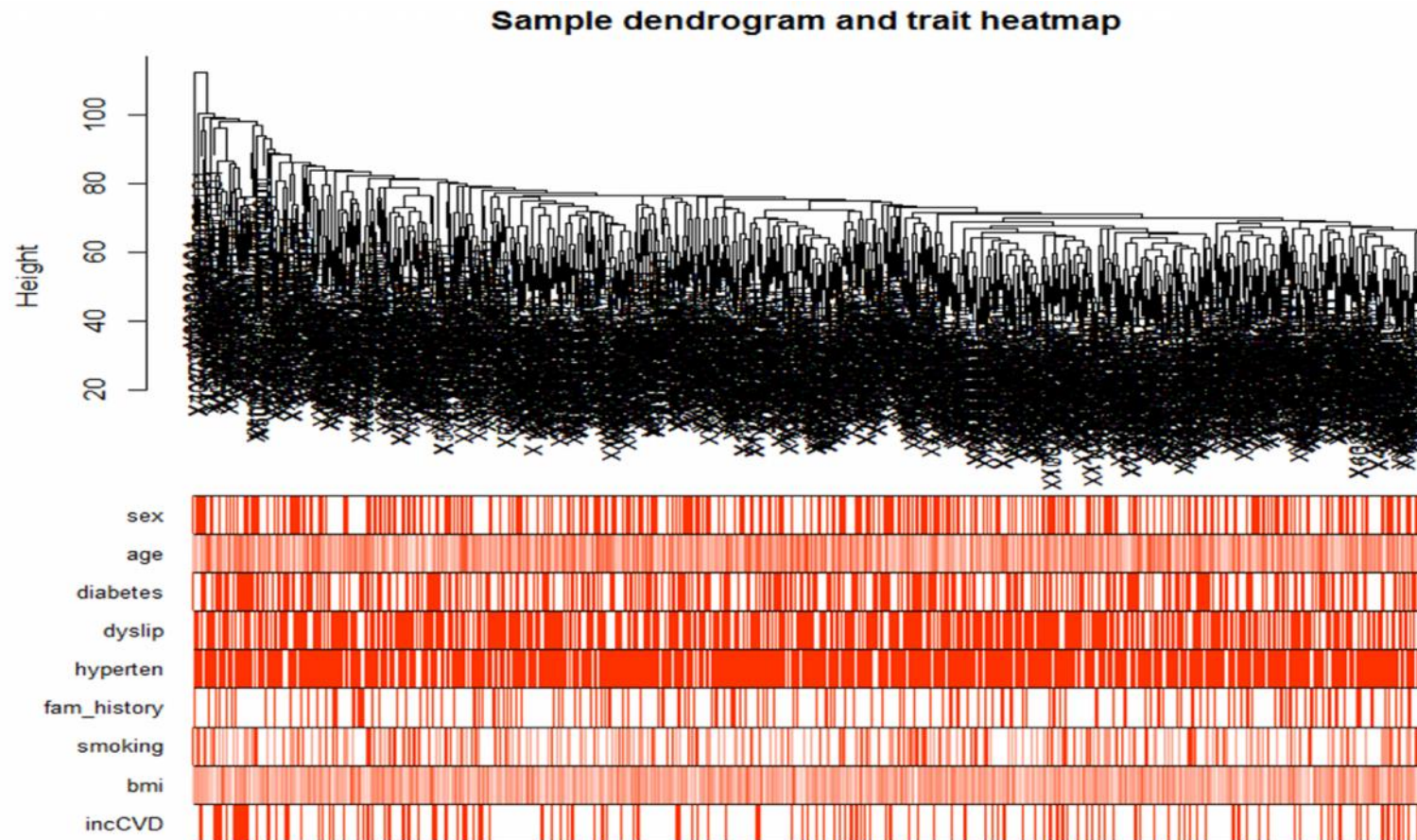
Sample clustering to detect sample outliers



X-axis: each sample (individual); Y-axis: measure of similarity between samples based on metabolites;

I did not exclude any sample based on this figure->**1017 samples for further analysis**

