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“α-synuclein transgenic models of Parkinson’s Disease”

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Lewy Body Diseases

Heterogenous group of disorders with parkinsonism and characteristic neuronal inclusions containing $\alpha$-synuclein and ubiquitin.

There is nigral degeneration plus loss of synapses in cortical and subcortical regions.

25%-50% cases develop dementia.

95% sporadic, rare mendelian forms are associated with mutations.
## Genetics of Parkinson's Disease

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosomal location</th>
<th>Protein</th>
<th>Inheritance Pattern</th>
<th>Atypical PD features</th>
<th>Lewy bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1</td>
<td>4q21</td>
<td>Alpha-Synuclein (^a)</td>
<td>AD</td>
<td>Early onset, Lower prevalence of tremor</td>
<td>Yes</td>
</tr>
<tr>
<td>PARK2</td>
<td>6q25.2-q27</td>
<td>Parkin</td>
<td>AR</td>
<td>Early or juvenile onset, More frequent dystonia and levodopa-induced dyskinesias, Slower disease progression</td>
<td>Mostly negative (^b)</td>
</tr>
<tr>
<td>PARK3</td>
<td>2p13</td>
<td>Unknown</td>
<td>AD</td>
<td>Dementia in some individuals, Rapid progression</td>
<td>Yes</td>
</tr>
<tr>
<td>PARK4c</td>
<td>4p15</td>
<td>Unknown</td>
<td>AD</td>
<td>Early onset, Rapid progression, Dementia, Autonomic dysfunction, Postural tremor</td>
<td>Yes</td>
</tr>
<tr>
<td>PARK5</td>
<td>4p14</td>
<td>UCH-L1</td>
<td>AD</td>
<td>None</td>
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<tr>
<td>PARK6</td>
<td>1p36</td>
<td>PINK1</td>
<td>AR</td>
<td>Early onset, Slow progression</td>
<td>Unknown</td>
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<tr>
<td>PARK7</td>
<td>1p36</td>
<td>DJ-1</td>
<td>AR</td>
<td>Early onset, Psychiatric symptoms, Slow progression</td>
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<tr>
<td>PARK8</td>
<td>12p11.2-q13.1</td>
<td>Dardarin</td>
<td>AD</td>
<td>None</td>
<td>No</td>
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<tr>
<td>PARK9</td>
<td>1p36</td>
<td>Unknown</td>
<td>AR</td>
<td>Juvenile onset, Spasticity, Supranuclear gaze paralysis, Dementia</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

AD, autosomal dominant; AR, autosomal recessive. \(^a\)Including mutations and wild-type multiplications. \(^b\)Lewy bodies reported in one individual with Parkin mutations. \(^c\)The initial PARK4 linkage to 4p15 could not be confirmed, and the PD phenotype in this family was subsequently linked to a PARK1 variant (alpha-synuclein triplication). Vila and Przedborski Nature Medicine 2004
$\alpha$-Synuclein

- 14 kDa, 140 amino acids
- cytoplasmic and nuclear
- abundant in synapses
- member of a protein family ($\alpha$, $\beta$, $\gamma$)
- involved in synaptic plasticity
  vesicular dopamine release

Chaperone functions

- Signaling molecules (MAPK, PKC, PLD)
- Mitochondrial proteins (BAD, BAX, Cyt C)
- Heat shock/proteosome molecules ($\beta$-syn, HSP70, Parkin, ubiquitin, UCHL1)
- Synaptic and cytoskeletal proteins
  (synphilin, NF, MAPs, tau, torsin, TH)
α-synuclein in neurodegenerative disorders

Familial Parkinson’s disease

Trojanowski et al

α-synuclein

A53T  Polymeropoulos et al
A30P  Kruger et al
E46K  Zarranz et al
WT multipl  Singleton et al

Presynaptic location

α-synuclein

synaptophysin

Iwai et al

Multiple system atrophy

Wakabayashi et al

Lewy body disease

Masliah et al

Takeda et al; Spillantini et al
Mechanisms of synaptic pathology in Alzheimer’s and Parkinson’s Disease

Early accumulation of synaptic molecules such as APP and α-synuclein at the synaptic site

Synaptic loss is the best predictor for the neurological deficits
α-synuclein and mechanisms of synapse loss

α-synuclein/NACP

Mutations
Polymorphisms

Oxidants
Toxins
Aβ1-42

conformational changes

polymers
oligomers

Inclusion formation
ubiquitinization isolation

↓
synaptic plasticity

Synapse loss

Parkin
UCH-L1
DJ-1

Lansbury et al
Kraft, Klein, et al
Walsh, Selkoe, et al
Hashimoto et al
Characteristics needed in tg models of Parkinson’s Disease (I).

