Dysfunctional HDL and Coronary Artery Disease (CAD)
PIs and Members of HDL Project

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Systems Biology of CVD

- DNA → Genomics (~20,000 Genes)
- mRNA → Transcriptomics (~100,000 Transcripts)
- Protein → Proteomics (~100,000 Native Proteins)
- Modified Proteins → Proteomics (>1,000,000 modified Proteins)
- Enzymatic reactions and Dietary sources
- Metabolites → Metabolomics (~3000-20,000 metabolites)

PHENOTYPE
Overview of the MS based Biomarker Discovery

Design and Validate high throughput platform for Discovery and Targeted analysis

Identify differentially regulated biomolecules in well-characterized models

Confirm markers by orthogonal technique

Identify markers in patients with Clinical Phenotypes
Recent Publications on Metabolomic and Proteomic Discovery from our group:

*Nature 2009*: Discovery metabolomics in Pca

*Nature Medicine 2009*: Protein targets in IPF

*JCI 2008, Cell Metabolism 2009*: Diabetic islet dysfunction using lipidomic techniques

*CVD, DC: Proteomics and metabolomics:*


*Circulation 2006, 2008 and 2011*
CVD: No. 1 Killer in China and the World

-----WHO 2002
CVD (35%)

--China Ministry of Health (2008-2009)
CVD (38%)
CVD Magnitude in the U.S.

- CVD - #1 cause of death in U.S. for men and women
- Accounts for 2,400 deaths daily, 1 every 37 seconds
- Claims more lives than the next four leading causes of death combined
- U.S. estimated cost of CVD exceeded $432 billion in 2007 accounting for > 16.5% of US total health care costs
- Michigan has 10th highest coronary death rate in the U.S.

Source: American Heart Association 2008 Heart and Stroke Statistical Update.  www.americanheart.org
Risk factors for CVD

Elevated Blood Pressure
Adverse Lipid Profile
Diabetes
Family History
Obesity
Sedentary Lifestyle
Smoking
Nutrition
AHA Statistics

From 1994 to 2004, death rates from CVD declined 24.7 percent in the US.

Claimed 871,500 lives in 2004 (1 of every 2.8 deaths)
HDL, LDL and CVD Risk
Prospective Cardiovascular Münster Study

19,698 subjects (aged 16–65 y)

Assmann et al.
Eur Heart J.
CHD Risk According to HDL-C Levels
Prospective Cardiovascular Münster Study

Assmann et al. Atherosclerosis. 1996
HDL Promotes Cholesterol Efflux from Macrophages
In 2007, the failure of Pfizer's torcetrapib (inhibiting CETP: Cholesteryl ester transfer protein) was associated with an increase in the number of cardiovascular events, despite a 72% increase in HDL cholesterol levels!!

Therefore, “quality/function” of HDL more important than “quantity”
Structure of HDL

Hydrophobic Core of Triglyceride and Cholesteryl Esters

Surface Monolayer of Phospholipids and Free Cholesterol

Apo A-I

Apo A-II

MPO-Oxidized HDL is a Novel Biomarker for CAD

Pennathur J Biol Chem 2004
PNAS 2004
### Table 3
Relationship between serum total protein and apoA-I NO₂Tyr and CitTyr content and CVD prevalence

