Lipid Metabolic Network, Mediterranean Diet and Cardiovascular Disease

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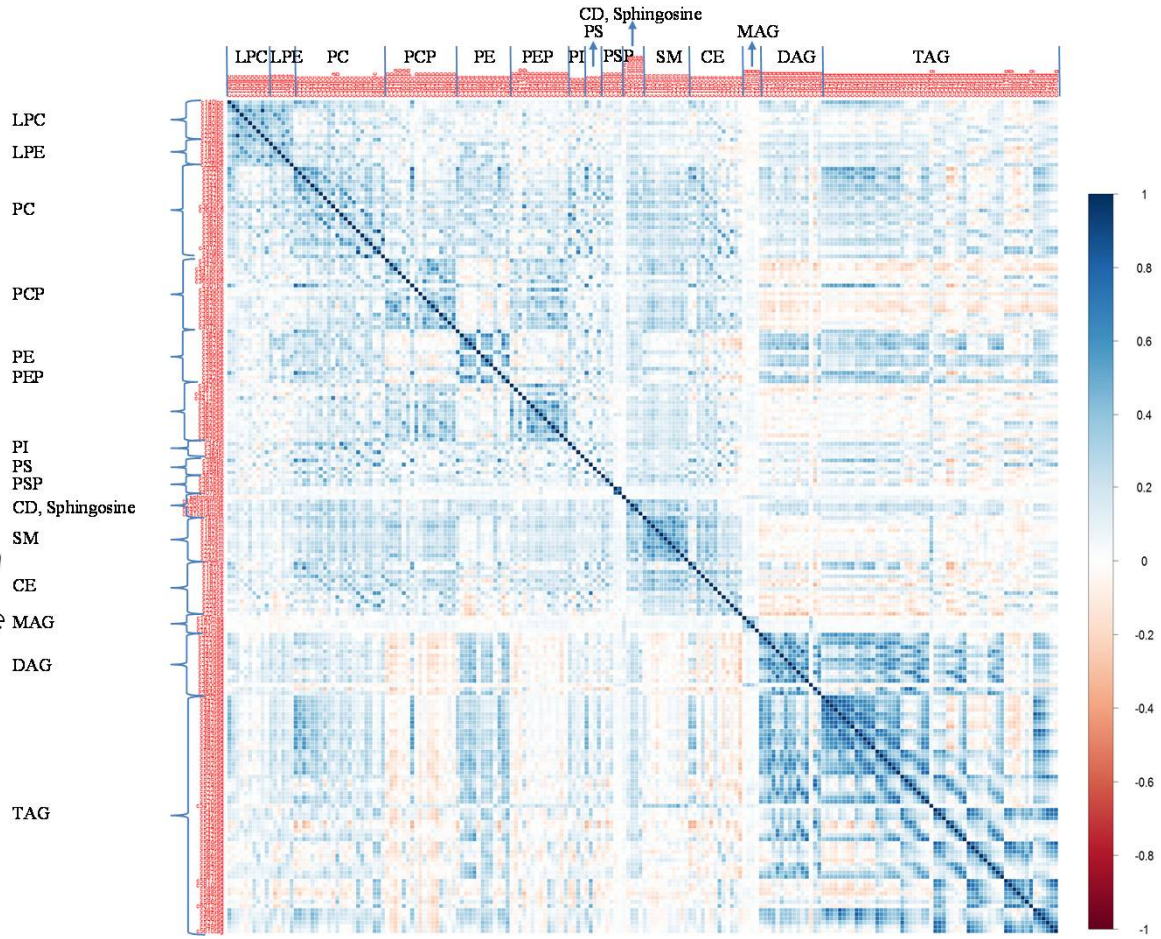
Lipid metabolites

- Glycerolipid:
  - *Diacylglycerols (DAG)*
  - *Monoacylglycerol (MAG)*
  - *Triacylglyceroles (TAG)*
- Phospholipid:
  - *Phosphatidylethanolamine (PE)*
  - *Lysophosphatidylethanolamine (LPE)*
  - *Phosphatidylethanolamine plasmalogen (PEP)*
  - *Phosphatidylserine (PS)*
  - *Phosphatidylserine plasmalogen (PSP)*
  - *Phosphatidylinositol (PI)*
  - *Phosphatidylcholine (PC)*
  - *Lysophosphatidylcholine (LPC)*
  - *Phosphatidylcholine plasmalogen (PCP)*
  - *hydroxylated phosphaticylcholine (HPC)*
- Cholesterol ester (CE)
- Sphingolipid:
  - *Sphingomyelins (SM)*
  - *Ceramide (CD)*
  - *Sphingosine*
- Untargeted lipids: ≈ 6,000



Partial correlation matrix of 200 targeted lipid metabolites in the sub-cohort in the PREDIMED Trial

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Lipidomics

- Lipidomics:
  - Hundreds of thousands
  - Structurally diverse
  - Intact lipid metabolites

- Additional information for CVD risk prediction beyond:
  - *Circulating fatty acids*
  - *Summary lipid markers*

