Networks to Identify New Mechanisms & Targets for Parkinson’s Disease

ESPT 2013
Pharmacogenomics: Cell to Clinic
September 28, 2013
Howard J Federoff, MD, PhD
Georgetown University
Inputs into Target Discovery

Clinical Presentation
GWAS Data Risk Variants
Monogenic Familial PD
Gene Expression Data- DEGs
Animal and Cellular Models

Biological Plausibility
Proof of Principle
Predictive in Model(s)
Pathobiologic Network

IND
Parkinson’s Disease

- Syndrome with multiple etiologies with NO DISEASE MODIFYING THERAPY
- Familial monogenic (~5% worldwide) and sporadic complex genetic forms
- 2nd most common neurodegenerative disease
- Most commonly, age-related slowly progressive disorder
  - Median age of onset 62 yo (except fPD)
  - More common in men
- Classic symptoms (TRAP)
  - resting Tremor
  - cogwheel Rigidity
  - Akinesia/bradykinesia
  - Postural instability
- Non-Motor symptoms: Depression, dementia
- Associated histological features:
  - protein inclusions
  - chronic inflammation (microglia and cytokines)
- Associated biochemical features:
  - Striatal dopamine deficiency
  - Mitochondrial respiratory chain defects (Complex I)
A Convergent Pathobiologic Model

Genetic Forms
*LRRK2, SNCA, GBA*

Toxicant
*Rotenone, Paraquat?*

Sporadic
*Gene : Environment*

Preclinical injury

Presynaptic injury and Dysfunction

Neurochemical Compensation, Metabolic Stress

DA Deficiency, Cell Death

Preclinical damage

Dopamine deficiency

Extra-CNS manifestations

CNS manifestations

## Meta-GWAS


<table>
<thead>
<tr>
<th>Chr Position (bp)</th>
<th>MAF in discovery phase</th>
<th>Minor/ major alleles</th>
<th>Candidate gene</th>
<th>Discovery phase</th>
<th>Replication phase</th>
<th>Combined PAR estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR (SE) per minor allele dose</td>
<td>Fixed effects p value</td>
<td>Random effects p value</td>
</tr>
<tr>
<td>chr1:154105678</td>
<td>1</td>
<td>0.02</td>
<td>T/C</td>
<td>1.67 (0.09)</td>
<td>1.02×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>5.70×10&lt;sup&gt;-10&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs6710823</td>
<td>2</td>
<td>0.19</td>
<td>A/G</td>
<td>1.28 (0.05)</td>
<td>1.35×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>1.61×10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs2102808</td>
<td>2</td>
<td>0.13</td>
<td>T/G</td>
<td>1.28 (0.04)</td>
<td>3.31×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>1.54×10&lt;sup&gt;-13&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs11711441</td>
<td>3</td>
<td>0.14</td>
<td>A/G</td>
<td>0.82 (0.04)</td>
<td>2.10×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>1.17×10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>chr4:9131311</td>
<td>4</td>
<td>0.28</td>
<td>C/G</td>
<td>1.21 (0.03)</td>
<td>1.80×10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>2.96×10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs1724655</td>
<td>4</td>
<td>0.45</td>
<td>C/A</td>
<td>0.87 (0.03)</td>
<td>1.85×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>0.001407</td>
</tr>
<tr>
<td>rs356219</td>
<td>4</td>
<td>0.39</td>
<td>G/A</td>
<td>1.30 (0.03)</td>
<td>7.90×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>1.11×10&lt;sup&gt;-8&lt;/sup&gt;</td>
</tr>
<tr>
<td>chr6:32588205</td>
<td>6</td>
<td>0.15</td>
<td>G/A</td>
<td>0.70 (0.06)</td>
<td>2.58×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>1.44×10&lt;sup&gt;-10&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs1491942</td>
<td>12</td>
<td>0.21</td>
<td>C/G</td>
<td>1.19 (0.03)</td>
<td>3.23×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>5.24×10&lt;sup&gt;-10&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs2817488</td>
<td>12</td>
<td>0.46</td>
<td>A/G</td>
<td>1.16 (0.03)</td>
<td>4.43×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>2.99×10&lt;sup&gt;-10&lt;/sup&gt;</td>
</tr>
<tr>
<td>rs2942168</td>
<td>17</td>
<td>0.22</td>
<td>A/G</td>
<td>0.76 (0.03)</td>
<td>1.62×10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>3.91×10&lt;sup&gt;-10&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Only loci with p<5×10<sup>-4</sup> in the meta-analysis are shown. The SNP with the smallest p value per locus on the basis of a fixed effects meta-analysis is shown. Webappendix pp 15–31 provide additional details for the associated loci described above. An expanded version of this table that shows all p values less than 1×10<sup>-6</sup> from the discovery phase of analyses is available upon request. C= chromosome. MAF= minor allele frequency. OR= odds ratio. PAR= population-attributable risk. P index=I² index of heterogeneity. P p value= heterogeneity p value.
Meta-GWAS Network
Meta Gene Expression in Sporadic PD  
GPEX Consortium

