Pharmacogenetics to Pharmaco-omics
Precision Medicine
and
Drug Response

- Introduction
- Pharmacogenetics
- Pharmacogenomics
- Pharmaco-omics
- Conclusions
Application of “omic” science to study variation in drug response phenotypes.

Critical component of Precision Medicine.
Evolution of Pharmacogenetics-Pharmaco-omics

Pharmacogenetics (candidate genes)

Pharmacogenomics (genome-wide studies)

Pharmaco-omics (Multiple “omics”)
Pharmacogenetics to Pharmaco-omics
Precision Medicine
and
Drug Response

Goals

- Avoid adverse drug reactions
- Maximize drug efficacy
- Select responsive patients
- Drugs as molecular probes
Pharmacogenetics to Pharmaco-omics

Precision Medicine and Drug Response

- Introduction
- **Pharmacogenetics**
- Pharmacogenomics
- Pharmaco-omics
- Conclusions
Julie Axelrod
Lack of Uptake of Catecholamines after Chronic Denervation of Sympathetic Nerves

Denervation of sympathetically innervated structures has been known to cause supersensitivity of these organs to catecholamines\(^1\) and depletion of these hormones\(^2\). However, no conclusive explanation has been offered for these findings. To clarify these phenomena further we compared the uptake of injected \(\text{DL } \beta^{-3}\text{H-noradrenaline}\) and \(\text{DL } \beta^{-3}\text{H-adrenaline}\) by intact and denervated tissue.
Dr. Julius Axelrod, NHIMT Researcher, Shares Nobel Prize With Two Others

Surrounded by colleagues and press photographers, Dr. Julius Axelrod joins in a revelry at an impromptu celebration after receiving word that he had been named co-winner of the Nobel Prize in Medicine or Physiology. Dr. Axelrod is the second Federal scientist to be so honored.
Catecholamine Biosynthesis and Metabolism

**Dopamine β-Hydroxylase**

Dopamine $\rightarrow$ Norepinephrine

- Dopamine: $\text{HO-CHCH}_2\text{NH}_2\text{C}_6\text{H}_4\text{HO}$
- Norepinephrine: $\text{HO-CHCH}_2\text{NH}_2\text{C}_6\text{H}_4\text{OH}$
Catecholamine Biosynthesis and Metabolism

Dopamine Catabolism

Dopamine

\[
\begin{align*}
\text{PAPS} & \quad \text{PAP} \\
\text{SULT1A3} & \quad \text{MAO} & \quad \text{COMT} \\
\text{Dopamine-3-O-Sulfate} & \quad 3,4\text{-Dihydroxy-phenylacetaldehyde} & \quad 3\text{-Methoxy-tyramine}
\end{align*}
\]
DBH and COMT
Sib-Sib Correlations

Science 181: 943-45, 1973
Nature 242: 490-491, 1974
COMT Frequency Distribution
Thermal Stability and Genotype


COMT Polymorphisms – 1947 Publications by 2017
Metabolism of 6-Mercaptopurine

1. Thiopurine Methyltransferase (TPMT)
2. Xanthine Oxidase (XO)
3. 2,8-Dihydroxy-6-Methylmercaptapurine
Human RBC TPMT

298 Unrelated Adults

% Of Subjects Per 0.5 Units of Activity

TPMT Activity, Units/ml RBC

Amer. J. Human Genetics, 32:651-62, 1980
Pharmacogenetics to Pharmaco-omics

Precision Medicine and Drug Response

• Introduction
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SSRI Pharmacogenomics and Pharmaco-omics

Variation in SSRI Therapeutic Outcomes and SSRIs as Molecular Probes for MDD Molecular Mechanisms
Week 0
- Consent
- Clinical assessment
- Start at escitalopram 10 mg or citalopram 20 mg
- DNA and baseline metabolomics blood draw

Week 4
- Clinical assessment
- Potential dose increase to 20 mg or 40 mg, depending on symptoms.
- Blood draw for metabolomic and plasma drug level assays

Week 8
- Clinical assessment
- Blood draw for metabolomic and plasma drug level assays

Follow-up phone call at Weeks 24

Mayo PGRN Citalopram-Escitalopram Clinical Trial
Mayo PGRN Citalopram-Escitalopram Clinical Trial Outcomes

