Biomarker discovery and validation in Parkinson’s Disease

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Assistant Professor of Neurology
September 19, 2014
That's my hospital.

That's my lab.

That's my family.

That's why I do what I do.
• 60-year-old R handed man presents with a 1-year history of left arm stiffness. He’s noted dragging of his left foot while running and deterioration of his tennis game. Over the past 3 months his wife has noticed that his left hand intermittently shakes.

• On exam, he has a mild decrease in his facial expression and rapid alternating movements L>R, cogwheel rigidity at the L wrist and decreased arm swing and L foot dragging on gait assessment.
Parkinson’s disease

• First described by James Parkinson, 1817.

“Involuntary tremulous motion, with lessened muscular power, ... with a propensity to bend the trunk forward, and to pass from a walking to a running pace; the senses and intellect being uninjured.”
Parkinson’s disease: The problem

- Average prevalence is 200-300 per 100,000 – so, in US with population 313M, that’s nearly 1M people
- Prevalence increases with age, and in people over 70 is greater than 500 per 100,000 – so, likely to be a bigger problem as population ages
- No treatments to slow progression, only to ameliorate symptoms
World Population by Age

Source: United Nations data

Age in Years

Females Males

Population in millions
Parkinson’s Disease: The Problem

Symptomatic treatment only. No therapies to slow down neurodegeneration.

Likely long prodromal phase.

Heterogeneous disease course, making prognostication difficult.

In current clinical trials, outcome measures are clinical. Minimal demonstration of target engagement, minimal ability to provide fine-scale measurement of outcomes.
Parkinson’s Disease: The Problem

Biomarkers in easily-assayed biofluids would be very helpful for understanding prognosis, for diagnostic confirmation or pre-symptomatic diagnosis, and for surrogate endpointing/proof of target engagement in clinical trials.

But how do you find them?

What biofluid?
The traditional approach: Specific genes and proteins

Decide *a priori* what genes or proteins are likely to lead to central processes in a disease → pursue experiments based on them.

Sometimes you’re right…
…but sometimes you’re wrong.
Another approach: Assay gene/protein expression, genetic variation at a **genomic/proteomic** scale

Made possible by recent technologies.

Whole picture may be a little fuzzier, but:

1. You’re getting a whole picture.
2. You’re not biased…and may make unexpected discoveries.
Parkinson’s Disease: The Problem

What technology might one use to find protein biomarkers using an unbiased approach?

- Multiplex immunoassays
  - EGF as a marker for cognitive status in PD
  - ApoA1 as a marker for PD risk?

- Mass spectrometry
- Aptamer-based probes

What biofluid?
- The potential for blood-based markers
Discovery screen for **plasma-based biomarkers** of clinical states of interest in PD

102 protein analytes simultaneously quantitated by multiplex immunoassay.

Linear regressions used to identify analytes that correlated with continuous clinical measures of interest such as:

