Serum metabolomics profiles predict coronary heart disease in the general population

Tanja Zeller, Daniela Börnigen, Francisco Ojeda, Mahir Karakas, Giovanni Veronesi, Torben Jorgensen, Licia Iacoviella, Hugh Tunstall-Pedoe, Kari Kuulasmaa, Veikko Salomaa, Stefan Blankenberg

– for the BiomarCaRE investigators

Late breaking science 04. Sweet Spot in Cardiometabolic Care
Current cardiovascular risk assessment

- is based on classical risk factors
- does not comprise environmental and genetic factors reflecting the heterogeneous etiology of coronary heart disease (CHD)

Identification of novel biomarkers
- e.g. molecular markers
- is needed to improve risk estimation
- Small molecules (< 1.5 kDa) such as carbohydrates, amino acids, fatty acids, nucleosides, phospholipids
- Intermediates and products of the metabolism that are critical for maintenance of cellular and organism level
- Changes in metabolites are a consequence of genetic, transcriptomic and proteomic variations
- Thereby ultimate responses to modifications linked to pathological conditions

What are metabolites?

- Genomics
- Transcriptomics
- Proteomics
- Metabolomics
- Phenotype
Objectives

- To study the association between circulating metabolites and incident coronary heart disease
- To investigate the performance of a combination of coronary heart disease-associated metabolites across large population-based cohorts from the BiomarCaRE consortium
Biomarker for Cardiovascular Risk Assessment in Europe

- Assess predictive value of existing and emerging biomarker in CVD
- N > 300,000 individuals from over 30 cohorts with long-term follow-up (up to 28 y)
- Central harmonized data base of phenotypes and endpoints and central biomarker laboratory
- Incident CHD events: first fatal or non-fatal CHD during follow-up

Metabolome project - BiomarCaRE case-cohort

- CHD cases and random subsample of the remaining population
- N = 10,741 individuals in total
- N = 2,166 CHD cases
- Targeted metabolite detection by Biocrates mass spectrometry assay

- Acylcarnitines
- Amino Acids
- Biogenic Amines
- Glycerophospholipids
- Spingolipids
- Monosaccharide


- DanMONICA (Denmark): n=4,364
- FinRisk (Finland): n=2,754
- MONICA-KORA (Germany): n=1,689
- SHHEC (Scotland): n=3,977
- Brianza (Italy): n=694
- Moli-Sani (Italy): n=2,325
- MONICA (Denmark): n=2,754
### Work flow and statistical methods

- **N = 10,741 individuals (2,166 CHD cases)**
- **141 metabolites for statistical analyses**
- **Adjustments for BMI, SBP, antihypertensives, diabetes, total cholesterol, gender, smoker, center, age**
- **Multivariable Cox regression analyses for each metabolite**
- **Significance level: \( p \leq 0.05 \) and Bonferroni correction**
- **Kaplan-Meier survival analyses accounting for case-cohort design (age used as time scale)**
  - Cox proportional hazard models according to thirds

#### Selection for BiomarCaRE CHD case-cohort
(subcohort and all CHD cases, \( N = 12,928 \))
Further exclusion criteria: prevalent CVD, HF, AF, no FU available, > 50% missing data, PCA-based outliers

\[ N \text{ total} = 10,741 \]
2,166 CHD cases and 8,575 non CHD cases

<table>
<thead>
<tr>
<th>Brianza</th>
<th>DanMONICA</th>
<th>FinRisk</th>
<th>KORA</th>
<th>Moli-Sani</th>
<th>SHHEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(182/327)</td>
<td>(691/2,741)</td>
<td>(274/1,497)</td>
<td>(205/880)</td>
<td>(138/1,226)</td>
<td>(674/1,904)</td>
</tr>
</tbody>
</table>

#### Metabolite Data Processing
Quality control and normalization of metabolite data
Metabolites exclusion criteria: >30% of measurements missing and >50% of measurements < LoD

\[ N \text{ total} = 141 \text{ metabolites} \]

#### Statistical analyses
Association of 141 metabolites (log-transformed) and incident CHD
(Multivariable Cox proportional Hazard models)

