Biomarker Discovery in Heart Failure through Proteomics & Systems Biology

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Biomarkers & Systems Biology

- Biomarkers to address clinical needs in heart failure
- DNA susceptibility markers
- Expressome & microRNA
- Cardiac proteome and proteomic biomarkers
- Functional insights & validation
Disease & Symptoms in HF

Asymptomatic Dis Prog

NYHA I - IV

LVEF (Remodeling)

C.O.

Symptoms

Time (Years)
Syst vs Diast HF: Diff Disease with the Same Prognosis?

Figure 1. Adjusted Survival Curves for Patients with Heart Failure with Reduced or Preserved Ejection Fraction over the Year after the First Hospital Admission.

Biomarker Discovery

Sources for biomarker discovery
- Blood
- Cardiac biopsy
- Cell-based sources

Strategies for screening
- GWAS
- Gene microarray

Large cohort validation
- Risk of heart failure by biomarker tertiles
- Probability of heart failure (%) vs. Time (months)

### Table 6. Association of Parental Heart Failure with the Risk of Heart Failure in Offspring According to the Cause of Heart Failure in Parents and Offspring.∗

<table>
<thead>
<tr>
<th>Cause of Heart Failure in Offspring</th>
<th>No Parental Heart Failure</th>
<th>Parental Ischemic Heart Failure</th>
<th>Parental Nonischemic Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases/ No. at Risk (%)</td>
<td>HR (95% CI)†</td>
<td>No. of Cases/ No. at Risk (%)</td>
</tr>
<tr>
<td>Any cause</td>
<td>51/1516 (3.4)</td>
<td>1.00‡</td>
<td>21/327 (6.4)</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic cause</td>
<td>26/1516 (1.7)</td>
<td>1.00‡</td>
<td>11/327 (3.4)</td>
</tr>
<tr>
<td>P value</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonischemic cause</td>
<td>25/1516 (1.6)</td>
<td>1.00‡</td>
<td>10/327 (3.1)</td>
</tr>
<tr>
<td>P value</td>
<td>0.046</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HR denotes hazard ratio, and CI confidence interval. Percentages may not sum to the totals because of rounding.

†Hazard ratios were determined from models adjusted for age, sex, systolic blood pressure, body-mass index, total cholesterol:high-density lipoprotein cholesterol ratio, left ventricular mass, and the presence or absence of hypertension treatment, diabetes, and valve disease.

‡This group served as the reference group.

§Data from offspring in whom nonischemic heart failure developed during the follow-up period were censored as nonevents at the time of the development of heart failure.

¶Data from offspring in whom ischemic heart failure developed during the follow-up period were censored as nonevents at the time of the development of heart failure.

GWAS & Effect Size

MiR423-5p As a Circulating Biomarker for Heart Failure

Anke J. Tijsen,* Esther E. Creemers,* Perry D. Moerland, Leon J. de Windt, Allard C. van der Wal, Wouter E. Kok, Yigal M. Pinto

Rationale: Aberrant expression profiles of circulating microRNAs (miRNAs) have been described in various diseases and provide high sensitivity and specificity. We explored circulating miRNAs as potential biomarkers in patients with heart failure (HF).

Objective: The goal of this study was to determine whether miRNAs allow to distinguish clinical HF not only from healthy controls but also from non-HF forms of dyspnea.

Methods and Results: A miRNA array was performed on plasma of 12 healthy controls and 12 HF patients. From this array, we selected 16 miRNAs for a second clinical study in 39 healthy controls and in 50 cases with reports of dyspnea, of whom 30 were diagnosed with HF and 20 were diagnosed with dyspnea attributable to non–HF-related causes. This revealed that miR423-5p was specifically enriched in blood of HF cases and receiver-operator-characteristics (ROC) curve analysis showed miR423-5p to be a diagnostic predictor of HF, with an area under the curve of 0.91 (P<0.001). Five other miRNAs were elevated in HF cases but also slightly increased in non-HF dyspnea cases.

Conclusion: We identify 6 miRNAs that are elevated in patients with HF, among which miR423-5p is most strongly related to the clinical diagnosis of HF. These 6 circulating miRNAs provide attractive candidates as putative biomarkers for HF. (Circ Res. 2010;106:1035-1039.)

