Genes, Environment & Parkinson’s Disease: An Epidemiologist’s Perspective On The Causes

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Consultant: Adamas, Neurocrine, Cynapsus, Ultragenyx Pharmaceuticals; DMC Service: Biotie, Voyager, Intec
PD Incidence Increases with Age

Estimated Age-Specific PD Incidence
Kaiser Permanente of N. California, 1994 - 1995

PD Incidence per 100,000 p-y

Male
Female

Age (years)

<30 30-39 40-49 50-59 60-69 70-79 80+
Life Expectancy Is Expected to Increase World Wide

Figure III.2. Life expectancy at birth for the world and major areas, 1950-2100

Consequently, the global burden of Parkinson’s disease is expected to increase

Change in number of people with Parkinson’s disease in the world’s most populous nations from 2005 to 2030*

*Among individuals over 50 in the world’s ten most and Western Europe’s five most populous nations

Source: Dorsey et al, Neurology 2007;68:384-6
Consequences for Society

Costs:

- Direct costs of health care
- Indirect costs:
  - Loss of years worked, lost societal contributions
  - Mental & physical costs
  - Affects person with PD & family members, colleagues, friends
Can We Bridge the Gap?

Knowledge gaps

Unmet needs
Why Study Etiology?

- May lead to better treatments
- May identify persons at risk
- May lead to prevention of disease or slowing of disease progression
What Causes Parkinson’s Disease?
Cluster of subacute parkinsonism in young narcotics addicts

**Similar to PD:**
- Same signs as PD
- Progressive worsening in some
- Improves with L-dopa
- Same side effects from L-dopa

**BUT**
- MPTP injection is rare
- Not a likely cause of PD

The toxicologic effects of MPTP suggested that similar chemicals, present in the environment, could cause PD.
Is Parkinson’s disease an environmental disorder?
“These findings favor monogenic autosomal dominant inheritance and show reason to argue against a multifactorial etiology or heteroplasmy.”

Duvoisin & Johnson Brain Pathology 1992

Is Parkinson’s Disease a monogenic disorder?
Is Parkinson’s disease an inherited disorder?
Twins: Mother Nature's Controlled Study

- MZ twins share ~100% of genes
- DZ twins share ~50% of genes

**Hypothesis:** If Parkinson’s disease is primarily a genetic disorder, then concordance in MZ twins should be > than in DZ twins.

**Results:** MZ & DZ concordance similar; 
*Except* young onset MZ > DZ

**Conclusion:** Environment is an important contributor to the cause of PD

*Tanner, et al, JAMA, 1999*
Inherited parkinsonism is rare, but yields clues to the cause of typical Parkinson’s Disease

• Current evidence suggests only ~ 10% of all PD is caused by a single genetic defect
• In many, inherited parkinsonism begin at an earlier than expected age
• In many, inherited parkinsonism has different clinical features than “typical” PD

<table>
<thead>
<tr>
<th>Approved Symbol</th>
<th>Approved Name</th>
<th>Previous Symbols</th>
<th>Synonyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNCA</td>
<td>synuclein, alpha (non A4 component of amyloid precursor)</td>
<td>PARK1, PARK4</td>
<td>NACP, PD1, alpha-synuclein</td>
</tr>
<tr>
<td>PARK2</td>
<td>parkinson protein 2, E3 ubiquitin protein ligase (parkin)</td>
<td></td>
<td>PD1, AR-JP, parkin</td>
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<tr>
<td>PARK3</td>
<td>Parkinson disease 3 (autosomal dominant, Lewy body)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCHL1</td>
<td>ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)</td>
<td>PARK5</td>
<td>PGP9.5, Uch-L1</td>
</tr>
<tr>
<td>PARK7</td>
<td>parkinson protein 7</td>
<td></td>
<td>DJ-1, DJ1</td>
</tr>
<tr>
<td>LRRK2</td>
<td>leucine-rich repeat kinase 2</td>
<td>PARK8</td>
<td>ROCO2, DKFZp443H2111, FLJ</td>
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<td>ATP13A2</td>
<td>ATPase type 13A2</td>
<td>PARK9</td>
<td>HSA9947, CLN12</td>
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<tr>
<td>PARK10</td>
<td>Parkinson disease 10 (susceptibility)</td>
<td></td>
<td>AAOPD</td>
</tr>
<tr>
<td>PARK11</td>
<td>Parkinson disease 11 (autosomal recessive, early onset)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARK12</td>
<td>Parkinson disease 12 (susceptibility)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTRA2</td>
<td>HtrA serine peptidase 2</td>
<td>PRSS25</td>
<td>OM1, PARK13</td>
</tr>
<tr>
<td>PLA2G6</td>
<td>phospholipase A2, group VI (cytosolic, calcium-independent)</td>
<td></td>
<td>iPLA2, PNPLA9, PARK14</td>
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<tr>
<td>FBXO7</td>
<td>F-box protein 7</td>
<td></td>
<td>FBX7, Fbx, PARK15</td>
</tr>
<tr>
<td>PARK16</td>
<td>Parkinson disease 16 (susceptibility)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