<table>
<thead>
<tr>
<th>Gene set</th>
<th>N Genes Annotation</th>
<th>sNES</th>
<th>P value</th>
<th>sNES</th>
<th>P value</th>
<th>sNES</th>
<th>P value</th>
<th>N</th>
<th>sNES</th>
<th>P value</th>
<th>N</th>
<th>sNES</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Transport Chain</td>
<td>95 Broad</td>
<td>-1.583</td>
<td>&lt;1x10⁻⁵</td>
<td>-1.496</td>
<td>1.46x10⁻²</td>
<td>-1.420</td>
<td>1.0x10⁻³</td>
<td>410</td>
<td>-1.519</td>
<td>&lt;1x10⁻⁶</td>
<td>218</td>
<td>-1.580</td>
<td>&lt;1x10⁻⁶</td>
</tr>
<tr>
<td>MAP00190 Oxidative phosphorylation</td>
<td>46 GenMAPP</td>
<td>-1.572</td>
<td>&lt;1x10⁻⁵</td>
<td>-1.716</td>
<td>4.70x10⁻²</td>
<td>-1.132</td>
<td>2.26x10⁻⁵</td>
<td>410</td>
<td>-1.388</td>
<td>&lt;1x10⁻⁵</td>
<td>218</td>
<td>-1.586</td>
<td>2.66x10⁻⁷</td>
</tr>
<tr>
<td>MAP00620 Pyruvate metabolism</td>
<td>31 GenMAPP</td>
<td>-1.529</td>
<td>3.36x10⁻³</td>
<td>-1.644</td>
<td>2.37x10⁻²</td>
<td>-1.062</td>
<td>4.84x10⁻³</td>
<td>410</td>
<td>-1.332</td>
<td>&lt;1x10⁻⁵</td>
<td>218</td>
<td>-1.541</td>
<td>5.32x10⁻⁷</td>
</tr>
<tr>
<td>VOXPHOS</td>
<td>87 BioCarta</td>
<td>-1.527</td>
<td>1.34x10⁻⁷</td>
<td>-1.451</td>
<td>2.26x10⁻²</td>
<td>-1.389</td>
<td>1.0x10⁻⁵</td>
<td>410</td>
<td>-1.471</td>
<td>&lt;1x10⁻⁵</td>
<td>218</td>
<td>-1.524</td>
<td>7.92x10⁻⁸</td>
</tr>
<tr>
<td>Mitochondr</td>
<td>447 Broad</td>
<td>-1.464</td>
<td>6.76x10⁻⁷</td>
<td>-1.761</td>
<td>1.43x10⁻²</td>
<td>-1.247</td>
<td>4.50x10⁻⁴</td>
<td>410</td>
<td>-1.376</td>
<td>3.11x10⁻⁷</td>
<td>218</td>
<td>-1.479</td>
<td>5.54x10⁻⁷</td>
</tr>
<tr>
<td>Krebs-TCA Cycle</td>
<td>29 BioCarta</td>
<td>-1.447</td>
<td>3.38x10⁻⁷</td>
<td>-1.633</td>
<td>3.02x10⁻²</td>
<td>-1.184</td>
<td>1.34x10⁻³</td>
<td>410</td>
<td>-1.359</td>
<td>6.22x10⁻⁵</td>
<td>218</td>
<td>-1.462</td>
<td>8.71x10⁻⁷</td>
</tr>
<tr>
<td>Human mitoDB 6 2002</td>
<td>428 Broad</td>
<td>-1.427</td>
<td>3.38x10⁻⁷</td>
<td>-1.750</td>
<td>1.23x10⁻²</td>
<td>-1.271</td>
<td>4.51x10⁻⁴</td>
<td>410</td>
<td>-1.373</td>
<td>&lt;1x10⁻⁵</td>
<td>218</td>
<td>-1.445</td>
<td>5.32x10⁻⁷</td>
</tr>
<tr>
<td>GO 0005739</td>
<td>170 GO</td>
<td>-1.369</td>
<td>3.72x10⁻⁵</td>
<td>-1.758</td>
<td>2.94x10⁻²</td>
<td>-1.230</td>
<td>3.91x10⁻⁴</td>
<td>410</td>
<td>-1.322</td>
<td>&lt;1x10⁻⁵</td>
<td>218</td>
<td>-1.391</td>
<td>3.19x10⁻⁶</td>
</tr>
<tr>
<td>PGC</td>
<td>425 Broad</td>
<td>-1.366</td>
<td>6.75x10⁻⁶</td>
<td>-1.576</td>
<td>4.96x10⁻⁵</td>
<td>-0.884</td>
<td>1.46x10⁻⁷</td>
<td>410</td>
<td>-1.165</td>
<td>1.27x10⁻⁷</td>
<td>218</td>
<td>-1.379</td>
<td>2.93x10⁻⁶</td>
</tr>
<tr>
<td>ChREBP Pathway</td>
<td>20 Broad</td>
<td>-1.280</td>
<td>3.34x10⁻⁵</td>
<td>-2.100</td>
<td>1.19x10⁻²</td>
<td>-0.799</td>
<td>2.93x10⁻⁵</td>
<td>410</td>
<td>-1.127</td>
<td>1.58x10⁻⁵</td>
<td>218</td>
<td>-1.341</td>
<td>6.92x10⁻⁶</td>
</tr>
<tr>
<td>Urea cycle Pathway</td>
<td>7 KEGG</td>
<td>-1.262</td>
<td>6.77x10⁻⁵</td>
<td>-1.671</td>
<td>1.46x10⁻²</td>
<td>-0.576</td>
<td>1.05x10⁻⁴</td>
<td>410</td>
<td>-0.994</td>
<td>0.00002212</td>
<td>218</td>
<td>-1.239</td>
<td>7.94x10⁻⁵</td>
</tr>
<tr>
<td>MAP00252 Alanine and aspartate metabolism</td>
<td>21 GenMAPP</td>
<td>-1.165</td>
<td>3.39x10⁻⁵</td>
<td>-1.831</td>
<td>1.80x10⁻²</td>
<td>-0.462</td>
<td>1.80x10⁻¹</td>
<td>410</td>
<td>-0.908</td>
<td>0.00015813</td>
<td>218</td>
<td>-1.213</td>
<td>0.00013384</td>
</tr>
</tbody>
</table>

