On the Relationship of \(\alpha\)-synuclein and Parkinson’s Disease

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Group Meeting–Literature Review  
Nov. 30\textsuperscript{th}, 2021
Neurodegenerative Diseases

Alzheimer's Disease
Parkinson's Disease
Multiple Sclerosis
Amyotrophic Lateral Sclerosis
Huntington's Disease

healthy neuron

→

degenerated neuron
Parkinson's Disease

death of dopaminergic neurons in substantia nigra

PD disease pathology

Dopamine biosynthesis

L-phenylalanine

\[
\text{Dopamine} \xrightarrow{2x \text{[O]}} \text{L-phenylalanine} + \text{CO}_2
\]

Parkinson's Disease

death of dopaminergic neurons in substantia nigra

PD disease pathology

substantia nigra

dopaminergic pathways

memory

reward

motivation

motor function

Hornykiewicz, O. Pharmacological Reviews 1966, 18, 925.
**Parkinson’s Disease**

Lewy Bodies

- Insoluble protein aggregates
- Appearance precedes cell death
- Histological Hallmark of PD
- Not diagnostically relevant

Lewy Bodies in nerve cells of the substantia nigra

Parkinson’s Disease Pathology

- 2nd most common neurodegenerative disease
- 10 million cases worldwide, ~ 1 million cases in US
- 90% of cases currently have no validated genetic link
- No cure or mechanism based treatment

### Motor Symptoms
- Stiffness (rigidity)
- Slowless (bradykinesia)
- Resting tremor

### Non-Motor Symptoms
- Cognitive problems
- Mood disturbances
- Psychosis
- Difficulty sleeping

Diagnosis based on having **2 out of 3** motor symptoms
Parkinson’s Disease Pathology

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Kalia, L. V.; Lang, A. E. Lancet 2015, 386, 896
State of the Art Treatments

Sinemet®

Levodopa
biosynthetic dopamine precursor

Carbidopa
inhibitor of peripheral Levodopa metabolism

DRD3 Agonist
increases dopamine production in healthy neurons

Pramipexole

MAO-B Inhibitor
reduces dopamine metabolism in the brain

Selegiline

State of the Art Treatments

Levodopa: biosynthetic dopamine precursor

Carbidopa: inhibitor of peripheral Levadopa metabolism

Sinemet®

Lack of long-term efficacy

Severe Side Effects
  - dyskinesia
  - motor fluctuations
  - extreme behavior

~ 5 years

State of the Art Treatments

Currently zero approved disease modifying or neuroprotective treatments available

- dyskinesia
- motor fluctuations
- extreme behavior

Lack of longterm efficacy

~ 5 years

Limiting Factor in Novel Treatments

healthy neuron ➔ ? ➔ degenerated neuron

What is the mechanism for selective dopaminergic cell death?

James Parkinson (1755-1824)

1817

“… an important object proposed to be obtained by [this essay], is the…attention of those who humanely employ anatomical examination in detecting the causes and nature of diseases…

By their benevolent labors…appropriate modes of relief, or even cure, can be pointed out”
Historical Perspective


1817

1865

Jean-Martin Charcot refers to the Shaking Palsy as “Parkinson’s Disease”

Jean-Martin Charcot
1825-1893

Historical Perspective


1817

Jean-Martin Charcot refers to the Shaking Palsy as “Parkinson’s Disease”

1865

1888

William Gowers (1845-1915)

first documented visual representation of PD phenotype

1817

Jean-Martin Charcot refers to the Shaking Palsy as “Parkinson’s Disease”
1865

1888

Lewy Bodies discovered and subsequently found in SN in PD
1913-1919

Lewy bodies in substantia nigra (SN)

Fredrich Lewy (1885-1950)
Konstantin Tretiakoff (1892-1958)
Historical Perspective

- Jean-Martin Charcot refers to the Shaking Palsy as “Parkinson’s Disease” (1865)
- Lewy Bodies discovered and subsequently found in SN in PD (1913-1919)
- First demonstration that L–DOPA has efficacy in treating PD symptoms (1961)