1. Motor and memory deficits
2. Synaptic damage in the striato-nigral system
   Dopaminergic fiber loss in the C-P
   Neuronal loss in the SN
3. Synaptic damage limbic system
   Damage to axons CA3,
   synapse loss in temporal, cingulate
4. Formation of Lewy bodies and Lewy neurites
   \( \alpha \)-synuclein, p-\( \alpha \)-syn, ubiquitin, NF
   electrodense and fibrillar aggregates
   by EM
Characteristics needed in tg models of Parkinson’s Disease (II).

5. α-syn accumulation in the CNS
   - biochemical evidence of α-syn in the detergent insoluble fraction
   - evidence of α-syn accumulation in the membrane fractions
   - evidence of α-syn oligomers in synaptosomes
   - double labeling and confocal analysis of synapses
   - presence of oxidized and pho-129
Summary of PD tg animals available at UCSD

PDGF \( \beta \)-\( \alpha \)synuclein wt (line D) abundant inclusions
PDGF \( \beta \)-\( \alpha \)synuclein wt (line M) glial inclusions
PDGF \( \beta \)-\( \alpha \)synuclein A53T (line 8) severe motor deficits
PDGF \( \beta \)-\( \alpha \)synuclein wt GFP fluorescent inclusions in vivo imaging and screening

mThy1-\( \alpha \)synuclein wt (line 61) high expression, SN path
mThy1-\( \alpha \)synuclein A30P (line 44) high expression
mThy1-\( \beta \)synuclein (line 11) blocks \( \alpha \)-syn pathology

PDGF \( \beta \)- hasynuclein wt homozygous
PDGF \( \beta \)- hasynuclein A53T homozygous (in breeding)
PDGF \( \beta \)- hasynuclein wt x mThy1-\( \alpha \)synuclein wt
PDGF \( \beta \)- hasynuclein A53T x mThy1-\( \alpha \)synuclein wt
Neurological and cellular alterations in \( \alpha \)-synuclein tg mice

**Transgenic models**

**PDGF-SYN**

- Motor deficits
- \( \alpha \)-syn in neurons and synapses

**mThy1 or mPrP-SYN**

- Loss of synaptic terminals

**Non-tg**
- \( \alpha \)-synuclein tg
Behavioral alterations in a-syn tg mice (Fleming, Chesselet, et al)

1. impairment in motor performance and coordination.
2. reduction in spontaneous activity
3. motor deficits became more apparent with age starting at 3 months
4. fine motor skills were altered at 4 months
5. sensorimotor deficits started at 6 months
6. no memory deficits in the water maze unless when challenged with Abeta or paraquat
PDGFb hα-synuclein transgenic

<table>
<thead>
<tr>
<th>Neocortex</th>
<th>Hippocampus</th>
<th>S. Nigra</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Line D</td>
<td></td>
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<tr>
<td></td>
<td>F</td>
<td>G</td>
<td>H</td>
</tr>
<tr>
<td>Line A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>L</td>
<td>M</td>
</tr>
<tr>
<td>Line M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line M</td>
<td></td>
<td></td>
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<td>O</td>
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</tbody>
</table>
α-synuclein in glial cells

- Synuclein accumulates in glial cells in LBD, MSA and AD
- Significant microgliosis in acute models (MPTP)
- But less apparent in tg models, some reported astrogliosis
Comparison of neuropathological alterations in LBD and α-synuclein tg mice

