Animal models of Parkinson’s disease: what can we learn from them?

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Ideal animal model that can mimic PD

- Motor & non-motor phenotype
- Alleviation of motor features with Ldopa replacement
- Pathological features: nigrostriatal degeneration associated with dopamine deficiency
- Accumulation of α-synuclein aggregates including Lewy bodies
- Aging effect: gradual worsening of phenotypic features correlated with pathological progression involving different neuronal pathways
Ideal animal model does not exist

Unicorn

Orynx with 1 deformed horn
Current animal models

- Toxin-based
  - 6 hydroxydopamine
  - MPTP
  - Rotenone
  - Paraquat
  - Maneb
  - Isoquinoline derivatives
  - Methamphetamine
  - Reserpine

- Gene-based
  - Transgenic
  - Knockin
  - Knockout
  - Genes
    - α-synuclein
    - LRRK2
    - Parkin
    - PINK1
    - DJ1

- Viral vector-based
  - Lentiviral vectors
  - Recombinant adeno-associated VV
    - ↑ or ↓ gene expression
  - Genes
    - α-synuclein
    - LRRK2
    - PINK1
    - Parkin?
    - DJ1?
Limitations of toxin-based model

- Most toxins used do not exist in nature
- Most toxins are administered parenterally and at high doses
- Rapid onset of deficits
- Unable to replicate the progressive loss of dopaminergic neurons & non-motor features
- Some pathological features of PD not present, e.g., Lewy body pathology and other brain areas not involved
Limitations of gene-based models

- These animals are not humans
- These animals kept in captivity are exposed to a homogenous environment whereas humans are exposed to different environments
- Expression of the mutated gene (level & distribution) in the model different from human
- Similar species with the same mutation can have differences in phenotype
  - mice can be different from rats
- Even within the same model & mutation, phenotype can be different
  - different promoters driving different gene expression
Current approaches to using animal models of PD

• Combine the findings of different models depending on the questions being asked based on a sound hypothesis

• Exploring for biomarkers & earliest pathological features

• Targeting pathogenic processes
  ➢ buildup of α-synuclein & effects from its toxic moieties (oligomers, protofibrils & fibrils)
  ➢ propagation of α-synuclein in CNS
  ➢ processes upstream & downstream of α-synuclein toxic moieties
Leucine-rich repeat kinase 2 (LRRK2) is one of the commonest mutant protein in familial and sporadic PD, (10% of familial PD; and 1-3% of sporadic PD). Majority of patients with LRRK2 mutation carry one of three pathogenic substitutions: G2019S, R1441C, or R1441G.

- Widely expressed throughout the brain, particularly high in brain dopaminoceptive areas.
- Patients with LRRK2-associated PD demonstrate similar clinical features to idiopathic PD.
# LRRK2 knockin mouse models

