PROGRESS: Beginning to Understand the Genetic Predisposition to PSC

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Primary Sclerosing Cholangitis
PSC

• In 1924, Delbet described the first case of PSC

• Early 1980’s first PSC case-series reports in medical literature
  Drs. R. Wiesner, N. LaRusso, Mayo Clinic, USA
  Dr. R. Chapman, United Kingdom

• To date, etiology of PSC remains unknown

• No medical therapy available
What is the cause of PSC?
Proposed Pathogenesis of PSC?

Genetic Predisposition

Environment
PSC and IBD

~1 x 10^6 cases in US

IBD

~30,000 cases in US

75% PSC with IBD

25% PSC with no IBD
PSC is a Heterogeneous Disease
How can we find the causes of PSC?
PSC is a Complex Disease

Gene A

Gene B

...Gene X

Environmental risks

PSC
Rationale for Studying Genetic Predisposition to PSC

Identify genetic susceptibility of PSC

- Novel diagnostics
  - Prevention

- Biological defect
  - New therapies

- Assess disease progression
PROGRESS
(PSC Resource Of Genetic Risk Environment & Synergy Studies)

Established in 2005

• To better understand the cause(s) and pathogenesis of PSC

• To improve prediction and therapy of PSC
PROGRESS
(PSC Resource Of Genetic Risk Environment & Synergy Studies)

- Whole blood collection
  biochemical testing
  DNA isolation
  cell-line creation

- Questionnaire data

- Family information (draw pedigrees)
PROGRESS

Study Requirements

• Read and sign a consent form

• Complete a questionnaire and a family information form

• Provide a sample of your blood

• Recipients of liver transplant are not excluded

• No need to visit Mayo Clinic to participate
### PROGRESS - Database Enrollment

**PSC Proband Demographics Form**

#### Study ID#
- [ ] Exclude From Study
- [ ] Pedigree has been created

**Study Group**: [ ]
**Recruitment Source**: [ ]
**Mony Clinic #**: [ ]

### Personal Information

- **Initials**: [ ]
- **FirstName**: [ ]
- **MI**: [ ]
- **LastName**: [ ]
- **Nickname**: [ ]
- **Sex**: [ ]

- **Date of Birth**: [ ]
- **Race**: [ ]
- **Specify Other Race**: [ ]

- **Home Phone #**: [ ]
- **Alt. Phone #**: [ ]
- **Deceased**: [ ]
- **DOD**: [ ]

- **Street Address**: [ ]
- **City**: [ ]
- **State**: [ ]
- **Zip Code**: [ ]
- **Region**: [ ]

### Recruitment Status

**Consent Form**
- [ ] Mailed
- [ ] Received
- [ ] Response

**Medical Questionnaire**
- [ ] Mailed
- [ ] Received

**Specimen Kit**
- [ ] Mailed
- [ ] Received

**Pediatric Kit**
- [ ]

### Follow Up Contacts

<table>
<thead>
<tr>
<th>#</th>
<th>Study ID #</th>
<th>Date</th>
<th>Reason for Follow-Up</th>
<th>Results / Notes</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>#</th>
<th>StudyID</th>
<th>Aik Pess</th>
<th>ALT</th>
<th>AMA</th>
<th>Thy Roc Ab</th>
<th>Thypert Ab</th>
<th>TSH</th>
<th>T4</th>
<th>Bilirubin</th>
<th>Creatinine</th>
<th>LabsType</th>
</tr>
</thead>
</table>

### Sample Processing
- [ ] DNA
- [ ] SC
- [ ] SEV

**Notes**: [ ]

## Lab Results
### PROGRESS - Database Phenotypes

#### Personal Information
- **Study ID#**
- **Exclude From Study**
- **Deceased**
- **DOD**

- **Study Group**
- **Recruitment Source**
- **Mayo Clinic #**

<table>
<thead>
<tr>
<th>Initials</th>
<th>First Name</th>
<th>MI</th>
<th>Last Name</th>
<th>Sex</th>
<th>Date of Birth</th>
</tr>
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<tr>
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</tr>
</tbody>
</table>

#### PSC History
- **KNL Chart Review**
- **Year of Dx**
- **Evidence of PSC**
- **Disease Location**
- **ERCP**: Date
- **MRCP**: Date
- **PTC**: Date