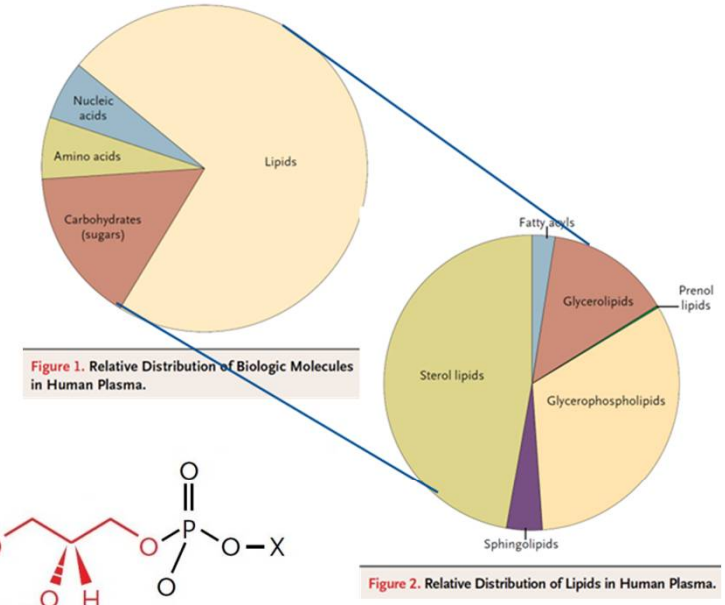
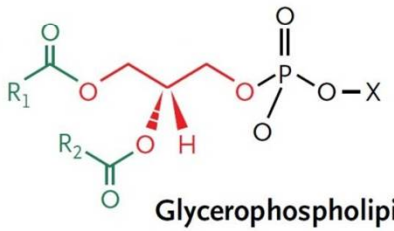
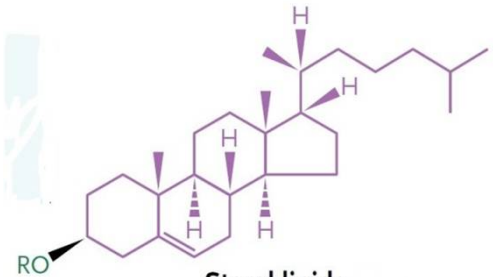


Figure 1. Relative Distribution of Biologic Molecules in Human Plasma.

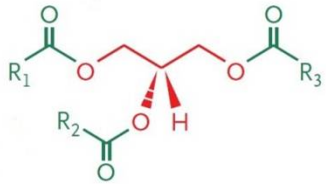
Figure 2. Relative Distribution of Lipids in Human Plasma.



