Alternative pre-mRNA Splicing and Gene Expression Patterns in Midbrain Lineage Cells Carrying Familial Parkinson's Disease Mutations

Yeon J. Lee[1,2,10,11], Khaja Syed[1,10,11], Oriol Busquets[3,9,10,12], Hanqin Li[1,8,12], Jesse Dunnack[1,10], Helen S. Bateup[1,6,7,10], Frank Soldner[3,4,5,9,10], Dirk Hockemeyer[1,6,8,10], Donald C. Rio[1,2,10]

Affiliations:

[1] University of California, Berkeley, Molecular and Cell Biology, Berkeley, CA, 94720; [2] University of California, Berkeley, California Institute for Quantitative Biosciences, Berkeley, CA, 94720;[3] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Rose F. Kennedy Center, Albert Einstein College of Medicine, 1410 Pelham Parkway South, Bronx, NY 10461; [4] Department of Genetics, Albert Einstein College of Medicine, 1301 Morris Park Ave., Bronx, NY 10461; [5] Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, 1301 Morris Park Ave., Bronx, NY 10461; [6] University of California, Berkeley, Department of Neuroscience, Berkeley, CA 94720; [7] Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, CA 94720; [8] Innovative Genomics Institute, University of California, Berkeley, CA, 94720; [9]Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, 1301 Morris Park Ave., Bronx, NY 10461; [10] Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815.

Author list footnotes:

[11, 12]authors contributed equally

Corresponding authors e-mail addresses: [*]Correspondence: don_rio@berkeley.edu.

Abstract

Parkinson's disease (PD) arises from genetic and environmental factors. Human genetics has identified mutations in ~20 inherited familial genes linked to monogenic forms of PD. To investigate the effects of individual familial PD mutations, human pluripotent embryonic stem cells (hPSCs) carrying 12 distinct familial PD mutations were differentiated into midbrain lineage cells, including dopaminergic (mDA) neurons. Global gene expression and pre-mRNA splicing patterns were analyzed in midbrain cultures carrying pathogenic PD mutations in the *PRKN*, *SNCA*, *LRRK2*, *PINK1*, *DNAJC6*, *FBXO7*, *SYNJ1*, DJ1, *VPS13C*, *ATP13A2* and *GBA1* genes. We have grouped the analysis of these familial PD mutations to genes expressed in mDA neurons and whose pre-mRNA splicing changes are linked to known PD defects in transport, cytoskeleton, lysosomes and mitochondria. Importantly, we have also shown that subsets of these splicing changes overlap with changes found in PD patient postmortem brains. Mutation-specific pre-mRNA isoforms may function as both diagnostic biomarkers for familial PD-associated genotypes and promising therapeutic targets.

Introduction

Parkinson's disease (PD) affects 2-3% of individuals over 65 years of age and is the second most common neurodegenerative disease (Poewe et al. 2017; Aarsland et al. 2021). PD is characterized by severe deterioration of motor function caused by loss of dopaminergic neurons in the substantia nigra and is accompanied by deficient dopamine release in the caudate/putamen (Kouli et al. 2018). A hallmark of PD is the intracellular aggregation of the protein α-synuclein into histologically detectable inclusions called Lewy bodies (Poewe et al. 2017; Aarsland et al. 2021). Cells outside the brain, including cells throughout the central and peripheral autonomic nervous system are also affected as the disease progresses (Poewe et al. 2017; Mendoza-Velasquez et al. 2019; Chen et al. 2020).

Lewy body diseases include dementia with Lewy body disease (DLB) and Parkinson's disease with dementia (PDD). In PD, PDD and DLB, the protein α-synuclein abnormally accumulates in the brain in aggregates, or clumps, called Lewy bodies. Key insights into the mechanism of the disease came from human genetic studies that have identified 20 genes to cause familial monogenetically inherited forms of PD (Poewe et al. 2017; Aarsland et al. 2021). Many of these genes, including *SNCA*, which encodes α-synuclein itself, point to defects in the lysosomal-autophagy system (LAS) (Winslow et al. 2010; Navarro-Romero et al. 2020). α-synuclein oligomers inhibit macro-autophagy, contributing to protein accumulation in PD (Winslow and Rubinsztein 2011; Fellner et al. 2021). Similarly, *LRRK2* mutations linked to familial PD impair the function of lysosome-associated membrane protein (LAMP)-positive compartments and cilia (O'Hara et al. 2020; Khan et al. 2021; Alessi and Pfeffer 2024), potentially by disrupting autophagy within these structures or affecting autophagosome transport (Boecker et al. 2021). Other mutant genes linked to PD affect lysosome or mitochondrial function, as well as lipid and protein sorting (Plotegher and Duchen 2017; Navarro-Romero et al. 2020). The proposed underlying mechanisms include defects in proteostasis, calcium homeostasis, mitochondrial and

autophagy dysfunction, oxidative stress, altered axonal transport and neuroinflammation (Lehtonen et al. 2019; Wilson et al. 2023).

Dysregulated alternative pre-mRNA splicing is emerging as a new and common mechanism implicated in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease, PD and spinal muscular atrophy (SMA) (Li et al. 2021; Nikom and Zheng 2023; Rogalska et al. 2023). We have developed a platform to study the effects of familial, hereditary PD mutations on pre-mRNA splicing patterns and gene expression changes in *in vitro* cultured human embryonic stem cell (hPSC)-derived dopaminergic (DA) neurons. We have previously developed a genome-editing platform using Cas9 and prime editing to engineer many of the most common familial PD mutations into hPSCs (Li et al. 2022b; Busquets et al. 2025). Using cells from this resource, we examined the effects on gene expression and pre-mRNA splicing patterns caused by common familial PD mutations in the *PRKN, SNCA, LRRK2, PINK1, DNAJC6, FBXO7, SYNJ1, PARK7/DJ1, VPS13C, ATP13A2 and GBA1* genes (Polymeropoulos et al. 1997; Kruger et al. 1998; Sasaki et al. 2004; Isaacs et al. 2017). Following differentiation of multiple independent cell clones carrying these mutations, we carried out deep bulk short-read RNA sequencing (RNA-seq) using the Illumina platform to determine pre-mRNA patterns, using an annotation-free method called the Junction Usage Model (JUM) for comparison of splicing patterns (Wang and Rio 2018) between the mutant and edited wild type (EWT) control cells (cells that received editing reagents in parallel but did not create the desired mutations). We found that cells carrying the 12 familial PD mutations exhibited many changes in spliced transcript isoforms from pathways governing cell projections, the cytoskeleton and GTPase regulation and sometimes impacting splicing factors themselves. There were also changes at the level of gene expression with different degrees of overlap with genes whose splicing patterns changed depending on the mutation. Furthermore, we compared our RNA-seq data to existing datasets from the anterior cingulate cortex, a region connected to the substantia nigra, derived from postmortem brain tissue of patients with PD, PDD, and DLB (Feleke et al. 2021).