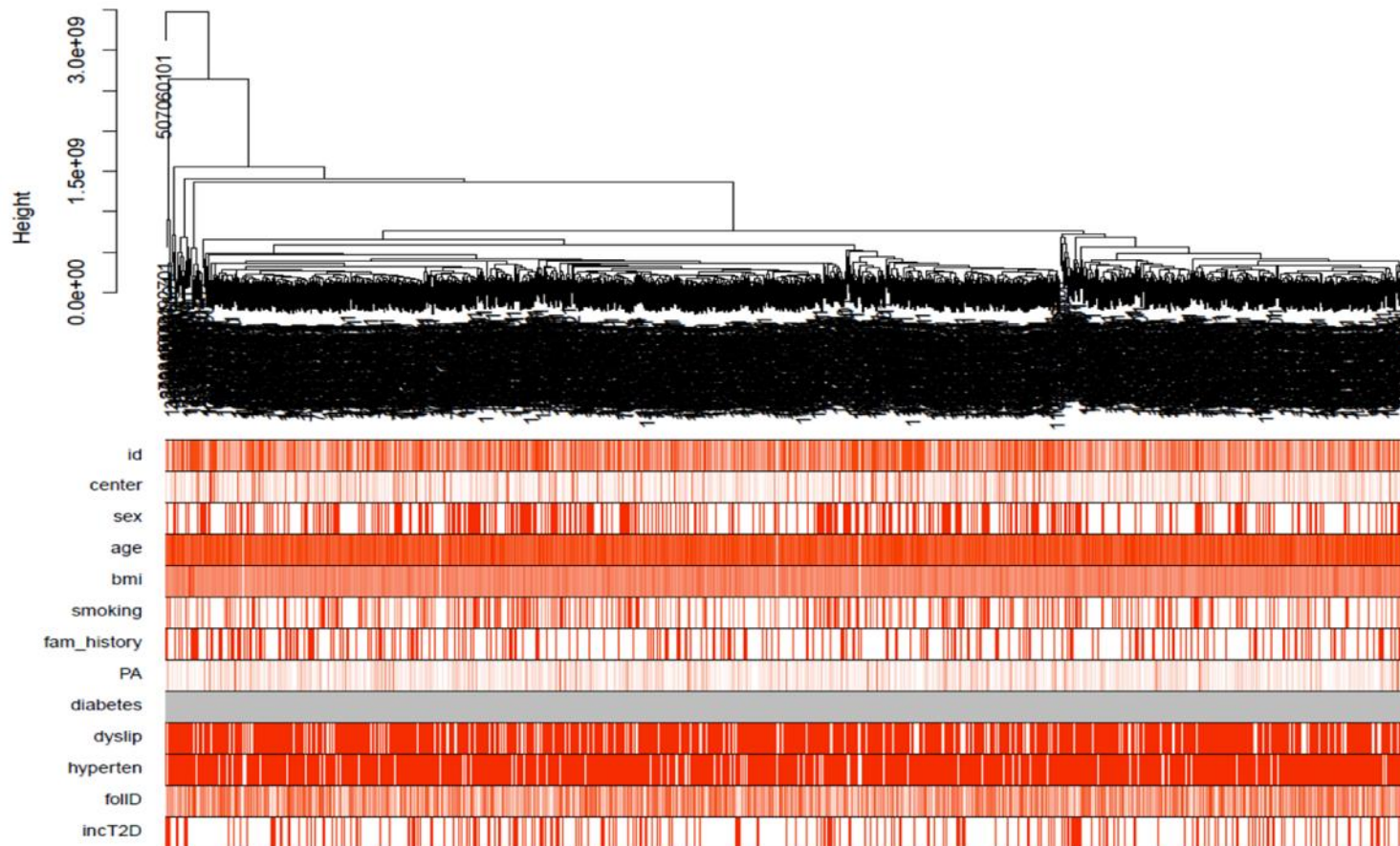
# Incorporating metabolite and trait data-CVD project



The upper plot: X-axis: each sample (individual); Y-axis (Height): measure of similarity between samples based on metabolites;

The lower heat-map: each row represents a trait, each column represents a sample (individual); white means a low value, red a high value, grey a missing value. E.g., most individuals had hypertension at baseline, and thus the hypertension row is almost red.

# Incorporating metabolite and trait data-T2D project



The upper plot: X-axis: each sample (individual); Y-axis (Height): measure of similarity between samples based on metabolites;

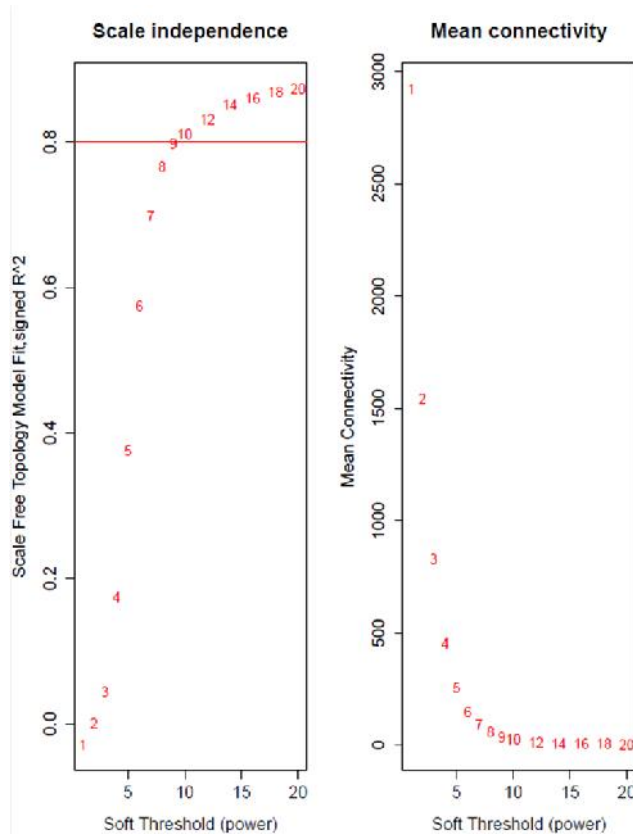
The lower heat-map: each row represents a trait, each column represents a sample (individual); white means a low value, red a high value, grey a missing value. E.g., most individuals had hypertension at baseline, and thus the hypertension row is almost red.



