PHENOTYPING FAMILIES OF A HYPERTENSIVE PATIENT

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BHF Glasgow Cardiovascular Research Centre
Advantages of a Family-based Design

- Related subjects are more likely to share disease-causing genetic variants
- Better suited to detect rare variants in case of locus and allele heterogeneity
- Controlling for the effects of shared environment
- Robust to population stratification
InGenious HyperCare Network of Excellence
Index Patient

- Hypertension diagnosed < 50 years
- Caucasian
- Age 18-60
- Index patient on treatment with \( \geq 2 \) drugs or \( SBP \geq 160 / \)
- \( DBP \geq 95 \) on two different occasions if untreated
- At least three 1st degree relatives of whom at least one should be affected (< 50 years) and at least one from a different generation
Examples of Possible Family Structures

- ▲: affected
- ◇: unaffected
- ↓: index patient
Two Typical Families

Family #12

Family #16
Recruitment

Index Patient Identification and Approach

Evaluation of family size and blood pressure history

Contact with available family members and organization of study visits

Study visit in recruitment centre

242 individuals meeting inclusion criteria/ 3240 outpatient visits

81 families with sufficient size

31 families agreed to participate

26 completed, 2 incomplete, 3 drop outs

Three hypertension clinics per week

Email or Phone

15 months
Routine Investigations

- Urine strip test for protein and blood
- Serum creatinine and electrolytes
- Blood glucose - ideally fasted
- Blood lipid profile (at least total and high density lipoprotein (HDL) cholesterol) – ideally fasted for consideration of triglycerides
- Electrocardiogram
<table>
<thead>
<tr>
<th>Markers</th>
<th>CV predictive value</th>
<th>Availability</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrocardiography</td>
<td>++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Echocardiography</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Carotid Intima-Media Thickness</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Arterial stiffness [Pulse wave velocity]</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ankle-Brachial index</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Coronary calcium content</td>
<td>+</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Cardiac/Vascular tissue composition</td>
<td>?</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Circulatory collagen markers</td>
<td>?</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Cerebral lacunae/White matter lesions</td>
<td>?</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Est. Glomerular Filtration Rate or Creatinine Clearance</td>
<td>+++</td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>+++</td>
<td>++++</td>
<td>+</td>
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</tbody>
</table>
Routine Investigations

LVH

cIMT

PWV

cPP

LVH, g/m²

IMT, mm

PWV, m/s

cPP, mmHg

Normotensives

Hypertensives
Cardiovascular Continuum

Altered protein expression

Oxidative Stress

Risk factors

Early tissue dysfunction

Oxidative and mechanical stress
Inflammation

Atherothrombosis and progressive CV disease

Pathological remodeling

Target organ damage

End-organ failure (CHF, ESRD)

Death

Tissue injury (MI, stroke, renal insufficiency, peripheral arterial insufficiency)

Dzau V et al. Circulation 2006
<table>
<thead>
<tr>
<th></th>
<th>CAD</th>
<th>Control</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>89</td>
<td>64</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age (y)</td>
<td>67 ± 9</td>
<td>62 ± 8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Male (%)</td>
<td>69 (77.5)</td>
<td>37 (57.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body Mass Index, kg/m²</td>
<td>29.8 ± 5.0</td>
<td>25.7 ± 3.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Waist:Hip ratio</td>
<td>0.93 ± 0.12</td>
<td>0.88 ± 0.09</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>138 ± 21</td>
<td>137 ± 18</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77±11</td>
<td>81±10</td>
<td>=0.05</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.04 ± 0.86</td>
<td>5.81 ± 1.14</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>1.96 ± 0.72</td>
<td>3.57 ± 0.98</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.17 ± 0.27</td>
<td>1.54 ± 0.42</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.47 [0.23-38.53]</td>
<td>0.94 [0.20-10.19]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White cell count (10⁶ cells/mL)</td>
<td>7.59 ± 1.67</td>
<td>5.85 ± 1.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>30.5 ± 6.9</td>
<td>32.7 ± 6.9</td>
<td>NS</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>6.1 ± 2.8</td>
<td>6.0 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>62.7 ± 10.0</td>
<td>60.3 ± 10.4</td>
<td>NS</td>
</tr>
</tbody>
</table>
Superoxide Determination by EPR