- cognitive status
- PD motor severity
- rate of decline
- age at onset
<table>
<thead>
<tr>
<th>Plasma protein</th>
<th>$R^2$</th>
<th>Dir</th>
<th>Model 1 P-value</th>
<th>Model 2 P-value</th>
<th>Model 3 P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD40 ligand</td>
<td>0.192</td>
<td>+</td>
<td>0.006 **</td>
<td>0.023 *</td>
<td>0.054</td>
</tr>
<tr>
<td>EGF</td>
<td>0.231</td>
<td>+</td>
<td>&lt;0.001 ***</td>
<td>0.008 **</td>
<td>0.006 **</td>
</tr>
<tr>
<td>ENA78</td>
<td>0.171</td>
<td>+</td>
<td>0.015 *</td>
<td>0.057</td>
<td>0.142</td>
</tr>
<tr>
<td>FAS</td>
<td>0.174</td>
<td>-</td>
<td>0.013 *</td>
<td>0.022 *</td>
<td>0.473</td>
</tr>
<tr>
<td>GROalpha</td>
<td>0.172</td>
<td>+</td>
<td>0.014 *</td>
<td>0.114</td>
<td>0.041 *</td>
</tr>
<tr>
<td>HBEGF</td>
<td>0.159</td>
<td>+</td>
<td>0.025 *</td>
<td>0.029 *</td>
<td>0.017 *</td>
</tr>
<tr>
<td>PAI1</td>
<td>0.174</td>
<td>+</td>
<td>0.013 *</td>
<td>0.052</td>
<td>0.142</td>
</tr>
<tr>
<td>PDGF</td>
<td>0.165</td>
<td>+</td>
<td>0.019 *</td>
<td>0.044 *</td>
<td>0.104</td>
</tr>
<tr>
<td>RANTES</td>
<td>0.166</td>
<td>+</td>
<td>0.019 *</td>
<td>0.074</td>
<td>0.050 *</td>
</tr>
<tr>
<td>Stem cell factor</td>
<td>0.156</td>
<td>+</td>
<td>0.029 *</td>
<td>0.125</td>
<td>0.091</td>
</tr>
<tr>
<td>Thrombospondin1</td>
<td>0.177</td>
<td>+</td>
<td>0.011 *</td>
<td>0.023 *</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Model 1: $\text{DRS} \sim \text{Age} + \text{Gender} + \text{Analyte}$
Model 2: $\text{DRS} \sim \text{Age} + \text{Gender} + \text{Duration} + \text{Analyte}$
Model 3: $\text{DRS} \sim \text{Age} + \text{Gender} + \text{UPDRS}_{\text{motor}} + \text{Analyte}$
P-values for individual analytes shown. Analytes evaluated one at a time.

*Chen-Plotkin et al, Ann Neurol, 2011*
EGF as a **predictor** of cognitive decline in PD

**HR 8.34, 95% CI 4.26-122.90**

Adjusting for age, gender, baseline DRS score (Cox proportional hazards) does not affect this relationship.
Can we (or other people) replicate this?

Replication cohort
113 PD patients

**Table:**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>0.039</td>
<td>0.035</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>3.755</td>
<td>0.054</td>
</tr>
<tr>
<td>UPDRS</td>
<td>&lt;0.001</td>
<td>0.997</td>
</tr>
<tr>
<td>EGF:Gender</td>
<td>0.046</td>
<td>0.035</td>
</tr>
<tr>
<td>EGF:UPDRS</td>
<td>0.001</td>
<td>0.291</td>
</tr>
<tr>
<td>Gender:UPDRS</td>
<td>0.133</td>
<td>0.166</td>
</tr>
<tr>
<td>EGF:Gender:UPDRS</td>
<td>0.001</td>
<td>0.410</td>
</tr>
</tbody>
</table>

Age-adjusted DRS ~ EGF * Gender * UPDRS
R² = 0.2824, P-value = 0.0008

Red=females
Blue=males
Solid=low UPDRS
Dashed=high UPDRS
EGF as a biomarker

Technically robust to Change in test platform

Replication cohort
113 PD patients

Values consistent across freeze-thaw cycle

Correlation coefficient (2 reads same day): 0.996
Correlation coefficient (2 reads sep by freeze/thaw): 0.931

Red=females
Blue=males
Solid=low UPDRS
Dashed=high UPDRS

Correlation coefficient  (2 reads same day): 0.996
Correlation coefficient  (2 reads sep by freeze/thaw): 0.931
11 proteins showed nominal correlations with age at PD onset. Adjusting for age at plasma sampling and sex. P-values for individual analytes shown. Analytes evaluated one at a time. 

N= 152 PD patients in discovery cohort.  

