Applying our knowledge of genetic factors in Parkinson's Disease: Linking pathophysiological mechanisms to sporadic cases & driving the discovery of novel therapeutics

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Are the Common Parkinson’s Disease Mutations Relevant to Sporadic Disease?

- Genetic mutations in the leucine-rich repeat kinase 2 (LRRK2) and the glucocerebrosidase (GBA) genes have been linked to Parkinson’s disease (PD)
  - Together comprising the most common known causes of PD

- Can we see evidence for changes in levels or activity of these enzymes in sporadic PD?

- These data have the potential to provide a critical link, which is currently missing, between LRRK2, GBA and sporadic Parkinson’s disease.
Mutations in LRRK2 are the Most Common Cause of Autosomal Dominant Parkinson’s Disease

Hypothesis:
• Increased kinase activity, driven by G2019S and other pathogenic mutations, drives pathogenic events leading to Parkinson’s disease.

LRRK2 mutation carriers develop disease that is clinically and pathologically similar to sporadic PD

Mutations account for approximately 1% of ‘sporadic’ and 4% of familial cases
Is LRRK2 Activity Relevant to Sporadic Parkinson’s Disease (PD)?

Can we see evidence for ↑ kinase levels and/or activity in sporadic PD?

Are biochemical forms known to be associated with higher LRRK2 activity elevated in PD brains?

Do biological processes that we associate with PD impact kinase levels and/or activity?

Grant from MJFF (2013 Biosamples) provided access to the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND) http://www.brainandbodydonationprogram.org/
Absence of LRRK2 (knock-out) Protects From α-Synuclein-Induced Dopaminergic Neurodegeneration

Daher et al PNAS 2014
Efficacy Models: G2019S Enhances rAAV2-α-Syn Neurodegeneration

- G2019S-BAC transgenic rats have normal DA cells counts in the SNpc
- G2019S-BAC transgenic enhances the lesion caused by rAAV2-WT-α-Syn by about 50%
PF-06447475: A Potent, Selective, Brain-Penetrant LRRK2 Kinase Inhibitor

1 mM ATP  WT LRRK2 IC$_{50}$ = 8 nM
1 mM ATP GS LRRK2 IC$_{50}$ = 34 nM
Whole cell assay (reduction of pS935 wt LRRK2 in HEK293 cells) IC$_{50}$ = 56 nM
ActivX huPBMC LRRK2 IC$_{50}$ = 15 nM
Rat liver microsomes CL$_{IA,s}$ = 131 mL/min/kg
RRCK AB = 27.2
MDR1 ER = 1.02
rat C$_{bu}$/C$_{pu}$ = 1

IC$_{50}$ values of ‘hits’ (>50% inhibition at 1uM)
LRRK2 = 15 nM; LOK = 810 nM (54x); MST1 = 140 nM (9x); MST2 = 110 nM (7x); MST3 = 990 nM (66x);
MST4 = 580 nM (39x); PIP4K2C = 750 nM (50x);
RSK1 = 710 nM (47x); SLK = 550 nM (37x)

Henderson et al 2015
J Med Chem
PF-06447475 Protects From α-Synuclein and LPS-Induced Dopaminergic Neurodegeneration in Wild Type Rats (i.e. inhibiting endogenous LRRK2)

All experiments were run fully blinded:
Control = calcium carbonate
PF-06447475 = Our 'tool' LRRK2 kinase inhibitor

AAV-2 αSyn viral model

LPS intracranial injections

Daher et al JBC 2015

Unpublished data
PF-06447475 Effectively Blocks Dopaminergic Neuron Loss Caused by rAAV2-WT-αSyn in G2019S tg Rats

Control

PF-06447475 effectively blocks the larger lesion size caused by rAAV2-WT-α-syn on a G2019S-LRRK2 background.

Daher et al JBC 2015

Collaboration with Andy West (UAB) – funded by MJFF
Several Genes Associated with PD Modulate Vesicle Trafficking & the Autophagy/Lysosomal Pathway

VPS35

GAK

α-synuclein

MAPT

LRRK2

ATP13A2

GBA
LRRK2 Modulates the Morphology of Lysosomes in Primary Astrocytes: G2019S Mutation Results in Large Lysosome

Henry et al HMG 2015
LRRK2 Localizes to Lysosomes in Astrocytes and its Kinase Activity Regulates the Size of the Lysosome
Inhibition of LRRK2 by PF-06447475 Can Rescue the G2019S Enlarged Lysosomes & Normalize Protein Degradation

Measurements of long lived protein degradation

Henry et al HMG 2015
Glucocerebrosidase beta, acid (GBA; GCase) – Homozygous Mutations Cause Gaucher Disease

• GBA is a lysosomal enzyme (497 amino acids, ~60kDa), membrane associated
  • Transport from ER to lysosomes is mediated by LIMP2
  • Requires activators: SapC, phosphatidylserine, Bis(monoacylglycerol)phosphate (BMP)
  • Sap C promotes of lysosomal hydrolysis & protects GCase from proteolytic degradation
  • Ubiquitously expressed