Whole blood → Mononuclear cells (5*10^6/mL)

Counts (AU)

Time (min)

0 2 4 6 8 10

0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 *10^6

MAXIMUM

BASAL

\[
\text{CPH} + \text{O}_2^- \rightarrow 3.2 \times 10^3 \text{M}^{-1}\text{s}^{-1} \rightarrow \text{CP}
\]
Intra-assay variability, CV = 10%

Inter-assay variability, CV = 10%

Inter-observer variability = 3%

y = 0.97x + 2.4
Superoxide Release from White Cells

**Basal $\cdot O_2^-$ (nmol/min/10^6 cells)**

- Control
- CAD

**Maximum $\cdot O_2^-$ (nmol/min/10^6 cells)**

- Control
- CAD

* $P < 0.001
Superoxide Release from Whole Blood

Control CAD

Whole blood $\cdot \text{O}_2^-$ (nmol/min)

* $P < 0.001$
Correlation

$r$ (Pearson) = 0.689

$P = 0.001$

$\text{log } [\text{Whole blood } \cdot \text{O}_2^- \text{ (nmol/min)}]$

$\text{log } [\text{Basal } \cdot \text{O}_2^- \text{ (nmol/min/10}^6 \text{ cells)}]$
Cardiovascular Continuum

Risk factors

Oxidative and mechanical stress
Inflammation

Early tissue dysfunction

Atherothrombosis and progressive CV disease

Tissue injury (MI, stroke, renal insufficiency, peripheral arterial insufficiency)

Pathological remodeling

Target organ damage

End-organ failure (CHF, ESRD)

Death

Dzau V et al. Circulation 2006
• Established methods include assessment of flow-mediated dilation, intraarterial infusion of ACh and pulse wave analysis-based methods

• These tests are user dependent and/or invasive and require special skills
Endo-PAT2000
Endo-PAT2000

Control arm

Control

CAD

Baseline pulse amplitude

occlusion

Post occlusion hyperaemia
RHI vs Baseline Pulse Amplitude

p = 0.017

CAD vs controls

RHI

controls

CAD
Cardiovascular Continuum

Endo-PAT2000

Control arm

CAD arm

Baseline pulse amplitude

occlusion

Post occlusion hyperaemia
Cardiovascular Continuum

RHI vs Baseline Pulse Amplitude

**controls CAD**

RHI

- **p=0.017**

Average baseline pulse amplitude, right arm

- **r= -0.449**
- **p=0.003**
Cardiovascular Continuum

RHI throughout Pregnancy

- Non-pregnant controls
- Week 16
- Week 28
- Post-partum
Cardiovascular Continuum

Tissue injury
(MI, stroke, renal insufficiency, peripheral arterial insufficiency)

Atherothrombosis and progressive CV disease

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Death

Altered protein expression

Dzau V et al. Circulation 2006
Systems Biology and "Omics"

- DNA
- mRNA
- miRNAs
- Protein
- Metabolites
- small molecules
- Genomics
- Transcriptomics
- Proteomics
- Metabolomics

