Genetic Mutations in Parkinson’s disease – LRRK2 Biology

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**Parkinson’s disease – Sporadic and Familial**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mode of inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-synuclein</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Parkin</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>UCH-L1</td>
<td>Unclear, dominant?</td>
</tr>
<tr>
<td>PINK-1</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>DJ-1</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>LRRK2</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>ATP13A2</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>FBX07</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>VPS35</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>eIF4G1</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>DNAJC6</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>SYNJ1</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>DNAJC13</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>CHCHD2</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>GIGYF2</td>
<td>Unclear, dominant?</td>
</tr>
<tr>
<td>Omi/HtrA2</td>
<td>Unclear, Susceptibility gene?</td>
</tr>
<tr>
<td>PLA2G6</td>
<td>Unclear, Susceptibility gene?</td>
</tr>
<tr>
<td>BST1</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>GAK</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>GBA</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>HLA-DRB5</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>LRRK2</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>MAPT</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>NR4A2</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>POLG</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>SCA2,3,8,17</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>Synphilin-1</td>
<td>Susceptibility gene</td>
</tr>
<tr>
<td>a-synuclein</td>
<td>Susceptibility gene</td>
</tr>
</tbody>
</table>

~85% of PD is Sporadic without any known familial inheritance  
~15% of PD is Familial (inherited)

Familial Mutations are in genes that are ubiquitously expressed, often at concentrations much higher than in DA neurons or the brain.  
Example: one of the highest concentrations of $\alpha$-syn is in red blood cells.

**Autosomal Dominant:** 1 copy of gene mutated, **Autosomal Recessive:** 2 copies of mutant gene

**Time Line**
1976 – MPTP Intoxication  
1999 – first genetic cause of PD described – A53T mutation in $\alpha$-Syn

Due to compensation of the DA system during development, conditional adult Tg and KO are required to observe PD pathology or phenotypes.
### Autosomal Recessive PD (ARPD)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parkin (PARK 2)</strong></td>
<td>RING-Type E3 ligase. Remains in an autoinhibited state until activated. Is recruited to OMM during mitochondrial damage where it ubiquitinates OMM proteins and autophagy adaptors involved in mitophagy. Ubiquitinates substrates such as PARIS, Miro, and AIMP2, key factors involved in mitochondrial biogenesis, mitochondrial transport, and parthanatos, respectively.</td>
</tr>
<tr>
<td><strong>PINK1 (PARK 6)</strong></td>
<td>Serine/threonine kinase. Localizes to the OMM in depolarization conditions where it phosphorylates a number of substrates including parkin, ubiquitin, and autophagy adaptors involved in mitophagy. Phosphorylates Miro, a key protein in mitochondrial trafficking. It also phosphorylates PARIS priming it for ubiquitination by parkin. May aid in mitigating oxidative stress by regulating calcium efflux from the mitochondria.</td>
</tr>
<tr>
<td><strong>DJ-1 (PARK 7)</strong></td>
<td>Endogenous redox sensor that self-oxidizes in oxidative stress conditions.</td>
</tr>
<tr>
<td><strong>ATP13A2 (PARK 9)</strong></td>
<td>5 P-Type ATPase. Localizes to lysosomal and late-endosomal membranes. Mutations in ATP13A2 cause lysosomal dysregulation as well as reduced autophagic flux and mitochondrial clearance. Thought to be involved in the oxidative stress response through the regulation of cations, such as Zn²⁺.</td>
</tr>
<tr>
<td><strong>FBXO7 (PARK 15)ᵃ</strong></td>
<td>Member of the Skp1-Cullin-F-Box type E3 ligase. Works with PINK1 to recruit parkin to OMM in mitochondrial depolarization conditions. Is required for Mfn1 ubiquitination and subsequent recruitment of autophagy machinery to the mitochondria.</td>
</tr>
<tr>
<td><strong>DNJC6 (PARK 19)ᵃ</strong></td>
<td>Encodes Auxilin, a member of the Hsp40 chaperone family. Works in tandem with SYNJ1 to recycle synaptic vesicles.</td>
</tr>
<tr>
<td><strong>SYNJ1 (PARK 20)ᵃ</strong></td>
<td>Encodes synaptotagmin-1, a phosphoinositide phosphatase. Works in tandem with DNAJC6 to recycle synaptic vesicles.</td>
</tr>
<tr>
<td><strong>PLA2G6 (PARK 14)ᵃ</strong></td>
<td>Calcium-independent phospholipase A2, group VI. Mutations in PLA2G6 cause aberrant ER calcium signaling which may impede mitophagy and the oxidative stress response.</td>
</tr>
</tbody>
</table>

(2017) PMID: 28445716
Autosomal Dominant - $\alpha$-Synuclein

- Point mutations in a single amino acid at A30P, E46K or A53T
- Duplication and triplication are risk factors.
- Can self-aggregate/oligomerize and fibrillize, part of the synuclein protein family (β- and γ-synuclein)
- May modulate synaptic plasticity and dopaminergic neurotransmission (vesicle release)
- Major component of Lewy bodies and neurites (the pathological hallmark of PD)
Autosomal Dominant- VPS35

Vacuolar protein sorting-associated protein 35 is the largest protein in the multimeric retromer complex involved in protein transport from endosomes to the trans-Golgi network.

- VPS35 ablation in dopaminergic neurons results in PD-associated deficits in mice, such as α-synuclein accumulation, loss of dopamine neurons and motor impairment that are reversed by overexpression of VPS35. PMID: 25533483, 26321632.

