Understanding Parkinson’s Disease Using Patient Neurons Derived From Induced Pluripotent Stem Cells (iPSC)

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Disclosure Information

- Scientific founder and scientific advisory board member of Lysosomal Therapeutics INC. (LTI)
- Research was supported in part using funds provided by LTI

LTI is a company that uses lysosomal biology to develop treatments for neurodegenerative disease. None of the materials or reagents shown in this presentation are propriety, and are therefore not directly related to the commercial interests of the company.
Protein Misfolding Diseases

1a) Mechanisms that initiate protein misfolding and aggregation

1b) Downstream toxicity of protein aggregates

2) Therapeutic strategies to reduce aggregates

3) Lysosomal Clearance
Neurodegenerative Diseases are Characterized by the Presence of Protein Aggregates

<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein</th>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s Disease</td>
<td>a-beta, tau</td>
<td>cognition, memory deficits</td>
</tr>
<tr>
<td>Frontotemporal Dementia</td>
<td>Tau, TDP-43</td>
<td></td>
</tr>
<tr>
<td>Progressive Supranuclear Palsy</td>
<td>tau</td>
<td>movement, balance disturbance</td>
</tr>
<tr>
<td>Parkinson’s Disease</td>
<td>α-synuclein</td>
<td></td>
</tr>
<tr>
<td>ALS</td>
<td>ubiquitin, SOD, TDP-43, FUS</td>
<td>atrophy, muscle weakness</td>
</tr>
<tr>
<td>SMBA</td>
<td>Androgen Receptor</td>
<td></td>
</tr>
<tr>
<td>Huntington’s Disease</td>
<td>huntingtin</td>
<td>dyskinesia, involuntary movements</td>
</tr>
</tbody>
</table>

Forman et al, Nat. Med., 2004
Parkinson’s Disease - Chronic, Age-related Movement disorder that affects 1% of population over the age of 60 yrs

• Degeneration of circumscribed regions of the nervous system, including midbrain dopamine neurons that mediate movement.
First Clues into the Etiology of Parkinson’s Disease (PD) Came from Genetic Analysis of a Rare Familial Case

Point Mutation identified resulting in Alanine -> Threonine Conversion (A53T) in \( \alpha \)-synuclein protein sequence

Mutation in the \( \alpha \)-Synuclein Gene Identified in Families with Parkinson’s Disease


SCIENCE • VOL. 276 • 27 JUNE 1997
α-Synuclein

- Enriched in presynaptic terminals (Maroteaux et al 1988, George and Clayton 1999)
- Binds preferentially to acidic phospholipids (Perrin et al 2000)
- Associates with lipid rafts in vivo; required for synaptic localization (Fortin et al 2004)
- Aids SNARE proteins in vesicle fusion at the presynaptic terminal (Chandra et al 2005, Burre et al 2010)

α-Synuclein knock-out mice do not develop a phenotype, suggesting that mutations cause toxicity through a gain-in-function mechanism
Familial-linked Point Mutations Enhance α-Synuclein Aggregation Kinetics

Mutations in α-synuclein (A53T, A30P, E46K, and H50Q) increase the rate of oligomerization and/or fibril formation in vitro.

Fibrils generated from recombinant A53T α-synuclein

Protein Aggregation (AUFS)

NATURE MEDICINE NOVEMBER 1998

Kelly A. Conway, James D. Harper & Peter T. Lansbury

Greenbaum et al, J. Biol. Chem, 2005
α-Synuclein in Lewy Bodies of both Sporadic and Familial Parkinson’s Disease

Lewy Bodies Contain Fibrillar Polymers Comprised of \( \alpha \)-Synuclein That Have Amyloid Properties and are Biochemically Insoluble

**Ultrastructural Analysis**

EM of a Lewy Body

From Baba et al. Am J. Pathol 1998

**Immuno-EM:**

Fibrillar \( \alpha \)-Syn from inclusions

From Giasson et al. J.Biol Chem 1999

**Biochemical Analysis**

Western blot for \( \alpha \)-synuclein

Hypothesis:

- PD is caused by increased synthesis or reduced degradation of $\alpha$-synuclein
- Reducing $\alpha$-synuclein will provide therapeutic benefit in PD
SNCA triplication causes autosomal dominant, early onset PD (age 20-30), with prominent dementia.