- **No Depression**
  - Baseline: n=0
  - Last visit: n=212

- **Mild Symptoms**
  - Baseline: n=40
  - Last visit: n=173

- **Moderate Symptoms**
  - Baseline: n=216
  - Last visit: n=56

- **Severe Symptoms**
  - Baseline: n=172
  - Last visit: n=21

- **Very Severe Symptoms**
  - Baseline: n=35
  - Last visit: n=1

QIDS-C16 Score

Number of Subjects at both Baseline and Last Visit Evaluations

- Baseline
- 8 weeks
# Mayo PGRN SSRI Clinical Trial
## Initial 529 Patients

<table>
<thead>
<tr>
<th>Characteristic and Measure</th>
<th>Adherent EA Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Last visit (N = 499)</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>39.91 (±13.8)</td>
</tr>
<tr>
<td>Female gender</td>
<td>312 (62.5%)</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>QIDS-C Baseline</td>
<td>15.08 (±3.47)</td>
</tr>
<tr>
<td>QIDS-C Week 4</td>
<td>8.46 (±4.41)</td>
</tr>
<tr>
<td>QIDS-C Week 8</td>
<td>6.24 (±4.05)</td>
</tr>
<tr>
<td>HAMD Baseline</td>
<td>22.31 (±5.08)</td>
</tr>
<tr>
<td><strong>Baseline Medication</strong></td>
<td></td>
</tr>
<tr>
<td>Citalopram</td>
<td>155 (31.2%)</td>
</tr>
<tr>
<td>Escitalopram</td>
<td>342 (68.8%)</td>
</tr>
<tr>
<td><strong>Outcomes</strong></td>
<td></td>
</tr>
<tr>
<td>Remitter (QIDS ≤ 5)</td>
<td>206 (41.3%)</td>
</tr>
<tr>
<td>Response (%QIDS ≤ 50%)</td>
<td>287 (57.5%)</td>
</tr>
</tbody>
</table>

Citalopram Biotransformation

Citalopram $\xrightarrow{CYP2C19}$ Monodesmethylcitalopram $\xrightarrow{CYP2D6}$ Didesmethylcitalopram

Citalopram Pharmacokinetic GWAS


Plasma S-CT GWAS

rs1074145  
\( p = 4.1 \times 10^{-9} \)  
**CYP2C19**

Plasma S-DDCT GWAS

rs1065852  
\( p = 2.0 \times 10^{-16} \)  
**CYP2D6**
MDD and SSRI Pharmacogenomics

Goals

Biomarkers for SSRI Pharmacokinetics and SSRI Pharmacodynamics and SSRI Mechanisms
Lack of Replication

• Possible explanation - phenotypic heterogeneity

• Possible approach - use pharmacometabolomics to inform genomics
Pharmacogenetics to Pharmaco-omics

Precision Medicine

and

Drug Response

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THE DEPARTMENT OF MOLECULAR PHARMACOLOGY & EXPERIMENTAL THERAPEUTICS IS SEEKING AN EXCEPTIONAL BIOLOGIST WITH AN EMPHASIS ON MULTI-DIMENSIONAL OMIC DATA, INCLUDING GENOMICS + PROTEOMICS OR METABOLOMICS, IN PRECISION MEDICINE.

This position is an institutionally funded faculty position at the Assistant, Associate or full Professor level providing 100% salary support. The candidate will be expected to maintain an internationally recognized, extramurally funded program of research within the Department of Molecular Pharmacology & Experimental Therapeutics, a basic department with strong ties to the Mayo Clinic Cancer Center and the Mayo Clinic Center for Individualized Medicine. Mayo Clinic provides a collaborative environment, bringing researchers and clinicians together to take cutting edge research from the bench to the bedside and back. Resources available at the Mayo Clinic include several major NIH-funded initiatives such as the NCI-funded Comprehensive Cancer Center, NIH-funded Center for Clinical and Translational Science and various NCI-funded SPORE programs. Also available are Mayo supported hybrid Centers, including the Center for Individualized Medicine where omics technologies are applied to help achieve individualized therapy and diagnosis.