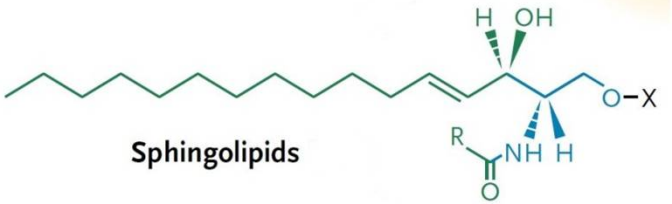
Glycerophospholipids



Sterol lipids

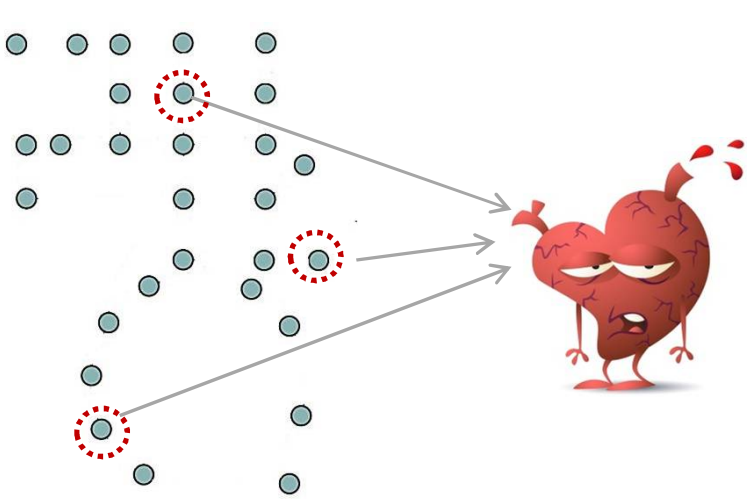


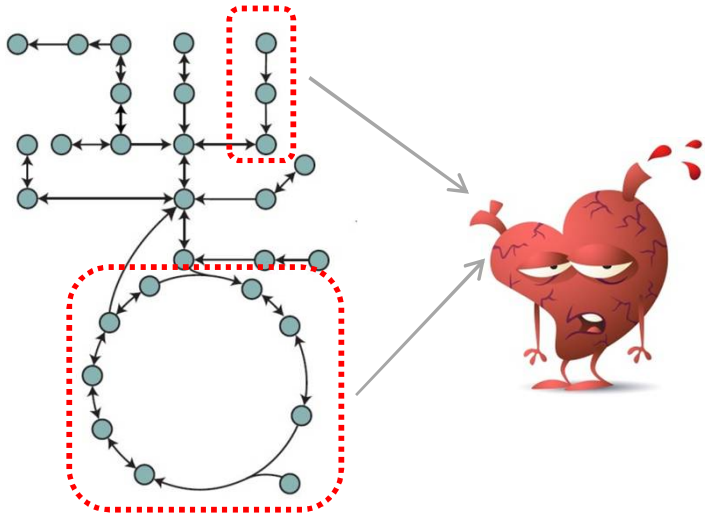
Glycerolipids



Sphingolipids

Analytical approaches in metabolomics studies





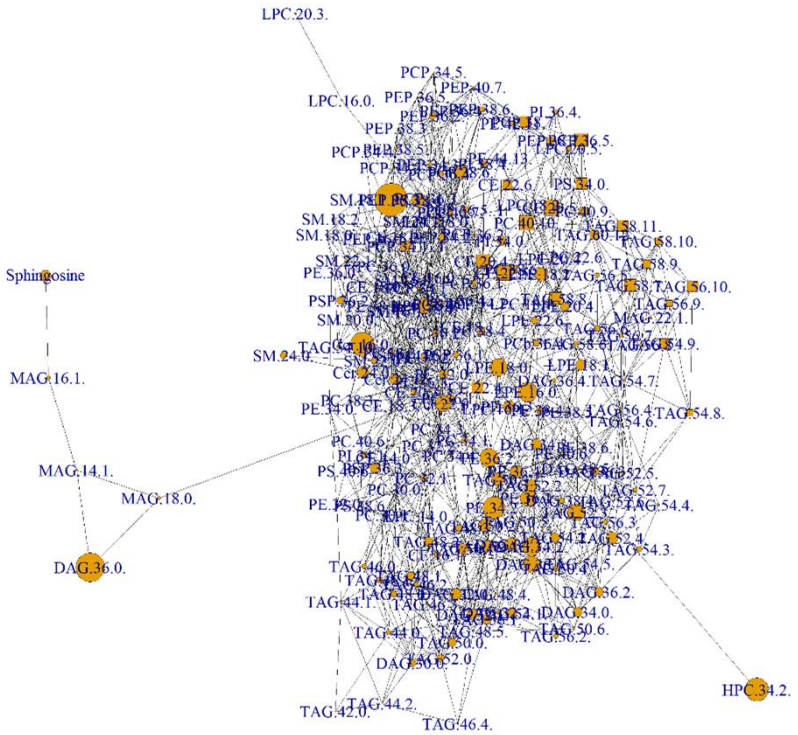
- **Single-point approaches**
  - *Analyzing unit: individual metabolite*
  - *A large number of independent statistical tests*
  - *Stringent multiple-comparison correction*
  - *Generally assumes no prior information*
- **Network/pathway analysis**
  - *Analyzing unit: Pathway*
  - *Consider interactions and dependences in pathways*
  - *Relax multiple-testing burden*
  - *Consider prior biological knowledge*

Photo source: Medical care Kerala & Fearnley. IJE 2016

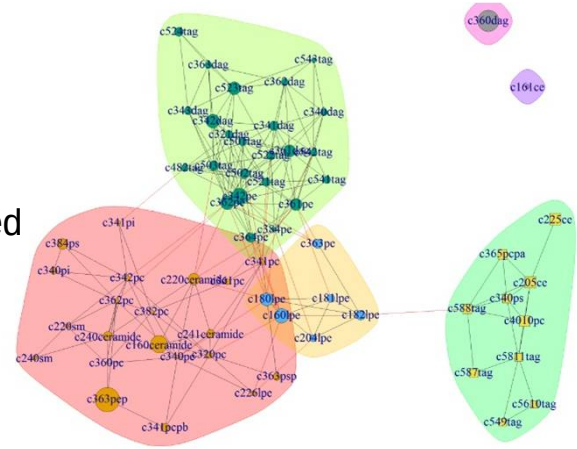
Usefulness of network/pathway analysis

- Network/pathway analysis:
  - *Pathway network building*
  - *Dimensionality reduction*
  - *Metabolic pathway detection*
  - *Utilize pathway topological information in regression model*