Lewy body Disease

α-synuclein tg mice
α-Synuclein-positive Neuronal Inclusions in Human α-SYN Mice Are Ubiquitinated and Some Are Found in Dopaminergic Neurons of the Substantia Nigra

hSYN/Ubiquitin

hSYN/Tyrosine Hydroxylase
Dopaminergic Deficits in 12-Month-Old α-SYN TG Mice

Tyrosine Hydroxylase Immunoreactivity

A

Nontg

Basal Ganglia
S. Nigra

SYN tg

Basal Ganglia

B

% area covered by TH positive fibers

Nontg
SYN tg

C

Basal Ganglia

DOPA
EPI
NOREPI

pg/ml

Nontg
SYN tg

D

SYN tg

Basal Ganglia
S. Nigra

E

number of TH+ cells per sq mm

Nontg
SYN tg

F

S. Nigra

DOPA
EPI
NOREPI

pg/ml
More α-Synuclein Is Not Necessarily More Pathogenic

Lines A, M, D

A

B

C

PDGF-SYN

Lines 44 and 61

D

E

F

mThy-1-SYN

Neocortex

Basal ganglia
Ultrastructural characteristics of the inclusions in the α-synuclein tg mice
Oxidative stress in α-synuclein tg mice

Mitochondrial and axonal alterations (MPTP+)

Others findings on oxidative stress
- Rotenone (Greenamyre)
- Reversible with Vitamin E
- Mitochondrial damage
- Increased oxidative stress markers
- α-synuclein oxidation (Ischiropoulos)
- Cytochrome C and H2O2 promotes α-synuclein aggregation
- aSyn KO is resistant to MPTP

Nitrosylation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>NAC</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>65</td>
<td>140</td>
</tr>
</tbody>
</table>
Neuropathological, Biochemical, and Neurological Alterations in α-SYN Transgenic Mice

Line D (WT)  Line A (WT)  Line 8 (A53T)

---

**Total α-syn (Syn-1 Antibody)**

- Line D (WT)
- Line A (WT)
- Line 8 (A53T)
- Nontg

**Oligomer α-syn**

**Monomer α-syn**

**Human α-syn (72-10 Antibody)**

---

Rotarod

- Nontg
- Line A (WT)
- Line D (WT)
- Line 8 (A53T)
Post-transcriptional alterations in α-synucle mice

Phospho α-syn (Ser 129)

Iwatsubo et al

Parkin-KO cross

HSP-tg cross

GSK3β-tg
Fyn-tg
CKII-tg
Crosses?

NAC

Ubiquitination
0-glycosylation

Fyn/syk Tyr 125

CKII/GSK3β Ser 129

N
Lys 35 65 140

140
Some Other Human $\alpha$-Synuclein Animal Models with PD-like Pathological and/or Neurological Alterations

<table>
<thead>
<tr>
<th>Transgenic Mice</th>
<th>Transgenic Flies or Worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giasson et al. 2002</td>
<td>Auluck et al. 2002</td>
</tr>
<tr>
<td>Gispert et al. 2003</td>
<td>Feany &amp; Bender 2000</td>
</tr>
<tr>
<td>Kahle et al. 2002</td>
<td>Lakso et al. 2003</td>
</tr>
<tr>
<td>Lee et al. 2002</td>
<td>Takahashi et al. 2003</td>
</tr>
<tr>
<td>Van der Putten et al. 2000</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viral Vectors in Rats or Monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirk et al. 2002</td>
</tr>
<tr>
<td>Kirik et al. 2003</td>
</tr>
<tr>
<td>Klein et al. 2002</td>
</tr>
<tr>
<td>Lo Bianco et al. 2002</td>
</tr>
</tbody>
</table>
Progression of neuropathological alterations in a-synuclein tg mice

- a-synuclein oligomerization
- inclusions
- Synapse loss
- Dopa deficits
- Motor deficits
- Astro/microgliosis
- Neuronal loss

Age (months): 0 3 6 9 12 15 18 21 24
Summary

- PDGFb --> moderate levels, neocortical and limbic system, inclusions, motor, Dopa loss, mimics both LBD and MSA
- moThy1--> high global expression, subcortical predilection, SN involvement
- moPrP --> very high levels, subcortical involvement, spinal cord
- A53T more toxic than WT or A30P
- DBA more susceptible than C57/BL6
- Important to identify oligomers and detergent insoluble aggregates
Lessons from studies in transgenic mice

- Synaptic pathology occurs early, is the best correlate to the clinical deficits and precedes neuronal loss.

- Protein misfolding and aggregation into oligomers appear to be centrally involved in neurodegeneration. (Inclusions are useful diagnostic markers but not the cause.)