• Gaucher disease is caused by mutations in GBA1
  • Nearly 300 pathogenic changes (point mutations, splice-site mutations, deletions, insertions & recombinant alleles) have been identified – result in misfolded proteins, reduced activity and accumulation of substrates predominantly in monocyte-derived macrophages
  • GCase activity in GD patients is typically only 10-20% of normal (carriers have ~50%)
  • Treatment: Enzyme replacement therapy (imiglucerase, velaglucerase alfa, taliglucerase alfa – Protalix/Pfizer): increase levels by ~27%; or substrate reduction therapy (misglustat)
Compelling Human Genetics: GBA Mutations Represent a Significant Risk Factor for Both PD and DLB

<table>
<thead>
<tr>
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<th>Parkinson’s disease</th>
<th>Dementia with Lewy bodies</th>
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<tbody>
<tr>
<td>Odds ratio (when fully sequenced)</td>
<td>5.43 (6.51)</td>
<td>8.28 (14.20)</td>
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<tr>
<td>Age of onset for GBA mutation carriers</td>
<td>3.9 years earlier (54.9 vs. 58.8)</td>
<td>5.4 years earlier (63.5 vs. 68.9)</td>
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<td>Most common mutations</td>
<td>L444P (33%); N370S (22%)</td>
<td>N370S (41%), L444P (13%)</td>
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<td>% mutations carriers (sporadic PD)</td>
<td>7%</td>
<td>7%</td>
</tr>
<tr>
<td>% mutations carriers (controls)</td>
<td>&lt; 1% for N370S &amp; L444P</td>
<td>&lt; 0.01%</td>
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Hypothesis: Reduced GBA Activity is Causative to PD and DLB Via α-Synuclein Accumulation

- Reciprocal relationship between GBA activity and α-synuclein
  - Knockdown of GBA1, GBA1 mutations, inhibition by CBE or treatment with glucocerebrosidase (substrate) all enhance accumulation and/or oligomerization of α-synuclein
  - Up-regulation of α-synuclein levels decrease glucocerebrosidase protein and activity levels in cell-free systems, cell and mouse models, and post-mortem brains of Parkinson’s disease patients with and without GBA1 mutations

- Emerging hypothesis linking GBA dysfunction to inflammation
Are the Common Parkinson’s Disease Mutations Relevant to Sporadic Disease?

Glucocerebrosidase Deficiency in Substantia Nigra of Parkinson Disease Brains

Matthew E. Gegg, PhD,1 Derek Burke, MSc,2,3 Simon J. R. Heales, PhD,2,3 J. Mark Cooper, PhD,1 John Hardy, PhD,4 Nicholas W. Wood, PhD, MD,5 and Anthony H. V. Schapira, Dsc, MD1

Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson’s disease

Karen E. Murphy,1,2 Amanda M. Gysbers,1 Sarah K. Abbott,3,4 Nahid Tavebi,5 Woojin S. Kim1,2
GBA Activity is $\downarrow$ in $\alpha$-Syn A53T Transgenic Mice & $\alpha$-Syn Pathology is $\downarrow$ by GBA Over-Expression

Gaucher animal models present features of synucleinopathies

- Homozygous $Gba^{D409V/D409V}$ mouse (Grabowski)
  - Heterozygous $Gba^{D409V/WT}$ present less aggregates and no memory deficits (gene dosage effect).
- $Gba^{D409V/D409V}$ mouse line sponsored by the MJFF (https://www.jax.org/strain/019106).

PNAS 2013, 110, 537-42

• Human and preclinical data demonstrate a critical link between known common genetic causes of PD and sporadic disease

• Data provide a platform to develop a deeper mechanistic understanding of the pathophysiology & drug discovery
Patient Stratification – Key for Success

Genetics – Rare variants e.g. LRRK2 and GBA vs. common variants

PET Imaging – DAT / VMAT, TSPO, Tau, αSyn?, LRRK2?

CSF Biomarkers – Exosome LRRK2, GBA, inflammation markers, miRNA, oligomeric αSyn, Tau, dynamic proteomics

In vivo imaging – Far-red GBA activity probes, fMRI, networks

Blood Biomarkers – Oligomeric αSyn, ‘omics’ (tbd), others?

New / improved clinical end-points – wearable devices, cognition, psychosis, sleep
Acknowledgements

Hirst Lab
Anastasia Henry
Christine Oborski
Elie Needle
Harry Samaroo
Kathy Welch
Paula Loos
Peter Buckett
Sakshi Bhargava
Weisong Shan
Yi Chen
Zdenek Berger

Clinical Research
Ashley Winslow
Sara Paciga

WWMC
Antonia Stepan
Jaclyn Henderson
Paul Galatsis

Research Statistics
Dmitri Volfson

Banner Sun Health Research Institute, Sun City, AZ
Thomas P Beach
Geidy Serrano

Mayo Clinic, Scottsdale, AZ
Charles H Adler

University of Alabama, AL
Andy West
Laura Volpicelli-Daley