# Signed Network construction

## (choosing the soft-thresholding power)

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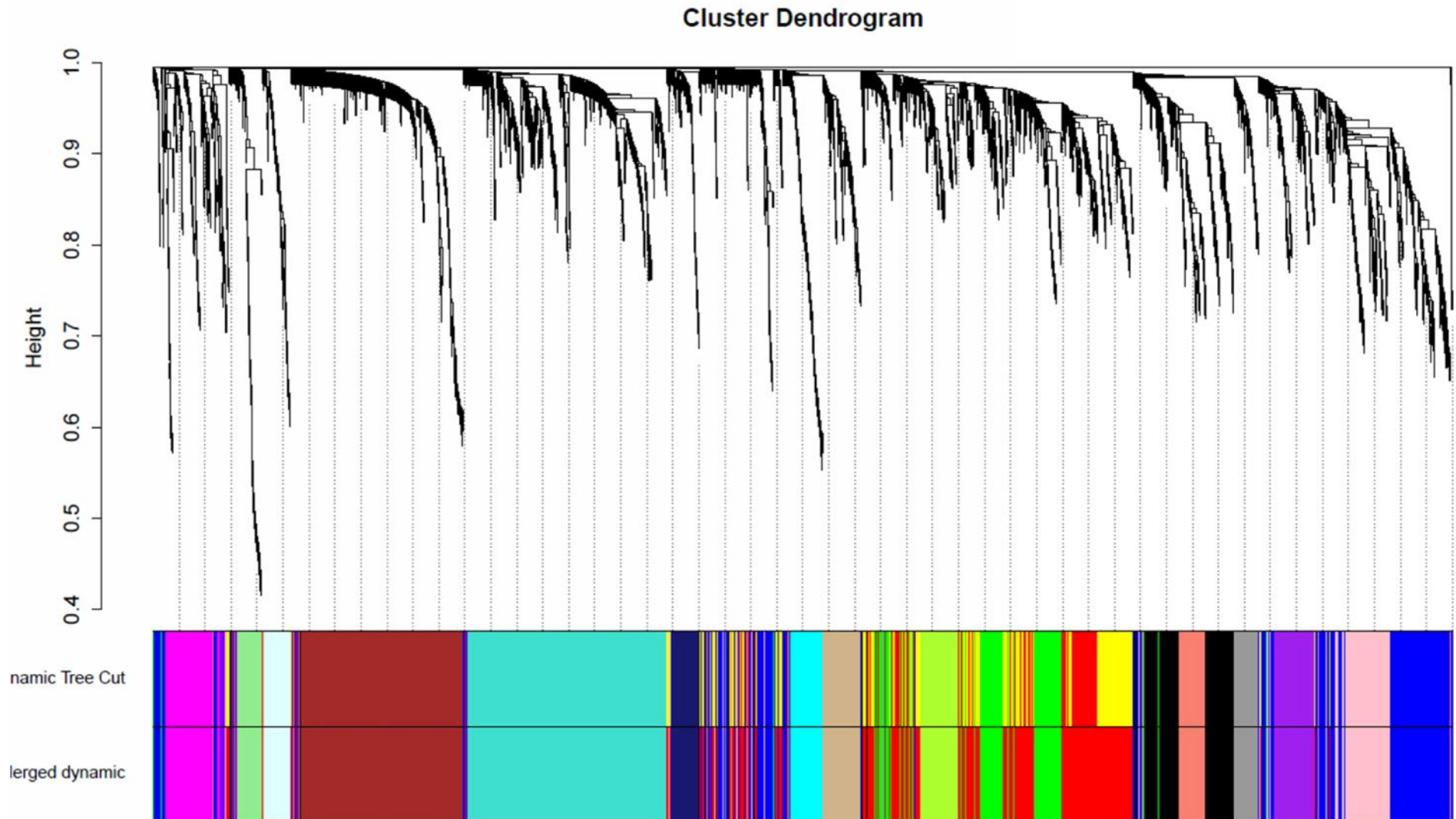
I choose the **power 9**, the lowest power for which the scale-free topology fit index reaches 0.80 (the default value is 0.90) with a relatively decent mean connectivity

Analysis of network topology for various soft-thresholding powers. The left panel shows the scale-free fit index (y-axis) as a function of the soft-thresholding power (x-axis). The right panel displays the mean connectivity (degree, y-axis) as a function of the soft-thresholding power (x-axis).

# Signed Network construction-CVD project

(I choose the minimum module size=100)

