Population Research with MS-based Lipidomics and Proteomics

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Outline of the Talk

• Shotgun Lipidomics
• MRM-Proteomics

Agilent 1290 Infinity II LC system

Agilent 6495 Triple Quadrupole MS
Cardiovascular Disease
Mortality in Women versus Men

Deaths by cause, women, 2004, United Kingdom
- Respiratory disease 14%
- Injuries & poisoning 3%
- Other cancer 14%
- Colo-rectal cancer 2%
- Breast cancer 4%
- Lung cancer 4%
- Other CVD 9%
- Stroke 12%
- Coronary heart disease 15%

Deaths by cause, men, 2004, United Kingdom
- Respiratory disease 13%
- Injuries & poisoning 5%
- Other cancer 19%
- Colo-rectal cancer 3%
- Breast cancer 4%
- Lung cancer 7%
- Other CVD 8%
- Stroke 8%
- Coronary heart disease 21%

Scotland General Register Office (2005)
Northern Ireland General Register Office (2005)
www.heartstats.org
Cardiovascular Risk Factors

Modifiable vs Non-modifiable
Framingham Risk Score
Most Events Occur in Patients at “Intermediate” Risk
(Courtesy of Prof. Steve Humphries, UCL)

57yrs
LDL 3.30
HDL 1.05
TG 1.76
SYS 138
Smoker
Fam Hist
21%
Give Statin

57yrs
LDL 3.16
HDL 1.20
TG 1.64
SYS 138
Smoker
Fam Hist
18%
Lifestyle only

http://www.qrisk.org
Lack of Simple Blood Tests for Future Events
Clinical Utility of Genetic Testing?
(Courtesy of Prof. Steve Humphries, UCL)

Risk alleles common but all have modest effect – OR 1.3-1.1
Mass Spectrometry

Bruneck Study on Cardiovascular Disease


Baseline 1st FU 2nd FU 3rd FU 4th FU

n = 936  n = 826  n = 701  n = 584  n = 468
Shotgun Lipidomics
Assessment of Molecular Lipid Species

By Mass: Isobaric lipid species
(mass difference 57.5 mu, 77 parts per million)

By Fragmentation Pattern
Shotgun Lipidomics
Assessment of Lipid Classes

1. full MS for TAGs
2. 184.1 for IPCs
3. 184.1 for PCs
4. 141.0 for IPEs
5. 141.0 for PEs
6. 185.0 for IPS
7. 185.0 for PS
8. 213.0 for SMs
9. 369.3 for CEs
Shotgun Lipidomics
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Q1 | Selection
---|---
Q2 | Fragmentation
Q3 | Fragment selection

MRM Signal

Intensity vs. Time
Shotgun Lipidomics
Assessment of Lipid Classes

1. full MS for TAGs
2. 184.1 for IPCs
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→ Triacylglycerol
→ Phosphatidylcholine (PI)
Shotgun Lipidomics
Assessment of Lipid Classes

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Triacylglycerol
Phosphatidylcholine (PI)

Selection
Fragmentation
Fragment selection

MRM Signal
Intensity
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→ Triacylglycerol
→ Phosphatidylcholine (PI)
→ Phosphatidylethanolamine (NL)
Triacylglycerol
Phosphatidylcholine (PI)
Phosphatidylethanolamine (NL)
Phosphatidylserine (NL)

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Shotgun Lipidomics
Assessment of Lipid Classes
Shotgun Lipidomics
Assessment of Lipid Classes

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→ Triacylglycerol
→ Phosphatidylcholine (PI)
→ Phosphatidylethanolamine (NL)
→ Phosphatidylserine (NL)
→ Sphingomyelin (NL)

Q1: Selection
Q2: Fragmentation
Q3: Fragment selection

MRM Signal

Intensity

Time
Shotgun Lipidomics
Assessment of Lipid Classes

1. full MS for TAGs
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→ Triacylglycerol
→ Phosphatidylcholine (PI)
→ Phosphatidylethanolamine (NL)
→ Phosphatidylserine (NL)
→ Sphingomyelin (NL)
→ Cholesteryl ester (PI)
Shotgun Lipidomics
Assessment of Conjugated Fatty Acids

