Lipid Metabolic Network, Mediterranean Diet and Cardiovascular Disease

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Lipid metabolites

- **Glycerolipid**:
  - Diacylglycerols (DAG)
  - Monoacylglycerol (MAG)
  - Triacylglycerols (TAG)

- **Phospholipid**:
  - Phosphatidylethanolamine (PE)
  - Lysosphatidylethanolamine (LPE)
  - Phosphatidylethanolamine plasmalogen (PEP)
  - Phosphatidylserine (PS)
  - Phosphatidylserine plasmalogen (PSP)
  - Phosphatidylinositol (PI)
  - Phosphatidylcholine (PC)
  - Lysosphatidylcholine (LPC)
  - Phosphatidylcholine plasmalogen (PCP)
  - Hydroxylated phosphatidylcholine (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
Lipidomics

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

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

Quehenberger, NEJM, 2011
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

Subnetwork 1
Average unsaturation = 0.07

Subnetwork 2
Average unsaturation = 0.15

Subnetwork 3
Average unsaturation = 0.06

Subnetwork 4
Average unsaturation = 0.04

Unsaturation = # of double bond/# of carbons
Step 3: Network-based regression

- Subnetwork score: $\sum_n W_i X_i$
  - $X_i$: Concentration of lipid metabolite
  - $W_{ij}$: 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
MedDiet and subnetwork

2 intervention groups combined

Subnetwork 1
- MeDiet
- Control

Subnetwork 2
- MeDiet
- Control

Subnetwork 3
- MeDiet
- Control

Subnetwork 4
- MeDiet
- Control

P_{interaction} = 0.35

P_{interaction} = 0.03

P_{interaction} = 0.69

P_{interaction} = 0.13

HR per 1-SD subnetwork score
**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 bonds

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

% changes in the risk of cardiovascular disease associated with 1-SD increment in pathway scores
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 phosphatidylcholines

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

% changes in the risk of cardiovascular disease associated with 1-SD increment in pathway scores
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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