Taken together, our data supports the idea that the pre-mRNA splicing pattern defects we observe in hPSC-derived midbrain DA neurons (mDA) *in vitro* are relevant to PD, that these distinct transcript isoforms should serve as useful biomarkers and might be amenable to RNA therapeutics (Anthony 2022; Li et al. 2022a; Rogalska et al. 2023).

Results

Differentiation of dopaminergic (DA) neurons from human embryonic stem cells (hPSCs) carrying familial PD mutations.

To study the effects of PD-linked mutations on splicing and gene expression, we differentiated a subset of the Isogenic Stem Cell Collection to Research Parkinson's Disease (iSCORE-PD) collection (Busquets et al. 2025) into mDA cells. Multiple clonally independent isogenic stem cell lines were generated carrying common familial PD mutations including *PRKN* (X3DEL), *SNCA* (A30P and A53T), *LRRK2* (G2019S), *PINK1* (Q129X), *DNAJC6* (FS), *FBXO7* (R498X), *SYNJ1* (R258Q), *DJ1* (X1_5DEL), *VPS13C* (W395C), *ATP13A2* (FS) *and GBA1* (IVS2) using CRISPR *Cas9* or prime editing techniques, as previously reported (Figure 1A) (Li et al. 2022b; Busquets et al. 2025). hPSCs were differentiated into mDA cells, which are the primary cell type that degenerates in PD using a modified version of a reported protocol (Kim et al. 2021b), which generated dopamine neurons from hPSCs by boosting WNT signaling during early midbrain lineage patterning. After the initial patterning for 11 days, cells were differentiated over 3 weeks. The resulting differentiated mDA cells robustly expressed the key differentiation markers *FOXA2* and *MAP2*, across subclones of familial PD mutant-expressing cells (Figure 1B; (Busquets et al. 2025)). Gene expression analysis from bulk RNA seq data of the differentiated mDA cells at day 35 confirmed the expression of key neuronal markers (e.g.

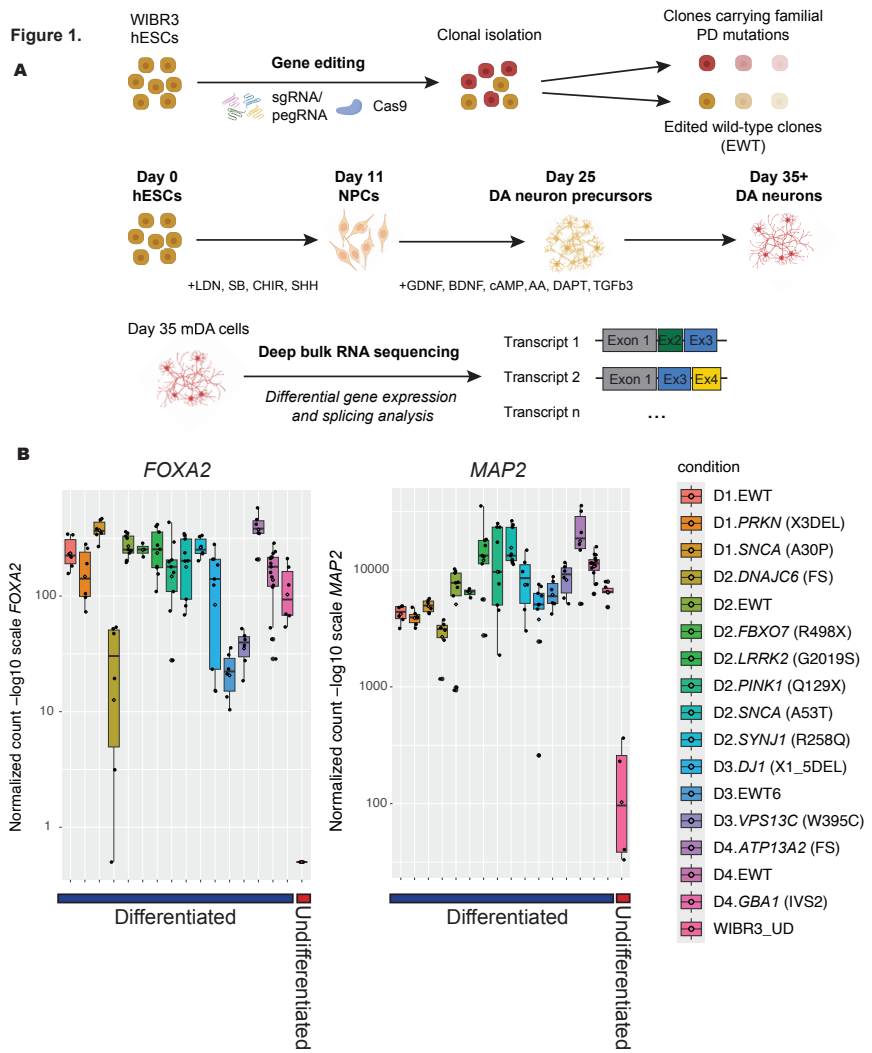


Figure 1. Platform for the medium-throughput genome-editing of human embryonic stem cells (hPSCs) carrying relevant familial Parkinson's mutations and differentiation of these cells to midbrain lineage cells. A. Schematic diagram of editing pipeline and expansion into clonal hPSC lines. B. Normalized gene counts for neuronal markers including *FOXA2* (*forkhead box A2*) and *MAP2* (*microtubule-associated protein 2*), in 35d differentiated edited wild-type (EWT), familial *PRKN, SNCA, LRRK2, PINK1, DNAJC6, FBX7, SYNJ1,* DJ1*, VPS13C, ATP13A2 and GBA1* mutant cells, in comparison to undifferentiated WIBR3 hPSCs. D1–D4 denote four independent sets of cell differentiation.

FOXA2 and *MAP2*) compared to undifferentiated WIBR3 hPSC cells, indicating robust differentiation within these hPSC-derived cell lines expressing familial PD mutations (Figure 1B). Other single-cell RNA-seq data indicate that these cultures generated ~15-20% mature DA neurons (Syed, manuscript in preparation). The deep sequencing analysis allowed for a simultaneous assessment of global gene expression and alternative pre-mRNA splicing patterns (Supplemental Fig. S2-S14; Supplemental Tables 2-3, 6). Notably, we observed minimal overlap between genes with altered expression levels and those exhibiting differential splicing

(Supplemental Fig. S14; Supplemental Tables 2 and 3). This divergence between transcriptional and post-transcriptional regulation is consistent with prior observations in PD patient brain biopsies (Feleke et al. 2021). Our subsequent analysis focused on a curated set of genes known to be expressed in both our hPSC-derived neurons and PD patient cortical neurons (Feleke et al. 2021), specifically targeting pathways central to PD pathogenesis including vesicle trafficking, cytoskeletal integrity, mitochondrial dynamics, and lysosomal function (Supplemental Fig. S1).