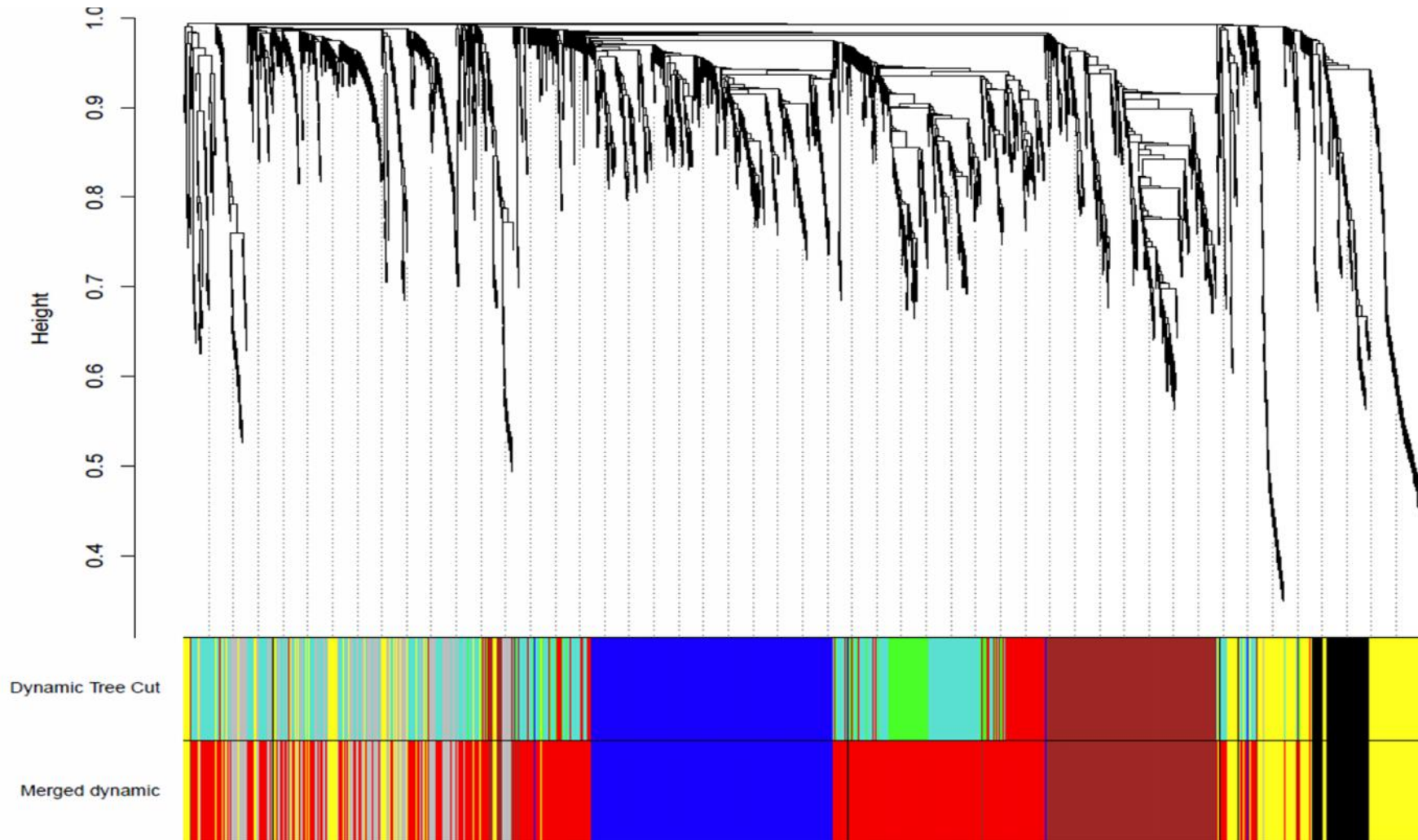
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# Signed Network construction-T2D project

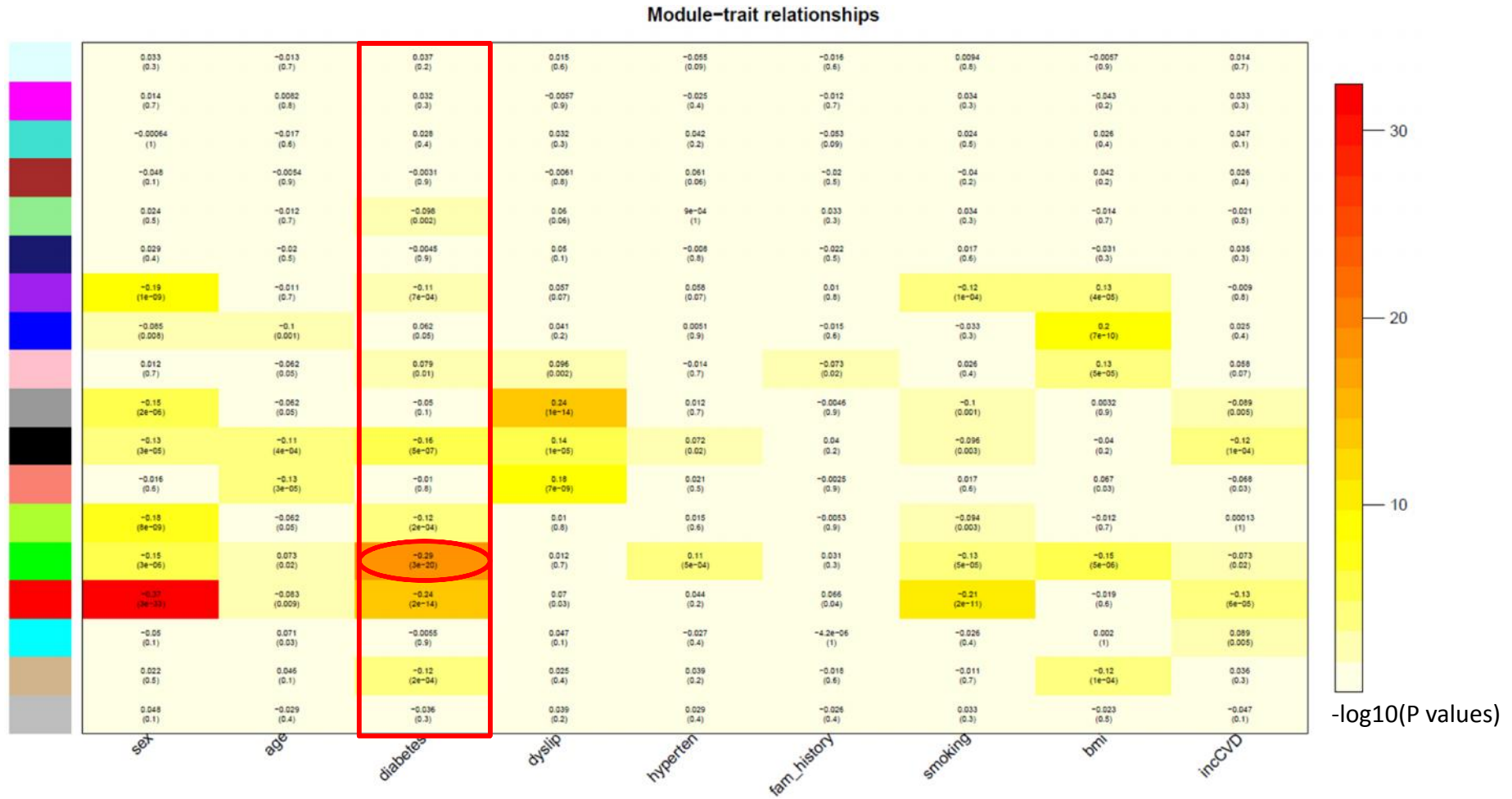
(I choose the minimum module size=100)