Kaplan-Meier survival curves
Cox proportional hazard models according to groups of metabolites
Cox proportional hazard models of comparison metabolites to classical risk factors
## Results – Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individuals</td>
<td>N 10,741</td>
<td>4,157 (38.7)</td>
<td>6,584 (61.3)</td>
</tr>
<tr>
<td>Age</td>
<td>years 56.5 (49.2, 62.2)</td>
<td>57.9 (50.2, 62.6)</td>
<td>55.8 (48.1, 61.8)</td>
</tr>
<tr>
<td>Daily smoker</td>
<td>N (%) 3,416 (31.8)</td>
<td>1,203 (28.9)</td>
<td>2,213 (33.6)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>N (%) 562 (5.2)</td>
<td>184 (4.4)</td>
<td>379 (5.8)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>N (%) 5,169 (48.1)</td>
<td>1,898 (45.7)</td>
<td>3,271 (49.7)</td>
</tr>
<tr>
<td>Body-mass-index</td>
<td>(kg/m2) 26.2 (23.7, 29.1)</td>
<td>25.8 (22.9, 29.3)</td>
<td>26.4 (24.2, 29.0)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>(mm/Hg) 135.0 (121.0, 150.0)</td>
<td>134.0 (119.5, 150.0)</td>
<td>135.0 (123.0, 151.0)</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>(mg/dL) 232.0 (201.1, 263.0)</td>
<td>235.9 (208.8, 270.7)</td>
<td>226.0 (200.0, 255.2)</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>(mg/dL) 53.0 (44.1, 64.2)</td>
<td>60.6 (50.7, 71.5)</td>
<td>49.1 (41.4, 58.4)</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>(mg/dL) 146.2 (121.4, 174.7)</td>
<td>149.3 (123.2, 179.4)</td>
<td>144.1 (120.2, 171.3)</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
<td>N (%) 1,795 (16.7)</td>
<td>795 (19.1)</td>
<td>999 (15.2)</td>
</tr>
<tr>
<td>Incident CHD</td>
<td>N (%) 2,166 (20.2)</td>
<td>703 (16.9)</td>
<td>1,463 (22.2)</td>
</tr>
<tr>
<td>CHD fatal</td>
<td>N (%) 677 (6.3)</td>
<td>220 (5.3)</td>
<td>457 (6.9)</td>
</tr>
</tbody>
</table>
Out of 141 metabolites 24 associated with incident CHD (p ≤ 0.05)

4 metabolites significant (at Bonferroni correction)

3 metabolites classes

Mainly phosphatidylcholines (lipoprotein species)

Inverse relation to CHD: Low levels of metabolites are harmful

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Hazard Ratio (95% CI)</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>PC ae C40:6</td>
<td>1.34 (1.19, 1.52)</td>
<td>3.0 x 10^{-6}</td>
</tr>
<tr>
<td>PC ae C38:6</td>
<td>1.23 (1.11, 1.36)</td>
<td>4.6 x 10^{-5}</td>
</tr>
<tr>
<td>PC aa C38:5</td>
<td>1.21 (1.10, 1.34)</td>
<td>1.1 x 10^{-4}</td>
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<tr>
<td>PC aa C38:6</td>
<td>1.15 (1.07, 1.24)</td>
<td>1.8 x 10^{-4}</td>
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<tr>
<td>PC aa C40:6</td>
<td>1.17 (1.07, 1.27)</td>
<td>4.3 x 10^{-4}</td>
</tr>
<tr>
<td>PC ae C34:3</td>
<td>1.21 (1.08, 1.36)</td>
<td>7.3 x 10^{-4}</td>
</tr>
<tr>
<td>PC ae C36:5</td>
<td>1.15 (1.05, 1.26)</td>
<td>1.8 x 10^{-3}</td>
</tr>
<tr>
<td>PC ae C44:5</td>
<td>1.21 (1.07, 1.37)</td>
<td>1.9 x 10^{-3}</td>
</tr>
<tr>
<td>PC ae C38:5</td>
<td>1.18 (1.06, 1.32)</td>
<td>2.5 x 10^{-3}</td>
</tr>
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<td>1.23 (1.08, 1.41)</td>
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<td>PC aa C40:5</td>
<td>1.17 (1.05, 1.30)</td>
<td>3.4 x 10^{-3}</td>
</tr>
<tr>
<td>PC ae C38:0</td>
<td>1.21 (1.06, 1.37)</td>
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<tr>
<td>PC aa C36:5</td>
<td>1.14 (1.04, 1.24)</td>
<td>3.6 x 10^{-3}</td>
</tr>
<tr>
<td>Met</td>
<td>1.04 (1.01, 1.08)</td>
<td>5.3 x 10^{-3}</td>
</tr>
<tr>
<td>PC aa C36:4</td>
<td>1.15 (1.04, 1.27)</td>
<td>5.9 x 10^{-3}</td>
</tr>
<tr>
<td>PC ae C44:6</td>
<td>1.18 (1.04, 1.33)</td>
<td>8.8 x 10^{-3}</td>
</tr>
<tr>
<td>PC ae C36:4</td>
<td>1.15 (1.03, 1.28)</td>
<td>1.1 x 10^{-2}</td>
</tr>
<tr>
<td>PC aa C36:6</td>
<td>1.16 (1.03, 1.30)</td>
<td>1.3 x 10^{-2}</td>
</tr>
<tr>
<td>lysoPC a C18:2</td>
<td>1.19 (1.03, 1.37)</td>
<td>1.5 x 10^{-2}</td>
</tr>
<tr>
<td>PC ae C42:5</td>
<td>1.19 (1.03, 1.37)</td>
<td>1.9 x 10^{-2}</td>
</tr>
<tr>
<td>His</td>
<td>1.24 (1.03, 1.49)</td>
<td>2.0 x 10^{-2}</td>
</tr>
<tr>
<td>SM C18:1</td>
<td>1.10 (1.01, 1.19)</td>
<td>2.9 x 10^{-2}</td>
</tr>
<tr>
<td>lysoPC a C20:4</td>
<td>1.17 (1.02, 1.35)</td>
<td>2.9 x 10^{-2}</td>
</tr>
<tr>
<td>Gln</td>
<td>1.06 (1.00, 1.12)</td>
<td>3.8 x 10^{-2}</td>
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</table>
# Results – Levels of phosphatidylcholines associate with incident CHD