- VPS35/retromer is responsible for endosome-to-golgi retrieval of lysosome-associated membrane glycoprotein-2a (Lamp2a) mediates α-synuclein degradation via chaperone-mediated autophagy (CMA). Thus, VPS35 deficits can lead to accumulation of α-synuclein.

- VPS35 may increase LRRK2 mediated Rab phosphorylation. PMID: 29743203

- VPS35 has a role in the lysosomal degradation of parkin substrate aminoacyl tRNA synthetase complex-interacting multifunctional protein 2 (AIMP2), of which accumulation leads to poly(ADP-ribose) polymerase-1 (PARP1)-dependent cell death. PMID: 28383562
LRRK2 (leucine-rich repeat kinase 2)

**Familial:** Mutations in the LRRK2 gene are the most common cause of late onset PD with clinical and neurochemical overlap with idiopathic disease including α-syn pathology.

**Sporadic PD:** LRRK2 mutations occur in high frequency, from 1%-7% of PD patients of European origin and 20%-40% of PD in Ashkenazi Jews and North African Arabs. Major risk factor for PD.

Mutations increase kinase activity and neurodegeneration is kinase dependent – viral and transgenic mouse models.

Enriched in ribosomes. Also at synaptic vesicles, lysosomes, golgi, cytoskeleton.

Suggested action on Rab GTPases (Rab 10, Rab 35 ..) may contribute to α-syn propogation via VPS35.
LRRK2 is widely expressed

1. Brain
2. Lung
3. Heart
4. Kidney
5. Gut
6. Muscle
7. Skin
8. Blood
It’s role in Inflammation and innate immunity, LRRK2 is also linked to:
Inflammatory Bowel disease
Leprosy
Crohn’s disease

Although mechanistic insight into how LRRK2 modulates the inflammatory response is in its infancy, two major inflammatory pathways (TLR and NFAT) have been biochemically linked to LRRK2 action.
Day Jobs versus Dirty Deeds

Protein Synthesis (G2019S, I202T)  
s15 / 4E-BP1

Cytoskeleton & Neurite Outgrowth (G2019S, R1441C/G, Y1699C)  
ERM protein / Tubulin phosphorylation

Synaptogenesis (R1441C)  
Protein Kinase A

Synaptic Vesicle Endocytosis (G2019S)  
EndoA Phosphorylation

Lysosomal Positioning & Autophagy (G2019S, R1441C/G, Y1699C)

Golgi Sorting / Retromer Function (G2019S, I202T, R1441C, Y1699C)

Adapted from Martin et al, J. Neurochem 2014, PMID: 25251388
LRRK2 GTPase and Kinase activity

Inactive → Cell survival

Active → Cell death

GEF → GTP → ArfGAP1 → Pi

GDP → GDP → ArfGAP1, P

LRRK2-Repeats → GTPase → COR → Kinase → WD

LRRK2-Repeats → GTPase → COR → Kinase → WD
LRRK2 GS Neurodegeneration is kinase dependent
LRRK2 Substrate Discovery

A

LRRK2 Tandem Affinity Purification (TAP)

Enrichment of LRRK2 TAP phosphoproteins by Immobilized Metal Affinity Chromatography (IMAC)

Eluting proteins extracted from SDS PAGE gel and identified by LC-MS/MS

LRRK2

Hexokinase 2

Histone H4

LC/MS/MS

LRRK2 (normalized)

Lysate

Nuc

Mito

Ribo

s15

250

150

100

15

***
Ribosome – Protein Synthesis

1. **43S preinitiation complex** (small ribosomal subunit)
   - elf5
   - elf1
   - elf3
   - elf2 ternary complex
   - elf1A

2. **LRRK2 phosphorylate s15**
S15 is a Pathologic Substrate for LRRK2 GS

A Human Dopamine Neurons

<table>
<thead>
<tr>
<th></th>
<th>DAPI</th>
<th>GFP</th>
<th>TH</th>
<th>GFP/TH</th>
<th>TUNEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2019S</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD s15</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>G2019S + s15</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>G2019S + TA s15</td>
<td></td>
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</tr>
</tbody>
</table>

**G2019S s15**

**GFP positive**

**TUNEL positive (%)**

**Neuronal injury (%)**

*** ns **

**G2019S**

**WT TA**

**TD TA**

**s15**

**+**

**-**
Parkinson’s disease and RNA Translation – Protein Synthesis

Although mechanistic insight into how altered RNA translation and protein synthesis is impacted in Parkinson’s disease is in its scientific infancy, multiple studies are emerging that identify this as an important feature in disease.
Drug Trials - Current

**IND Phase 1 Safety Trials:** 1ST Biotherapeutics, Inc - 1ST-102 c-Abl Inhibitor

**Phase 1 Safety Trials:** Denali Therapeutics – DNL-201 LRRK2 inhibitor

**Phase 2 Trials:** Sun Pharma Advanced Research Company Limited – K0706 c-Abl inhibitor

Michael J. Fox Foundation – NILO PD c-Abl inhibitor
Parkinson’s disease Neurotherapeutic Pipeline

- Exploratory Research
- Target Validation
- Drug Discovery
- Lead Optimization
- Candidate
- Pre-IND
- IND
- Phase I
- Phase II
- Phase III
- Drug Approval
- Pre-Clinical Development
- Clinical Trials
- Pipeline

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