HO

OH

NH

2

levodopa (L–DOPA)

Donaldson, I. M. L. J. R. Coll. Physicians Edinb 2015, 45, 84
1817


1865

Jean-Martin Charcot refers to the Shaking Palsy as “Parkinson’s Disease”

1888


1913-1919

Lewy Bodies discovered and subsequently found in SN in PD

1961

First demonstration that L–DOPA has efficacy in treating PD symptoms

1990

2 forms of PD defined:
1) Genetic PD
2) Idiopathic PD
Historical Perspective

α-synuclein linked to both genetic and sporadic forms of PD

1997

Two Key Breakthroughs in 1997

Polymeropoulos et. al. 
June 1997

human chromosome 4

Spillantini et. al. 
August 1997

α-synuclein gene (SNCA) 4q21–q22

mutated in familial PD

α-synuclein aggregates are the main component of Lewy Bodies in Parkinson’s Disease

Two Key Breakthroughs in 1997


human chromosome 4

α-synuclein gene (SNCA) 4q21–q22
mutated in familial PD

“… a presynaptic protein with no known function”
Two Key Questions

what is the native function of $\alpha$-synuclein?

is $\alpha$-synuclein neurotoxic?
On the Link Between α-synuclein and Parkinson’s Disease

I. Evidence for aSyn neurotoxicity
   - Evidence for aSyn toxicity
   - aSyn structural polymorphism

II. Link between aSyn function and neurodegeneration
   - Synaptic vesicle trafficking
   - Autophagy/lysosomal disfunction
   - Prion hypothesis

III. Outlook
Genetic Evidence for α-synuclein Neurotoxicity

normal SNCA  →  2x SNCA  →  3x SNCA

expression of endogenous α-synuclein

severity of disease phenotype

“allelic” dose response relationship links expression and pathology

Oliveira, L. M. A., et. al. npj Parkinsons Dis. 2021, 7, 65
Evidence for Insoluble α-synuclein Aggregates as Neurotoxic Entities

wild type mouse → α-synuclein aggregate → PD phenotype

α-synuclein aggregates are neurotoxic

30 dpi 90 dpi 180 dpi

Wire hang

α-synuclein aggregate mechanism?

$\alpha$-synuclein: Structure and Polymorphism

- Micelle bound monomer
- Tetramer
- Pre-amyloid fibrils
- Aggregated fibrils

**α-synuclein: Structure and Polymorphism**

- Generally considered to be **non-toxic** but non-innocent in neurodegeneration.
α-synuclein: Structure and Polymorphism

resists aggregation
and proposed to be a non toxic aSyn
“resting state”

tetramer

$\alpha$-synuclein: Structure and Polymorphism

pre-amyloid fibrils

proposed to be a toxic form of aSyn.
in vivo evidence for “prion like” behavior

**α-synuclein: Structure and Polymorphism**

Main component of **Lewy Bodies**. Less neurotoxic than pre-amyloid fibril.

Wang, W. *et al.* PNAS 2011, 108, 17797,
Abeliovich, A. *et al.* Nature 2016, 539, 207,
On the Link Between α-synuclein and Parkinson’s Disease

I. Evidence for aSyn neurotoxicity

- *Evidence for aSyn toxicity*
- *aSyn structural polymorphism*

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III. Outlook
\(\alpha\)-synuclein: Native Function

- native function not fully understood
- monomers/tetramers localize to presynaptic terminal
- involved in synaptic vesicle trafficking

\(\alpha\)-Syn KO mice
- no functional defects
- increased dopamine release
- no increase in cytosolic [dopamine]

overexpressed \(\alpha\)-Syn
- neurodegeneration
- decreased dopamine release
- altered vesicle distribution

$\alpha$-synuclein: SNARE Complex Formation

SNARE Complex
- formation mediates exocytosis
- responsible for release of neurotransmitters
- $a$Syn alters synaptic vesicle trafficking

α-synuclein: SNARE Complex Formation

can aSyn inhibit SNARE complex formation and prevent dopamine release?