Risk

Disease
Urinary Proteomics


Zimmerli LU et al. Mol Cell Proteomics 2008
### Patients

<table>
<thead>
<tr>
<th>Study cohort</th>
<th>Samples</th>
<th>CAD</th>
<th>Control</th>
<th>Primary Usage</th>
<th>Secondary Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomarker Discovery</strong></td>
<td>586</td>
<td>204</td>
<td>382</td>
<td></td>
<td></td>
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<tr>
<td>CAD [9,10] (N=120†)</td>
<td>183</td>
<td>151</td>
<td>32</td>
<td>CAD markers</td>
<td>SVM modeling</td>
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<tr>
<td>Additional controls [14] (N=153)</td>
<td>229</td>
<td>0</td>
<td>229</td>
<td>SVM modeling</td>
<td>n.a.</td>
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<tr>
<td>TRENODY, baseline [9,12] (N=17†)</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>Medication markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>TRENODY, follow-up [9,12]</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>Medication markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>Fenofibrate, baseline [13] (N=26)</td>
<td>26</td>
<td>0</td>
<td>26</td>
<td>Medication markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td>Fenofibrate, follow-up</td>
<td>26</td>
<td>0</td>
<td>26</td>
<td>Medication markers</td>
<td>SVM modeling</td>
</tr>
<tr>
<td><strong>Blinded cohort (N=138)</strong></td>
<td>138</td>
<td>71</td>
<td>67</td>
<td>Validation</td>
<td>n.a.</td>
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<tr>
<td>HIB 0 mg (N=55‡)</td>
<td>55</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
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<tr>
<td>HIB 300 mg</td>
<td>48</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
</tr>
<tr>
<td>HIB 600 mg</td>
<td>45</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
</tr>
<tr>
<td>HIB 900 mg</td>
<td>45</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Drug interference</td>
<td>n.a.</td>
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<tr>
<td><strong>Long-term treatment effects</strong> [16]</td>
<td>44</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>IRMA-2 Irbesartan baseline (N=11†)</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>IRMA-2 Irbesartan follow-up</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>IRMA-2 Placebo baseline(N=11†)</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td>IRMA-2 Placebo follow-up</td>
<td>11</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Therapy monitoring</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Total (N=623)</strong></td>
<td>961</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
### Patients: Blinded Cohort

<table>
<thead>
<tr>
<th></th>
<th>CAD</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>64±9</td>
<td>62±8</td>
<td>0.094</td>
</tr>
<tr>
<td><strong>Sex (m/f)</strong></td>
<td>56/15</td>
<td>41/26</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>29.5±5.0</td>
<td>26.0±3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>139±25</td>
<td>138±19</td>
<td>0.756</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>78±12</td>
<td>82±11</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>Heart rate (min⁻¹)</strong></td>
<td>64±12</td>
<td>68±13</td>
<td>0.039</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>4.1±0.8</td>
<td>5.7±1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>2.0±0.7</td>
<td>3.5±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>1.2±0.3</td>
<td>1.5±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>1.8 [0.7;4.9]</td>
<td>1.3 [0.5;3.7]</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Urinary albumin/creatinine ratio (mg/mmol)</strong></td>
<td>1.1 [0.3;46.6]</td>
<td>1.0 [0.3;4.9]</td>
<td>0.073</td>
</tr>
<tr>
<td><strong>Active smoking (y/n)</strong></td>
<td>7/64</td>
<td>5/62</td>
<td>0.423</td>
</tr>
<tr>
<td><strong>Statin (y/n)</strong></td>
<td>63/8</td>
<td>8/59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Aspirin (y/n)</strong></td>
<td>61/10</td>
<td>9/58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Beta blocker (y/n)</strong></td>
<td>59/12</td>
<td>5/62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>ACEI/ARB (y/n)</strong></td>
<td>42/29</td>
<td>6/61</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
238 Biomarker Panel

Training

238 Biomarkers

AUC 95%
238 Biomarker Panel

CAD negative

CAD positive

Mass

Mass

CE-time

CE-time
Comparison with Previous Models

**Training**

AUC 95%

**Test**

- Zimmerli: AUC 68%
- v.z. Muhlen: AUC 77%
- New panel: AUC 87%

Sensitivity: 79%
Specificity: 88%
Identification of Proteins

• Collagen type 1
• Collagen type 3
• Alpha-1-antitrypsin (AAT)
• Granin-like neuroendocrine peptide precursor (ProSAAS)
• Membrane associated progesterone receptor component 1
• Sodium/potassium-transporting ATPase gamma chain
• Fibrinogen-alpha-chain
• We have established urinary proteomics and new methods to assess oxidative stress and endothelial function for routine use in clinical studies.

• Follow-up of patients will demonstrate whether these new markers are useful predictors of cardiovascular risk.