Identification of unique splicing signatures in hPSC-derived dopaminergic neurons carrying PD-associated mutations

During neuronal differentiation, alternative splicing can modulate diverse processes such as signaling activity, centriolar dynamics and metabolic pathways (Su et al. 2018). RNA-seq data can provide insights into the regulation of alternative splicing by revealing the usage of known or unknown (unannotated) splice sites as well as the expression level of exons. Using 35 day-differentiated mDA cells carrying common familial PD mutations in the *SNCA, LRRK2, PRKN, PINK1, PARK7/DJ1, ATP13A2, DNAJC6, FBXO7, SYNJ1, VPS13C and GBA1* genes or wild-type genotypes, we prepared total RNA, poly(A)-mRNA selected and then generated random-primed cDNA libraries from 1-3 independently derived subclonal cell lines in technical triplicates. These libraries were sequenced on the Illumina NovaSeq S1 or S4 platform with between 200-300M paired-end 150bp (PE150) reads. The large datasets coming from this experiment were analyzed using the JUM software (Wang and Rio 2018) to compare and quantitate pre-mRNA splicing pattern differences between the mutant and edited wild type (EWT) control cells. The ratio between reads including or excluding exons, also known as the percent-spliced-in index (PSI), indicates how efficiently sequences of interest are spliced into mature transcripts. We observed thousands of splicing events that differed between the EWT and mutant cell lines with the PSI cut-off set to > 5%, in canonical splicing patterns and in a more complex category that JUM denotes as “composite”, which is a combination of splicing patterns beyond a simple

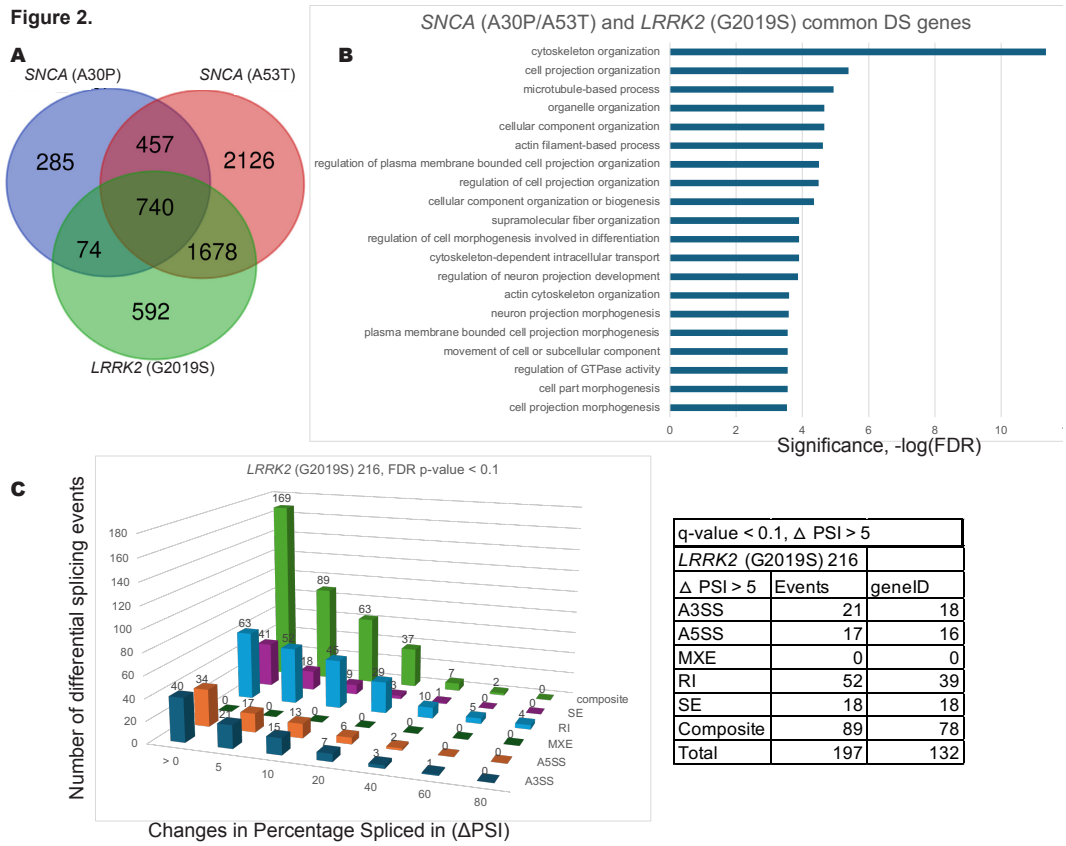
splicing event within the same splicing structure (Wang and Rio, 2018). RNA-seq data derived from 12 different familial mutant cell lines were analyzed for RNA splicing and gene expression changes compared to EWT controls (Supplemental Tables 2-3, 6). We also compared the splicing signatures for each PD-associated mutation to a published bulk PD patient biopsy tissue RNA-sequencing datasets containing 24 postmortem human brain cortex samples to determine the disease relevance of the observed splicing changes (Feleke et al. 2021). In our analyses described below we had grouped the PD mutations into related categories, such as the autosomal dominant forms (*SNCA, LRRK2*) that are associated with lysosomal dysfunction, the autosomal recessive forms *(PRKN, PINK1, and DJ1*) that are associated with mitochondrial dysfunction and the mutations in the *ATP13A2, DNAJC6, FBXO7 or SYNJ1* genes that are associated with complex or atypical forms of parkinsonism, as well as common features found in data from the *VPS13C* and *GBA* mutations.

Common splicing pattern changes in genes linked to trafficking, mitochondria and lysosomes in the SNCA (A30P/A53T) and LRRK2 (G2019S) mutants

Alpha-synuclein (SNCA) is a prominent component in Lewy bodies and aggregation of this protein is commonly used as a marker for PD (Kruger et al. 1998). There are multiple mutations and genetic variations in the alpha-synuclein (*SNCA*) gene that drive Parkinson's disease (PD) pathology. While the A53T mutation is a well-known example, others include the previously mentioned A30P mutation and the E46K mutation, as well as gene duplications and triplications (Polymeropoulos et al. 1997; Kruger et al. 1998; Singleton et al. 2003; Chartier-Harlin et al. 2004; Zarranz et al. 2004). The mutations in *LRRK2* that are associated with aberrant kinase activity is a common and important inherited mutation linked to PD, where a series of kinase inhibitor molecules have been identified (Lesage and Brice 2009; Taymans and Greggio 2016). The G2019S mutation leads to a hyperactive form of the kinase (Jaleel et al. 2007; West et al. 2007; Greggio and Cookson 2009). The mutations in both *SNCA* and *LRRK2* that we examined

are autosomal dominant.