**Volta M, Melrose H.** *Biochem Soc Transc* 2017; 45: 113-122

<table>
<thead>
<tr>
<th>Study</th>
<th>Mutation</th>
<th>In vitro only?</th>
<th>DA system</th>
<th>Behavior phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mouse line ‘Jie Shen’</strong>&lt;br&gt;Tong et al. 2009 [42]</td>
<td>R1441C</td>
<td>No</td>
<td>Reduced sensitivity to DA-mediated inhibition of firing</td>
<td>Reduced sensitivity to D2R-dependent locomotion inhibition</td>
</tr>
<tr>
<td>Nichols et al. 2010 [57]</td>
<td>R1441C</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Parisladou et al. 2014 [52]</td>
<td>R1441C</td>
<td>No</td>
<td>NA</td>
<td>Reduced sensitivity to D1R-mediated locomotion stimulation</td>
</tr>
<tr>
<td><strong>Mouse line ‘Novartis’</strong>&lt;br&gt;Herzig et al. 2011 [40]</td>
<td>G2019S</td>
<td>No</td>
<td>No alterations</td>
<td>No alterations</td>
</tr>
<tr>
<td>Longo et al. 2014 [58]</td>
<td>G2019S</td>
<td>No</td>
<td>NA</td>
<td>Hyperkinetic phenotype</td>
</tr>
<tr>
<td><strong>Mouse line ‘Melrose and Farrer’</strong>&lt;br&gt;Döbsch et al. 2010 [68]</td>
<td>G2019S</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Yue et al. 2015 [43]</td>
<td>G2019S</td>
<td>No</td>
<td>Age-dependent reduction in extracellular DA levels in the striatum</td>
<td>Early hyperactivity followed by anxiety-like behaviors</td>
</tr>
<tr>
<td><strong>Mouse line ‘Shu-Leong Ho’</strong>&lt;br&gt;Liu et al. 2014 [70]</td>
<td>R1441G</td>
<td>No</td>
<td>Increased sensitivity to reserpine-induced reduction in striatal DA uptake from striatal synaptosomes</td>
<td>Increased sensitivity to reserpine-induced motor deficits</td>
</tr>
<tr>
<td>Ito et al. 2016 [71]</td>
<td>R1441G</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Mouse line ‘Eli-Lilly’</strong>&lt;br&gt;Steger et al. 2016 [59]</td>
<td>G2019S</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ito et al. 2016 [71]</td>
<td>G2019S</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Transgenic vs knockin mutant animals

• Transgenic
  – Exogenous structural & regulatory gene sequences inserted into genome
  – Gene expression different from endogenous WT in levels & in distribution

• Knockin
  – Specific gene sequence of genome is modified at the endogenous chromosomal position of a given gene
  – Backcrossing of founder animals to ensure mutant mice have similar genetic background as WT
  – Gene expression in mutant animals similar to endogenous WT in terms of level & distribution
Dopamine transporter (DAT) binding with $^{11}$C-methylphenidate imaged with PET

Healthy control  Non-symptomatic LRRK2 Mutation carrier  Symptomatic LRRK2-PD

LRRK2 R1441G mice are more liable to dopamine depletion and locomotor inactivity

Hui-Fang Liu, Song Lu, Philip Wing-Lok Ho, Ho-Man Tse, Shirley Yin-Yu Pang, Michelle Hiu-Wai Kung, Jessica Wing-Man Ho, David B. Ramsden, Zhong-Jun Zhou, & Shu-Leong Ho

ACTN 2014; 1(3) 199-208
Inherited versus environmental factors

- Inherited factors
- Environmental factors
Rotenone

- Odourless, colourless organic pesticide
- Occurs naturally in seeds and stems of several plants
- Lipophilic mitochondrial toxin
- Inhibits electron transfer from Complex I to ubiquinone in ETC
- ↑reactive oxygen species & ↓ATP production
- Causes parkinsonism in primates, used in experimental models of parkinsonism

*Pachyrhizus erosus* (Jicama vine)
What is the crucial factor which is missing in most current animal experimental models of PD?

Aging!!!
↓ Dopamine uptake in mutant synaptosomes
• ↓ striatal synaptosomal mitochondrial levels
• ↓ synaptic vesicular proton pump protein (V-ATPase H)
• Mutant LRRK2 mice were more susceptible to chronic low dose of rotenone
Link between α-synuclein & PD

- Multiple point mutations found in SNCA gene in autosomal dominant PD
- Duplications & triplications of its wild-type allele found in familial PD
- GWAS studies linked SNPs in SNCA gene to ↑ risk of PD
- α-synuclein undergo prion-like propagation, initiating & spreading pathological process originating at striatal dopaminergic terminals
- Human α-synuclein species from PD-derived Lewy bodies are pathogenic & initiate PD-like process in rodents & primates
α-synuclein

**Physiological**

- α-syn 
  - Monomers 
  - Tetramers
  - Binds to tubulin and enhances microtubule formation
  - Regulates the fusion and clustering of presynaptic vesicles
  - Involved in exocytosis and presynaptic dopamine release