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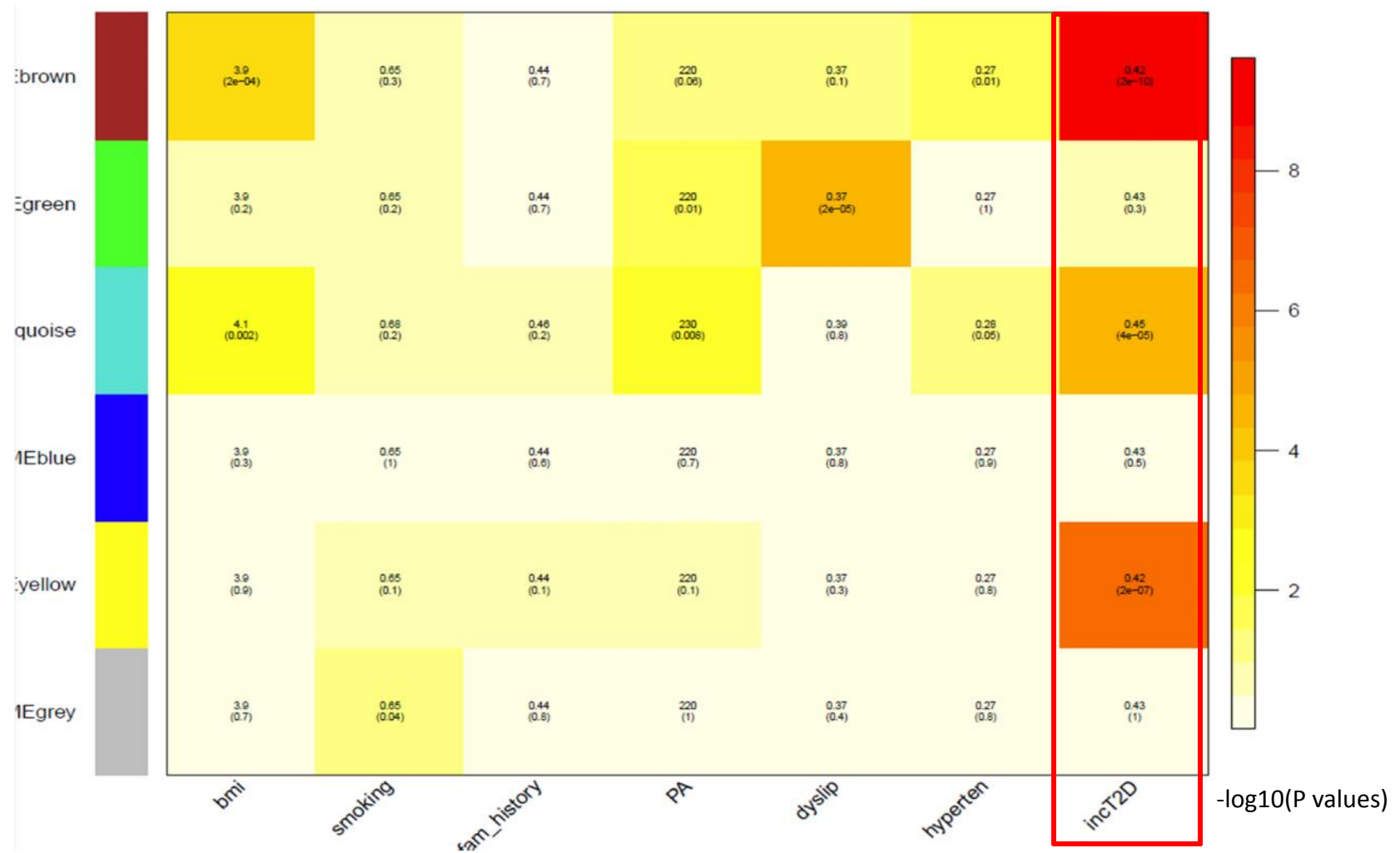