Top candidates were MIP1-beta and ApoA1.
Plasma ApoA1 and age at onset in PD

HR 0.742, p<0.001

Qiang et al, Ann Neurol, 2013

Lower plasma ApoA1 is correlated with earlier age at PD onset. Lower plasma ApoA1 is also correlated with more severe PD.
Replication in UW cohort (n=187)

Tertile N Apolipoprotein A1 mg/mL
Median (full range)
1 62 0.57 (0.06-0.65)
2 62 0.78 (0.66-0.89)
3 63 1.53 (0.90-4.00)
Hazard Ratio 0.647
P-value <0.001***

Note: Different cohort
Different technical assay

Qiang et al, Ann Neurol, 2013
The problem with correlational studies

One possible solution: Make predictions based on your model and then seek ancillary approaches / data to prove/disprove the model.
Prediction #1: PD individuals will have lower ApoA1 levels than non-PD individuals.
Prediction #2: In prospective epidemiological cohort studies, individuals with higher ApoA1 will be less likely to develop PD.
Prediction #3: In asymptomatic individuals, high ApoA1 individuals will have better DA neuron integrity.
Prediction #4: If there are genetic determinants of ApoA1 levels, the genotypes that lead to lower ApoA1 will be enriched in PD.
Prediction #1: PD individuals will have lower ApoA1 levels than non-PD individuals.

True in a cohort of 301 PD, 80 normal controls, 165 other neurodegenerative disease patients.

Persists after adjusting for age and sex.
Prediction #2: In prospective epidemiological cohort studies, individuals with higher ApoA1 will be less likely to develop PD.

**Prospective Study of Statin Use and Risk of Parkinson Disease**

Xiang Gao, MD, PhD; Kelly C. Simon, ScD; Michael A. Schwarzschild, MD, PhD; Alberto Ascherio, MD, DrPH

Gao et al, Arch Neurol, 2012

38192 men, 90874 women in Health Professional Follow-Up Study and Nurses’ Health Study

644 incident PD cases in 12 years

Risk of PD lower among statin users (RR 0.74, p=0.049)
Prediction #2: In prospective epidemiological cohort studies, individuals with higher ApoA1 will be less likely to develop PD.

43,810 statin users in Taiwan, government policy required discontinuation of statin use if LDL goal was reached. Continuation of lipophilic statins was associated with decreased risk of developing PD (HR 0.42 (95% CI 0.27-0.64)).
Prediction #3: In asymptomatic individuals, high ApoA1 individuals will have better DA neuron integrity.

Estimated that up to 50% of substantia nigra dopaminergic neurons are already lost at the time of clinical diagnosis of PD. (Fearnley and Lees, Brain, 1991)

Suggests there is a long prodromal phase.

Parkinson’s Associated Risk Study (PARS) cohort is comprised of individuals at high risk of developing PD. Dopaminergic terminal integrity has been measured in these subjects by SPECT (DaT) imaging.
Refined Prediction #3: In PARS cohort, high ApoA1 individuals will have better DA neuron integrity.

N=134, 50 with DaT<80%, 84 with DaT>80%

Qiang et al, Ann Neurol, 2013
Prediction #4: If there are genetic determinants of ApoA1 levels, the genotypes that lead to lower ApoA1 will be enriched in PD.
**Prediction #4:** If there are genetic determinants of ApoA1 levels, the genotypes that lead to lower ApoA1 will be enriched in PD.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>1930</td>
<td>0.168</td>
</tr>
<tr>
<td>Normal Control</td>
<td>997</td>
<td>0.180</td>
</tr>
</tbody>
</table>

**A-dominant model**

<table>
<thead>
<tr>
<th></th>
<th>AA/AG (%)</th>
<th>GG (%)</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>579 (30.0)</td>
<td>1351 (70.0)</td>
<td>( X^2 = 1.63 )</td>
</tr>
<tr>
<td>Normal Control</td>
<td>322 (32.3)</td>
<td>675 (67.7)</td>
<td>( P = 0.101 )</td>
</tr>
</tbody>
</table>

**Co-dominant model**

<table>
<thead>
<tr>
<th></th>
<th>AA (%)</th>
<th>AG (%)</th>
<th>GG (%)</th>
<th>C-Atest</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>70 (3.6)</td>
<td>509 (26.4)</td>
<td>1351 (70.0)</td>
<td>( X^2 = 1.15 )</td>
</tr>
<tr>
<td>Normal Control</td>
<td>36 (3.6)</td>
<td>286 (28.8)</td>
<td>675 (67.7)</td>
<td>( P = 0.142 )</td>
</tr>
</tbody>
</table>

Swanson et al, Mov Disord, 2014

...then you look for the association.
Prediction #1: PD individuals will have lower ApoA1 levels than non-PD individuals.
Prediction #2: In prospective epidemiological cohort studies, individuals on ApoA1-increasing drugs will be less likely to develop PD.
Prediction #3: In asymptomatic individuals, high ApoA1 individuals will have better DA neuron integrity.
Prediction #4: If there are genetic determinants of ApoA1 levels, the genotypes that lead to lower ApoA1 will be enriched in PD.