Figure 2.



q-value < 0.1, Δ PSI > 5		
LRRK2 (G2019S) 216		
Δ PSI > 5	Events	geneID
A3SS	21	18
A5SS	17	16
MXE	0	0
RI	52	39
SE	18	18
Composite	89	78
Total	197	132

D

Gene	Protein	Core Function	Impact on Organelles
Motor Proteins & Adaptors (Microtubules & Transport)			
DYNC1H1	Dynein Heavy Chain	Component of the Dynein motor (retrograde transport)	Essential for moving lysosomes toward the cell center (nucleus) and distributing mitochondria
DCTN1	Dynactin Subunit 1	Regulator of the Dynein motor complex	Required to link Dynein to both lysosomal and mitochondrial cargo
KIF2A	Kinesin Family Member	Kinesin motor protein (anterograde transport).	Moves mitochondria and late endosomes/lysosomes toward the cell periphery
BICD2	BICD Related Protein	Adaptor protein for Dynein	Helps link the Dynein motor directly to endosomes and lysosomes
CLIP1 / CLIP2	Cytoplasmic Linker Associated Protein	Microtubule Scaffolding and Plus-End Tracking Proteins	Stabilize the growing ends of microtubules and also act as scaffolding to link motor proteins (like Dynein) to the cargo, impacting organelle transport
Organelle/Cytoskeleton Anchors & Regulators			
DMD	Dystrophin	Links actin to the cell membrane.	Anchors mitochondria near sites of high energy demand
PCLO	Piccolo	Scaffolding protein primarily found at synapses	Regulates the transport and docking of vesicles, which includes associated lysosomes and mitochondria
PDCD6IP	Programmed Cell Death 6 Interacting Protein	Crucial protein in the autophagy pathway	Essential for delivering damaged mitochondria (mitophagy) and other cellular components to lysosomes for degradation
BIN1	Bridging Integrator 1	Involved in shaping and anchoring T-tubules	Indirectly impacts mitochondrial placement and fusion/fission processes through membrane dynamics
VPS54	Vacuolar Protein Sorting 54	Component of the Garrick complex	Crucial for retrograde trafficking between endosomes and the Golgi network, directly affecting the maturation and movement of lysosomes

Figure 2. Mutations in *SNCA* and *LRRK2* differentially affect gene splicing in autosomal dominant Parkinson's disease, highlighting aberrations in cytoskeletal organization. (A) Comparative analysis of differentially spliced genes in Autosomal Dominant *SNCA* and *LRRK2* Parkinson's Disease Mutants. (B) GO-enrichment analysis of 2,492 common differentially spliced genes in *SNCA* and *LRRK2* PD familial mutants, with the regulation of cell projection organization (GO:0031344) being the most enriched GO term. (C) Deconvolution of differential splicing events for the genes involved in the cytoskeleton organization (GO:0007010). The *LRRK2* (G2019S) clonal line 216 is shown as an example. (D) A table of a few key cytoskeletal genes that are differentially spliced among the 209 genes belonging to the cytoskeleton organization (GO:0007010) category.

We first analyzed and compared the JUM splicing pattern changes from the bulk *SNCA* (A30P), *SNCA* (A53T) and *LRRK2* (G2019S) RNA-seq data (Fig. 2A and Supplemental Fig. S2-S4). There is overlap in splicing changes between the *SNCA* (A30P), *SNCA* (A53T) and *LRRK2* (G2019S) mutant cells and transcript isoform changes (Fig. 2A). Based on this observed overlap, we focused our subsequent analysis on genes associated with cytoskeletal organization and function (Fig. 2B). This specific gene set was selected based on two criteria: their confirmed expression in both control and PD patient cortical neurons (Supplemental Fig. S1; (Feleke et al. 2021)) and their common differential signature across the three mutant cell lines (Fig. 2A). Some of these genes are interesting given the defects in cilia and vesicle trafficking linked to PD and the involvement of Rab proteins in PD (Bonet-Ponce and Cookson 2019; Bellucci et al. 2022; Komori and Kuwahara 2023; Pfeffer 2023; Tian et al. 2024) (Supplemental Fig. S2-S4). An example of these JUM data is shown for this cytoskeletal grouping (GO:0007010) for the genes (132) and type of altered splicing events in the *LRRK2* (G2019S) clonal mutant line 216 (Fig. 2C). The complete detailed analyses of these data are shown in Supplemental Tables 1 and 2. Interestingly, many of the genes in this GO term cluster whose splicing patterns are changed are linked to trafficking, mitochondria and lysosomes (Fig. 2D). Finally, there is overlap in splicing patterns from the mutant human pluripotent stem cells and transcript isoforms altered in patient samples from three Lewy body diseases (Supplemental Fig. S2D-S4D). Alterations in the splicing isoforms produced from these genes in the PD mutant cells could affect organelle transport or mitochondrial/lysosomal function.

Common splicing pattern changes in genes linked to trafficking, GTPase signaling, lipid signaling, mitochondria and the cytoskeleton in the PRKN gene exon 3 deletion (X3DEL), PINK1 (Q129X) and DJ1 (X1-5DEL) mutants

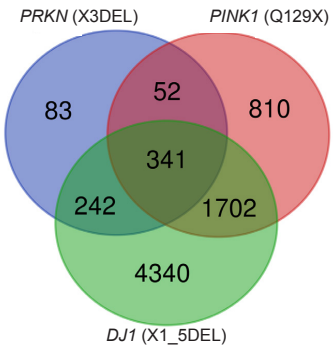
PRKN is a ubiquitin ligase acting to ensure mitochondrial quality control. PINK1 is a protein kinase involved in mitochondrial quality control. The *PINK1* Q129X1 mutation is genetically linked to PD (Valente et al. 2004; Beilina et al. 2005; Ibanez et al. 2006; Ishihara-Paul et al.

2008). DJ1 is critical for mitochondrial function and mitophagy (Canet-Aviles et al. 2004; Dodson and Guo 2007; Thomas et al. 2011). Loss-of-function mutations in *DJ1* impair ATP production and exacerbate oxidative stress via dysfunctional mitochondrial accumulation and reduced antioxidant capacity, contributing to neurodegeneration in Parkinson's disease. DJ1, encoded by the *PARK7* gene, is a protein that protects cells from oxidative stress and mutations in the *PARK7* gene have been linked to early onset PD (Bonifati et al. 2003a; Bonifati et al. 2003b; Pankratz et al. 2006).