Step 1: Global network construction

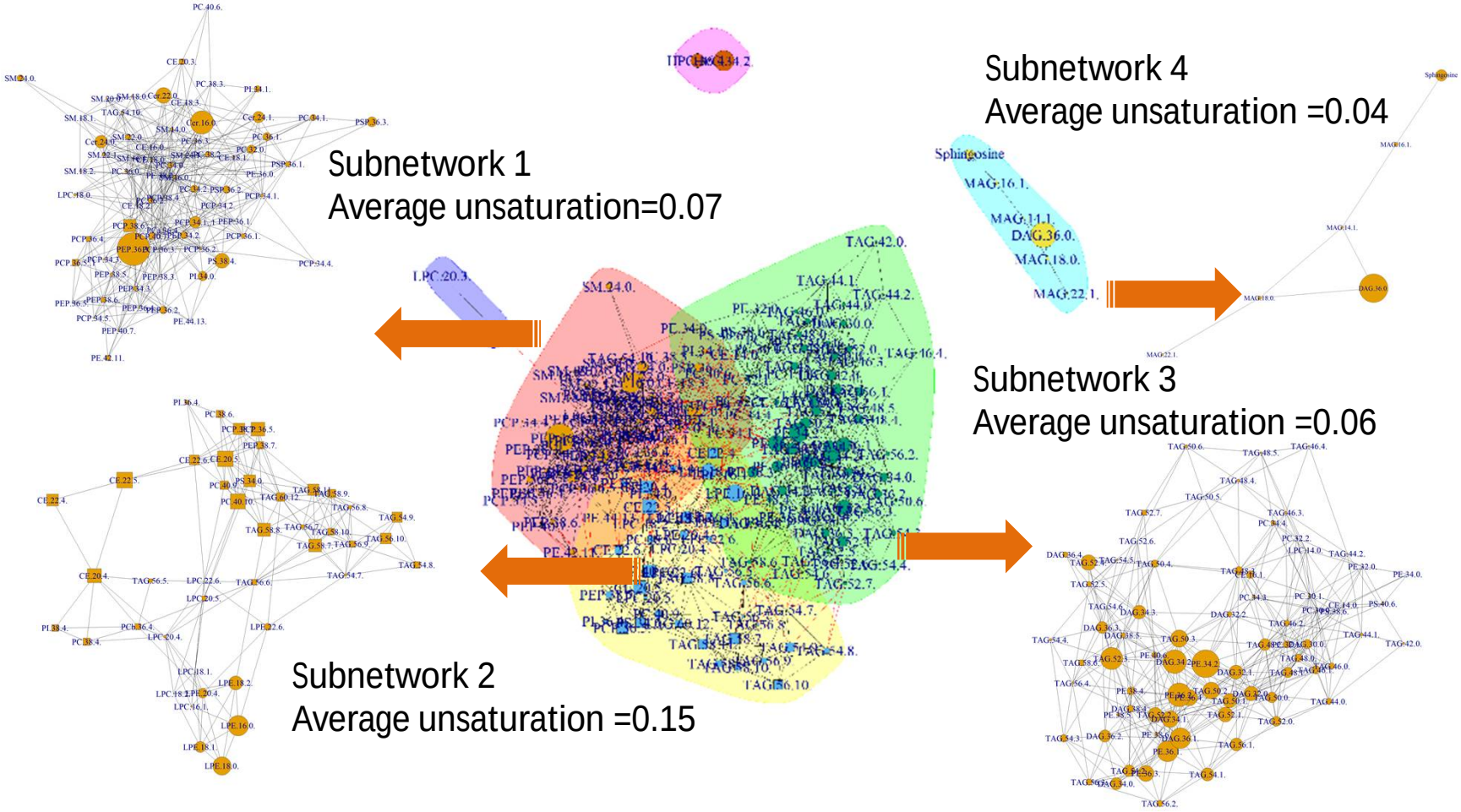


Remove lipid metabolites not significantly associated with CVD



Size of vertex: Proportion to –log (P-value) of hazard ratio of CVD
Square vertex: HR <1.00
Circle vertex: HR ≥1.00

Step 2: Major subnetwork detection



Unsaturation =# of double bond/# of carbons

Step 3: Network-based regression

- *Subnetwork score* $= \sum_n W_i X_i$