<table>
<thead>
<tr>
<th></th>
<th>Frequency of CVD per tertile</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>P for trend</td>
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<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein NO₂Tyr</td>
<td>30.8%</td>
<td>33.3%</td>
<td>69.2%</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>ApoA-I NO₂Tyr</td>
<td>32.0%</td>
<td>44.0%</td>
<td>72.0%</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Total protein CitTyr</td>
<td>40.0%</td>
<td>50.0%</td>
<td>58.3%</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>ApoA-I CitTyr</td>
<td>20.0%</td>
<td>48.0%</td>
<td>80.0%</td>
<td>&lt;0.001</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio (95% CI) of CVD per tertile</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
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</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein NO₂Tyr</td>
<td>1.0</td>
<td>1.1 (0.4–3.6)</td>
<td>5.1 (1.6–16.4)</td>
</tr>
<tr>
<td>ApoA-I NO₂Tyr</td>
<td>1.0</td>
<td>1.7 (0.5–5.3)</td>
<td>5.5 (1.6–13.4)</td>
</tr>
<tr>
<td>Total protein CitTyr</td>
<td>1.0</td>
<td>1.5 (0.5–4.6)</td>
<td>2.1 (0.7–6.6)</td>
</tr>
<tr>
<td>ApoA-I CitTyr</td>
<td>1.0</td>
<td>3.7 (1.1–13.0)</td>
<td>16.0 (4.0–64.0)</td>
</tr>
</tbody>
</table>

*Top:* Frequencies of CVD prevalence within each tertile of the entire cohort. *Bottom:* Odds ratios and 95% confidence intervals for the second and third tertiles, compared with the lowest (first) tertile, as predictors of CVD. CI, confidence interval.
Oxidation of HDL by MPO inhibits two steps of reverse cholesterol transport.
HDL Proteomics reveals unique Proteins in CAD

- apoC-IV
- PON1
- Complement C3
- apoA-IV
- apoE
- apoL1
- SAA1
- β-2-glycoprotein I
- Complement C4B
- apoC-II
- apoM
- LCAT
- CETP
- α-2-antiplasmin
- α-1-acid-glycoprotein 2
- PLTP
- Angiotensinogen
- apoB-100
- α-2-HS-glycoprotein
- apoF
- Complement C4
- apoD
- α-1-antitrypsin
- apoC-I
- apoC-III
- apoA-I
- SAA
- apoA-II
- SAA2
- SAA4
- Serum albumin
- Vitronecrtin
- HRP
- Clusterin

Enriched in control subjects

Enriched in CAD subjects

- Peptide index

JCI 2007
Significance

- HDL has protective effects on CVD (Reverse Cholesterol Transport)
- MPO modifies HDL making it dysfunctional
- MPO modified HDL and alterations in HDL protein cargo are measurable and predict CVD
- Steady state levels of HDL may not determine the functionality of HDL in reverse cholesterol transport
- Proteins involved in HDL metabolism and function are potential targets for the development of novel therapeutics for atherosclerosis

*It is not known if MPO modified HDL and its protein cargo can predict HDL function CVD regardless of total HDL level*
Hypothesis

• We postulate that *myeloperoxidase-oxidized dysfunctional HDL and HDL proteome* will predict increased CVD risk in patients irrespective of their plasma HDL levels.

• Additionally, we hypothesize that *oxidized HDL and HDL proteome will alter with therapies that improve CV outcomes*.
Use a MS proteomics approach to determine if atherogenic proteins are associated with HDL isolated from humans with established CAD irrespective of HDL level
Overall Approach

• Targeted Analysis of HDL
  – Normal Subjects
  – CAD Subjects

• Untargeted analysis of protein digests with 2-D LC MS/MS

• Correlate with measures of HDL function
Aim 1: Determine MPO and oxidized HDL levels in study subjects with and without CVD

- Utilize MS to test whether 3-chlorotyrosine and 3-nitrotyrosine in HDL are elevated in CVD
- We predict that the degree of oxidation will predict phenotypic expression of CVD, irrespective of circulating HDL levels
Aim 2: Determine if HDL proteome is altered in CVD subjects

- Discover specific proteins in HDL important in the pathogenesis and diagnosis of CVD
- We predict that protein content of HDL from patients with active CVD will differ
- Quantifying changes in protein composition may lead to novel predictors of cardiovascular risk.
Aim 3: Determine if MPO oxidized HDL and HDL proteome predict HDL function in all groups of patients

• Assess if changes in MPO oxidation and protein content and other inflammatory biomarkers predict HDL cholesterol efflux capacity

• We predict that HDL oxidation markers, MPO, and the HDL proteome will accurately correlate with measures of HDL function
PKU Cardiovascular Disease Trial