PGC-1α and the electron transport chain were downregulated  
Meta-Gene Expression Analysis Network
Multi-Dimensional Network
PD Therapeutic Target

PGC-1α (*PPARGC1A*)

- Coactivator of PPARg
- Responsive to oxidative insults and regulates ROS defenses
- Master regulator of mitochondrial content
- Induced by cold exposure and aerobic exercise
- SIRT1 activates PGC-1α through acetylation
- LOF results in enhanced sensitivity to oxidative stressors
- Excessive PGC-1α is toxic
- Candidate target for neuroprotection and anti-inflammation?
Biological Plausibility
PGC-1a Loss of Function & MPTP

Cell 127, 397–408, October 20, 2006

PGC-1a LOF renders DAN More vulnerable to complex I inhibitor MPTP
Systemic LPS Model of SNpc DAN Injury

Zheng et al., PLOS ONE, vol 8, Issue 8, 2013
PGC-1α Loss of Function & LPS

WT: PGC-1α+/+
KD: PGC-1α-/-

IP Saline or LPS

Collect liver and brain for TNFα and IL1β qRT-PCR

Liver

**

TNFα gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

**

IL-1β gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

Brain

TNFα gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

**

IL-1β gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

PGC-1α regulates CNS inflammation

Liver

IP Saline or LPS

Collect liver and brain for TNFα and IL1β qRT-PCR

Liver

**

TNFα gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

**

IL-1β gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

Brain

TNFα gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

**

IL-1β gene expression (WT LPS compared to WT saline; KD LPS compared to KD saline)

WT saline WT LPS KD saline KD LPS

PGC-1α regulates CNS inflammation
Linking data elements: What is the mechanism for PGC-1α downregulation?
PGC-1a Promoter Methylation in Primary Neurons

Palmitate (Inflammatory mediator)

Palmitate promotes PGC-1a methylation and downstream changes
Linking data elements: Does α-synuclein (SNCA) mediated neuroinflammation cause PGC-1α promoter methylation?
Neuroinflammation in Young Human Mutant SNCA Transgenic Mice

Iba immunocytochemistry (1 month)

Mouse model of human synucleinopathy manifests early neuroinflammation
PGC-1α Promoter methylation in DMSYN+/+ Mice

Palmitate interacts with α-synuclein to promote PGC-1α methylation in SNpc and downstream changes
PGC-1α Promoter methylation: Sporadic PD