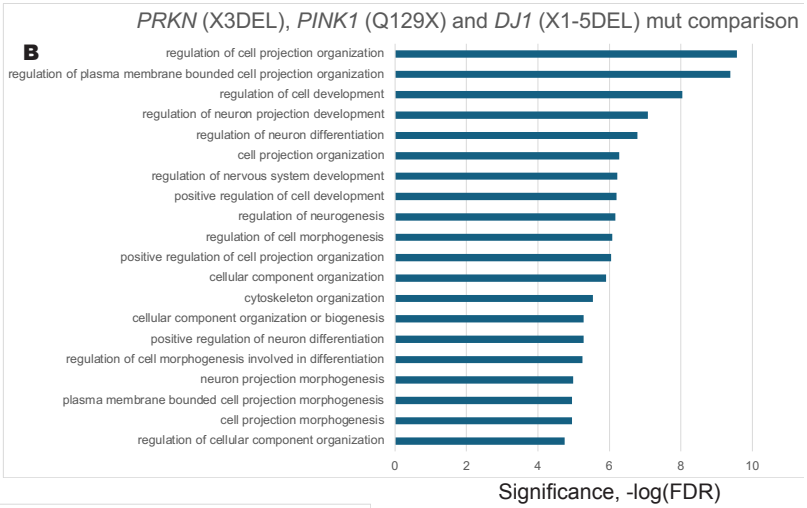
We analyzed and compared the JUM splicing pattern changes from the bulk *PRKN* gene exon 3 deletion (X3DEL), *PINK1* (Q129X) and *DJ1* (X1-5DEL) RNA-seq data (Fig. 3A and Supplemental Fig. S5-S7). There is overlap in splicing isoform changes between the *PRKN gene exon 3 deletion (X3DEL), PINK1 (Q129X) and DJ1 (X1-5DEL)* mutant cells (Fig. 3A). This analysis identified genes with GO terms for cell projection organization and cytoskeletal organization (Fig. 3B) and for genes that were expressed in control and PD patient cortical neurons (Fig. 3D and Supplemental Fig. S1;(Feleke et al. 2021)) and that were in common between the three mutant cell lines (Fig. 3B). An example of these JUM data is displayed for this GO term (GO:0031344) grouping and the genes (156) and type of altered splicing events in the *DJ1 (X1-5DEL)* clonal mutant line 2860 (Fig. 3C). The complete detailed analyses of these data are shown in Supplemental Tables 1 and 2. Many of the genes in this GO term cluster whose splicing patterns are changed are linked to trafficking, GTPase signaling, lipid signaling and the cytoskeleton (Fig. 3D). Again, there is overlap in splicing patterns from the mutant human pluripotent stem cells and transcript isoforms altered in patient samples from three Lewy body diseases (Supplemental Fig. S4D-S7D). Alterations in the splicing isoforms produced from these genes in the PD mutant cells could affect membranes, vesicle transport and mitochondrial damage.

Figure 3.

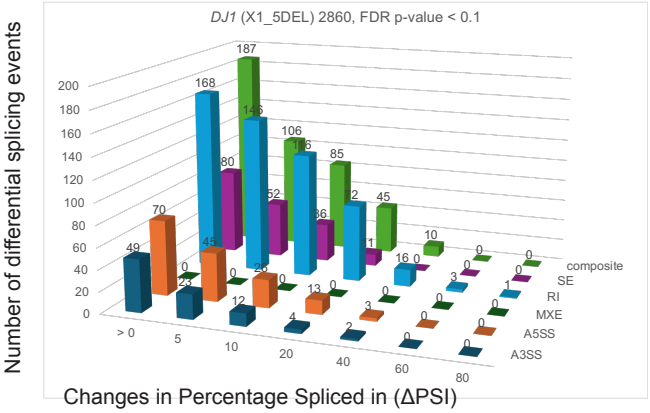
A



B



C



q-value < 0.1, Δ PSI > 5		
DJ1 (X1_5DEL) 2860		
Δ PSI > 5	Events	geneID
A3SS	23	20
A5SS	45	37
MXE	0	0
RI	146	82
SE	52	40
Composite	106	82
Total	372	156

D

Gene	Protein	Core Function	Impact on Organelles
DNM1L	Dynamin 1 Like	Fission/Fusion Regulation	DNM1L encodes Drp1, the master GTPase that orchestrates mitochondrial and peroxisomal fission.
KIF1A	Kinesin Family Member 1A	Transport/Positioning	Anterograde motor protein responsible for axonal transport of organelles and cargo along microtubules toward the cell periphery.
PARK2	Parkin RBR E3 Ubiquitin Protein Ligase	Quality Control (Mitophagy)	Encodes Parkin, an E3 ubiquitin ligase that is essential for recognizing and tagging damaged mitochondria.
MAP2 / MAP4 / MAP6	Microtubule-Associated Protein 2 / 4 / 6	Microtubule-Associated Proteins	Structural proteins that bind, stabilize, and bundle microtubules to regulate cytoskeletal dynamics and cell morphology.
PLCE1	Phospholipase C Epsilon 1	Lipid Signaling	Phospholipase that hydrolyzes PIP2 to generate IP3 and DAG second messengers, linking signals to Ca2+ release.
PIK3R1	Phosphoinositide-3-Kinase Regulatory Subunit 1	Lipid Signaling	The regulatory subunit p85a of PI3K that controls the PI3K/Akt/mTOR signaling pathway central to cell growth and survival.
WASH1	Wiskott-Aldrich Syndrome Protein and SCAR Homolog 1	Actin Polymerization	Key subunit of the WASH complex that activates the Arp2/3 complex to form branched actin networks on endosomes, regulating membrane traffic and recycling.
ARHGAP24 ARHGAP4 ARHGEF7	Rho GTPase Activating Protein 24 / 4, and Rho Guanine Nucleotide Exchange Factor 7	Rho GTPase Signaling	Regulators of the Rho GTPase signaling pathway that control actin cytoskeleton dynamics for cell migration and polarity.
CDC42	Cell Division Cycle 42	Rho GTPase Regulator	Key regulator of the actin cytoskeleton; controls membrane dynamics and vesicle movement near the plasma membrane, impacting endocytosis toward the lysosome.

Figure 3. Shared perturbations in *PRKN*, *PINK1*, and *DJ1* familial Parkinson's disease mutants demonstrated by GO-enrichment and differential splicing of genes regulating cell projection organization. (A) Comparison of the *PRKN*, *PINK1*, and *DJ1* mutations that cause an atypical autosomal recessive (AR)-Parkinsonism phenotype. (B) GO-enrichment analysis of 2,337 common differentially spliced genes in *PRKN*, *PINK1*, and *DJ1* PD familial mutants. This analysis reveals the regulation of cell projection organization (GO:0031344) being the most significantly enriched GO term. (C) Deconvolution of differential splicing events for the genes involved in the regulation of cell projection organization (GO:0031344). The *DJ1* (X1_5DEL) clonal line 2860 is shown as an example. (D) A table summarizing key cytoskeletal genes that are differentially spliced among the 163 genes in the regulation of cell projection organization (GO:0031344) category.