- Accumulation of \( \alpha \)-syn oligomers is more widespread and includes axons and dendrites.

- In variants of LBD the damage is not limited to the S. Nigra but also involves the neocortex and hippocampus.
α-synuclein might disturb multiple neuronal functions

- Mitochondrial dysfunction/Oxidative stress
- Plasma membrane damage
- Interference with Signal transduction
- Endosomal/Lysosomal leakage
- Proteasomal dysfunction
- Nuclear toxicity
- ER stress
- Golgi fragmentation

Bax
CytoC
Caspase

α-synuclein might disturb multiple neuronal functions
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## Comparison of various a-synuclein tg mouse models

<table>
<thead>
<tr>
<th>Group</th>
<th>Promoter</th>
<th>Construct</th>
<th>Neuropath</th>
<th>Motor</th>
<th>Biochem</th>
<th>Age of onset</th>
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<tbody>
<tr>
<td>Lee MK 2002</td>
<td>MoPrP</td>
<td>ha-syn (wt) ha-syn (A53T) ha-syn (A30P)</td>
<td>a-syn subcortex Axonal degeneration Gliosis</td>
<td>Severe def Premature death</td>
<td>Detergent insoluble</td>
<td>10m</td>
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<tr>
<td>Giasson 2002</td>
<td>MoPrP</td>
<td>ha-syn (wt) ha-syn (A53T) homozygous</td>
<td>Gliosis a-syn accumulat Spinal cord Axonal degeneration Gliosis</td>
<td>Severe, complex</td>
<td>Detergent insoluble</td>
<td>8-12m</td>
</tr>
<tr>
<td>Van der Putten 2000</td>
<td>moThy-1</td>
<td>ha-syn (wt) ha-syn (A53T)</td>
<td>a-syn motor neur Spinal cord Axonal degeneration</td>
<td>Early onset</td>
<td>Detergent Insoluble ?</td>
<td>1-2m</td>
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<tr>
<td>Kahle 2002</td>
<td>Proteolipid</td>
<td>ha-syn (wt)</td>
<td>Accumul a-syn OLIGOS No myelin loss</td>
<td>NA</td>
<td>Detergent Insoluble</td>
<td>12m ?</td>
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</table>
a-synuclein models for selective neuronal vulnerability (II)

<table>
<thead>
<tr>
<th>Group</th>
<th>Vector</th>
<th>Construct</th>
<th>Neuropathological Features</th>
<th>Motor</th>
<th>age</th>
<th>Age of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirk 2002</td>
<td>rAAV</td>
<td>ha-syn (wt) A53T</td>
<td>Neuron loss DOPA loss a-syn accumulat</td>
<td>Mild Motor deficits</td>
<td>Adult Rat SN</td>
<td>2-3m</td>
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<tr>
<td>Klein 2002</td>
<td>rAAV</td>
<td>ha-syn (wt) A30P</td>
<td>Neuron loss a-syn accumulat</td>
<td>No Motor deficits</td>
<td>Young Rat SN</td>
<td>12m</td>
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<tr>
<td>Lo Bianco 2002</td>
<td>Lenti</td>
<td>ha-syn (wt) A30P A53T</td>
<td>Neuron loss a-syn accumulat</td>
<td>?</td>
<td>Rat SN</td>
<td>6w</td>
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a-synuclein models for selective neuronal vulnerability (I)

<table>
<thead>
<tr>
<th>Group</th>
<th>Promoter</th>
<th>Construct</th>
<th>Neuropath</th>
<th>Motor</th>
<th>Biochem</th>
<th>Age of onset</th>
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<tbody>
<tr>
<td>Richfield 2002</td>
<td>Rat TH</td>
<td>ha-syn (wt) ha-syn (A53T/A30 P)</td>
<td>a-syn accumulat MPTP tox</td>
<td>Alter motor response to Meth</td>
<td>NA</td>
<td>9-12m</td>
</tr>
<tr>
<td>Matsuoka 2002</td>
<td>Rat TH</td>
<td>ha-syn (wt) ha-syn (A53T;A30 P)</td>
<td>a-syn in SN</td>
<td>none</td>
<td>NA</td>
<td>12m</td>
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