Molecular Pathology of Neurodegenerative Diseases

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Course:
Recent Advances in Neurodegenerative Disorders

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Loss of Brain Neurons With Ageing in Parkinson's and Alzheimer’s Diseases

1. Normal aging
2. Disease states; Parkinson’s and Alzheimer’s diseases
Life expectancy at birth in the United States in 1901 was 49 years. At the end of the 20th century it was 77 years, an increase of greater than 50%. Similar gains have been observed throughout the world.

Life expectancy in India and The People's Republic of China was around 40 years at mid-20th century. At century's close it had risen to around 63 years.
The prevalence of Alzheimer's disease (AD) and Parkinson’s disease (PD) of the adult population over 60 years of age is ~10% and ~2%, respectively.
Every five years after the age of 65, the risk of acquiring the disease approximately doubles, increasing from 3 to as much as 69 per thousand person years.

<table>
<thead>
<tr>
<th>Age</th>
<th>Incidence (new affected) per thousand person–years</th>
</tr>
</thead>
<tbody>
<tr>
<td>65–69</td>
<td>3</td>
</tr>
<tr>
<td>70–74</td>
<td>6</td>
</tr>
<tr>
<td>75–79</td>
<td>9</td>
</tr>
<tr>
<td>80–84</td>
<td>23</td>
</tr>
<tr>
<td>85–89</td>
<td>40</td>
</tr>
<tr>
<td>90–</td>
<td>69</td>
</tr>
</tbody>
</table>
Neurodegenerative disorders

Neurodegeneration

**Stroke:**
- Oxygen deprivation
- Glucose deprivation
- Glutamate/neurotoxins release

**Traumatic brain and spinal-cord injury:**
- Physical damage
- Glutamate/neurotoxin release
- Ischemia

**Aging Malnutrition**

**Alzheimer’s disease:**
- β-amyloid
- Presenilins
- Apolipoprotein E

**Parkinson’s disease:**
- α-synuclein, Parkin, LRRK2 etc
- Toxins (rotenone, iron)

**Huntington’s disease:**
- Huntingtin (polyglutamine)

**Amyotrophic Lateral Sclerosis (ALS)**
- Mitochondrial membrane permeabilization
- Synaptic dysfunction
- Neuritic degeneration
- Neuronal death

**Oxidative stress**
**Metabolic impairment**
**Ion dyshomeostasis**
**DNA damage**

**Decline in growth factors levels**

- Mitochondrial (complex I deficiency)
- Ubiquitin-proteasome system dysfunction
- Abnormal protein folding and aggregation

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AN ESSAY ON THE SHAKING PALSY.

BY JAMES PARKINSON, MEMBER OF THE ROYAL COLLEGE OF SURGEONS.

LONDON:
PRINTED BY WHITTINGHAM AND HOWLAND,
Goswell Street,
FOR SHERWOOD, NEELY, AND JONES,
Paternoster Row.
1817.
Wilhelm von Humboldt

“You have seen ... that my handwriting ... has become larger, more distinct and legible ... it is a victory of my will power over my hand ... Thus in ageing one comes back to childhood's writing, because indeed childish are these large [Latin] letters without connecting parts and the lines ... that it is better to have the letters attached to the top line ...”

Tegel, April 15, 1834
Parkinson’s disease

“Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards and to pass from a walking to a running pace: the senses and intellect being uninjured.”

James Parkinson, “An assay on the Shaking Palsy”, 1817

<table>
<thead>
<tr>
<th>A</th>
<th>Parkinson’s disease</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Normal SN pars-compacta</td>
<td>SN pars-compacta in PD</td>
</tr>
<tr>
<td>C</td>
<td>Lowy body in a SN neuron</td>
<td></td>
</tr>
</tbody>
</table>

Substantia nigra degeneration in PD and Lewy bodies.

From: Silvia Mandel¹, Ilan Halperin², Amos D Korczyn,³ and Micaela Morelli⁴
Neuroimaging with FD-PET assesses the capacity of dopaminergic neurons to decarboxylate and store levodopa/dopamine.