**Pathological**

- Protofibrils, oligomers or ribbons
- Fibrils
- Lewy bodies and Lewy neurites
  - Lewy body
  - Lewy neurite

- Causes mitochondrial dysfunction, leading to increased oxidative stress
- Overwhelms the calcium-buffering capacity of the cell
- Induces ER stress
- Disrupts ER and Golgi trafficking
- Impairs proteostasis, including protein degradation by the ubiquitin–proteasome and autophagy–lysosomal systems
- Facilitates the pathological aggregation of other proteins (e.g. amyloid-β or tau)
- Promotes neuroinflammation
- Impairs microtubule formation and axonal transport
- Causes presynaptic dysfunction and abnormal neurotransmitter release

**Haematopoietic system**

- Lymphocyte
  - Possible functions outside the CNS (e.g. lymphocyte development)

**Mitochondrion**

- ER–Golgi network
- Autophagy-lysosomal system
- Neurofibrillary tangle
- Amyloid-β plaque
The Formation of Highly Soluble Oligomers of α-Synuclein Is Regulated by Fatty Acids and Enhanced in Parkinson’s Disease

Ronit Sharon, Ifat Bar-Joseph, Matthew P. Frosch, Dominic M. Walsh, James A. Hamilton, and Dennis J. Selkoe*
Center for Neurologic Diseases
Harvard Medical School
Brigham and Women’s Hospital
Boston, Massachusetts 02215

Accumulation of misfolded proteins as insoluble aggregates occurs in several neurodegenerative diseases. In Parkinson’s disease (PD) and dementia with Lewy bodies (DLB), α-synuclein (αS) accumulates in insoluble inclusions. To identify soluble αS oligomers that precede insoluble aggregates, we probed the cytosols of mesencephalic neuronal (MES) cells, normal and αS-transgenic mouse brains, and normal, PD, and DLB human brains. All contained highly soluble oligomers of αS whose detection was enhanced by delipidation. Exposure of living MES neurons to polyunsaturated fatty acids (PUFAs) increased αS oligomer levels, whereas saturated FAs decreased them. PUFAs directly promoted oligomerization of recombinant αS. Transgenic mice accumulated soluble oligomers with age. PD and DLB brains had elevated amounts of the soluble, lipid-dependent oligomers. We conclude that αS interacts with PUFAs in vivo to promote the formation of highly soluble oligomers that precede the insoluble αS aggregates associated with neurodegeneration.
Age-dependent accumulation of amyloid-like oligomers in LRRK2\textsuperscript{R1441G} knockin mutant mouse brain

(A) Age-dependent changes in total amyloid-like oligomer levels (Dot-blots)

(i) Dot blot sample distribution

(ii) Amyloid-like oligomer level (Soluble striatal lysates)

(iii) Total αSyn level (Soluble striatal lysates)

(iv) Total αSyn level

(v) Relative levels of A11 oligomer (normalized to total α-Syn)

(vi) Growth prediction of oligomer accumulation

Ho PWL, Leung CT, Lui HF, Pang SYY, Lam CSC, Xian JW, Li LF, Kung MHW, Ramsden DB, Ho SL
Age-dependent accumulation of α-synuclein oligomers in LRRK2\textsuperscript{R1441G} knockin mutant mouse brain

(i) Immunohistochemistry of α-synuclein oligomers

Ho PWL et al., Autophagy 2019.
Unwanted protein clearance

• Ubiquitin proteosome system
• Autophagy
  – Macroautophagy
  – Chaperone-mediated autophagy
  – Microautophagy
Different forms of autophagy process
Chaperone-mediated autophagy (CMA) pathway machinery

Xilouri M, Stefanis L. Mol Cell Neurosci 2015

- α synuclein
- LRRK2
Chaperone-mediated autophagy (CMA) pathway machinery
Xilouri M, Stefanis L. Mol Cell Neurosci 2015

(A) Physiological conditions
substrate protein

mutant LRRK2

mutant UCH-L1

wild-type or mutant alpha-synuclein

(B) Parkinson’s Disease

Lysosome

CMA substrates

aberrant alpha-synuclein species

Functions
a) protein synthesis
b) removal of damaged or aggregated-prone proteins
c) neuronal survival
CMA-specific LAMP2A accumulation in LRRK2<sup>R1441G</sup> knockin mutant mouse brain indicating CMA impairment

**LAMP2A (CMA substrate receptor)**

- Flow cytometry showing levels of LAMP2A staining in wildtype and LRRK2<sup>R1441G</sup> mice.
- Bar graph showing mean intensity of LAMP2A staining.