Saturated / Mono- and Polyunsaturated
Network View of Plasma Lipids
135 Molecular Lipid Species of 9 Different Classes

1. full MS for TAGs
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Stegemann et al. Circulation, 2014
Network View of Plasma Lipids
135 Molecular Lipid Species of 9 Different Classes

Stegemann et al. Circulation, 2014
Network View of Plasma Lipids
135 Molecular Lipid Species of 9 Different Classes

Module
“Total Cholesterol”

Stegemann et al. Circulation, 2014
Unwarranted Focus on Lipid Classes?
Molecular Lipid Species and CVD Risk

-value

High Risk

Low Risk

Stegemann et al. Circulation, 2014
Incremental Predictive Value for CVD Risk
(10-year follow-up, 2000-2010)

Non-cases

CVD cases

Intermediate risk group

Reclassified to lower risk group

Reclassified to higher risk group

Willeit P et al. JACC, 2014
Consideration of molecular lipid species on top of conventional risk factors resulted in a significant improvement in risk stratification (measured by a C-index change).

### Risk discrimination

<table>
<thead>
<tr>
<th>Model</th>
<th>C index (95% CI)</th>
<th>C index change (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall cohort:</strong> n=685, 90 cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional risk factors</td>
<td>[Reference]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ TAG(54:2), PE(36:5), CE(16:1)</td>
<td>[Reference]</td>
<td>0.0249 (0.0055, 0.0443)</td>
<td>0.012</td>
</tr>
<tr>
<td>+ above + SM(34:2), LPC(20:5), LPC(22:6)</td>
<td>[Reference]</td>
<td>0.0465 (0.0178, 0.0752)</td>
<td>0.002</td>
</tr>
<tr>
<td>Conventional risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ 3 lipids replacing HDL-C and T-C</td>
<td>[Reference]</td>
<td>0.0227 (0.0015, 0.0439)</td>
<td>0.036</td>
</tr>
<tr>
<td>+ 6 lipids replacing HDL-C and T-C</td>
<td>[Reference]</td>
<td>0.0448 (0.0151, 0.0745)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Stegemann et al. Circulation, 2014
Fatty Acid Chain Length of Complex Lipids

Bruneck Study, n=685, 90 CV Events

Stegemann et al. Circulation, 2014
Fatty Acid Chain Length of Complex Lipids
Shorter Fatty Acids with Higher Risk

Phosphatidylcholine

Myristate (14:0)
Palmitate (16:0)
Palmitoleate (16:1)
Olate (18:1)

Myristoleate (14:1)
Stearate (18:0)
Lipid profiling identifies a triacylglycerol signature of insulin resistance and improves diabetes prediction in humans

Eugene P. Rhee,1,2 Susan Cheng,3,4,5 Martin G. Larson,3,6 Geoffrey A. Walford,7,8 Gregory D. Lewis,2,5 Elizabeth McCabe,3,5 Elaine Yang,2 Laurie Farrell,5 Caroline S. Fox,3,9,10 Christopher J. O’Donnell,3,5,10 Steven A. Carr,2 Ramachandran S. Vasan,3,11 Jose C. Florez,2,7,8 Clary B. Clish,2 Thomas J. Wang,3,5,12 and Robert E. Gerszten2,5,12,13
Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study

- **A** Even-chain fatty acids
  - Overall ($I^2=88.1\%, p<0.0001$)
  - Hazard ratio (95% CI): $1.43 (1.29-1.58)$

- **B** Odd-chain fatty acids
  - Overall ($I^2=54.1\%, p=0.033$)
  - Hazard ratio (95% CI): $0.70 (0.66-0.74)$

- **C** Long- and very-long-chain fatty acids
  - Overall ($I^2=88.9\%, p<0.0001$)
  - Hazard ratio (95% CI): $0.70 (0.59-0.84)$
Non-Essential Fatty Acids
Hepatic De Novo Lipogenesis versus Diet

Stegemann et al. Circulation, 2014
Non-Essential Fatty Acids
Hepatic De Novo Lipogenesis versus Diet

De novo Lipogenesis

Acetyl-CoA → polymerization

Myristate (14:0) → elongation

16:0 → Δ9 desaturation

16:1 → elongation

18:0 → Δ9 desaturation

18:1n-9 → beta-oxidation

16:1n-9

Stegemann et al. Circulation, 2014
Non-Essential Fatty Acids
Hepatic De Novo Lipogenesis versus Diet

