Getting involved in Parkinson’s disease

DEVELOPING AND EXPLOITING C. ELEGANS MODEL FOR PARKINSON’S DISEASE

Supported by the PD society

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DNA damage check-point pathway of C. elegans
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The long way to Dundee

Imp, Vienna, Austria

Cold Spring Harbor, New York

MPI, Munich, Germany

University of Dundee
Talk

- Brief definition of Parkinson’s Disease (PD)

- Human Parkinson’s Disease genetics
  
  *Human genetics*

- Exploring *C. elegans* as a model for Parkinson’s Disease
Why is it so difficult to give this talk

- I have family members affected by neurodegenerative diseases
- Hope for a cure, follow up on internet and literature
- Why is it so difficult to find a cure against neurodegenerative disease as opposed to “say infectious diseases” eg. “antibiotics”
- Why is basic science so important and relevant to disease models even if these models do not appear to be directly connected to affected patients at the first glance.
- Please bare in mind that I am not a medical doctor
Parkinson’s Disease (PD): motor symptoms

- Clinical signs:
  - resting tremor
  - bradykinesia
  - rigidity

Parkinson J. (1817) An essay on the shaking palsy, Sherwood, Neely and Jones, London
- Frequent neurodegenerative disease: 2% population > 65 years

- No curative or preventive treatment

- Effective symptomatic treatment (L-Dopa)

- Largely unknown ethiology
Neuronal loss in the **Substantia Nigra**

**Neuropathology:**
death of the neuromelanin containing neurons of the **Substantia Nigra**

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*Brissaud E. (1895) Leçons sur les Malades Nerveuses*

*Tretiakoff C. (1919) Thesis*
Lewy Bodies are aggregates of:
- organelles
- proteins

- Not present in all parkinsonian patients

- Found in other neurodegenerative diseases:
  Synucleinopathies (DLB, LBAD; MSA)

Dopaminergic death / L-dopa treatment

- Dopamine deficit in the striatum caused by the progressive and selective death of the dopaminergic neurons of the Substantia Nigra

- Symptomatic treatment: L-Dopa (levodopa)

Fundamental Problem:

How to prevent death of dopaminergic neurons?

we ideally want to take a system apart, see what is wrong
fix it and reassemble again, eg broken car

Why is this so difficult for PD compared to infectious disease:

symptoms appear rather late

hard to determine what is cause and consequences

hard to identify molecules involved in PD Disease

hard to look into the brain of patients

hard to find model systems
How can we start to address what is wrong in PD disease? (taking advantage of rare disease variants)
PD patients entering the clinic

FAMILIAL
(rare)

SPORADIC
(no family history)
(most common)

AUTOSOMAL RECESSIVE (AR)
Early Onset

AUTOSOMAL DOMINANT (AD)

“NOT GENETIC”
## Mutations in 6 genes cause Parkinson’s Disease

<table>
<thead>
<tr>
<th>Locus</th>
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<th>Gene</th>
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<td>Dominant</td>
<td>± dementia</td>
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<td>12-14</td>
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</table>
How can we start to address what is wrong in PD disease? (taking advantage of rare disease variants)
• The Case of the Frozen Addicts: How the solution of an extraordinary medical mystery spawned a revolution in the understanding and treatment of Parkinson's disease


• This book dramatically recounts the discovery of the cause of a local outbreak of sudden, severe parkinsonism in a group of young adults in northern California and how this discovery led to greater insight into Parkinson's disease. Langston is the Bay Area neurologist who reported the event and led a team of investigators to pinpoint the toxicant responsible for the acute loss of dopamine-containing neurons in the substantia nigra, causing the parkinsonism in these patients. The story unfolds and builds suspense as Langston and his colleagues determine that the toxicant is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP was the unwanted product resulting from INTOXICATION

Toxins like MPTP and 6-OHDA are directly imported into dopaminergic neurons and cause their degeneration
Why do we use model organisms?

C. elegans worms as a model for Parkinsons disease?
Nobel Prices for medicine on “worm” research?

2002
Sydney Brenner
John Sulston
Bob Horvitz

2006
Craig Mello
Andy Fire
Life cycle of *Caenorhabditis elegans*

The newly hatched larva contains all somatic cells of the adult. The gonads develop during the four larval stages L1 to L4.
Why worms as a model system?