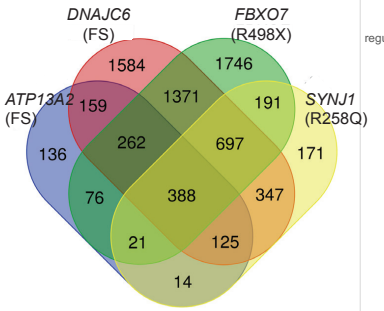
Common Splicing Pattern Changes in Genes linked to the Cytoskeleton, Cilia, and Mitochondria in the ATP13A2, DNAJC6, FBXO7, and SYNJ1 Mutants

The *ATP13A2* (*PARK9*) gene encodes a lysosomal ion transport protein that functions in acidification of lysosomes. Mutations in the *ATP13A2* gene are linked to Kufor-Rakeb Syndrome, a form of early onset PD (Ramirez et al. 2006; Park et al. 2015; Fujii et al. 2023). S8F), including the *ATP13A2* gene itself (Supplemental Table S6). Studies have reported decreased ATP13A2 protein levels in the substantia nigra dopaminergic neurons and frontal cortex of PD patients compared to controls (Kitada et al. 1998; Dehay et al. 2012; Fujii et al. 2023). DNAJC6 is a co-chaperone protein implicated in vesicle transport and lipid homeostasis. It is found mutated in early onset PD patients (Edvardson et al. 2012; Olgiati et al. 2016). FBXO7 is a ubiquitin ligase that regulates the cell cycle and mediates mitochondrial clearance and mutations in this gene have been linked to PD (Di Fonzo et al. 2009; Zhou et al. 2018). SYNJ1 is a lipid phosphatase involved in endosomal trafficking and vesicle recycling and mutations in *SYNJ1* have been linked to PD and several neurological disorders (Drouet and Lesage 2014; Soukup et al. 2018; Choudhry et al. 2021).

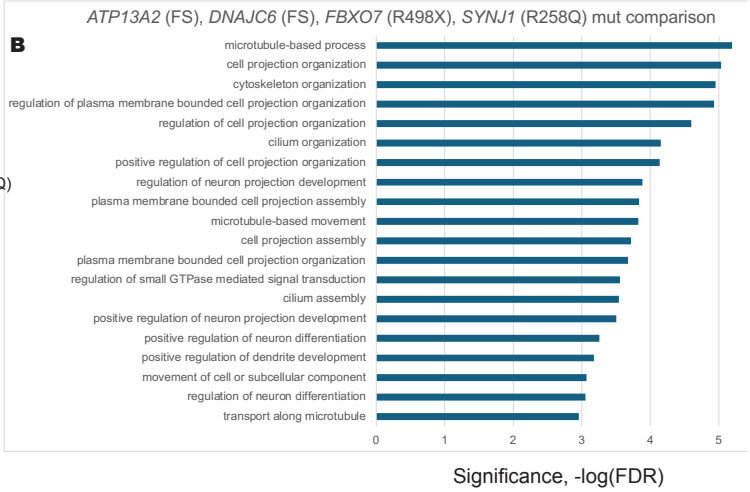
The JUM splicing pattern changes from the bulk *ATP13A2* Frameshift (FS)*, DNAJC6* (FS)*, FBXO7* (R498X)*, SYNJ1* (R258Q) RNA-seq data (Fig.4 and Supplemental Fig. S8-S11) was performed. There is overlap in splicing changes between the *ATP13A2* Frameshift (FS), *DNAJC6* (FS), *FBXO7* (R498X), *SYNJ1* (R258Q) gene transcripts in mutant cells and transcript isoforms (Fig. 4A). This analysis identified genes with GO terms for cell projection organization, cytoskeletal organization and cilia (Fig. 4B) for genes that were expressed in control and PD patient cortical neurons (Supplemental Fig. S1; (Feleke et al. 2021)) and that were in common between the four mutant cell lines (Fig. 4A). An example of this JUM data is displayed for this GO term (GO:0007017) grouping for the number (201 genes) and type of altered splicing events in the *SYNJ1* (R258Q) clonal mutant line A5 (Fig.4C). The complete detailed analysis of these

Figure 4.

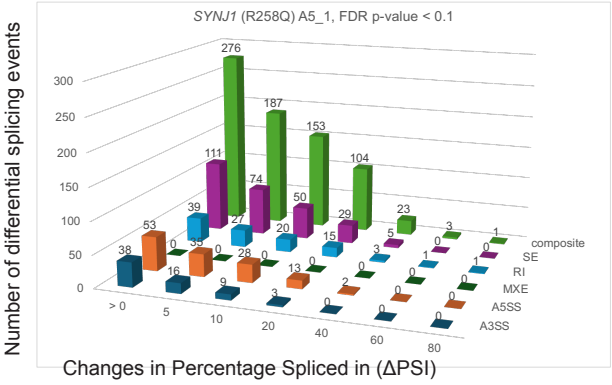
A



B



C



q-value < 0.1, Δ PSI > 5		
SYNJ1 (R258Q) A5_1		
Δ PSI > 5	Events	geneID
A3SS	16	16
A5SS	35	33
MXE	0	0
RI	27	24
SE	74	62
Composite	187	141
Total	339	201

D

Gene ID	Protein	Core Function	Impact on Organelles
AAAS	ALADIN	Component of the nuclear pore complex (NPC).	Critical for regulating mitochondrial fission/fusion dynamics; mutations cause severe mitochondrial disease (Triple A syndrome).
BIN1	Bridging Integrator 1	Membrane curvature sensing and tubulation (via BAR domain).	Plays a central role in endocytosis and autophagy. Involved in membrane remodeling necessary for both processes.
ARHGEF11	Rho Guanine Nucleotide Exchange Factor 11 (GEF-H1)	Cytoskeletal regulator that activates the RhoA GTPase.	RhoA signaling is essential for the fusion of autophagosomes with lysosomes (autolysosome formation), the final step in degradation.
ROCK1	Rho-associated coiled-coil containing protein kinase 1	Downstream kinase of RhoA/RhoB. Regulates actin-myosin contraction.	Regulates the overall size and distribution of lysosomes and is critical for controlling the flow of the autophagy pathway.
RIPOR2 / RICTOR	RPTOR-independent companion of mTORC2	Component of the mTORC2 complex, a master cell growth and metabolism regulator.	mTOR is the primary sensor of nutrient status, regulating autophagy and mitochondrial metabolism.
TBCK	TBC1 domain-containing kinase	Kinase that likely functions as a GTPase-activating protein (GAP).	Phosphorylates proteins involved in lysosomal trafficking and is a known cause of neurodevelopmental disorders associated with lysosomal dysfunction.
INF2	Inverted Formin 2	Regulator of actin polymerization and the actin cytoskeleton.	Functions to promote mitochondrial fission by driving actin polymerization at the mitochondrial surface.
DCTN1	Dynactin Subunit 1	Component of the Dynactin complex, an essential regulator of the Dynein motor.	Dynein is the primary motor protein for retrograde transport. Crucial for moving both lysosomes and mitochondria back to the perinuclear region.
KIF2A / KIF3A	Kinesin Family Member 2A/3A	Kinesin motor proteins (plus-end directed transport).	Kinesins are the motor proteins for anterograde transport, controlling the distribution and mobility of both organelles.
MARK2 / MARK3 / MARK4	Microtubule Affinity Regulating Kinase	Kinases that phosphorylate microtubule-associated proteins.	Regulates the mitochondrial fission/fusion balance by phosphorylating and controlling proteins involved in mitochondrial dynamics.