# Module-trait relationship-CVD project



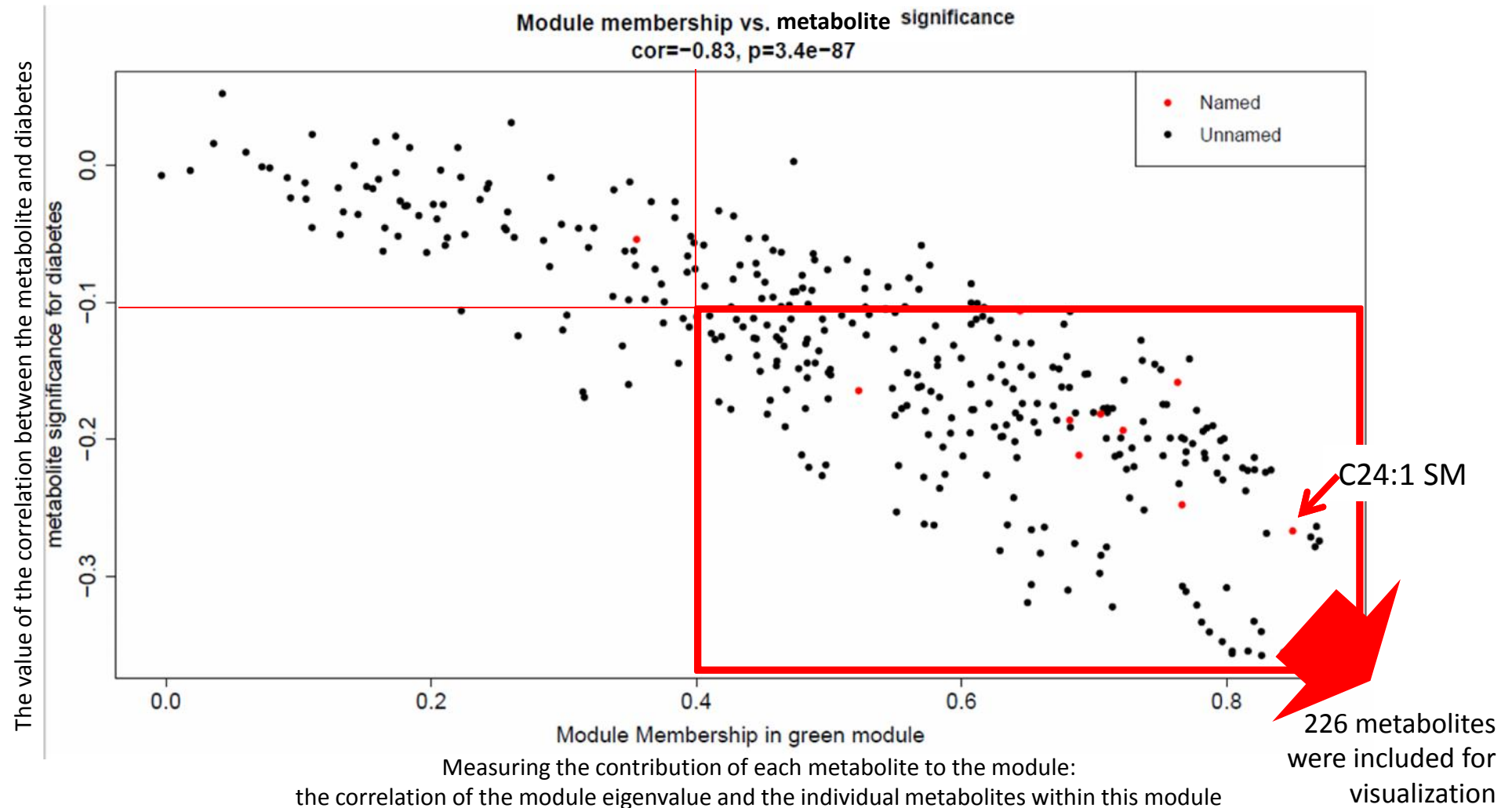
I use diabetes as the only outcome in the following analysis;  
I select the most significant module: GREEN for the further analysis