QUALIFICATIONS: Credentials of a successful candidate will include a doctoral degree in biomedical sciences (e.g., M.D. and/or Ph.D.), national recognition and experience in applying multiple omic technologies, especially genomics + proteomics or metabolomics, and a strong track record of publication. Consideration will be given to applicants performing research into any aspect of applied proteomics and metabolomics in precision medicine, but individuals investigating determinants of drug sensitivity/resistance through a combination of multiple omics and biochemical studies are particularly encouraged to apply. Established
Metabolomics Informed PGx

Genomics

Transcriptomics

Proteomics

Metabolomics

Clinical Phenotypes
Mayo PGRN
Citalopram-Escitalopram Clinical Trial

LCECA Metabolomics

• 918 patient samples (290 subjects, 3 timepoints)
• 37 LCECA metabolites assayed
• Dr. Wayne Matson, Bedford, MA
Plasma Pharmacometabolomics

Challenges

• Merging metabolomics and genome-wide genomics

• Relationship of peripheral metabolites to CNS function
Pharmacometabolomics Informed Pharmacogenomics

1. Associate Metabolite Levels With Clinical Outcomes
2. Perform GWAS for Metabolite Levels to Identify Genes Associated with Metabolite Concentrations
3. Functionally Validate the Genes Identified during GWAS
4. Replicate the Genes/SNPs in Other Studies
Plasma Serotonin and Change in Plasma Serotonin were Associated with SSRI Clinical Response

Association of Plasma Serotonin Concentration with Clinical Outcomes in Mayo Study

<table>
<thead>
<tr>
<th>Plasma Serotonin</th>
<th>Clinical Outcomes</th>
<th>Remission</th>
<th>Response</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 weeks</td>
<td>8 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td>$p = 0.012$</td>
<td>$p = 0.028$</td>
<td>$p = 0.007$</td>
</tr>
<tr>
<td>Changes after 4 weeks</td>
<td></td>
<td>$p = 0.011$</td>
<td>$p = 0.041$</td>
<td>$p = 0.026$</td>
</tr>
<tr>
<td>Changes after 8 weeks</td>
<td></td>
<td>$p = 0.069$</td>
<td>$p = 0.147$</td>
<td>$p = 0.037$</td>
</tr>
</tbody>
</table>

Remission: post-treatment QIDS < 5 or HAMD < 7.
Response: >50% reduction in QIDS or HAMD.

Plasma Serotonin Concentrations After SSRI Therapy

- Plasma Serotonin concentrations, and change in plasma serotonin, were highly associated with SSRI outcomes.
- Higher baseline serotonin and greater change were both associated with better SSRI outcomes.

Sero\-tonin-Kynure\-nene Balance and Major Depressive Disorder

L-Tryptophan

- Serotonin
  - (Serotonin Neurotransmission)
- Kynurenine
  - (Glutamate Neurotransmission)
Baseline Plasma Serotonin GWAS

**ERICH3**

$p = 9.28 \times 10^{-8}$

**TSPAN5**

$p = 7.84 \times 10^{-9}$

Baseline Serotonin Concentrations by ERICH3 and TSPAN5 SNP Genotypes

**ERICH3 and TSPAN5 Locus Zoom Plots**

Chromosome 1 Locus Zoom

- rs696692
- $p = 9.28E-08$

Chromosome 4 Locus Zoom

- rs11947402
- $p = 7.84E-09$

TSPAN5 SNPs are cis-eQTLs

SK-N-BE(2) Neuroblastoma cells

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Fold Change</th>
<th>TSPAN5 Knockdown (72 hrs)</th>
<th>TSPAN5 Over-expression (72 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC6A4</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPH1</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPH2</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDC</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAOA</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAOB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR1A</td>
<td>***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR3A</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR3B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTR7</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC18A2</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.005
N=3; Mean±SEM

**ERICH3 nsSNP Expression**

Culture Media Serotonin

**ERICH3** and **TSPAN5 KD** and **OE**

**SK-N-BE(2) Neuroblastoma Cells**

- **Serotonin Concentration**
  - Control KD: [Serotonin] ng/mL
  - TSPAN5 KD: [Serotonin] ng/mL
- **KD and OE Efficiency**
  - Control KD: Relative mRNA of TSPAN5/GAPDH
  - TSPAN5 KD: Relative mRNA of TSPAN5/GAPDH
  - EV: Relative mRNA of ERICH3/GAPDH
  - ERICH3 OE: Relative mRNA of ERICH3/GAPDH

Significance levels:
- * p < 0.05
- ** p < 0.01
- *** p < 0.001
### ERICH3 SNPs and Clinical Outcomes in SSRI GWAS

**SSRI Response at Four or Six Weeks P Values**

<table>
<thead>
<tr>
<th></th>
<th>PGRN-AMPS</th>
<th>ISPC</th>
<th>STAR*D</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs11580409 (ERICH3)</td>
<td>0.16</td>
<td>0.022</td>
<td>0.041</td>
</tr>
</tbody>
</table>