- **Concurrent Disease Assessment**
  - **Chart Reviewed**
  - **Last Clinic Visit**
  - **IBD**: Year, Type
  - **CCA**: Year, Location
  - **OLT**: Date

- **Notes**

*PSC Proband Disease Phenotyping*
PROGRESS Enrollment by State

Mayo Clinic - Rochester, MN
U Indiana, IN
Virginia Mason Clinic, WA
U Pittsburgh, PA

Mt Sinai Medical Center, NY
Virginia Commonwealth U, VA
Johns Hopkins U, MD
U Toronto, ON, Canada
## PROGRESS: Recruitment by Medical Center

<table>
<thead>
<tr>
<th>Medical Center</th>
<th>Consent</th>
<th>DNA</th>
<th>Questionnaire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayo Clinic</td>
<td>807</td>
<td>651</td>
<td>661</td>
</tr>
<tr>
<td>U. Indiana</td>
<td>106</td>
<td>105</td>
<td>95</td>
</tr>
<tr>
<td>U. Toronto, CA</td>
<td>51</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>U. Pittsburgh</td>
<td>42</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>V.M. Clinic</td>
<td>40</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>V.C.U.</td>
<td>18</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Mt. Sinai, NY</td>
<td>33</td>
<td>29</td>
<td>22</td>
</tr>
<tr>
<td>Johns Hopkins</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total collaboration(^a)</strong></td>
<td>292</td>
<td>280</td>
<td>220</td>
</tr>
<tr>
<td><strong>Total (all centers)</strong></td>
<td>1,099</td>
<td>931</td>
<td>881</td>
</tr>
</tbody>
</table>

\(^a\) Includes collection of 300 PSC DNAs from G.H and P. D. and 50 PSC DNAs from Poland.
Aim 1: To expand PROGRESS, by:

- Continuing recruitment of PSC patients at Mayo Clinic
- Initiating referral of patients to PROGRESS by our external collaborators
- Fostering existing relationships with international PSC and IBD study groups
PROGRESS NIDDK Grant - Specific Aims

Aim 2: Genomic Wide Association Studies (GWAS)

Susceptibility to PSC

- Unaffected Controls (5000)
- PSC Patients (2000)
- UC Patients no PSC (1000)

Susceptibility to PSC in UC

Replication

- 1900 PSC – 3000 Controls
- 1330 PSC with UC – 1000 UC no PSC

*Existing genotypes; IBDGC: IBD genetics consortium; NOPSC: Norwegian PSC Research Center; UKPSC: UK PSC Consortium*
To determine environmental risk factors for PSC by performing a study of 1000 patients and 1000 controls utilizing the self-administered questionnaire data collected by PROGRESS.
Outcome in PSC-UC Patients Homozygous for MMP3 rs522616 and rs650108 Genetic Variants

Juran et al., Liver International  2011
Immunochip Experiment

• 196,524 Single Nucleotide Polymorphisms (SNPs)

• 186 genetic loci with known autoimmune diseases associations
## International PSC Immunochip Study

<table>
<thead>
<tr>
<th>Origin</th>
<th>Cases</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td>Belgium</td>
<td>163</td>
<td>1,425</td>
</tr>
<tr>
<td>Canada</td>
<td>323</td>
<td>0</td>
</tr>
<tr>
<td>Finland</td>
<td>308</td>
<td>504</td>
</tr>
<tr>
<td>France</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>852</td>
<td>5,435</td>
</tr>
<tr>
<td>Netherlands</td>
<td>255</td>
<td>3,421</td>
</tr>
<tr>
<td>Norway</td>
<td>504</td>
<td>1,412</td>
</tr>
<tr>
<td>Poland</td>
<td>43</td>
<td>541</td>
</tr>
<tr>
<td>Spain</td>
<td>27</td>
<td>284</td>
</tr>
<tr>
<td>Sweden</td>
<td>282</td>
<td>2,665</td>
</tr>
<tr>
<td>UK</td>
<td>1,121</td>
<td>8,970</td>
</tr>
<tr>
<td>USA</td>
<td>533</td>
<td>681</td>
</tr>
</tbody>
</table>

Total: 4,456 cases and 25,338 controls
Exome Sequencing of a Family
Hypothesis and AIM

• We hypothesized that families with multiple members affected by PSC might carry rare genetic polymorphisms.