Myristate
Palmitate

14:0
16:0
16:1
18:0
18:1
16:1n-9

acetyl-CoA

polymerization

elargonation

Δ9 desaturation

elargonation

Δ9 desaturation

beta-oxidation

Stegemann et al. Circulation, 2014
Non-Essential Fatty Acids
Hepatic De Novo Lipogenesis versus Diet

Stegemann et al. Circulation, 2014
Non-Essential Fatty Acids
Hepatic De Novo Lipogenesis versus Diet

Stegemann et al. Circulation, 2014
Non-Essential Fatty Acids
Hepatic De Novo Lipogenesis versus Diet

acetyl-CoA → polymerization → 14:0 → elongation → 16:0 → elongation → 18:0 → 18:1n-9 → beta-oxidation → 16:1n-9

Saturated & monounsaturated fatty acids with stronger association to CVD

Stegemann et al. Circulation, 2014
Plaque-enriched Cholesteryl Esters
Relative Distribution Compared to Control Arteries

Diet and de novo Lipogenesis

- Saturated FA (16:0, 18:0)
- Monosaturated FA (16:1, 18:1)
- PEMT or CDP-Choline pathways
- ACAT2
- Cholesteryl Esters (16:1-CE)
- DGAT1/2
- Triglycerides (54:2-TG)

- VLDL
  - 16:1-CE
  - 54:2 TG
  - 36:5-PE

- LDL
  - 16:1-CE
  - 54:2 TG
  - 36:5-PE

Inflammation
Atherogenesis

Altered PRR Signaling
Multiple Reaction Monitoring
MS-based Protein Quantitation
Multiple Reaction Monitoring

Nanoflow LC-MS

Bruneck 2000 Plasma (n=701)
(10uL/sample)

Denature, Reduction, Alkylation

+ reference peptide (Heavy)

Tryptic Digestion (over night)

Purification (C18 spin plate)

+ iRT Standard

Buffer exchange

Nano flow LC-MS

Thermo RSILCnano
(75um x 15cm, 0.3uL/min, 70min)

+ TSQ Vantage

(Scheduled SRM for target proteins, 850 transitions)

Data analysis
Multiple Reaction Monitoring
High Flow LC-MS

High flow LC-MS

Agilent 1290 Infinity II LC System
(2.1mm x 25cm, 350ul/min, 27min)
+ 6495 Triple Quadrupole MS
(Dynamic MRM for target proteins, 765 transitions)

Data analysis
Agilent 1290 Infinity II
Shift <0.05 min over 750 Injections;
Total Run Time: 14 Days

Peak width 0.2min
Agilent 6495 Triple Quadrupole-MS
RSD of Heavy Peak Area 18.8% in 700 Samples
Scatter Plot for 100 Plasma Proteins
Detectability in High Flow Method

Transferrin
Platelet factor 4
Dynamic Range Limitation
LC-MS/MS Compared to Alternative Techniques

Anderson et al., Mol Cell Prot 2002
Framingham Risk Score

Most Events Occur in Patients at “Intermediate” Risk

(Courtesy of Prof. Steve Humphries, UCL)
Do apolipoproteins reflect the molecular lipid composition?
Are we actually measuring the ‘right’ apolipoproteins?
Loss-of-Function Mutations in APOC3 and Risk of Ischemic Vascular Disease


**Figure 2.** Mean Plasma Levels of Nonfasting Triglycerides as a Function
Loss-of-Function Mutations in APOC3, Triglycerides, and Coronary Disease

The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute

**Figure 2.** Association of APOC3 Loss-of-Function Mutations with Risk of Coronary Heart Disease among 110,970 Participants in 15 Studies.
Conclusions

- The bulk of CVD risk is not explained by traditional risk factors.
- Currently, we just monitor lipid classes (total cholesterol, total triglycerides) as well as HDL and LDL cholesterol.
- The focus is on “quantity” rather than “molecular composition”.
- Molecular lipid species and additional apolipoproteins may complement the Framingham risk scores in a stratified medicine approach for diagnosis and treatment of CVD.
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Prof. Johann Willeit

http://www.vascular-proteomics.com