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Pier Mastroberardino - Erasmus MC
Takao Yagi - Scripps

Current Funding:
NINDS
NIEHS
Veterans Administration
American Parkinson Disease Association
Michael J. Fox Foundation
# Parkinson’s Disease

| Prevalence:         | 1% of people over age 55  
<table>
<thead>
<tr>
<th></th>
<th>(1 million in North America)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance:</td>
<td>Sporadic and Familial</td>
</tr>
<tr>
<td>Etiology:</td>
<td>Environmental toxins</td>
</tr>
<tr>
<td></td>
<td>Complex I defects?</td>
</tr>
<tr>
<td></td>
<td>Single gene mutations</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-synuclein dupli- &amp; triplications</td>
</tr>
</tbody>
</table>
| Cardinal Signs:     | Tremor, rigidity, bradykinesia,  
|                     | postural instability         |
| Other Signs:        | Shuffling gait, masked facies,  
|                     | deceased blink rate          |
Parkinson’s Disease

Classical Pathology:

• Loss of dopamine neurons in the substantia nigra pars compacta
• Lewy bodies/neurites
• Loss of neurons in locus ceruleus, dorsal vagal nucleus, dorsal raphe and nucleus basalis of Meynert
• Microglial activation
Parkinson's Disease

Degeneration of nigrostriatal dopamine neurons

- Nerve Terminals
- Caudate & Putamen
- Cell Body
- Substantia nigra
Lewy Bodies

The pathological hallmark of Parkinson’s disease. Among the proteins they contain:

- Phosphorylated neurofilament proteins
- Ubiquitin
- \( \alpha \)-Synuclein
- Parkin
- Proteasome subunits
Parkinson’s Disease

Biochemical Pathology in Substantia Nigra:

• Loss of reduced glutathione (GSH)
• Increased levels of malondialdehyde & lipid hydroperoxides
• Oxidative DNA & protein damage
• Oxidative (nitrative) modification of $\alpha$-synuclein
• Iron accumulation
Parkinson’s Disease
Etiology

Genetic Susceptibility + Environmental Exposure

α-synuclein
parkin

Mendelian Genetics

MPTP
Toxic Exposure
<table>
<thead>
<tr>
<th>Table</th>
<th>Different monogenic forms of parkinsonism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Acronym</strong></td>
</tr>
<tr>
<td>Monogenic</td>
<td>confirmed</td>
</tr>
<tr>
<td>PARK1/PARK4</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK8</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK2</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK6</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK7</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>PARK9</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Monogenic</td>
<td>single cases</td>
</tr>
<tr>
<td>PARK5</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>PARK13</td>
<td>Unknown</td>
</tr>
<tr>
<td>Not assigned</td>
<td>Unknown</td>
</tr>
<tr>
<td>Not assigned</td>
<td>Unknown</td>
</tr>
<tr>
<td>Not assigned</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Klein & Schlossmacher, 2007*
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Mode of inheritance</th>
<th>Locus</th>
<th>Gene/protein</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monogenic confirmed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARK1/PARK4</td>
<td>Autosomal dominant</td>
<td>4q21-q23</td>
<td>SNCA/α-synuclein</td>
<td>3 missense mutations, whole gene duplications/triplications in &lt;10 families</td>
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<tr>
<td>PARK8</td>
<td>Autosomal dominant</td>
<td>12q12</td>
<td>LRRK2/dardarin</td>
<td>&gt;50 variants, &gt;16 of them pathogenic</td>
</tr>
<tr>
<td>PARK2</td>
<td>Autosomal recessive</td>
<td>6q25.2-q27</td>
<td>Parkin</td>
<td>&gt;100 different mutations [gene dosage alterations and small sequence changes, rarely large deletions]</td>
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<tr>
<td>PARK6</td>
<td>Autosomal recessive</td>
<td>1p35-p36</td>
<td>PINK1</td>
<td>40 small sequence change, rarely large deletions</td>
</tr>
<tr>
<td>PARK7</td>
<td>Autosomal recessive</td>
<td>1p36</td>
<td>DJ-1</td>
<td>10 mutations [point mutations and large deletions]</td>
</tr>
<tr>
<td>PARK9</td>
<td>Autosomal recessive</td>
<td>1p36</td>
<td>ATP13A2</td>
<td>3 different mutations that lead to premature protein truncation</td>
</tr>
<tr>
<td>Monogenic single cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARK5</td>
<td>Autosomal dominant</td>
<td>4p14</td>
<td>UCHL1/ubiquitin carboxy-terminal hydrolase I</td>
<td>1 mutation found in single family</td>
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<tr>
<td>PARK13</td>
<td>Unknown</td>
<td>2p12</td>
<td>Omi/HtrA2</td>
<td>1 point mutation in four families; disease-associated variant</td>
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<tr>
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<td>Unknown</td>
<td>5q23.1-q23.3</td>
<td>Synphilin-1</td>
<td>1 missense mutation in two patients</td>
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<tr>
<td>Not assigned</td>
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<td>2q22-q23</td>
<td>NR4A2/Nurr1</td>
<td>3 different mutations, 1 of them in coding region</td>
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<tr>
<td>Not assigned</td>
<td>Unknown</td>
<td>15q25</td>
<td>POLG/DNA polymerase γ</td>
<td>1 family with compound heterozygous mutations</td>
</tr>
</tbody>
</table>