→ Normal protein products of these genes are all likely involved in protein degradation & /or cellular response to toxicant injury or oxidative stress
Studying Twin Pairs Discordant for PD Can Yield Clues Regarding Causes
Head Injury and PD Risk in WWII Veteran Twins
Goldman, Tanner et al, Annals of Neurology 2006

Subjects: 93 discordant pairs with complete information → 26 pairs with at least one head injury
Results: 14.7% with head injury; 7.8% hospitalized

Head injury 37.4 yrs (mean) before PD onset
→ Increased Risk of PD with head injury

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>3.0</td>
<td>1.14-9.2</td>
<td>0.023</td>
</tr>
<tr>
<td><strong>MZ</strong></td>
<td>3.3</td>
<td>0.86-19</td>
<td>0.092</td>
</tr>
<tr>
<td><strong>DZ</strong></td>
<td>2.7</td>
<td>0.64-16</td>
<td>0.23</td>
</tr>
</tbody>
</table>

→ PD risk further increased with > 1 head injury:
1 injury: OR 2.6 (1.07,6.5; p = 0.035)
2 injuries: OR 5.1 (0.54, 48; p = 0.16)

Test for trend 0.042

*McNemar’s
Mild-moderate head injury associated with PD in >70% of studies.

2-3 fold increased risk

Biologic Plausibility:
- Triggers chronic inflammatory process
- Oxidative stress
- Protein aggregation
- Mitochondrial damage
- Disrupts Blood Brain Barrier

BUT only some people with head injuries develop PD
Why?
Gene-Environment Interaction in PD

Gene: α-synuclein
Environment: Head injury
PD Pathology: Lewy Bodies

Lewy Bodies are mostly aggregated $\alpha$-synuclein protein.

Lewy bodies in a neuron from the substantia nigra in PD
Head-Injury

- Blood-brain barrier breakdown
- $\alpha$-Synuclein aggregation
  - Microglial activation
  - Release of inflammatory cytokines: Interleukins IL-1, IL-6; TNF-$\alpha$; COX-2

$\alpha$-Synuclein in human brain after injury
$\alpha$-Synuclein in mouse striatum after moderate cortical impact
Research Question

Does \( \alpha \)-synuclein gene variant modify the effect of head injury on PD risk?
Alpha-Synuclein Gene Variant is Associated with *Small* Increase in PD Risk

Gene Variant 2 makes more alpha-synuclein protein than Variant 1
**BOTH** Head Injury & Synuclein Gene Variant

Parkinson’s Disease: A Complex Disorder

Genetics loads the gun

Environment pulls the trigger

Digital Art by Bob Gilman
Environmental Chemical Exposures and Parkinson’s Disease
Since the 1990s, over 20 case-control studies have shown an association of PD and pesticides. PD risk is usually about twice as high in pesticide exposed persons.

BUT

- Broad chemical categories
- Few specific agents identified
SEARCH Study: Case Control Study of Occupational Risk Factors
Tanner et al, Arch Neurol, 2009;66(9):1106-1113

519 PD cases, 511 controls in 8 MD centers

Lifelong, job-task-based occupational histories; other risk factors

**Paraquat**: OR* = 2.8
(95% C.I.: 0.8, 9.7)

**2,4-Diphenoxyacetic acid**
(2,4-D) : OR* = 2.6
(95% C.I.: 1.03, 6.48)

Mechanism:

? Alpha Synuclein aggregation

*adjusted for age, gender, smoking*
New Diseases Associated with Agent Orange

October 13, 2009 Secretary Shinseki decided to establish service-connection for Vietnam Veterans with B cell leukemias, such as hairy cell leukemia; Parkinson’s disease; and ischemic heart disease. This is based on an independent study by the Institute of Medicine showing an association with exposure to Agent Orange. Vietnam veterans with these diseases may be eligible for disability compensation and health care benefits.
FAME Study: PD in Agricultural Health Study  
Tanner, Kamel et al, 2011

