Association of Fibrosis Risk in HCV Patients with a Missense SNP in Gene CPT1A

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1. Celera Diagnostics, Alameda, California
2. University of California at San Francisco, California
3. Stanford University, California
4. University of Illinois, Chicago
5. Virginia Commonwealth University, Virginia
Natural History of HCV

- **Normal Liver**
- **Acute hepatitis C**
- **Recovery 15%**
- **Chronic hepatitis 85%**
- **Cirrhosis Up to 20%**
- **Hepatocellular Carcinoma 1-5%**

10 to 40+ years
Previously Identified Major Risk Factors

• Correlation with
  – Male gender (OR = 2.66)
  – Daily alcohol > 50 g (OR = 1.49)
  – Age > 40 at infection (OR = 1.08)
  – Steatosis

• No correlation with
  – HCV viral load or genotype
  – Cause of infection
  – Ethnicity

\[
\text{Very poor predictors for fibrosis}
\]

Hypothesis: other host factors, such as genetics, may play a role

Objectives

• To identify genetic markers which predict patients predisposing to developing liver fibrosis
Study Subjects

1,625 HCV Patients Enrolled

- **Discovery (N=537)**
  - UCSF Teresa L. Wright N = 537

- **Replication (N=698)**
  - VCU Mitchell L. Shiffman N = 483
  - Stanford Ramsey C. Cheung N = 100
  - UIC Thomas J. Layden N = 115

- **Validation (N=390)**
  - Sutter Natalie Bzowej N = 240
  - Ottawa Curtis Cooper N = 150
Fibrosis Enrollment Criteria

• Inclusion criteria
  – Adults (age 18 – 75)
  – Chronic HCV infection (HCV RNA positive)
  – Liver biopsy before any treatment

• Exclusion criteria
  – Other causes of chronic liver diseases (hepatitis B, Wilson’s disease, hemochromatosis, autoimmune hepatitis)
  – Co-infection with HIV
  – Hepatocellular carcinoma
<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UCSF</td>
<td>VCU</td>
</tr>
<tr>
<td># of patients</td>
<td>433 / 537</td>
<td>483</td>
</tr>
<tr>
<td>Gender (% Male)</td>
<td>83.7%</td>
<td>57.8%</td>
</tr>
<tr>
<td>Age</td>
<td>52.7</td>
<td>50.8</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>69.0%</td>
<td>70.6%</td>
</tr>
<tr>
<td>AA</td>
<td>14.5%</td>
<td>26.3%</td>
</tr>
<tr>
<td>Others</td>
<td>16.6%</td>
<td>3.1%</td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>21.6%</td>
<td>17.4%</td>
</tr>
<tr>
<td>1</td>
<td>24.6%</td>
<td>34.2%</td>
</tr>
<tr>
<td>2</td>
<td>26.0%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16.8%</td>
<td>23.6%</td>
</tr>
<tr>
<td>4</td>
<td>11.0%</td>
<td>24.8%</td>
</tr>
<tr>
<td>mean</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Duration_infection</td>
<td>27.3</td>
<td>21.9</td>
</tr>
<tr>
<td>Fibrosis rate (Stage/Duration)</td>
<td>0.08</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Functional Genome Scan

> 20,000 SNPs

Cover 12,500 known and predicted genes (40% genome)

Botstein and Risch *Nature Genetics* 33, 228 (2003).
Association Study

Genome scan in DISCOVERY samples

\[ P < 0.05 \text{ in discovery} \]

Primary hits

Genotype in REPLICATION samples

\[ P < 0.05 \text{ in discovery and replication} \]

\( \sim 60 \) Replicated hits

CPT1A SNP (Ala175Thr)
## Association of CPT1A A175T with Advanced Fibrosis

**Major homozygote:** CC  
**Carriers:** CT  TT

<table>
<thead>
<tr>
<th>Study</th>
<th>Genotype</th>
<th>Cases (Bridging Fib. or Cirrhosis)</th>
<th>Controls (Mild or No-fibrosis)</th>
<th>Adjusted for sex, ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCSF (N=537)</td>
<td>TT+TC CC</td>
<td>3.3%</td>
<td>11.2%</td>
<td>OR 0.3 (3.4) 95% CI 0.1 - 0.9 P 0.025</td>
</tr>
<tr>
<td>VCU (N=483)</td>
<td>TT+TC CC</td>
<td>8.5%</td>
<td>13.3%</td>
<td>OR 0.6 (1.7) 95% CI 0.3 - 1.1 P 0.044</td>
</tr>
<tr>
<td>Stanford (N=100)</td>
<td>TT+TC CC</td>
<td>0.0%</td>
<td>12.9%</td>
<td>OR 0.1 (10) 95% CI -inf - 1.0 P 0.023</td>
</tr>
<tr>
<td>UIC (N=115)</td>
<td>TT+TC CC</td>
<td>4.3%</td>
<td>4.3%</td>
<td>OR 0.8 (1.2) 95% CI 0.1 - 5.4 P 0.423</td>
</tr>
</tbody>
</table>

**Combined P** 0.004

- Adjusted for sex, ethnicity.
Carnitine Palmitoyl Transferase 1A (liver)

*A Key Enzyme Regulating Fatty Acid Oxidation*

1. What is the impact of A175T on fatty acid oxidation?
2. Is the association with fibrosis via steatosis – another risk factor?
Function of CPT1A A175T

1. A175T is not critical to the enzymatic activity of CPT1A*
2. A175T is not associated with steatosis grade in UCSF samples

Summary

• Conclusion
  – 1,625 HCV patients were enrolled from 6 centers
  – Functional genome scan was performed
  – SNP A175T in CPT1A was associated with decreased risk of advanced fibrosis in multiple sample sets
  – The association with fibrosis was not mediated by steatosis

• Future work
  – Investigate the mechanism of A175T in fibrogenesis pathway
Acknowledgements

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Scott Friedman

University of California, San Francisco
A PUBLIC UNIVERSITY DEDICATED TO SAVING LIVES AND IMPROVING HEALTH

Teresa L. Wright
Linda Yee
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Alexander Monto

VCU

Mitchell L. Shiffman

STANFORD UNIVERSITY

Ramsey C. Cheung

UIC

Thomas J. Layden