  - $\boldsymbol{X_i}$*: Concentration of lipid metabolite*
  - $\boldsymbol{W_{ij}}$*: Network topology structure weight*
- *Upweight hub metabolites*
- *Downweight non-hub metabolites*
- *Include subnetwork scores as exposures into regression model*

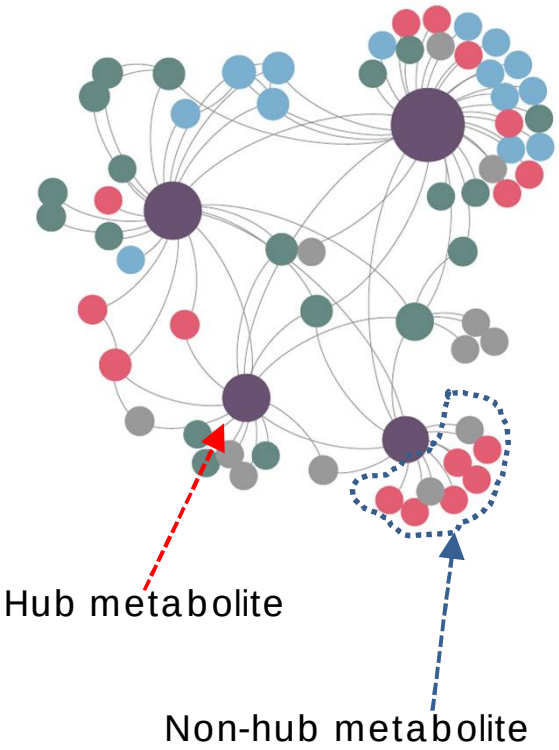
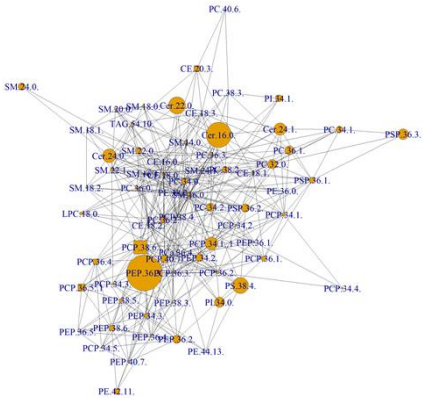
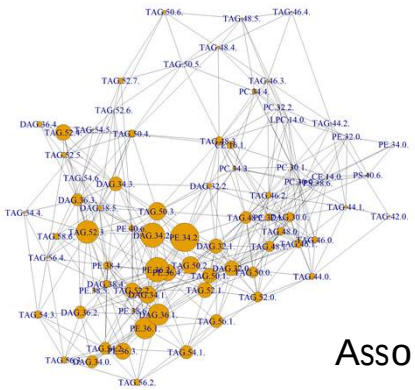
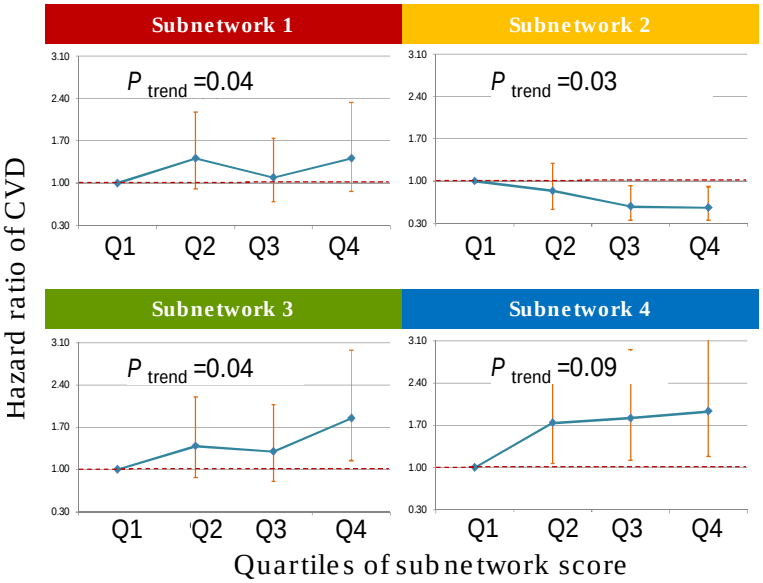