SNCA duplication has a later onset, not completely penetrant, and resembles idiopathic PD, with mild dementia.

This indicates that increased dosage of α-Synuclein is sufficient to cause PD, and implies that α-synuclein aggregates found in idiopathic PD are cytotoxic.
Aggregation of \( \alpha \)-Synuclein Occurs \textit{in vitro} and is Accelerated by Increasing Concentration

![Graph and Diagram]

1. Monomer
2. Oligomer
3. Fibril
Hypothesis:

PD is caused by reduced degradation of $\alpha$-synuclein: Link to the lysosomal system

$\alpha$-synuclein is normally degraded by lysosomes (Cuervo et al, Science, 2004)

- Disrupting lysosomal function may cause accumulation of the protein
Mutations in a Lysosomal Protein, Glucocerebrosidase (encoded by \textit{GBA1}) Increase Risk of Parkinson’s Disease and Accelerate Disease Progression

\textit{GBA1} mutations cause a rare lysosomal storage disorder called Gaucher Disease

- Glucocerebrosidase degrades Glucosylceramide (GluCer) in lysosomes

\begin{center}
\begin{tikzpicture}
    \node [anchor=west] (text1) at (0,0) {GluCer};
    \node [anchor=west] (text2) at (0,-1) {\textit{\beta\text{-}glucosidic linkage \downarrow \text{Degradation occurs in the lysosome}}};
    \end{tikzpicture}
\end{center}

- Mutations are loss-of-function, and result in build-up of GluCer causing Gaucher disease

- Visceral Symptoms Can be Treated by Enzyme Replacement Therapy

*The clinical phenotype and age-at-onset varies for Gaucher disease; Some patients show pathology in the nervous system, while others do not*
Lysosomal *GBA1* Mutant Allele Increases Risk of Parkinson’s Disease and Accelerates Disease Progression

- The association was first discovered in the clinic, where patients with Gaucher disease were occasionally noted to develop parkinsonism.

- An increased frequency of PD was seen in relatives of Gaucher probands.

- **Homozygote** *GBA1* mutants have a ca. **20->40-fold increased risk** for developing PD.

- **Heterozygote** *GBA1* mutants have a ca. **5-fold increased risk** for developing PD (Sidransky et al, 2009).

- At autopsy, characteristic synuclein-containing Lewy bodies are observed in the brain.

*Lewy bodies in Gaucher’s brain*  
Reduced GCase Enzyme and Activity Leads to Increased Levels of GluCer Substrates and α-Synuclein

Mazzulli et al, Cell, 2011
Mechanism of $GBA1$-linked PD

Reduced Lysosomal Proteolysis

Specificity for a-Synuclein

$\Rightarrow$ No change in Tau Protein

Mazzulli et al, Cell 2011
GluCer Directly Stimulates α-Synuclein Aggregation in vitro at pH 5.0

Purified GlcCer lipids + Purified α-synuclein → Incubate 37°C → Shake (1000rpm) → SEC / western blot to detect aggregates

Mazzulli et al, Cell 2011
GluCer interacts with α-synuclein in lysosomes to promote aggregation.
Improved Disease Models Through the Development of Induced Pluripotent Stem Cell of PD and GD Patients

GD or PD Patient

Skin fibroblasts from punch biopsy

Viral Reprogramming

Induced pluripotent stem cell

Tyrosine Hydroxylase / βIII-Tubulin

Patient neurons

DAPI

Oct4

Tra-1-60

SSEA-4

NANOG
Neurons Generated from Patient iPSC Cells Express Dopaminergic Midbrain Markers at Early Time Points (day 0-25)

Mazzulli et al, PNAS 2016
Characterization of Patient Midbrain Cultures Over Time

Mazzulli et al PNAS, 2016
Time-dependent Accumulation of α-Synuclein at the Cell Body of Patient Neurons

Mazzulli et al PNAS, 2016
Accumulation of Amyloidogenic Insoluble α-Synuclein in Patient Midbrain Neurons

Mazzulli et al. PNAS, 2016
α-Synuclein Inhibits the Trafficking and Activity of Lysosomal Hydrolases, Including Wild-Type GCase

Mazzulli et al, Cell 2011; PNAS 2016
Loss-of-GCase Function Occurs in Neurons that Accumulate α-Synuclein