YES
YES
YES
Maybe
**IMMEDIATE GOAL:** Replicate in many, many people.

**3-5 YEAR GOAL:** Pre-clinical model??, Possible human trial??
### Table A

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>Yrs of PD</th>
<th>Measurement</th>
<th>Age at Onset</th>
<th>UPDRS III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beta</td>
<td>P</td>
<td>Beta</td>
</tr>
<tr>
<td>UPenn Cohort 1</td>
<td>152</td>
<td>7</td>
<td>Luminex Assay</td>
<td>11.22</td>
<td>-21.98</td>
</tr>
<tr>
<td>U. Washington</td>
<td>187</td>
<td>9.45</td>
<td>ELISA</td>
<td>2.72</td>
<td>-3.75</td>
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<tr>
<td>UPenn Cohort 2</td>
<td>288</td>
<td>7</td>
<td>ELISA</td>
<td>48.11</td>
<td>14.54</td>
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<tr>
<td>Columbia U.</td>
<td>237</td>
<td>6</td>
<td>Immunoturbidimetry</td>
<td>0.024</td>
<td>-0.037</td>
</tr>
<tr>
<td>PPMI</td>
<td>154</td>
<td>1</td>
<td>Immunoturbidimetry</td>
<td>0.004</td>
<td>-0.035</td>
</tr>
<tr>
<td><strong>COMBINED</strong></td>
<td>1018</td>
<td>-</td>
<td>-</td>
<td>0.86</td>
<td>-1.57</td>
</tr>
</tbody>
</table>

### Graph B

- **Hazard Ratio**: 0.86
- **95% CI**: 0.79-0.94
- **P-value**: 0.0004

### Graph C

- **Hazard Ratio**: 1.12
- **95% CI**: 1.08-1.29
- **P-value**: 0.0004
What if you could make a blood test for PD? How would you do this?

-- Would probably need to test a lot of proteins
-- Would probably be a set of proteins rather than just one
94 proteins differentiate PD from Normal controls
But 94 proteins is probably too many to have on 1 blood test, 
So we need to reduce this number to those that are most discriminating.

One way to do this is stability selection. 
In this way, we found that an 8-biomarker panel gave us good results.
How good were the results? 
And did they work on an independent set of samples, too?
Summary

• We need to find new biomarkers in PD, and unbiased screening approaches may be promising.
• Blood-based markers would be especially amenable to widespread clinical translation.

• **Plasma EGF** may be a promising marker for cognitive status in PD.
  – Low EGF correlates with poorer performance on cognitive tests.
  – Cognitively normal low-EGF individuals are more likely to dement in the near term.

• **Plasma ApoA1** may be a promising marker for PD risk.
  – Low ApoA1 correlates with poorer DAT uptake in asymptomatic individuals.
  – Low ApoA1 correlates with earlier age at onset and higher UPDRS-III in PD.

• A novel aptamer-based screen suggests that a blood test based on 8 proteins may be able to diagnose PD with high accuracy.
Summary

**DISCOVERY → REPLICATION → VALIDATION → TRANSLATION**

Find new biomarkers

Aptamer-based screen

See if they replicate in more patients

See if they replicate in different cohorts
See if they make sense biologically

EGF and ApoA1

Move them to practical use
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Defne Amado

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Judy Qiang

University of Pennsylvania
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Howard Hurtig
Dan Weintraub
Rachel Goldmann Gross
Sharon Xie
Steven Arnold
Murray Grossman
Leslie Shaw
Viviana Van Deerlin
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Emory University
William Hu

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OUR PATIENTS AND THEIR FAMILIES