52,000 farmers, 32,000 spouses in Iowa & N Carolina screened for PD
112 PD cases, 368 controls
In-person examination, videotape, blood, dust, soil
Lifelong history: occupation, pesticides, other risks

**Paraquat** → Increased Risk of PD:
- All   OR = 2.3 (95% C.I. 1.45, 4.3)
- Men   OR = 2.5 (95% C.I. 1.3, 4.7)

**Rotenone** → Increased Risk of PD:
- All   OR = 2.3 (95% C.I.: 1.2, 4.3)
- Men   OR = 2.8 (95% C.I.: 1.4, 5.8)

Models adjusted for age, gender, state, ever smoking, ever pesticide use
Paraquat, GST-T1 and PD Gene-Environment Interaction

Risk of PD Associated with Joint Occurrence of Paraquat Exposure and a Variant of the GST-T1 Gene

Goldman et al, 2012
<table>
<thead>
<tr>
<th>Compound</th>
<th>Odds ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-hexane</td>
<td>1.27</td>
<td>0.40-4.07</td>
<td>0.69</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.28</td>
<td>0.49-3.31</td>
<td>0.61</td>
</tr>
<tr>
<td>Xylene</td>
<td>2.24</td>
<td>0.43-11.6</td>
<td>0.34</td>
</tr>
<tr>
<td>CCl$_4$</td>
<td>2.32</td>
<td>0.88-6.11</td>
<td>0.088</td>
</tr>
<tr>
<td>TCE</td>
<td>6.11</td>
<td>1.15-32.5</td>
<td>0.034</td>
</tr>
<tr>
<td>PERC</td>
<td>10.5</td>
<td>0.97-113</td>
<td>0.053</td>
</tr>
<tr>
<td>TCE or PERC</td>
<td>8.94</td>
<td>1.70-47.0</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Consistent with occupational cluster (Gash et al 2008) & TCE rat model (Liu et al, 2010)
Inverse Associations

Lower Parkinson’s Disease Risk
Cigarette Smoking & Tobacco Use

- Inverse association between smoking & PD in more than 40 studies
- Meta-analyses, pooled analysis: 40% lower risk
- Dose response: More smoking, lower risk
- Twin pairs: discordant: No PD in twin smoking more; concordant: later onset age in twin smoking more
- "Passive" tobacco smoke exposure lowers risk
- Smokeless tobacco lowers PD risk
- Fewer Lewy bodies post-mortem in heavy smokers

Ritz; 2007; Hernan 2002; Tanner 2002; Searles Nielsen 2012; Tsuang 2010;
Heavier smokers have a reduced risk of PD

(Age adjusted incidence (per 100,000 person-years) of PD is greatest in Never Smokers and lowest in those smoking >46 pack-years in the Honolulu Asian Aging Study)
Nicotine:
• Blocks nigral cell loss (hemitransection, MPTP)
• Increases growth factors

Cigarette smoke:
• Reduces MAO B activity
• Complex mixture of combustion products - other actions?

Gene-environment interactions: MAOB, SNCA, GST, NAT, NOS2A

Checkoway 1998; Hancock 2006; McCulloch 2008; Quik 2008; DePalma 2010; Miyake 2010
Nonsmoking Carriers of LRRK2 Gly2385Arg Have Increased Risk of PD

An example of gene-environment interaction
Nonsmoking Carriers of LRRK2 Gly2385Arg Have Increased Risk of PD

An example of gene-environment interaction

GA ↓ OR 2.06

GA EVER SMOKERS → OR 1.47, NS

GA NONSMOKERS → OR 5.76
Coffee, Tea, Caffeinated Beverages

- PD risk lower (~ 40%) in regular coffee or tea drinkers
- Independent of smoking
- Decaffeinated coffee use not associated
- Less consistent in women
- May be adenosine A2 receptor mediated
- Effect modifiers: postmenopausal hormone use; variants in CYP1A1, APOE, GRIN2A

Greater Midlife Coffee & Tea Drinking is Associated with a Lower Risk of PD  
(Ross et al, 2000)

Adjusted for age, pack-years of smoking

Test for Trend 1965 - p=0.001
Test for Trend 1971 - p=0.079
Uric Acid, Gout

- **High normal serum urate**: PD incidence reduced 40% if midlife serum uric acid above the median; 50% reduction in nonsmokers; Not consistently observed; primarily found in men
- **Gout**: Lower PD risk; not consistently observed
- **High urate diet**: Lower PD risk