PGC-1α methylation in PD SNpc trending upward
Screening for approved drugs that increase PGC-1α
### Approved drugs increasing PGC-1α mRNA

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Clinical usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Econazole</td>
<td>antifungal medication</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Osmotic diuretic agent</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>antipsychotic drug</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>antidiabetic and anti-inflammatory drug</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>antipsychotic drug</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>reduces cholesterol levels through PPARα</td>
</tr>
<tr>
<td>Flurandrenolide</td>
<td>an anti-inflammatory treatment for use on skin irritations</td>
</tr>
<tr>
<td>Flumethasone</td>
<td>a corticosteroid for topical use</td>
</tr>
<tr>
<td>Neomycin</td>
<td>aminoglycoside antibiotic</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>treat hypertension</td>
</tr>
<tr>
<td>Clobetasol</td>
<td>treat various skin disorders</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>therapy for hormone receptor-positive breast cancer</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>an oral selective estrogen receptor modulator (SERM)</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>treat high blood pressure</td>
</tr>
</tbody>
</table>

*Collaboration with Vanda Pharmaceuticals*
Fenofibrate Induces PGC-1α in MN9D cells and Promotes Neuroprotection

Fenofibrate increases PGC-1α and promotes neuroprotection in dopaminergic cells

Fenofibrate (uM)

PGC-1α expression (compared to control)

0 5 10 20

0.0 0.5 1.0 1.5 2.0

Fenofibrate (uM)

Mitochondrial content (normalized to cell number)

0 5 10 20

0 1 2 3 4 5

Fenofibrate (uM)

Cell Viability (compared to 6-OHDA/DMSO)

0 5 10 20

0 1 2 3 4 5
Fenofibrate Induces PGC-1α Upregulation in BV2 and Promotes an Anti-inflammatory Effect

Fenofibrate increases PGC-1α and inhibits inflammatory cytokine production in microglial line BV2
Is fenofibrate protective in PD models?
Fenofibrate Neuroprotection in MPTP Mice

TH+ Neurons in SNpc

TH Density in STR

Brain Res. 2007 Mar 2; 1135(1):77-84; Behav Pharmacol. 2010 May; 21(3):194-205
Retrospective analysis of fenofibrate usage suggests protection against PD

<table>
<thead>
<tr>
<th>Group</th>
<th>Year of birth</th>
<th>Year of PD onset</th>
<th>Age at PD onset</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>No lipid-lowering drug (n = 346)</td>
<td>1938.7</td>
<td>0.6</td>
<td>1993.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Statin before PD onset (n = 13)</td>
<td>1939.5</td>
<td>1.2</td>
<td>1956.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Fibrates before PD onset (n = 13)</td>
<td>1933.3</td>
<td>2.4</td>
<td>1993.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Statin after PD onset (n = 34)</td>
<td>1955.6</td>
<td>1.2</td>
<td>1995.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Fibrates after PD onset (n = 13)</td>
<td>1939.5</td>
<td>2.3</td>
<td>1991.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

NS: non significant; PD: Parkinson’s disease; SEM: standard error of the mean.

Fenofibrate use for dyslipidemia delayed PD onset by 8.7 years

Mutez et al., Pharmacological Research 60 (2009)
Summary

» PGC-1a is decreased in PD SNpc and DANs
» PGC-1a can be epigenetically modified by palmitate
» PGC-1a gene dosage alters DAN vulnerability
» Fenofibrate upregulates PGC-1a
» Fenofibrate anti-inflammatory effect requires PGC-1a
» Fenofibrate anti-inflammatory effect and PGC-1a upregulation are PPARα independent
» Fenofibrate protects against SNpc DAN loss and striatal innervation

Q: When (premotor, H&Y stage I) should fenofibrate be evaluated in PD?
Acknowledgements

» Juan Wang, PhD
» Liang Huang, PhD
» Hong Cao, PhD
» Bin Li, MD
» Xiaomin Su, PhD
» Danielle Phelps

» Massimo Fiandaca, MD
» Robert Padilla
» Subha Mahdavan, PhD
» Yuriy Gusev, PhD
» Amrita Cheema, PhD
» Mihael Polymeropoulos, MD

» Mark Mapstone, PhD
» Claudia Kawas, MD
» Eileen Johnson, MSN
» Derick Peterson, PhD
» Ming Tan, PhD

Support from NINDS, NIA, DOD
A Dual MD/MS Degree in Systems Medicine at Georgetown University

Biomedical Informatics

Translational Bioinformatics

Informatics Grandrounds

Clinical Statistics

Proteomics

Genomics

Metabolomics

Case Based Practicum