Figure 4. Differential Splicing Analysis Reveals Common Microtubule-Based Process Dysregulation in Parkinson's Disease Mutants that causes atypical AR-Parkinsonism Phenotype. (A) Comparison of the *ATP13A2*, *FBXO7*, *DNAJC6*, and *SYNJ1* mutations that cause an atypical AR-Parkinsonism phenotype. (B) GO-enrichment analysis of 3,651 common differentially spliced genes in *ATP13A2*, *FBXO7*, *DNAJC6*, and *SYNJ1* PD familial mutants. The analysis indicates the microtubule-based process (GO:0007017) as the most enriched GO term. (C) Deconvolution of differential splicing events for the genes involved in the microtubule-based process (GO:0007017). The *SYNJ1* (R258Q) A5_1 clonal line is presented as a representative example. (D) A table of a few key cytoskeletal genes that are differentially spliced among the 251 genes in the microtubules-based process category (GO:0007017) common to the *ATP13A2*, *FBXO7*, *DNAJC6*, and *SYNJ1* PD familial mutants.

cytoskeleton (Fig. 4D). Again, there is overlap in splicing patterns from the mutant human pluripotent stem cells and transcript isoforms altered in patient samples from three Lewy body diseases (Supplemental Fig. S8-S11). Alterations in the splicing isoforms produced from these genes in the PD mutant cells could affect membranes, vesicle transport, mitochondria and lysosomes.

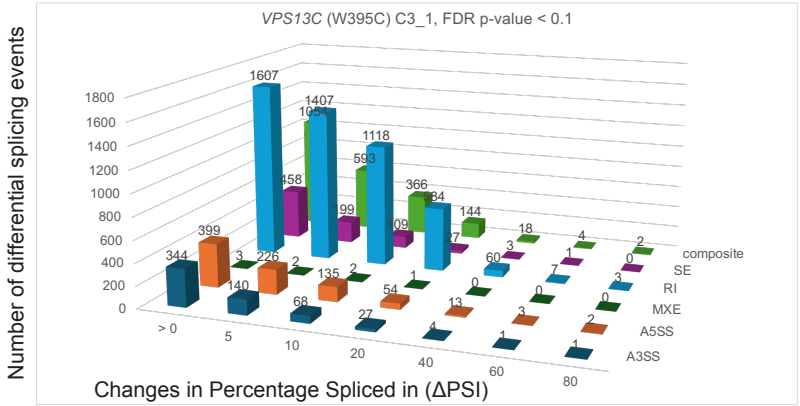
Splicing pattern changes in the VPS13C W395C mutant

The *VPS13C* gene encodes a large protein involved in vesicle sorting, mitochondrial-lysosome function and lipid transfer between membranes. Recessive mutations in this gene are linked to early onset PD (Lesage et al. 2016; Schormair et al. 2018; Smolders et al. 2021). Our bulk analysis of the RNA from cells carrying the *VPS13C* W395C mutation identified 4175 pre-mRNA splicing changes from two differentiated mutant cell clones (Supplemental Fig. S12A and S12B). In keeping with our idea that some common genes/pathways may link the familial PD genes we analyzed a set of 1386 genes linked to cellular component organization or biogenesis which should be expressed in mDA neurons (Fig. 5) Our splicing analysis showed 2567 splicing event changes and some of the affected transcripts are for genes that are linked to trafficking, mitochondria and lysosomes (Fig. 5B). Note there are many splicing events that involve intron retention which could trigger nonsense mediated mRNA decay and leading to defects in proteostasis (Fig. 5A).

From the bulk sequencing data, the genes whose pre-mRNA isoforms were altered in the *VPS13C* mutant cells were also linked to metabolic processes, chromatin modification and RNA splicing (Supplemental Fig. S12C). There could be a connection between mitochondrial function, ATP production and RNA splicing since the intron removal uses ATP at multiple steps. Comparison of the splicing events affected in *VPS13C* mutant differentiated hPSCs and the RNA-seq data from Lewy body patient brains indicates overlap between the transcripts whose splicing is affected, with DLB having the most overlap (Supplemental Fig. S12D). The genes

Figure 5. 1387 genes in GO:0071840 Cellular Component Organization or Biogenesis

A



q-value < 0.1, Δ PSI > 5		
VPS13C (W395C) C3_1		
Δ PSI > 5	Events	geneID
A3SS	140	131
A5SS	226	210
MXE	2	2
RI	1407	853
SE	199	183
Composite	593	526
Total	2567	1376

B

Gene	Protein	Core Function	Effect on Organelles
BICD1	Bicaudal D Homolog 1	Microtubule Motor Adaptor	Links lysosomes, Endosomes, and Autophagosomes to the Dynein motor complex for retrograde (minus-end) transport, crucial for positioning and fusion.
DCTN2	Dynactin 2 (p50)	Microtubule Motor Adaptor	Core subunit of the Dynactin complex, which activates Dynein. Essential for all MT-based Lysosome/Mitochondria transport.
FMNL2, FMNL3	Formin-like 2/3	Actin Regulator (Nucleates Actin)	Mitochondria Fission: Actin polymerization, driven by Formins, is required to constrict the mitochondrial outer membrane during fission.
PAK1, PAK3	p21-Activated Kinase 1/3	Kinase/GTPase Effector	Regulates Actin and MT dynamics. Involved in signaling pathways that control Mitochondrial membrane fusion/fission processes.
RHO C	Ras Homolog Family Member C	Small GTPase	Regulates Actin stress fibers. Rho GTPases control the stability of MTs and Actin, impacting organelle motility.
ABL1	c-Abl Tyrosine Kinase	Tyrosine Kinase	Regulates Actin dynamics and MT stability. ABL1 signaling is implicated in Autophagy regulation and Mitochondrial stress pathways.
ARHGEF7	Rho GEF 7 (PIX)	Rho-GEF (Rac/Cdc42 activator)	Activates GTPases that control the local Actin cytoskeleton at contact sites, including Mitochondria-ER tethering.
SHANK3	SHANK3	Synaptic Scaffold (Actin organizer)	Organizes the Actin cytoskeleton at the Postsynaptic Density. Its loss impairs Autophagosome and Lysosome clearance in neurons.
WEE1	WEE1 Homolog	Cell Cycle Kinase	Controls the Cell Cycle machinery. Cell Cycle proteins often regulate MT organization which, in turn, influences Mitochondrial and Lysosomal positioning.