# Module-trait relationship-T2D project

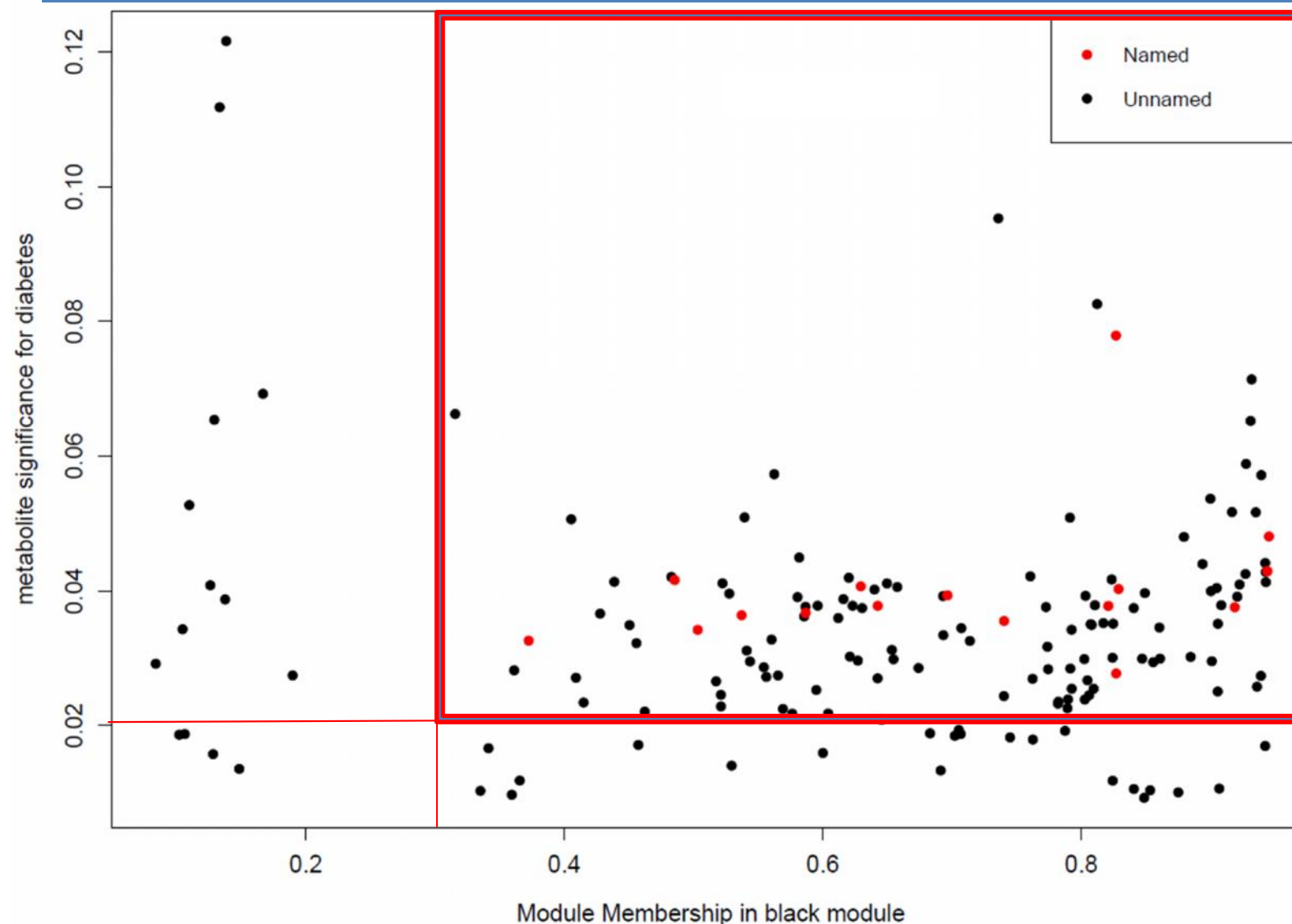


I use diabetes as the only outcome in the following analysis;  
 I select the most significant modules: Brown and Black for the  
 further analysis