α-synuclein Promotes SNARE Complex Formation

**HEK293 T Cells**

transfect with aSyn

![Diagram](image)

\[ \text{aSyn binds simultaneously to synaptic vesicles and surface protein Syb2 to facilitate SNARE formation} \]

**a-synuclein Promotes SNARE Complex Formation**

TKO mice (α, β, γ-synuclein)

**Hypothesis**

Age dependent *sequestration* of aSyn by Lewy Bodies drives *neurodegeneration*.

Burre, J. *et al.* 2010, 329, 1663
α-synuclein Oligomers Inhibit Vessicle Fusion

aSyn–SNARE interaction is dependent on aSyn morphology

Choi, B. K. PNAS 2013, 110, 4087
α-synuclein Oligomers Inhibit Vessicle Fusion

aSyn–SNARE interaction is dependent on aSyn morphology

Choi, B. K. *PNAS* 2013, 110, 4087
$\alpha$-synuclein Monomers Potentiate Oligomer Toxicity

**soluble monomer**

**prefibril oligomers**

\[\text{aSyn monomers potentiate oligomer driven inhibition of lipid mixing}\]

Yoo, G. *Sci. Rep.* 2021 11, 10955
$\alpha$-synuclein Monomers Potentiate Oligomer Toxicity

soluble monomer

prefibril oligomers

cooperative inhibition

Yoo, G. Sci. Rep. 2021 11, 10955
α-synuclein Monomers Potentiate Oligomer Toxicity

Soluble monomer

Prefibril oligomers

Competitive binders to vesicle receptors prevent initial binding by aSyn monomers and inhibit deleterious vesicle clustering

Yoo, G. Sci. Rep. 2021 11, 10955
Synaptic Vessicle Trafficking

Vessicle Trafficking Hypothesis

aSyn monomers potentiate aSyn oligomer inhibition of SNARE complex formation and exocytosis

↓

inhibited exocytosis prevents dopamine and other neurotransmitters from reaching their destinations, inhibiting motor function and ultimately leading to neuron death
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III. Outlook
Additional Genetic Risk Factors – GBA and GCase

- GBA mutation is the highest genetic risk factor for sporadic PD
- GBA codes for lysosomal protein GCase
- Autosomal recessive GCase mutations cause Gaucher’s Disease (GD)
- All mutations leading to GCase dysfunction increase PD risk

Glucocerebrosidase (GCase)

Glucosylceramide (GlcCer)

Glucose

Ceramide

Additional Genetic Risk Factors – GBA and GCase

- GBA mutation is the **highest genetic risk factor** for sporadic PD
- GBA codes for lysosomal protein **GCase**
- Autosomal recessive GCase mutations cause **Gaucher’s Disease (GD)**
- All mutations leading to **GCase disfunction increase PD risk**

*glucocerebrosidase (GCase)*

*α-Syn monomer* → *α-Syn degradation*

GCase and aSyn

A: Normal
- Normal GCase Function
- α-synuclein degradation

B: GD or heterozygous GCase mutation
- α-synuclein oligomers
- Disfunctional GCase
GCase and αSyn

- inhibition or deficient GCase enzyme activity
- increased concentration of αSyn polymorphs
- increase in lysosomal GlcCer concentration
- GlcCer stabilizes αSyn oligomers

can defective GCase activity be connected to neurodegeneration?

intra-nigral injection of AAV-aSyn

upregulation of aSyn in SN

dead of dopaminergic neurons (> 6 months)

co-injection of AAV-aSyn and AAV-GCase

overexpression of GCase prevents aggregation and increases clearance of aSyn

Rocha, E. M. et. al. Neurobiology of Disease 2015, 82, 495
GCase and aSyn

intra-nigral injection of AAV-aSyn

upregulation of aSyn in SN

dead of dopaminergic neurons (> 6 months)

control (healthy neurons) AAV-aSyn AAV-aSyn + AAV-GCase

co-injection of AAV-aSyn and AAV-GCase

Rocha, E. M. et. al. Neurobiology of Disease 2015, 82, 495
Intranigral injection of AAV-aSyn leads to upregulation of aSyn in SN, resulting in death of dopaminergic neurons (> 6 months). Co-injection of AAV-aSyn and AAV-GCase results in overexpression of GCase, which is neuroprotective in a rodent model of PD. 