Reduced Activity and Substrate Accumulation → Rescued by α-Syn Reduction

Mazzulli et al, PNAS, 2016
Reproducibility of Lysosomal Dysfunction in Lenti-overexpressed α-Synuclein Control Neurons

**Vector**

**α-syn**

**CBB**

**α-Syn Levels (fold change)**

**Lysosomal GCase Activity (AUC x 10^3)**

Control line 2

- vect: 0
- wt α-syn: +

Control line 3

- vect: 0
- wt α-syn: +
Time dependent Lysosomal Dysfunction and Neurodegeneration in SNCA Trp Neurons

Protein Accumulation → Lysosomal Dysfunction → Neurodegeneration
Therapeutic Strategy: Allosteric Activation of GCase

Discovery, Structure—Activity Relationship, and Biological Evaluation of Noninhibitory Small Molecule Chaperones of Glucocerebrosidase

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NIH 758
GCase Activator 758 Enhances Lysosomal Activity and Reduces Substrates in Patient Midbrain Neurons

758 Reduces α-Synuclein in iPS Midbrain Neurons Derived from Several Different Types of Synucleinopathy Cases

758 Reduces Amyloidogenic α-Synuclein in iPS Midbrain Neurons

758 Reverses \(\alpha\)-Synuclein-Induced Downstream Pathology: Restoration of Lysosomal Hydrolase Trafficking and Function

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Citation</th>
<th>Synuclein Pathology</th>
<th>DA phenotype</th>
<th>DA Neuron Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-wt-a-syn</td>
<td>Masliah et al, Science 2000</td>
<td>Hippocampus, Cortex, Brain stem*</td>
<td>Subtle reduction in dopamine levels*</td>
<td>Neurite degeneration*, no cell loss</td>
</tr>
<tr>
<td>Thy1-A30P-a-syn</td>
<td>Kahle et al, J. Neurosci, 2000; Neumann et al, JCI 2002</td>
<td>Cerebellum, Hippocampus, Cortex, Brain stem</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Prp-A53T-a-syn</td>
<td>Giasson et al, Neuron 2002; Lee et al, PNAS 2002</td>
<td>Spinal Cord, Cortex, Brain stem*</td>
<td>Hyperactivity</td>
<td>None</td>
</tr>
<tr>
<td>rTH-A30P-a-syn</td>
<td></td>
<td></td>
<td>-no change</td>
<td></td>
</tr>
<tr>
<td>LRRK2 R1441</td>
<td>Li et al Nat. Neurosci 2009</td>
<td>Tau pathology</td>
<td>-subtle change in DA levels; no change in striatal TH</td>
<td>None</td>
</tr>
<tr>
<td>LRRK2 WT overexpression</td>
<td></td>
<td>None</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Parkin knock-out</td>
<td>Goldberg et al JBC, 2003</td>
<td>None</td>
<td>Elevated DA, subtle changes</td>
<td>None</td>
</tr>
<tr>
<td>DJ-1 knock-out</td>
<td>Chen et al, JBC, 2005; Goldberg et al, Neuron, 2005</td>
<td>None</td>
<td>Elevated DA</td>
<td>None</td>
</tr>
<tr>
<td>DJ-1 knock-out</td>
<td>Chen et al, JBC, 2005; Goldberg et al, Neuron, 2005</td>
<td>None</td>
<td>Elevated DA</td>
<td>None</td>
</tr>
<tr>
<td>PINK1 knock-out</td>
<td>Kitada et al, 2007; Gispert et al, 2009</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>GBA1/A53T-a-syn</td>
<td>Fishbein et al, Brain 2014</td>
<td>Hippocampal inclusions</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

* Dramatic overexpression required
Human and Mice Exhibit Inherent Differences in Dopamine Metabolism

- Human substantia nigra is characterized by pigmented Neuromelanin-containing cells; mouse is not pigmented

Zecca et al, PNAS 2008
Sulzer et al, PNAS 2000
Zecca et al, PNAS 2008
Neuromelanin Accumulates in Cultures of Human iPS Midbrain Neurons

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Dimitri Krainc and lab
Loss-of-Function Mutations in Lysosomal GBA1 (N370S / c.dup84GG) Induce $\alpha$-Synuclein Accumulation in GD Midbrain Neurons

Mazzulli et al, Cell, 2011