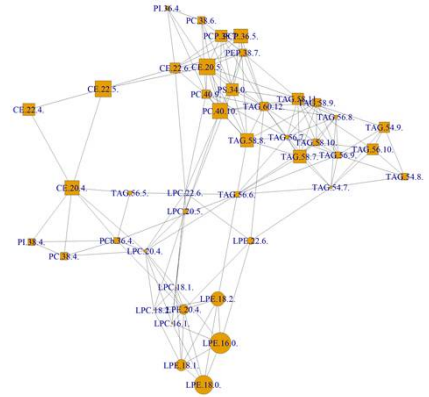
Photo source: Ann Emery. Social Network Analysis by Twitter

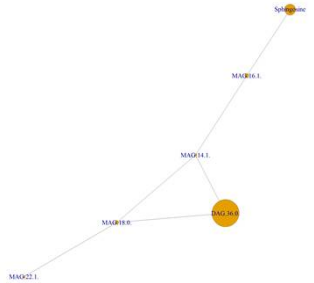
Major subnetworks and CVD











Associations between major subnetwork scores and CVD risk

Cox model stratified on intervention group and included age, sex, BMI, family Hx of CHD, smoking, HTN, DM, dyslipidemia & all 4 subnetwork scores

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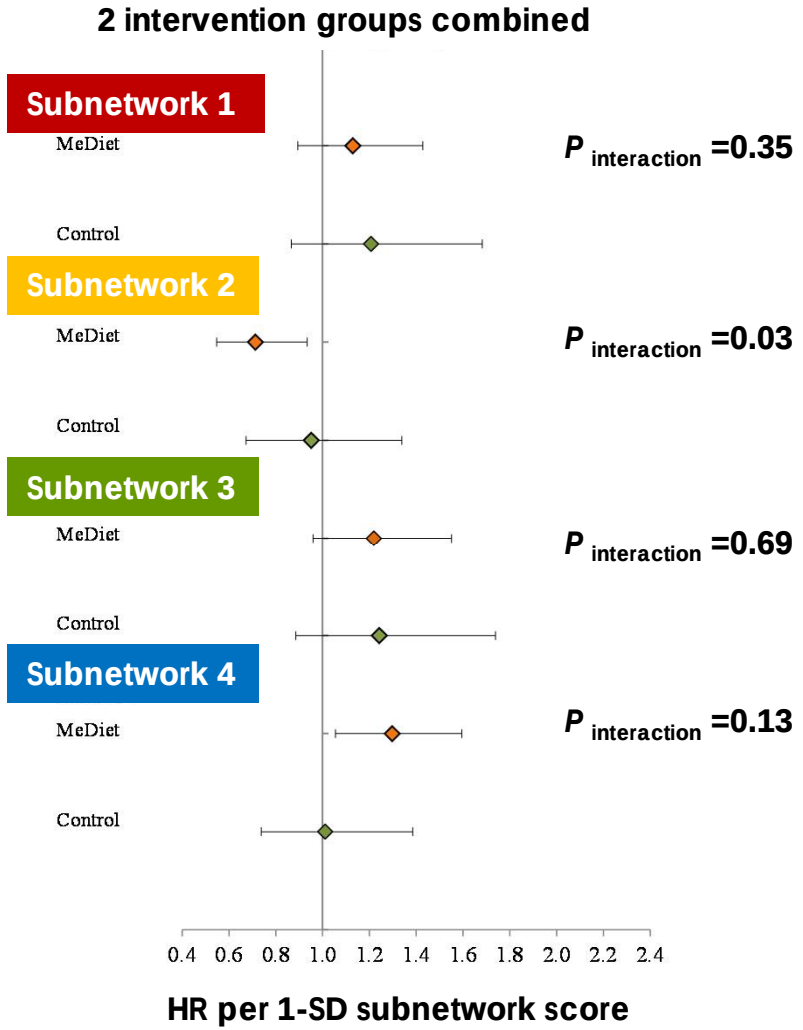
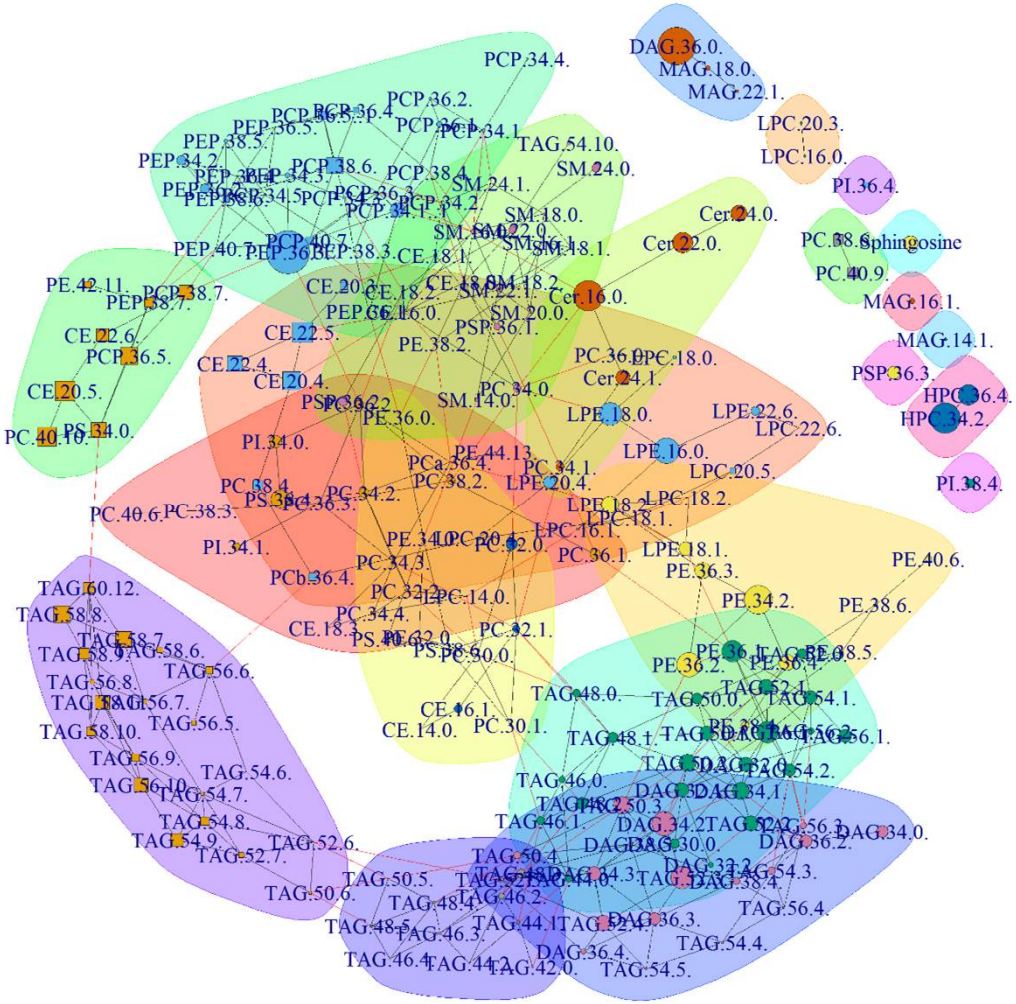
MedDiet and subnetwork



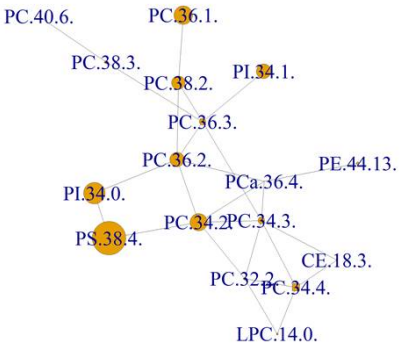
Step 4: Metabolic pathway detection

- Further removed paths: FDR adjustment
- Repeat Greedy Optimization algorithm
- Pathways:
  - Small-scope
  - Lipid metabolites closely connected within pathways
  - Potential biological functions