**GAPDH (CMA substrate)**

- Flow cytometry showing levels of GAPDH staining in wildtype and LRRK2<sup>R1441G</sup> mice.
- Bar graph showing mean intensity of GAPDH staining.

**Immunohistochemistry of LAMP2A in aged LRRK2<sup>R1441G</sup> mouse striatum**

- Images showing abnormal LAMP2A aggregation in the striatum of LRRK2<sup>R1441G</sup> mice compared to wildtype.

*Ho PWL et al., Autophagy 2019.*
A new method to monitor protein degradation in live cells - potential drug screening platform

Ho PWL et al., Autophagy 2019
Slower $\alpha$-synuclein degradation in LRRK2$^{R1441G}$ knockin mutant cells

Total cellular rate of protein clearance

**Target substrate to be degraded**

<table>
<thead>
<tr>
<th>CMA substrate</th>
<th>CMA recognition motif “VKKDQ”</th>
<th>CMA substrate</th>
<th>CMA recognition motif “KFERQ”</th>
<th>non-CMA substrate</th>
<th>Mutated “KFERQ” &gt; “KFSDA”</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) PAmCherry-SNCA-NE clearance</td>
<td>Wildtype MEFs</td>
<td>LRRK2$^{R1441G}$ mutant MEFs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B) PAmCherry-KFERQ-NE clearance</td>
<td>Wildtype MEFs</td>
<td>LRRK2$^{R1441G}$ mutant MEFs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C) PAmCherry-KFSDA-NE clearance</td>
<td>Wildtype MEFs</td>
<td>LRRK2$^{R1441G}$ mutant MEFs</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Total cellular clearance of PAmCherry-SNCA-NE protein**

- Wildtype MEFs
- LRRK2$^{R1441G}$ mutant MEFs

**Total cellular clearance of PAmCherry-KFERQ-NE protein**

- Wildtype MEFs
- LRRK2$^{R1441G}$ mutant MEFs

**Total cellular clearance of PAmCherry-KFSDA-NE protein**

- Wildtype MEFs
- LRRK2$^{R1441G}$ mutant MEFs

Ho PWL et al., Autophagy 2019
CMA protein degradation in our assay is mediated via LAMP2A & KFERQ

(A) siRNA-mediated knockdown of LAMP2A (mouse embryonic fibroblasts)

Mutagenesis of CMA recognition motif
"KFERQ" → "KFSDA" (KFERQ mutant):
1st & 2nd amino acid: unchanged
3rd amino acid: E (acidic) → S (polar)
4th amino acid: R (basic) → D (acidic)
5th amino acid: Q (polar) → A (hydrophobic)

Total LAMP2A levels (72hr post-transfection)

<table>
<thead>
<tr>
<th>siRNA</th>
<th>WT-Q</th>
<th>KI-Q</th>
<th>WT-mutQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAMP2A siRNA</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Scrambled siRNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LAMP2A (110kDa)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ACTIN (43kDa)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

MEF cell lines | WT-Q | KI-Q | WT-mutQ |
Lamp2a knockdown (% of control) | 73%  | 68%  | 46%     |

WT-Q: Wildtype MEFs (PAmCherry-KFERQ-NE)  
KI-Q: LRRK2[^1441G] MEFs (PAmCherry-KFERQ-NE)  
WT-mutQ: Wildtype MEFs (PAmCherry-KFSDA-NE)