- Simple
- Cheap to maintain and easy to propagate
- Amenable to genetic analysis (hermaphrodites/males)
- Short generation time
- Can be frozen away
- Simple, invariant development
- Complete wire diagram of the worm nervous system is available
- Fully sequenced, more than 50% of worm genes have human homologs
C. ELEGANS AS A MODEL FOR DOPAMINERGIC DEGENERATION AND PARKINSON’S DISEASE
**C. elegans**: a relevant and useful model for PD

- For studying the central nervous system:
  
  more than 50% of mammalian genes have orthologs in *C. elegans*
  
  many of the same neurotransmitters as in mammals
  
  302 neurons (vs. 1,000,000,000,000 in humans)
  
  all neuronal connections have been determined

  - For Parkinson’s Disease:
    
    similar dopamine metabolism
    conserved enzymes and transporters
    8 dopaminergic neurons in hermaphrodites
    
    how does *C. elegans* PD look like?

  - To mimic dopaminergic death:
    
    conservation of genes involved in PD
    sensitivity and specificity of toxin
C. ELEGANS
Dendritic part of *C. elegans* dopaminergic neurons
Most human PD genes are conserved in *C. elegans*

<table>
<thead>
<tr>
<th>Gene</th>
<th><em>C. elegans</em> gene</th>
<th>Strain</th>
<th>Function</th>
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<tbody>
<tr>
<td>SNCA</td>
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<td>tm1898</td>
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BY200: a strain with GFP dopaminergic neurons


assay dopaminergic degeneration

# Mutations in the α-SYNUCLEIN Gene

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<td>?</td>
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Increased Severity with SNCA Copy Number

**DOSE EFFECT:**

TRIPLICATION – diffuse Lewy Body disease
DUPPLICATIONS – typical PD

From Ibanez et al. in preparation
6-OHDA: a toxin for dopaminergic death

- BY200
- 6-OHDA + imipramine


- BY200 x ΔDAT-1
- 6-OHDA

Studying *PINK1* kinase in *C. Elegans*

*PINK1*: kinase genes
pink-1(tm1779) *C. Elegans* strains

- Are gene deletions in *C. elegans* associated with any phenotype?

- Do these genes protect dopaminergic neurons?
No overt phenotype upon 6-OHDA intoxication

- Preliminary results indicate no overt increase in 6-OHDA mediated neurodegeneration in pink-1(PINK1) and Irk-1 (LRRK2) knockouts
- pink-1/Irk-1 strains have to be tested

Louise Chapman
Neurotoxic intoxication to reveal susceptibility

Challenge worms with dopaminergic neurotoxin regimes:

- 6-OHDA (Nass et al., 2002)
- 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)
- Rotenone
- Paraquat

Look for factors (genes) that lead to enhanced or suppressed neurodegeneration
- Genetic screen for hypersensitivity to dopaminergic toxins
Screening for 6-OHDA Hypersensitivity or Resistance

Pdat-1::GFP worms mutagenesis

6-OHDA intoxication

Screen under low magnification fluorescence microscope for candidate mutants

Isolation, phenotype confirmation in subsequent generation and after back crosses
6-OHDA Resistance and Hypersensitivity Screens

Highly toxic 6-OHDA doses (50mM):

- Wild type
- Resistant

Non-toxic 6-OHDA doses (10mM):

- Wild type
- Hypersensitive

<table>
<thead>
<tr>
<th></th>
<th>Resistant</th>
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<td>10</td>
<td>5</td>
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<td>-</td>
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<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
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</table>
Mutagenised Pdat-1::GFP worms are on N2 background.
Back crossing aims at reducing the number of mutations in the genome.

- PCR
- Digest
- RFLP analysis

100% WT DNA

0% WT DNA

Mutant chromosome

WT chromosome

mutation

Hawaii chromosome

Cross

F1

wild type phenotype

Self-fertilization

F2

wild type phenotype

Select mutant phenotype

Recombination
The *gt1681* Mutant Is Hypersensitive to 6-OHDA (Loss of Dopaminergic Neurons)

![Graph showing the effect of 6-OHDA on wild-type and mutant worms. The graph illustrates the percentage of worms showing complete loss, partial loss, and no loss at different dosages of 6-OHDA.](image)
Rescue of the *tg1681* 6-OHDA Hypersensitivity by a TSP-17 Encoding Fosmid
Alignment of *C. elegans* tsp-17 with Orthologues
Where does TSP-17 act?

Evidence for TSP-17 acting in dopaminergic neurons
tsp-17 is expressed in the dopaminergic neurons

Pdat-1:: yfp:: 3’let858
Ptsp-17:: tsp-17::cfp:: 3’tsp-17
merge

pdat-1:: mcherry:: 3’let858
ptsp-17:: tsp-17::gfp:: 3’tsp-17