# Select metabolites within module-CVD project

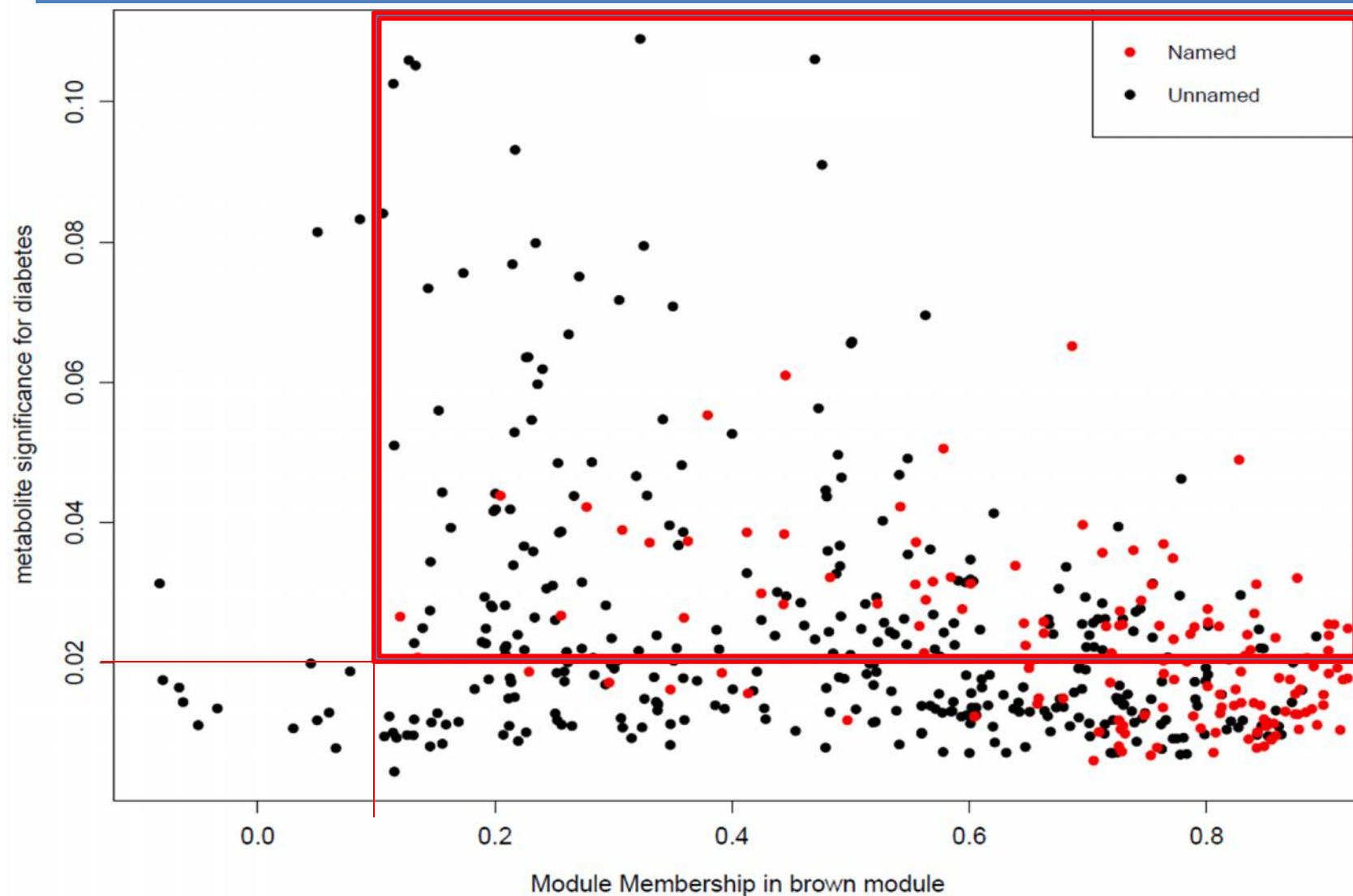


# Select metabolites within BLACK module-T2D project



Measuring the contribution of each metabolite to the module:  
the correlation of the module eigenvalue and the individual metabolites within this module

# Select metabolites within BROWN module-T2D project



Measuring the contribution of each metabolite to the module:  
the correlation of the module eigenvalue and the individual metabolites within this module

# Implications

## Prevalent T2D

C34:2 PC  
 C36:2 PC  
 C34:3 PC plasmalogen  
 C34:2 PC plasmalogen  
 C34:1 PC plasmalogen-A  
 C36:3 PC plasmalogen  
 C36:2 PC plasmalogen  
 C36:1 PC plasmalogen  
 C40:7 PC plasmalogen  
 C38:2 PE  
 C24:0 Ceramide (d18:1)  
 C14:0 SM  
 C16:1 SM  
 C16:0 SM  
 C18:2 SM  
 C18:1 SM  
 C18:0 SM  
 C20:0 SM  
 C22:1 SM  
 C22:0 SM  
 C24:1 SM  
 C24:0 SM  
 C16:0 CE  
 C18:2 CE  
 C18:1 CE  
 C54:10 TAG

## Incident T2D

### • Black module

C16.1.LPC  
 C16.0.LPC  
 C18.2.LPC  
 C18.1.LPC  
 C18.0.LPC  
 C20.4.LPC  
 C20.3.LPC  
 C22.6.LPC  
 C16.0.LPE  
 C18.2.LPE  
 C18.1.LPE  
 C18.0.LPE  
 C20.0.LPE  
 C22.0.LPE

### • Brown module

C14.0.LPC	C34.2.DAG	C14.0.CE..M.NH4.
C30.0.PC	C34.1.DAG	C16.1.CE..M.NH4.
C32.2.PC	C34.0.DAG	C34.2.DAG..M.NH4.
C32.1.PC	C36.3.DAG	C34.1.DAG..M.NH4.
C34.4.PC	C36.2.DAG	C36.3.DAG..M.NH4.
C34.1.PC	C36.1.DAG	C36.2.DAG..M.NH4.
C36.1.PC	C49.3.TAG	C50.3.TAG..M.NH4.
C34.0.PE	C49.2.TAG	C50.2.TAG..M.NH4.
C36.3.PE	C50.4.TAG	C50.1.TAG..M.NH4.
C36.2.PE	C50.3.TAG	C51.3.TAG..M.NH4.
C36.1.PE	C50.2.TAG	C51.2.TAG..M.NH4.
C40.6.PS	C50.1.TAG	C51.0.TAG..M.NH4.
C16.1.CE	C51.3.TAG	C52.4.TAG..M.NH4.
	C51.2.TAG	C52.3.TAG..M.NH4.
	C51.1.TAG	C52.2.TAG..M.NH4.
	C52.5.TAG	C53.3.TAG..M.NH4.
	C52.4.TAG	C53.2.TAG..M.NH4.
	C52.3.TAG	C54.4.TAG..M.NH4.
	C52.2.TAG	C54.3.TAG..M.NH4.
	C52.1.TAG	C54.2.TAG..M.NH4.
	C53.3.TAG	C55.3.TAG..M.NH4.
	C53.2.TAG	C56.4.TAG..M.NH4.
	C54.3.TAG	C56.3.TAG..M.NH4.
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	C54.1.TAG	
	C55.3.TAG	
	C55.2.TAG	
	C56.4.TAG	
	C56.3.TAG	
	C56.2.TAG	

*Thank  
you*