CIT SPECT reflects the density of dopamine transporters (DATs) on presynaptic dopamine terminals.


Figure 2. Neuroimaging Scans in Normal Controls and Patients With PD

Top, fluorodopa with positron emission tomography in healthy controls and patients with PD (provided courtesy of and with permission from David Brooks, MD). Bottom, 2-carbomethoxy-3-[4-iodophenyl]tropane (CIT) with single-photon emission computed tomography uptake in healthy controls and patients with Parkinson disease (provided courtesy of and with permission from Ken Marek, MD). Note that in PD, striatal uptake of these markers is asymmetrically reduced, more so in the posterior portion of the putamen. Note also that with advancing disease, uptake of these indices is further reduced, which can be quantified and serve as a surrogate marker of disease progression.
more than 90% of PD-patients show hyperechogenicity of the SN compared to healthy individuals and this could be related in part to an increased iron content of the SN.

Neurodegenerative disorders

Braak hypothesis

Periphery

Sense of smell One of the first areas of the Central Nervous System affected in the first stages of PD is the olfactory bulb and there is indeed evidence that the sense of smell is often affected before the motor symptoms of PD appear.

Lewy Bodies are observed to occur in the sympathetic ganglia, around the heart, in the pelvic plexus and adrenal medulla in PD.
The course of the disease can be subdivided into presymptomatic and symptomatic phases

Presymptomatic phase:  Autonomic deficit in the vagus

Symptomatic phase:
- Olfactory bulb, loss of sense of smell
- Predisposition to depression, with loss of serotonergic and noradrenergic neurons of raphe nucleus and locus ceruleous
- Parkinsonism with mid brain lesion of substantia nigra dopamine neurons
- Dementia, with cognitive deficit resulting from prefrontal cholinergic loss

Technion
The course of the disease can be subdivided into presymptomatic and symptomatic phases.
Clinical evidences about premotor phase

The clinical observations of premotor symptoms of PD are related to the novel neuropathological concept of emerging neurodegeneration, which starts in the enteric system and then rises via spinal cord and brainstem to nigral and subsequent cortical neurons.

Lewy Bodies (LBs) are observed in the heart, the pelvic plexus and adrenal medulla in PD.
Stage 1

Dorsal motor nucleus of the vagal nerve in the medulla oblongata.

dm X dorsal motor nucleus of the vagal nerve
irz intermediate reticular zone
LNs (arrowheads)

PD lesions in the Auerbach plexus of the enteric nervous system. (esophagus)

Start of LB pathology
LNss
mature LB
Stage 3

Dopaminergic melanoneurons in the substantia nigra, pars compacta

H Multiple LBs (arrows) Fill a nerve cell directly next to a healthy melanoneuron.
I–M Particulate α-synuclein (small arrows) aggregates to form LBs (larger arrows).
K, compare the normal melanoneuron, without α-synuclein particles, to the one right.
L two astrocytes have ingested α-synuclein-immunoreactive material plus neuromelanin (asterisk). The term “extraneuronal” neuromelanin can be applied to such material.
N In this nerve cell, it is difficult to distinguish the LB (arrow) from the surrounding α-synuclein particles.
O Here, a group of neuromelanin-containing macrophages mark the shape of the former nerve cell.
In these stages, patients manifest the full range of PD associated clinical symptoms. Severe damage to the autonomic, limbic, and somatomotor systems that began in the presymptomatic phase can become compounded by supervening functional deficits on the part of the cerebral cortex.
Cognitive dysfunction

- The present state of knowledge leads to the hypothesis that it is the **cumulative load of Lewy body** pathology that contributes to the development of cognitive decline.
- The brain pathology that develops involves first the **aminergic** then the **dopaminergic** and then the **cholinergic systems** and increases in severity with no signs of remission.
• Although Braak staging suggests that Lewy body formation begins in the medulla and dorsal nucleus of the vagus spreading upwards, dysfunction is consistently greatest in the ventrolateral nigral-dorsal putamen dopaminergic system.