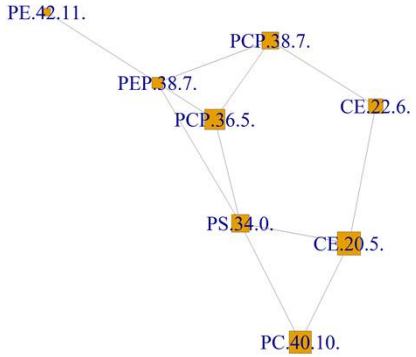


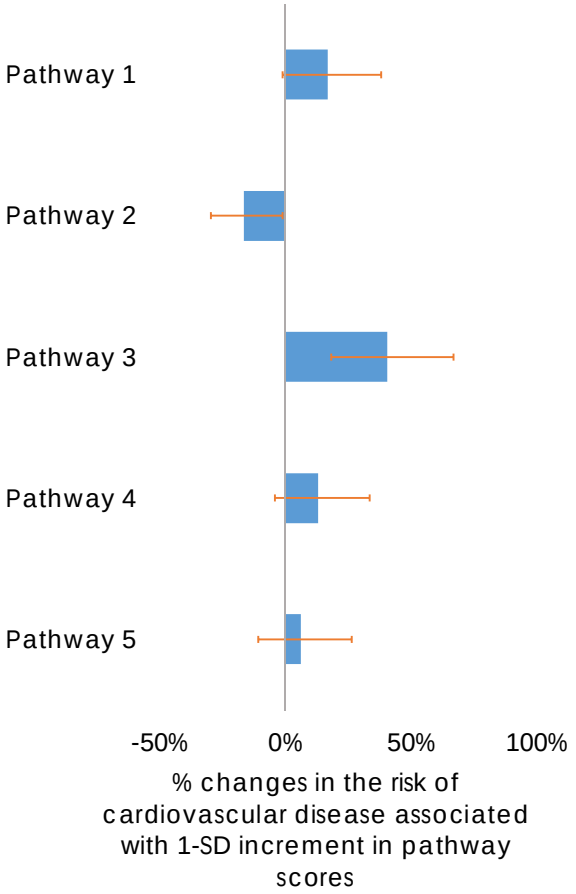
Metabolic pathways and CVD

Pathway 1: Phospholipids with fewer number of double

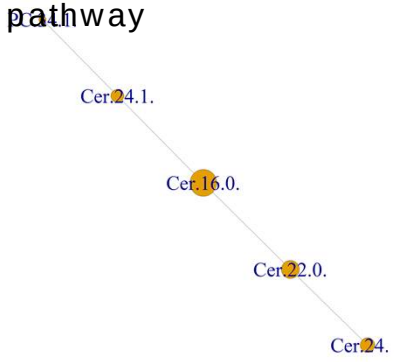


Pathway 2: Phospholipids and CEs with more double bonds

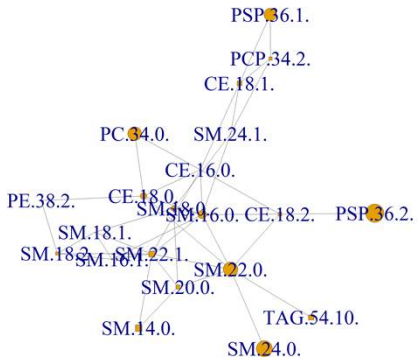


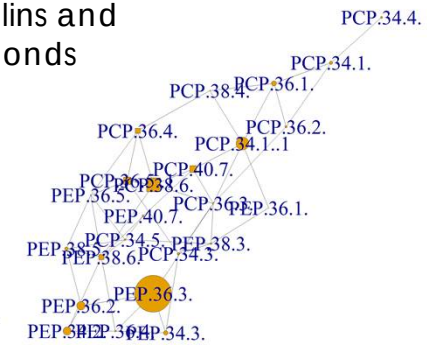


Pathway 3: Ceramide pathway



Pathway 4: Sphingomyelins and CEs with fewer double bonds

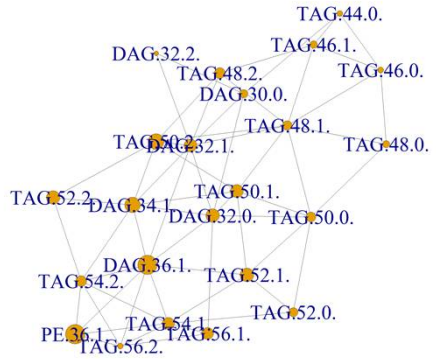




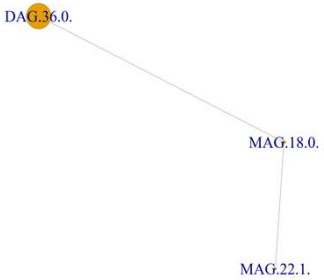
Pathway 5: Plasmalogen phospholipids

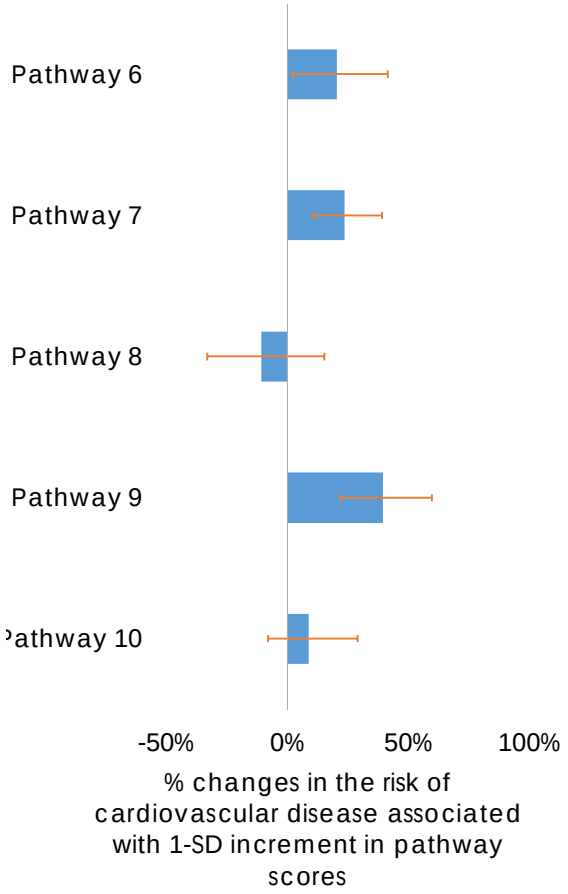
Metabolic pathways and CVD

Pathway 6: DAGs & TAGs with saturated fatty acyl chains

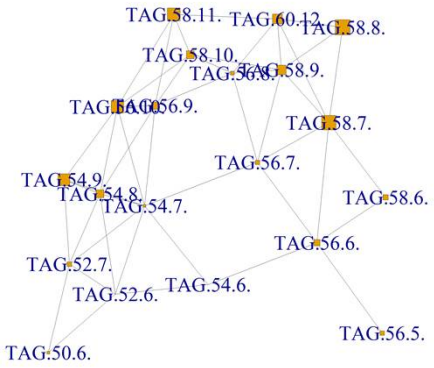


Pathway 7: DAG & MAGs with stearic acyl chain (18:0)