Key Words: MicroRNAs ■ plasma ■ heart failure ■ biomarker
HF MicroRNA Regulation

Tsijen, et al., Circ Res 2010; 106:1035-9
Searching for Proteomic Clues to Heart Failure

Fractionation simplifies samples being analyzed resulting in greater peptide identification: a 'byproduct' is a known subcellular location.
Detailed Proteomic Analysis of Multiple Tissues

A

All proteins clustered (4768)

Cytosol  Membrane  Mitochondria

B

Tissue-selective proteins (2018)

Brain  Heart  Kidney  Liver  Lung  Placenta

“Cardiac Unique” proteins
We have assembled a cardiac proteome of ~4600 proteins with ~300 proteins being “cardiac unique”
All cases, we have subcellular location information
Progression of Disease in R9C Transgenic Mouse

A) Survival (%)

B) Cardiac Shortening (%)

C) Images of hearts at 8wk, 16wk, 24wk showing WT and R9C

D) Images of heart tissue sections showing WT and R9C

40 Proteins Ranked Following False Discovery Rate (FDR)

A

Early Mid Late
8W 16W 24W

P-value

B

<table>
<thead>
<tr>
<th>Protein</th>
<th>8 wk +/- R9C</th>
<th>16 wk +/- R9C</th>
<th>24 wk +/- R9C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDIA1_MOUSE</td>
<td>48 146 39</td>
<td>288 89 449</td>
<td></td>
</tr>
<tr>
<td>GRP78_MOUSE</td>
<td>120 295 73</td>
<td>223 63 194</td>
<td></td>
</tr>
<tr>
<td>ANXA2_MOUSE</td>
<td>57 158 47</td>
<td>288 12 131</td>
<td></td>
</tr>
<tr>
<td>FHL1_MOUSE</td>
<td>111 228 125</td>
<td>349 23 70</td>
<td></td>
</tr>
<tr>
<td>TLN1_MOUSE</td>
<td>130 280 107</td>
<td>204 52 142</td>
<td></td>
</tr>
<tr>
<td>COF1_MOUSE</td>
<td>59 157 23</td>
<td>260 38 97</td>
<td></td>
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<tr>
<td>POSTN_MOUSE</td>
<td>130 280 107</td>
<td>204 52 142</td>
<td></td>
</tr>
<tr>
<td>DPY3_MOUSE</td>
<td>156 259 113</td>
<td>408 131 497</td>
<td></td>
</tr>
<tr>
<td>IBP7_MOUSE</td>
<td>0 13 0</td>
<td>21 2 2</td>
<td></td>
</tr>
<tr>
<td>CNN2_MOUSE</td>
<td>2 6 0</td>
<td>19 0 10</td>
<td></td>
</tr>
</tbody>
</table>

C

Proteomics data is validated by conventional methods and predicts biological changes on a large scale

Prediction of ER Stress Response Activation

A

Endoplasmic reticulum

\[ \uparrow [\text{Ca}^{2+}]_{i} \]

Stress

Unfolded proteins

GRP78

CRTC

GRP94

PERK

\[ \downarrow \]

Calpain

Caspase 12

Caspases 3 and 9

IRE1

P38 MapKinase

\[ \downarrow \]

CHOP

B

<table>
<thead>
<tr>
<th>8 wk</th>
<th>16 wk</th>
<th>24 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+ R9C</td>
<td>+/+ R9C</td>
<td>+/+ R9C</td>
</tr>
<tr>
<td>CRTC</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>GRP94</td>
<td>34</td>
<td>46</td>
</tr>
<tr>
<td>Hsp47</td>
<td>37</td>
<td>137</td>
</tr>
<tr>
<td>GR78</td>
<td>300</td>
<td>820</td>
</tr>
<tr>
<td>CHOP</td>
<td>( p )</td>
<td></td>
</tr>
<tr>
<td>ELF2</td>
<td>( m )</td>
<td></td>
</tr>
</tbody>
</table>

Enrichment Map of Targets Early vs Late in Disease

Apoptotic Pathway Interactions

Gelsolin: Regulator of Remodeling

Potential presence of proteins in blood plasma

Potential markers (blood or urine) for disease progression can be identified.
Biomarker Discovery to Application

Protein /Gene Candidate → Test Grade Reagents → Initial Clinical Validation

Regulatory Approval → High Grade Reagents Validation → Secondary Clinical Validation

Cost-Effective / Clin Decis’n Research → Guidelines/ Implementation → Outcomes Research & New Indications
MAPK Activation Reversed with Propranolol

Conclusions

• Systems biology approach permits discovery of novel pathophysiological pathways underlying chronic diseases

• Hypotheses generated can lead to new biological understanding and helps to develop novel biomarkers and Rx targets

• Validation across models and platforms critical, and testing in multiple populations

• Translation requires multidisciplinary collaboration and public/private partnership
Acknowledgement

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