• It would seem, therefore, that there is discordance between Braak staging and imaging observations suggesting that the presence of Lewy bodies does not necessarily equates to neuronal dysfunction.
Thus not only the disease is tissue progressive but also the process of neurodegeneration. This is characterized by the loss of melanin-containing dopamine neurons.

What is the cause?
Etiology of PD

1. Environmental factors
2. Gene factors, epigenetics
3. Environment-gene interactions

Pathogenesis of PD

1. Mitochondrial Dysfunction and Oxidative Stress
   • Abnormalities in complex I activity in PD may subject cells to oxidative stress and energy failure.
   • Increases production of the ROS superoxide, which may form toxic hydroxyl radicals or react with nitric oxide to form peroxynitrite. These molecules may cause cellular damage by reacting with nucleic acids, proteins, and lipids.
   • One target of these reactive species may be the electron transport chain itself, leading to mitochondrial damage and further production of ROS.
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2. Misfolding and Aggregation of Proteins
   - The abnormal deposition of protein in brain tissue is a feature of several age-related neurodegenerative diseases, including PD.
   - Although the composition and location (i.e., intra- or extracellular) of protein aggregates differ from disease to disease, this common feature suggests that protein deposition per se, or some related event, is toxic to neurons.
   - The presence of ROS would increase the amount of misfolded proteins, increasing the demand on the ubiquitin-proteasome system to remove them.
<table>
<thead>
<tr>
<th>Locus</th>
<th>Gene/Protein</th>
<th>Chromosome location</th>
<th>Inheritance</th>
<th>Suggested function</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK 1/4</td>
<td>SNCA/ α-synuclein</td>
<td>4q21-q23</td>
<td>AD</td>
<td>Synaptic function/vesicle trafficking</td>
</tr>
<tr>
<td>PARK 2</td>
<td>parkin</td>
<td>6q25-q27</td>
<td>AR</td>
<td>E3 ubiquitin ligase</td>
</tr>
<tr>
<td>PARK 5</td>
<td>UCH-LI</td>
<td>4p14</td>
<td>AD</td>
<td>Ubiquitin C-terminal</td>
</tr>
<tr>
<td>PARK 6</td>
<td>PINKI</td>
<td>1p35-p36</td>
<td>AR</td>
<td>Mitochondrial serine/threonine kinase</td>
</tr>
<tr>
<td>PARK 7</td>
<td>DJI</td>
<td>1p36</td>
<td>AR</td>
<td>Chaperone, oxidative stress response</td>
</tr>
<tr>
<td>PARK 8</td>
<td>LRRK2/dardarin</td>
<td>12p11.2-q13.1</td>
<td>AD</td>
<td>Protein kinase</td>
</tr>
<tr>
<td>PARK 9</td>
<td>ATP13 A2</td>
<td>1p36</td>
<td>AR</td>
<td>Lysosomal type 5 ATPase</td>
</tr>
<tr>
<td>PARK 13</td>
<td>OMI/HTRA2</td>
<td>2p12</td>
<td>AD</td>
<td>Serine protease</td>
</tr>
</tbody>
</table>
Alzheimer’s Disease

It is the 4th major cause of death in the developed world.

Age is the most important risk factor for AD; the number of people with the disease doubles every 5 years beyond age 65.

AD is the most common basis for senile dementia accounting for about 2/3 of the dementia cases.

Dementia - it is an acquired impairment in intellectual abilities, which result in a restriction in daily activities.

Dementia affects: memory, language, cognition, emotion, personality
The defining characteristic of AD

AD is characterized by:
Progressive loss of cognitive abilities, caused probably by the early onset of synaptic degeneration and the subsequent loss of neurons (whereby cholinergic neurotransmission is impaired at a particularly early stage).