Aim 1
Oxidized HDL Profile in subjects with low and high HDL

Aim 2
HDL Proteome Profile in subjects with low and high HDL

Compare with US cohort of Healthy and CVD subjects

Aim 3
Correlate HDL function with Oxidative and Proteomic HDL Profiles

PKU CVD Study
Training Plan

• Training period 1:
  – First year of the project
  – Study design, clinical database, Biomarker development, HDL isolation and collection and QC, project oversight

• Training period 2:
  – ~1 – 2 years, after data has been generated.
  – Marker Validation and next steps

• Skills
  – Analysis of HDL markers and proteomic methodologies by mass spectrometry
  – Clinical database development and QC
  – Proteome informatics and Statistics
  – Functional analysis: *in vitro* cell culture systems and *in vivo* validation of target protein markers
Study Subjects

• + CAD ↓ HDL
  n=20
  males: HDL-C<40
  females: HDL-C<50

• + CAD ↑ HDL
  n=20
  males: HDL-C>50
  females: HDL-C>60

• – CAD ↓ HDL
  n=20
  males: HDL-C<40
  females: HDL-C<50

• – CAD ↑ HDL
  n=20
  males: HDL-C>50
  females: HDL-C>60
Criteria for patient enrollment

- **Inclusion Criteria**
  - Males or females aged 30-75
  - Subjects with CAD will be identified by Coronary or CT Angiography.
    - ≥50% stenosis at least in one of the main branches of coronary arteries
  - Subjects with no CAD will be identified by Coronary or CT Angiography.
    - Normal coronary arteries or only mild lumen irregularities in the main branches of coronary arteries

- **Exclusion Criteria**
  - AMI (in the preceding 3 months)
  - Pregnant women and lactating women
  - Myocardial ischemia induced by non-CAD
  - Diabetes mellitus
  - Receiving cholesterol-lowering medications within 3 months
  - Heart failure (New York Heart Association class III or IV)
  - Chronic kidney disease: Scr>2mg/dl or eGFR< 30ml/min/1.73m²
  - Serum hepatic transaminases levels> 3 times upper normal limit or clinical evidence of active liver disease.
Flow chart for patient enrollment

1. **Subjects screening**
   - CAG/CT
   - CAD (40 cases)
   - no CAD (40 cases)

2. **HDL level**
   - CAD with high HDL (20 cases)
   - CAD with low HDL (20 cases)
   - no CAD with high HDL (20 cases)
   - no CAD with high HDL (20 cases)

3. **Informed Consent**
   - Blood sample collection
   - Clinical data collection

4. **Blood sample collection**
   - HDL Isolation
   - Plasma MPO
   - Plasma for biomarkers

5. **Clinical data collection**
   - Oxidized HDL
   - HDL proteome profile
   - HDL functional assessment
One Year Case Volume at PUHSC

- CAD
- CAD with high HDL
- CAD with low HDL

- no CAD
- no CAD with high HDL
- no CAD with low HDL

1800 cases:
- CAD: 70
- CAD with high HDL: 18
- CAD with low HDL: 72

2900 cases:
- no CAD: 26
- no CAD with high HDL: 72
- no CAD with low HDL: 70
Potential Impact of the study

• The proposed study will be the first to examine the roles of MPO-oxidized HDL and HDL proteome in vascular dysfunction in Chinese patients.

• It will be the first study to investigate biochemical and functional alterations in HDL occurs irrespective of HDL levels in persons prone for CAD

• Potential discovery of novel targets of HDL metabolism that can be explored in later studies for clinical utility and provide rationale for novel drug development.
高密度脂蛋白氧化及其蛋白质组分变化
与冠心病发病的相关性研究

研究方案

申请人：北京大学第三医院心内科
中方负责人：北京大学第三医院心内科 王韧

美方联系人：密歇根大学内科心血管医学 李刚

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Thank you!

“Your Heart is in our Hands” – J. DeDecker 2007