Rocha, E. M. et al. Neurobiology of Disease 2015, 82, 495
Clues from In Human Gauche’s Disease Therapy

control
ERT < 5 years
ERT > 5 years

aSyn levels in plasma

1.0
0.5
control
< 5 years
> 5 years

increased levels of GCase can reduce endogenous, human aSyn oligomers

Pchelina, S. N. et. al. Neuroscience Letters 2014, 583, 188
Lysosomal/Autosomal Function

Lysosomal Disfunction

Deficient GCase activity is responsible for build up of aSyn oligomers

\[-\]

aSyn oligomers initiate a positive feedback loop leading to increased aSyn levels that can interfere with several cellular functions
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III. Outlook
**Prions and Misfolded Proteins**

*Misfolded proteins* capable of transmitting their misfolded shape on to normal variants of the same protein.

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- **Balchin, D. et. al. Science 2016, 353, 42**
Prion Hypothesis in PD - Initial Evidence

Patient with Parkinson’s Disease

grafting of healthy dopaminergic neurons in to PD patients

Patient with grafted healthy dopaminergic neurons

grafted neurons before transplant

16 years

autopsy: Lewy Body Pathology

Prion Hypothesis in PD - Initial Evidence

Patient with Parkinson’s Disease

grafting of healthy dopaminergic neurons in to PD patients

Patient with grafted healthy dopaminergic neurons

native, monomeric, soluble αSyn

αSyn oligomers

autopsy:

Lewy Body Pathology

Preformed Fibrils Can Initiate Aggregation

wild type mouse
hippocampal neurons

Preformed Fibrils Can Initiate Aggregation

wild type mouse
hippocampal neurons

aSyn preformed fibrils (oligomers)

Preformed Fibrils Can Initiate Aggregation

**Preformed Fibrils Can Initiate Aggregation**

Preformed Fibrils Can Initiate Aggregation

Preformed Fibrils Can Initiate Aggregation

Lewy pathology from healthy WT neurons

Preformed Fibrils Can Initiate Aggregation

Lewy pathology from healthy WT neurons

fibrillar aSyn seeds propagate and initiate aggregation of endogenous aSyn

Preformed Fibrils Can Initiate Aggregation

Lewy pathology from healthy WT neurons

fibrillar aSyn seeds initiate defective neuron function and ultimately lead to cell death

Prion Hypothesis

Prion Like Propagation of Fibrils

aSyn oligomers are released in to the extracellular environment and taken up by healthy neurons

\[ \downarrow \]

aSyn oligomers initiate misfolding and aggregation of native, soluble, monomeric aSyn
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III. Outlook
Parkinson’s Disease Therapeutic Outlook

Goal: disease modifying therapy

Wong, Y. C. Nat. Med. 2017, 23, 1
Parkinson’s Disease Therapeutic Outlook

aSyn targeting antibodies

propagation ↓ aggregation ↓
clearance ↑

small molecule GCase activators

clearance ↑ aggregation ↓

siRNA

aSyn expression ↓ aggregation ↓

aSyn monomer stabilizers

propagation ↓ aggregation ↓

Wong, Y. C. Nat. Med. 2017, 23, 1
Parkinson’s Disease Therapeutic Outlook

no clinical success to date

Wong, Y. C. *Nat. Med.* 2017, 23, 1
“Ultimately, the nature and extent of the aSyn–PD connection can only be determined by carefully designed clinical experiments in humans” – MJFF 2021

“After all, a major question we still cannot completely discard…is: are aSyn inclusions relevant or simply a epiphenomenon? Time will tell.” – 2020

Outeiro, F. et. al. Journal of Neurochemistry 2020, 153, 433
Oliveira, L. M. A. et. al. NPJ Parkinson’s Disease 2021, 7, 65
Questions?

Nick Intermaggio
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