Cognitive symptoms
memory loss, language deterioration, impaired ability to mentally manipulate visual information, poor judgment, confusion, restlessness

Mood and behavioral symptoms such as:
Depression, Anxiety
Irritability,
Inappropriate behavior
Sleep disturbance
Psychosis, Agitation
Areas of the brain affected in AD

Intelligence, judgment, and behavior

Language

Memory
Brain atrophy - comparison between normal and diseased brain

A. The brain of a normal elderly person

B. The brain of a person with Alzheimer's disease

The clinical features of AD are accompanied by characteristic histological changes in the brain.
Pathologically AD is defined by:

1. Neuronal loss
2. Extracellular insoluble deposition of amyloid or senile plaques (composed mainly of Aβ)
3. Intracellular lesions: neurofibrillary tangles (composed mainly of hyperphosphorylated microtubule associated protein, tau)
Tau in healthy neurons and disease
APP (Amyloid Precursor Protein) processing

Amyloidogenic derivatives:
- sAPPβ
- Aβ
- β− CTF

Non-amyloidogenic derivatives:
- sAPPα
- α−CTF
Amyloid β-protein generation by normal proteolytic processing of β-amyloid precursor protein.
Amyloidogenic processing of β-amyloid precursor protein (APP) by β-site APP-cleaving enzyme (BACE) and the γ-secretase complex. In this pathway, full-length APP (left) is first processed by BACE, and the large ectodomain is secreted. The remaining membrane retained stub (CTFβ) binds to a docking site on the surface of the γ-secretase complex and is then transferred to the active site that includes transmembrane domains 6 and 7 of presenilin-1 (PS1) or PS2. PS1 and PS2 are both activated by presumed autoproteolytic cleavages, which create their N- and C-terminal fragments (NTF and CTF). These bind to each other and also to 3 other essential γ-secretase components, APH1a (or APH1b), PEN2 and nicastrin (NCT). All four proteins form the core complex required for γ-secretase activity (shown in dashed box). The two intramembrane aspartate residues in the NTF and CTF of presenilins (marked with a D) are a crucial part of the unusual catalytic site of the protease. The γ-secretase cleavage occurs in the middle of the membrane and liberates amyloid β-protein (Aβ) and the APP intracellular domain (AICD). The function of the AICD is unclear. b | Various proposed sites of intramembrane proteolysis by γ-secretase. The amino-acid sequence around the cleavage sites of APP is shown (numbers refer to the sequence of Aβ; shaded amino acids are in the transmembrane domain). γ-secretase cuts its substrates several times. The cleavage sites are referred to as ε, ζ and γ (from the C- to N-terminal). The γ-site is variable and can occur at least after amino acids 38, 40 and 42. This cleavage is highly relevant for the subsequent aggregation propensity of Aβ. Some γ-secretase-modifying drugs shift the cleavage at Aβ42 to amino acid 38, and the resultant peptide aggregates much less readily.
• **Diffusible soluble Aβ oligomers**
  (principal role most probably during the earliest, even presymptomatic stages of AD process)

vs

• **Fibrillary Aβ in plaques**
  (might contribute to surrounding neuronal injury)

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**Synaptic dysfunction**

It is possible that soluble Aβ oligomers bind to synaptic plasma membranes and interfere with the complex system of receptor and/or channel proteins and signalling pathways that are required for synaptic plasticity: NMDA (N-methyl-d-aspartate) or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole) receptors at synaptic plasma membranes in a manner that allows an initial LTP response but not its persistence.
Soluble Oligomers in Other Disorders

Soluble proteins of entirely different sequences can fold into β-sheet-rich structures:

• Parkinson’s disease
  - oligomeric assemblies of α-synuclein.
  Protofibril formation is accelerated by α-synuclein mutations that cause early onset PD; the A30P α-synuclein mutation promotes the formation of spherical PFs.

• Huntington’s disease
  Annular structures that are composed of polyglutamine (for example, the aggregation-causing repetitive sequence in huntingtin).