*Klein & Schlossmacher, 2007*
Parkinson’s Disease
Mutations and Mitochondria

**PINK1** - a nuclear-encoded, mitochondrial protein kinase (Valente et al, 2004; Rohe et al, 2004)

**Parkin** - mitochondrial quality control; knock-out results in disruption of mitochondrial function (Greene et al, 2003; Palacino et al, 2004)

**DJ-1** - under conditions of oxidative stress, DJ-1 translocates to mitochondria (Canet-Aviles et al, 2004)

**Omi** - a mitochondrial protease (Strauss et al, 2005)

**POLG** - mitochondrial DNA polymerase gamma
Parkinson’s Disease

Associated with pesticide exposure:
Tanner et al., Envir. Health Perspect, 2011
Parkinson's Disease

Etiology

MPTP
Rotenone
Pesticides

PINK1
DJ-1
Parkin

Mendelian Genetics

Toxic Exposure
MPTP

• In 1982, IV drug users present with an acute parkinsonian syndrome

• Astute medical detective work identifies the toxin as MPTP

• MPTP is metabolized to MPP\(^+\), a substrate for the dopamine uptake transporter (DAT)

• Mechanism of action is inhibition of mitochondrial respiration at complex I

• Mitochondrial dysfunction can cause a parkinsonian syndrome
Parkinson’s Disease

A defect in mitochondrial complex I

After the discovery of MPTP and its mechanism:

• 1989-92: A selective decrease in complex I activity in PD brains (Mizuno et al, Schapira et al)

• Complex I activity is reduced by 16 - 55% in platelets of PD patients (Yoshino et al, Parker et al, Mann et al, Haas & Shults et al)
Parkinson’s disease is associated with a systemic complex I defect, yet dopaminergic neurons of substantia nigra degenerate selectively. **Is the complex I defect relevant?**

**Hypothesis:** An experimentally-induced, chronic, systemic inhibition of complex I can reproduce the behavioral, neurochemical and neuropathological features of PD in an animal model.
Rotenone

• Classical high-affinity inhibitor of complex I of the mitochondrial electron transport chain

• A natural product - from several plant species

• Common pesticide; the “organic” (natural) alternative to synthetic pesticides

• Used to sample fish populations in reservoirs & kill nuisance fish in lakes

• Highly lipophilic; crosses biological membranes easily & independent of transporters
Rotenone causes selective nigrostriatal degeneration

Betarbet, Sherer et al, *Nature Neuroscience*
Rotenone causes selective nigrostriatal degeneration

Graphs showing:
- TH-positive neurons
- DA (ng/mg protein)
- Distance to trigger (cm)
Refinement of the rotenone model
(3 mg/kg/d)
Proof of concept: Systemic mitochondrial dysfunction can reproduce the selective neurodegeneration and pathological features of PD
The rotenone model provided the rationale and ‘biological plausibility’ for subsequent epidemiological studies.
FAME Study: PD in Agricultural Health Study  Tanner, Kamel et al

Nested, case-control study of 52,000 licensed pesticide applicators and 32,000 spouses

**Rotenone → Increased Risk of PD:**

<table>
<thead>
<tr>
<th></th>
<th>OR = 2.3 (95% C.I.: 1.2, 4.3)</th>
<th>OR = 2.8 (95% C.I.: 1.4, 5.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model adjusted for age, gender, state, ever smoking, ever pesticide use

Plant derivative (worldwide)  Crop insecticide (mid-1800’s - present)  Fish poison (ancient)

Home gardening  Pest Control

Tanner et al, Environ Health Perspect, 2011
How does rotenone cause neurodegeneration?
Oxygen $\rightarrow$ Superoxide anion $\rightarrow$ Hydrogen peroxide $\rightarrow$ Hydroxyl radical $\rightarrow$ Water

Reactive oxygen species (ROS)
Superoxide production in the mitochondria of rotenone-treated rats measured with electron spin resonance
Single-Cell Redox Imaging Demonstrates a Distinctive Response of Dopaminergic Neurons to Oxidative Insults

Maxx P. Horowitz, Chiara Milanese, Roberto Di Maio, Xiaoping Hu, Laura M. Montero, Laurie H. Sanders, Victor Tapias, Sara Sepe, Wiggert A. van Cappellen, Edward A. Burton, John Timothy Greenamyre, and Pier G. Mastroberardino
Why are dopamine neurons selectively vulnerable? Why does degeneration begin in nerve terminals? Is it dopamine itself?
What is the effect of cytosolic DAQ on mitochondria?
DAQ penetrates intact mitochondria and binds covalently to complex I subunits.
DAQ penetrates intact mitochondria and inhibits complexes I & II

The effect of DAQ is blocked by glutathione
Are these results relevant?

Can DA inhibit mitochondrial function in vivo?
Methamphetamine releases DA from vesicles

Relevance:
- alpha-synuclein increases cytosolic dopamine
- Complex I dysfunction increases cytosolic dopamine
Cytosolic DA inhibits mitochondrial respiration in intact cells
Cytosolic DA inhibits mitochondrial respiration in intact cells
Parkinson’s Disease
The Rotenone Model
✓ Systemic mitochondrial impairment
  ✓ Pesticide exposure
✓ Selective nigrostriatal dopamine cell loss
✓ Lewy body formation (α-synuclein accumulation)
  ✓ Oxidative damage
✓ Microglial activation (inflammation)
✓ Proteasome dysfunction
✓ Cardiac sympathetic denervation
✓ GI pathology/constipation
✓ Iron accumulation
We’ll do whatever it takes to cure Parkinson’s.
(So, who had the decaf?)