Proposed mechanism: Strong anti-oxidant; could be confounded by pharmacologic effect: drugs that can increase uric acid measurements include aspirin, caffeine, levodopa)

Davis 1996; Chen 2009; Weisskopf 2007
Medications: NSAIDs, Statins

- **Nonsteroidal anti-inflammatory drugs**: 15-20% lower risk, especially with ibuprofen, but not consistently found
  
  Putative Mechanism: Block inflammatory changes

- **Statins**: 25 – 50% lower risk of PD in those taking lipophilic statins (simvastatin, atorvastatin); not consistently found; May be confounded by association of high serum cholesterol & lower PD risk
  
  Putative Mechanism: Anti-inflammatory

Chen 2003; Wahner 2007; Rees 2011; Huang, 2015; Lee, 2013; Undela 2013
Medications:
Calcium Channel Blockers, Female Hormones

- **Calcium channel blockers**: 30% lower risk of PD; not consistently found; reverse causality possible (low BP in pre-PD)

  Putative Mechanism: ↓ glutamate toxicity & apoptosis

- **Oral contraceptives**: 40% lower risk

- **Post-menopausal hormones**: Higher PD risk in some; Age of use may be critical; confounded by greater risk with oophorectomy, early menarche

  Putative Mechanism: Estrogen neuroprotective

  Surmeier 2010; Ritz 2010; Lee 2014; Popat 2005; Rocca 2006; Greene
Physical activity

• Midlife moderate to vigorous physical activity ⇒ 40% reduced risk

• Mechanism: reduce inflammation, increase neurotrophic factors

• Animal models: physical activity protective vs. toxicants, TBI, reduces inflammation & oxidative stress

Chen 2005; Logroscino 2006; Thacker 2008; Zigmond 2014; Tanner & Comella 2015
Diet

**High saturated fats**: Increased risk
- Proposed mechanism: increased inflammation, lipid soluble toxicants

**High polyunsaturated fatty acids (PUFAs), fruits, vegetables ("Mediterranean diet")**: Lower risk

**High Intake Solanaceae**: Lower PD risk

**Effect modification**: High dietary PUFAs ➔ no ↑ PD risk in rotenone or paraquat exposed farmers

➔ *Inconsistencies in reports. Many study design differences.*

**High intake dairy products**:
Increased risk PD & lower nigral neuron density at post mortem
- Proposed mechanism: increased toxicant exposure (bioconcentrated toxicants, e.g., POPs)

Hellenbrand 1996; Park 2005; Chen 2007; Okubo 2012; Searles 2013; Kamel 2014; Abott 2016
Risk of Parkinson’s disease associated with the herbicide paraquat is attenuated by high dietary intake of polyunsaturated fatty acids

- Parkinson’s disease inversely associated with polyunsaturated fatty acids, notably α-linolenic acid (OR 0.4, 95% CI 0.2-0.8)

- Association of Parkinson’s disease with paraquat stronger in those with low intake of α-linolenic acid

<table>
<thead>
<tr>
<th>Intake Level</th>
<th>Paraquat Exposure</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High, -</td>
<td>1.0 (referent)</td>
<td></td>
</tr>
<tr>
<td>Low, +</td>
<td>1.3 (0.7-2.5)</td>
<td></td>
</tr>
<tr>
<td>High, -</td>
<td>1.4 (0.5-3.9)</td>
<td></td>
</tr>
<tr>
<td>Low, +</td>
<td>4.5 (1.7-12)</td>
<td></td>
</tr>
</tbody>
</table>

Kamel et al, 2012

- 89 confirmed cases and 336 matched controls in FAME
- Diet before diagnosis from a food frequency questionnaire
Microbiome

• Colonic bacteria differ in PD vs. HC → could cause pro-inflammatory response & increased intestinal permeability

• Variants in genes encoding peptidoglycan recognition protein associated with PD risk → regulate GI microbiota & could cause increased systemic inflammatory response

Keshavarzian, Mov Disord 2015; Goldman, Mov Disord 2014
Serum IgG antibody levels to periodontal microbiota are associated with incident AD

- Longitudinal cohort > 65 (Washington Heights-Hamilton Heights-Inwood Community Aging Project)
- Serum IgG for bacteria associated with periodontitis
- 5 year mean F/U