• Frontotemporal dementia and AD
  Microtubule associated protein, tau, which can form soluble oligomers.
Overview of the established Alzheimer’s genes and their functional relevance

<table>
<thead>
<tr>
<th>Gene (protein)</th>
<th>Chromosomal location</th>
<th>Mode of inheritance</th>
<th>Number of pathogenic mutations (affected families)</th>
<th>Mean familial onset age (range)</th>
<th>Relevance to AD pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP (Aβ-precursor protein)</td>
<td>21q21.3</td>
<td>Autosomal-dominant</td>
<td>15 (99)</td>
<td>51.5 years (55–60)</td>
<td>Increase in Aβ (Aββ/β)-levels; mutations close to γ-secretase site</td>
</tr>
<tr>
<td>PSEN1 (presenilin 1)</td>
<td>1q24.3</td>
<td>Autosomal-dominant</td>
<td>128 (33%)</td>
<td>44.1 years (24–68)</td>
<td>Increase in Aβ (Aββ/β)-levels; essential for γ-secretase activity</td>
</tr>
<tr>
<td>PSEN2 (presenilin 2)</td>
<td>1q21.42</td>
<td>Autosomal-dominant</td>
<td>7 (15)</td>
<td>57.1 years (46–71)</td>
<td>Increase in Aβ (Aββ/β)-levels; essential for γ-secretase activity (?)</td>
</tr>
<tr>
<td>APOE (apolipoprotein E, e4 allele)</td>
<td>19q13.22</td>
<td>Complex (risk increase)</td>
<td>n.a.</td>
<td>Onset-age modifier</td>
<td>Increase in Aβ-aggregation; via lipid/cholesterol transport (?)</td>
</tr>
</tbody>
</table>

Bertram and Tanzi Pharmacological Research 50:385-396, 2004
Increased Localized Brain Ferritin and Iron In Neurodegenerative Diseases

- Parkinson’s disease:
  - Substantia nigra pars compacta;
  - Melanized dopamine neurons, reactive microglia, oligodendrites, astrocytes
- Juvenile Parkinson’s disease (Parkin)
- Alzheimer’s disease
- Huntington Chorea (huntingtin)
- ALS
- Multiple Sclerosis
- Hallervorden-Spatz disease
- AIDS
- Multiple sclerosis
- Freidreich ataxia (Frataxin)
- Tardive dyskinesia?
- Aceruloplasminemia
- Mad Cow disease

Major levels of iron operation in AD & PD:

1. **Free iron induces oxidative stress**, because of its interaction with hydrogen peroxide (Fenton reaction), resulting in an increased formation of hydroxyl free radicals.

2. **Iron facilitates α-syn/Aβ protofibrils aggregation/fibrillization and toxicity**, as well as aggregation of hyperphosphorylated \( \tau \) (PHF\( \tau \)).

3. **Iron enhances endogenous APP translation and subsequent Aβ formation**.

4. **Activates the iron-dependent hypoxia-inducible factor (HIF)-1 prolyl-4-hydroxylase enzyme**, resulting in destabilization of HIF and concomitant decreased expression of various pro-survival genes.
Iron regulation of APP translation via a functional iron responsive element (IRE)

The first molecular biological support for the nature of APP as a metalloprotein has come from the studies of Rogers and coworkers (Rogers et al., 2002a) who described the existence of a functional iron responsive element (IRE-type II) in the 5' untranslated region (UTR) of APP mRNA encoding the Alzheimer's APP (+51 to 94 from the 5'-cap site).
Structural similarity in the placement of translational regulatory elements in the 146 nucleotide APP-mRNA 5'UTR and the L- and H-ferritin mRNA 5'UTRs. An overlapping IRE-like sequence is present upstream of IL-1 responsive acute box translational enhancers in APP-mRNA.

Ferroxidase (330-345)

- APP-mRNA
- IRE homology
- +101 +146 Functional Acute box

Ferroxidase (330-345)

- α-Synuclein-mRNA
- IRE homology
- +101 +146 Functional Acute box

Ferroxidase (56-70 H-subunit)

- L and H Ferritin mRNAs
- IRE
- +20 +53 +74 Acute box
- +199 H-Ferritin mRNA acute box
- IRE
- +20 +64 +139 Acute box
- +142 L-Ferritin mRNA acute box
Iron-induced neurodegeneration in AD via transcriptional activation of APP mRNA and suppression of hypoxia-inducible genes.