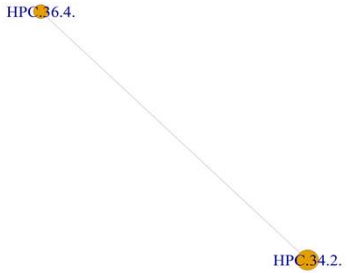


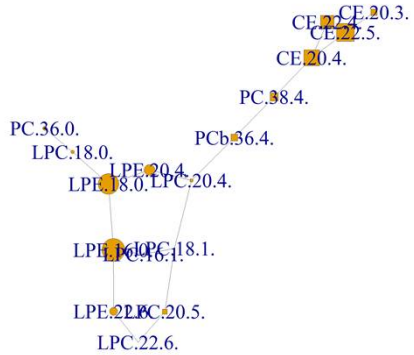


Pathway 8: TAGs with more double bounds



Pathway 9: 2 hydroxylated phosphaticylcholines





Pathway 10: Lyso-phosphalipids, PCs and CEs with more double bonds

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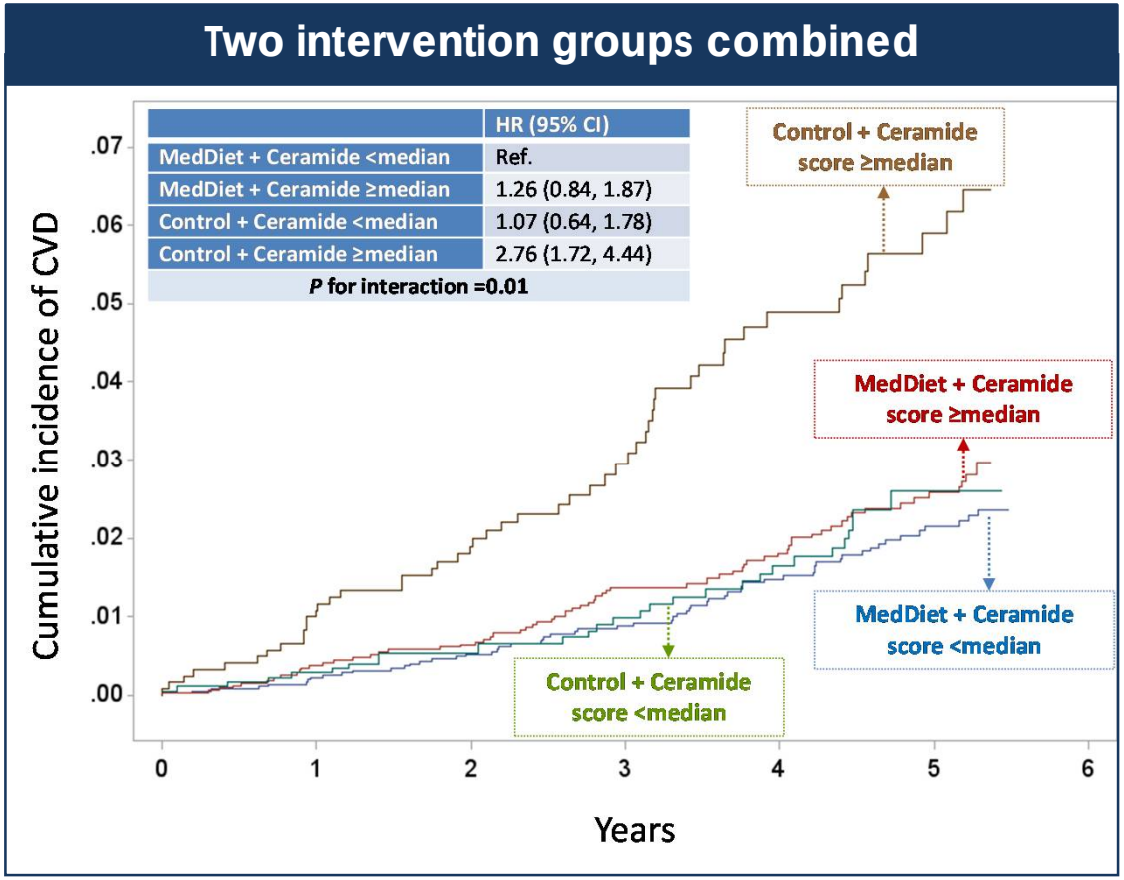
Interaction between MedDiet & ceramide pathway

- Mediterranean diet modified the deleterious effects of ceramide accumulation
- *P* for interaction =0.01



Adjusted cumulative incidence estimates according to joint groups defined by intervention group and ceramide score level

Wang et al Circulation 2017

Summaries & Conclusions

- 4 major subnetworks & 10 metabolic pathways were detected based on the topological structure
- Degree of unsaturation is major driving force underlying the network general architecture
- Divergent associations of the major subnetworks/metabolic pathways with CVD risk
- Novel pathways:
  - *HPC pathway*
  - *Pathway including DAGs & MAG with 18:0*
  - *Ceramide pathway*
- MedDiet intervention could potentially modify association between lipid pathways and CVD risk

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Thank You!