• We aimed to perform exome sequencing and analysis in this multiply-affected PSC family as a pilot to inform future large-scale efforts.
# A Novel Genetic Variant of ABCB4 Gene in Pedigree #5139

<table>
<thead>
<tr>
<th>SNP</th>
<th>gene_name1</th>
<th>daughter1</th>
<th>father</th>
<th>mother</th>
<th>daughter2</th>
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<tbody>
<tr>
<td>rs31653(A/G)</td>
<td>ABCB4</td>
<td>hom</td>
<td>hom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs31668(G/A)</td>
<td>ABCB4</td>
<td>hom</td>
<td>hom</td>
<td>hom</td>
<td>hom</td>
</tr>
<tr>
<td>rs2230029(T/C)</td>
<td>ABCB4</td>
<td></td>
<td></td>
<td></td>
<td>het</td>
</tr>
<tr>
<td>(G/A) R595X</td>
<td>ABCB4</td>
<td>het</td>
<td>het</td>
<td>het</td>
<td>het</td>
</tr>
<tr>
<td>rs2109505(T/A)</td>
<td>ABCB4</td>
<td>het</td>
<td></td>
<td>het</td>
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<tr>
<td>rs1202283(G/A)</td>
<td>ABCB4</td>
<td>het</td>
<td>hom</td>
<td></td>
<td>het</td>
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<tr>
<td>rs2302367(G/A)</td>
<td>ABCB4</td>
<td>het</td>
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<td>het</td>
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</tbody>
</table>
Defects in ABCB4 are known to cause a wide range of heritable cholestatic syndromes and contribute to cholelithiasis

- Progressive Familial Intrahepatic Cholestasis 3
- Intrahepatic Cholestasis of Pregnancy
- Low Phospholipid Associated Cholelithiasis
- PSC
Physiopathology of ABCB4 Deficiency

A. Bile salts
ABCB11
ABCB4
Phosphatidylcholine
Hepatocyte
Cholangiocytes (bile duct epithelia)

PC + BS – Mixed Micelles

B. Bile salts
ABCB11
ABCB4
Phosphatidylcholine

Lower PC – BS toxicity
Pedigree #5139

- Pedigree branch:
  - 200 57
  - 300 30/53
  - 401 52
  - 402 32
  - 403 35
  - 404 51
  - 405 48
  - 406 46

- Genetic markers:
  - Small duct
  - Ovarian ca

- Medical conditions:
  - Primary Sclerosing Cholangitis (PSC)
  - Orthotopic Liver Transplantation (OLT)
  - Inflammatory Bowel Disease (IBD)
  - Gallstone Disease (GSD)
Conclusions from Pedigree #5139

- The R595X mutation in ABCB4 is likely a strong contributor to the severe liver disease in this family.

- Exome sequencing of mother’s siblings will help to better define the contribution of the R595X mutation to PSC.

- Exome or Whole Genome Sequencing in the near future will improve the diagnosis and therapy of PSC.
PROGRESS Future Studies

• Whole Exome Sequencing in Selected TRIOs (affected patient and unaffected parents)

• Gene x Environment interaction studies

• Genomic-based disease outcome studies (prediction of disease progression)
Acknowledgements

- PSC patients and family members
- PSC Partners Seeking A Cure
- NIDDK RO1 grant (2011-2015)
- A. J. Sigismunda Palumbo Charitable Trust
- American Liver Foundation
- Mayo Clinic College of Medicine
- Division of Gastroenterology and Hepatology Mayo Clinic
Exome Sequencing

- **Exome enrichment**: Agilent SureSelect system

- **Sequencer**: Applied Biosystems SOLID v4
  (All 4 DNAs sequenced on single slide, 50bp run)

- **Alignment to reference genome** (hg18): BioScope

- **Polymorphism calling**: BioScope diBayes and SAMtools pileup

- **Filtering and Annotation**: In-house tools
Filtering

Filter #1: SNPs present in all 3 affected individuals
Non-synonymous cSNP or splice-site
Not in dbSNP, 1000 genomes freq <0.01
Total # SNPs – 84

Filter #2: SNPs in cholestasis candidate genes
Total # SNPs – 61

Overlap: 1 nonsense SNP/variant in MDR3 or ABCB4 (R595X)
Primary Sclerosing Cholangitis