(C) "KFERQ" motif is critical for LAMP2A-mediated CMA degradation

KFERQ: PAmCherry-KFERQ-NE as target substrate
KFSDA (mutated KFERQ): PAmCherry-KFSDA-NE as target substrate

(D) Total lysosomal activity assay after LAMP2A knockdown

Ho PWL et al., Autophagy 2019. In press
αSyn oligomer accumulation in LRRK2<sup>R1441G</sup> mutant neurons can be suppressed by CMA activator (AR7)

**Primary cortical neuronal culture**

(A) α-synuclein oligomer levels

**Long term (21 days) treatment of AR7 Protocol:**

- Primary cortical neurons
  - WT or R1441G
  - Conditioned medium
- +AR7 Day 9
- +AR7 Day 14
- +AR7 Day 21
- αSyn oligomer ELISA
- anti-αSyn
- HRP

**Wildtype**

Day 9: +AR7
Day 14: +AR7
Day 21: +AR7

**R1441G**

Day 9: +AR7
Day 14: +AR7
Day 21: +AR7

**(i)** Intracellular αSyn oligomer level (PBS-soluble lysates)

**(ii)** Extracellular αSyn oligomer level (Conditioned medium)

**AR7**

7-chloro-3-(p-tolyl)-2H-benzo[b][1,4]oxazine

Atypical retinoic acid receptor α antagonist

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*Ho PWL et al., Autophagy 2019*
Chaperone-Mediated Autophagy Markers in Parkinson Disease Brains

Lydia Alvarez-Erviti, PhD; Maria C. Rodriguez-Oroz, MS, PhD; J. Mark Cooper, PhD; Cristina Caballero, MD; Isidro Ferrer, MD; Jose A. Obeso, PhD, MD; Anthony H. V. Schapira, MD, DSc, FRCP, FMedSci

**Objective:** To investigate chaperone-mediated autophagy in the pathogenesis of Parkinson disease (PD).

**Design:** Postmortem observational study.

**Setting:** University Department of Clinical Neuroscience, Institute of Neurology, University College London.

**Subjects:** Postmortem samples from 7 PD, 6 Alzheimer disease (AD), and 8 control brains.

**Main Outcome Measure:** Lysosomal-associated membrane protein 2A (LAMP2A) and heat shock cognate 70 (hsc70) protein levels were compared in the substantia nigra pars compacta and amygdala of PD, AD, and control brain samples. To provide insight into the turnover of α-synuclein, degradation pathways for this protein were studied in a dopaminergic cell line.

**Results:** The expression levels of the chaperone-mediated autophagy proteins LAMP2A and hsc70 were significantly reduced in the substantia nigra pars compacta and amygdala of PD brains compared with age-matched AD and control brain samples. Lewy bodies in these regions contained autophagy-related proteins. We demonstrated that decreased LAMP2A levels in dopaminergic cell lines reduced chaperone-mediated autophagy activity and increased the half-life of α-synuclein.

**Conclusions:** These findings suggest that there is reduced chaperone-mediated autophagy activity in the PD brain, provide evidence for the role of autophagy in PD pathogenesis and Lewy body formation, and suggest that this pathway may be a suitable therapeutic target in PD.

Summary

- Ideal animal model of PD does not currently exist
- Best model depends on the question being asked
- Current models: toxin, gene-based or combination
- Various limitations of current models
- Aging should be involved in the model
- Current approach: integrate findings from various models to form a clearer & bigger picture, with the aim of elucidating pathogenesis, biomarkers & potential disease modifiers in PD
Summary: \( \text{LRRK2}^{R1441G} \) knockin mutant mice

- ↑brain & striatal α-synuclein oligomers with aging
- Age-related ↑α-synuclein oligomers & other substrates (eg, GAPDH) in part due to ↓protein degradation & ↓CMA
- Developed a new assay to quantify the rate of protein degradation
- ↓CMA-specific protein degradation in our assay was mediated via LAMP2A & KFERQ
- Specific CMA activation can ↓accumulation of α-synuclein oligomers → potential therapeutic target in PD