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<td>1.3 × 10^{-5}</td>
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<tr>
<td>1.09 (1.02, 1.15)</td>
<td>8.61 × 10^{-3}</td>
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<td><strong>PC ae C38:6</strong></td>
<td></td>
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<tr>
<td>1.11 (1.05, 1.16)</td>
<td>4.61 × 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>1.15 (1.05, 1.25)</td>
<td>1.47 × 10^{-3}</td>
<td></td>
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<tr>
<td>1.08 (1.02, 1.15)</td>
<td>7.65 × 10^{-3}</td>
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<tr>
<td><strong>PC aa C38:5</strong></td>
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<td>1.10 (1.05, 1.16)</td>
<td>1.07 × 10^{-4}</td>
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<tr>
<td>1.14 (1.06, 1.24)</td>
<td>6.64 × 10^{-4}</td>
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<td>1.54 × 10^{-2}</td>
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<td>2.06 × 10^{-2}</td>
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HR per 1 SD

- **Red**: overall
- **Green**: women
- **Blue**: men
Results – Low levels of phosphatidylcholines increase the probability of CHD

Age used as time scale

PC ae C40:6 $P = 0.037$

PC ae C38:6 $P < 0.001$

PC aa C38:5 $P < 0.001$

PC aa C38:6 $P < 0.001$

third 1 (low levels)
third 2
third 3 (high levels)
Results – Hazard ratios according to thirds of phosphatidylcholines levels

- **PC ae 40:6**
  - Third 3 (High levels): Hazard Ratio
  - Third 2 (Low levels): Hazard Ratio
  - Third 1 (High levels): Hazard Ratio

- **PC ae 38:6**
  - Third 3 (High levels): Hazard Ratio
  - Third 2 (Low levels): Hazard Ratio

- **PC aa 38:5**
  - Third 3 (High levels): Hazard Ratio
  - Third 2 (Low levels): Hazard Ratio

- **PC aa 38:6**
  - Third 3 (High levels): Hazard Ratio
  - Third 2 (Low levels): Hazard Ratio

Legend:
- Red circles: Basic adjustment (age, sex, center)
- Blue circles: Full adjustment (BMI, SBP, diabetes, gender, total cholesterol, smoker, center, age, antihypertensives)
Results – Combination of metabolites into a panel

Metabolite Panel

Hazard Ratio

1.50
1.40
1.30
1.20
1.10
1.00

Third 3
Third 2
Third 1

High levels
Low levels

Basic adjustment (age, sex, center)
Full adjustment (BMI, SBP, diabetes, gender, total cholesterol, smoker, center, age, antihypertensives)
Results – Strength of metabolite association is similar to that of classical risk factors

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<td>1.8 × 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Metabolite panel</td>
<td>1.10 (1.05, 1.16)</td>
<td>1.77 × 10^{-4}</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>1.13 (1.07, 1.20)</td>
<td>3.96 × 10^{-5}</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1.17 (1.10, 1.23)</td>
<td>3.51 × 10^{-8}</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.20 (1.16, 1.25)</td>
<td>1.43 × 10^{-19}</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1.33 (1.27, 1.39)</td>
<td>9.9 × 10^{-34}</td>
<td></td>
</tr>
</tbody>
</table>
Limitations

- Targeted approach for metabolite measurements
  - selected metabolites
  - known metabolites

- Data not available in all cohorts
  - lipid lowering medication
  - dietary parameter

- Studies needed to validate a possible improvement of score-based risk prediction
Largest population-based study evaluating the value of metabolites in CHD

Metabolites (phosphatidylcholines) associate inversely with incident CHD

Combination of metabolites shows similar strength than individual metabolites

Strength of metabolite association is similar to that of classical risk factors

Value of metabolomics for biomarker discovery and improved risk stratification
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