**PGRN-AMPS:** Mayo Clinic Pharmacogenomics Research Network-Antidepressant Medication Pharmacogenomics Study  
**ISPC:** International SSRI Pharmacogenomics Consortium  
**STAR*D:** Sequenced Treatment Alternatives to Relieve Depression

## Association of HAMD Scores with Baseline Metabolite Concentrations

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>r</th>
<th>P-value</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kynurenine</td>
<td>-0.157</td>
<td>0.008</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>3-Hydroxykynurenine</td>
<td>-0.143</td>
<td>0.015</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-0.134</td>
<td>0.023</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.106</td>
<td>0.072</td>
<td>Methionine</td>
</tr>
<tr>
<td>Serotonin</td>
<td>-0.099</td>
<td>0.093</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Guanosine</td>
<td>0.099</td>
<td>0.095</td>
<td>Purine</td>
</tr>
<tr>
<td>5-Hydroxytryptophan</td>
<td>0.098</td>
<td>0.097</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>(+)-delta-Tocopherol</td>
<td>0.094</td>
<td>0.112</td>
<td>Antioxidants</td>
</tr>
<tr>
<td>Xanthosine</td>
<td>0.097</td>
<td>0.159</td>
<td>Purine</td>
</tr>
<tr>
<td>Salicylic Acid</td>
<td>-0.082</td>
<td>0.163</td>
<td>Phenylalanine</td>
</tr>
</tbody>
</table>
Baseline Plasma KYN GWAS

B. Baseline Plasma Kynurenine Chromosome 8 Locus Zoom
- rs5743467
- $P = 8.18 \times 10^{-7}$

C. Baseline Plasma Kynurenine Chromosome 7 Locus Zoom
- rs17137566
- $P = 6.22 \times 10^{-6}$
Baseline Plasma Kynurenine

Relative KYN to Standard

T/T: n=110
C/T: n=78
C/C: n=19

AHR: rs17137566

DEFB1: rs5743467

G/G: n=2
C/G: n=7
C/C: n=0
DEFB1: Beta-Defensin 1

- Constitutively expressed in epithelial cells;
- Functions in anaerobic environment.

**DEFB1** (shown in purple) Antimicrobial Peptides

**Oxidized**

**Reduced**

*Nature* 2011 Jan 20;469(7330):309-10

36 AA; 3.9 KDa
### Mayo-PGRN AMPS

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>HAMD-17</th>
<th>QIDS-SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2702877</td>
<td>DEFB1</td>
<td>1.74E-04</td>
<td>1.25E-05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beta 0.9422</td>
<td>Beta 1.5987</td>
</tr>
</tbody>
</table>
DEFB1 and KYN Pathway Functional Genomics in Monocytic Cells

**KYN**

- [KYN] (µM)
  - Vehicle
  - LPS
  - LPS + DEFB1

**TRP**

- [TRP] (µM)
  - Vehicle
  - LPS
  - LPS + DEFB1

**K/T**

- KYN/TRP
  - Vehicle
  - LPS
  - LPS + DEFB1

**IDO1**

- Relative mRNA Levels
  - Vehicle
  - LPS
  - LPS + DEFB1

**TDO2**

- Relative mRNA Levels
  - Vehicle
  - LPS
  - LPS + DEFB1
AHR and KYN Pathway Functional Genomics in HepaRG Cells

AHR KD (mRNA Level)

AHR KD (Protein Level)

Kynurenine Concentration
AHR and KYN Pathway Functional Genomics in U87 MG Cells

(A) AHR KD (mRNA Level)

(B) AHR KD (Protein Level)

(C) Kynurenine Concentration
Pharmacogenetics to Pharmac-o-mics

Precision Medicine and Drug Response

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Predictive Algorithm - SSRI Response

Men
- QIDS: 72%
- HAMD: 68%

Women
- QIDS: 80%
- HAMD: 95.8%

Using both Clinical Symptoms and Metabolomics

Arjun Athreya
April 29, 2013

“In a few weeks the APA will release...DSM-5. Unlike our definitions of ischemic heart disease, lymphoma or AIDS, the DSM diagnoses are based on a consensus about clusters of clinical symptoms, not any objective laboratory measure. Patients with mental illness deserve better.”
Beyond the Genome

Goal

Understanding the causes of MDD and the mechanisms of drugs used to treat MDD.
Mayo Pharmacogenomics Laboratories - 2016
We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. Eliot
“Four Quartets”