<table>
<thead>
<tr>
<th>Periodontal pathogen</th>
<th>Hazard Ratio*</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. naeslundii</td>
<td>2.0</td>
<td>(1,4)</td>
</tr>
<tr>
<td>E. nodatum</td>
<td>0.4</td>
<td>(0.1,0.9)</td>
</tr>
</tbody>
</table>

*Adjusted for age, APOE, gender, education, high BP, stroke, smoking, DM; using pseudolikelihood risk estimator, robust variance estimator, with 1 case (1), control (10)

Nobels et al, PLOS ONE, 2014
Can Combined Effects of Several Environmental Factors Influence Risk of Parkinson’s Disease?
### Head Injury, Paraquat Use and Risk of PD

83 PD and 328 controls with complete data in FAME
- Head injury in 19%
- Paraquat used by 17%, all men

<table>
<thead>
<tr>
<th>Head injury</th>
<th>Paraquat Use</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
<td>1.2</td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Yes</strong></td>
<td><strong>Yes</strong></td>
<td><strong>4.2</strong></td>
</tr>
</tbody>
</table>

Head injury and paraquat use were synergistically associated with increased PD risk
Both cause oxidative stress
Joint effects are synergistic in a recent animal model (Hutson, 2011).
Purely Genetic PD is Rare
Purely Environmental PD is Rare

Most PD is likely due to the combined effects of genetic predisposition and environmental exposures.

This is a hopeful finding, because environment can be changed!
Is Secondary Prevention of Parkinson’s Disease Possible?

→ Identify persons “at risk” for PD before symptoms manifest

→ Intervene to prevent the development of PD
Some Factors Associated with a Lower Risk of Parkinson’s Disease – Possible Disease Modifying Treatments?

- Physical activity
- Cigarette smoking
  - Flavonoids?
  - PUFAs?
- Coffee & Tea Drinking
- Higher serum urate
- Higher Vitamin D
- Anti-inflammatory drugs (ibuprofen)
- Ca channel blockers
- Statins?
- Female gender; Estrogens?
- Statins?
- Vitamins D?
Why Have Trials of Disease Modifying Therapies Been Inconclusive?

Is the experimental treatment ineffective?

Is the intervention too late?
At PD diagnosis:
- 50% neuron loss in the substantia nigra
- 80% striatal dopamine deficit
Who May Be “At Risk” for PD?

- Persons with clinical features highly predictive of the onset of PD in the future: "prodromal" PD: e.g., RBD, hyposmia
- Persons with genetic susceptibility: primary risk genotypes
- Persons exposed to certain toxicants

DILEMMA: PREDICTIVE VALUE VERY LOW FOR MOST FEATURES
Reliable Biomarkers Needed!

Next Steps
Systematic Prospective Follow-up of Well-Characterized Populations
PPMI
The Parkinson’s Progression Markers Initiative: A Prospective Biomarkers Study

OBJECTIVES
- Standardized protocols
- Dataset/sample collection
- Biomarker verification studies
- Identify progression markers

POPULATIONS
- Early Untreated PD
- Matched Controls
- RBD
- LRRK2, SNCA Families
- Hyposmia

Ideal to identify a biomarker tool set to inform decisions at early stages of drug development and clinical testing

Frasier et al, 2010; Marek et al 2011
Is Preventing PD Possible?

Preliminary Results

Primary Prevention:

- Remove causative factors
- Disease process never initiated

Increased Risk of PD Was Not Observed in Farmers Using Gloves During Pesticide Application

Furlong, Tanner, Goldman, et al. 2014

1 Adjusted for age, sex, state, smoking
The Next Step: Identifying Risk in Populations: California Parkinson’s Disease Registry

[AB 2248, Frommer, Ch. 945, 2004]

- Pilot project in 4 counties funded by NETRP
- All PD cases -> reduce bias, findings generalizable
- Identify incidence, prevalence, disease clusters
- Link to environmental toxins
- Identify PD subgroups
- Identify biomarkers
Examples of Toxicant Monitoring in CA

http://www.atsdr.cdc.gov/substances/SubstanceMapResults.asp

Accessed 3/14/2012
Understanding Parkinson’s Disease
A Dynamic Multidisciplinary Process

Clinical Research:
Epidemiology, Clinical Trials

Clinicians:
Diagnosis, treatment

Basic Scientists:
Laboratory studies

DISEASE PREVENTION
Our Supporters

Research Study Participants & Families

The Valley Foundation
James & Sharron Clark

Neurotoxin Exposure Treatment (Parkinson’s) Research (NETPR)

THANK YOU!