Figure 5. Aberrant Splicing of Genes Governing Cellular Component Organization and Biogenesis in *VPS13C* Parkinson's Disease Mutant Cells. (A) Deconvolution of differential splicing events for the genes involved in the cellular component organization or biogenesis (GO:0071840). The *VPS13C* (W395C) mutant clonal line C3_1 is shown as an example. (B) A table of a few key cytoskeletal genes that are differentially spliced among the 1,387 genes in the cellular component organization or biogenesis (GO:0071840) in the *VPS13C* (W395C) PD familial mutant.

whose transcripts are affected at the level of pre-mRNA splicing in this data set are indicated in Supplemental Tables S1 and S2. DESeq2 analysis revealed differential gene expression profiles for the up-regulation of genes involved in cell adhesion and down-regulation of genes involved in splicing and regulation of gene expression. Down-regulation of mitochondrial genes is also predominant in the *VPS13C* W395C mutant (Supplemental Fig. S12E and S12F). Differentially expressed genes from the GO enrichment analysis are listed in Supplemental Table S3. Some studies suggest *VPS13C* plays a role in mitophagy (Lesage et al. 2016;

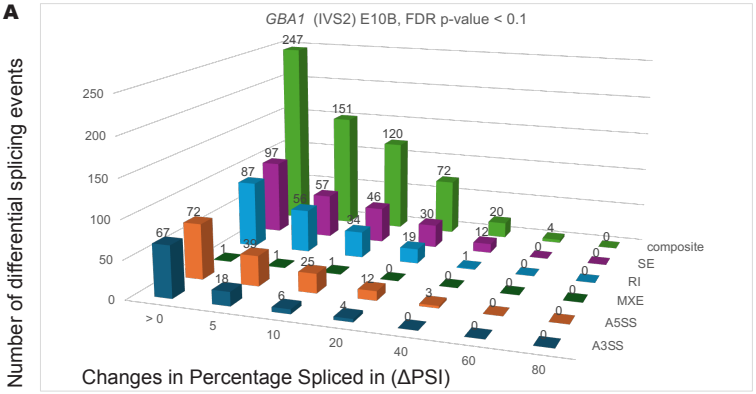
Schröder et al. 2024; Wang et al. 2025). Loss of *VPS13C* could impair mitochondrial homeostasis due to compromised lysosomes, leading to the accumulation of dysfunctional mitochondria and increased oxidative stress. Using GO terms for cellular component Organization or Biogenesis and comparing to the curated set of cortical neuron-expressed genes (Supplemental Fig. S1), we find splicing defects in gene transcripts linked to mitochondria and lysosomes such as *BICD1*, *ABL1*, and *PAK1/PAK3* (Fig. 5D).

Splicing pattern changes in the GBA1 IVS2 mutant

The *GBA1* gene encodes a lysosomal enzyme that maintains glycosphingolipid homeostasis (Sidransky et al. 2009; Huh et al. 2023). Mutations in the *GBA1* gene, such as the c.115+1G>A variant, are among the most common risk factors found in PD patients, as they can disrupt the gene's splicing pattern, leading to a non-functional glucocerebrosidase protein (Neumann et al. 2009; Onal et al. 2024). Using mDA cells homozygous for the *GBA1* intron 2 mutation, a variant associated with Gaucher disease that also confers a significant risk for Parkinson's disease (Sidransky et al. 2009), we identified 1,857 high-confidence pre-mRNA splicing alterations across two independent mutant clones (Supplemental Fig. S13A and S13B). Genes whose splicing was affected include cell projection, cytoskeletal organization and regulation of GTPase activity (Supplemental Fig. S13C). There is overlap between the splicing event changes detected in the *GBA1* mutant cell line and the altered transcript isoforms from Lewy body disease brain biopsy RNA-seq data (Supplemental Fig. S13D). The genes whose transcripts are affected at the level of pre-mRNA splicing are indicated in Supplemental Tables S1 and S2. Differential alternative splicing (AS) events between EWT and *GBA1* (FS) mutants, initially identified by JUM analysis, were validated through RT–PCR assays. These experiments confirmed the predicted splicing shifts across multiple targets (Supplemental Fig. S16). Expression profiling analysis indicated genes involved in mitotic cell cycle checkpoint and microtubule-based processes are up-regulated, while genes involved in regulation of trans-synaptic signaling and regulation of transport are down-regulated in the *GBA1* IVS2 mutant

Figure 6. 148 genes in GO:0031344 Regulation of Cell Projection Organization

A



q-value < 0.1, Δ PSI > 5		
GBA1 (IVS2) E10B		
Δ PSI > 5	Events	geneID
A3SS	18	17
A5SS	39	31
MXE	1	1
RI	56	38
SE	57	46
Composite	151	105
Total	322	148

B

Gene	Protein	Core Function	Effect on Organelles
KIF1A	Kinesin Family Member 1A	MT-based Anterograde Motor	Mitochondria/Vesicles: Directly transports Mitochondria and Synaptic Vesicle Precursors along MTs to the cell periphery.
MACF1	Microtubule-Actin Crosslinking Factor 1 (ACF7)	MT/Actin Cross-Linker	General Trafficking: Links MTs to Actin. Critical for coordinating the transport of various organelles (including Lysosomes) during cell migration and growth.
MYO9A	Myosin 9A	Actin-based Motor with GAP domain	Trafficking: Involved in Actin-based transport and membrane trafficking. Its GAP domain can regulate small GTPases involved in Endosome maturation.
NDEL1	Nudel	MT Dynamics Regulator	Mitochondria: Regulates Dynein motor function and MT stability. Crucial for organizing MTs that serve as tracks for mitochondrial and lysosomal movement.
CDC42	Cell Division Cycle 42	Small GTPase (Master Actin regulator)	Mitochondria/Endosomes: Regulates local Actin polymerization, which is essential for Mitochondrial fission and Endosome maturation/trafficking.
PAK3	p21-Activated Kinase 3	Kinase (Cdc42 Effector)	Mitochondria/Actin: Signals downstream of Cdc42. Controls Actin dynamics which are required for Mitochondrial morphology and distribution.
WASH1	WASP and Scar Homolog 1	Actin Nucleator (Arp2/3 activator)	Endosome/Lysosome: Component of the WASH complex that drives Actin polymerization to facilitate protein sorting and budding from Endosomesto Lysosomes.
RTN4	Reticulon 4 (Nogo)	ER Membrane Sculptor	Mitochondria/ER: Primarily ER protein that shapes the ER network. ER contact sites are critical for Mitochondrial fission and lipid exchange.
SRC	c-Src	Tyrosine Kinase	General Regulation: Phosphorylates many Actin-binding and Adhesion molecules. SRC signaling is implicated in Mitochondrial health and Autophagy.
AKT1	v-Akt Kinase	Signaling Kinase	Autophagy/Mitochondria: Key node in the PI3K/AKT pathway, which negatively regulates Autophagy (ULK1 inhibition) and supports Mitochondrial survival.
PCLO	Piccolo	Presynaptic Cytomatrix Scaffold	Synaptic Vesicles: Links the Synaptic vesicle cycle machinery to the underlying Actin cytoskeleton, regulating Vesicle docking and recycling (a form of Endocytosis).
IFT20	Intraflagellar Transport 20	IFT Machinery Component	Cilia/Vesicle Trafficking: Involved in trafficking proteins into the primary cilium. Also implicated in non-ciliary Vesicle transport from the Golgi to the cell periphery (general trafficking).

Figure 6. Aberrant Splicing Driven by *GBA1* Mutations Targets Genes Involved in Cell Projection Organization. (A) Deconvolution of differential splicing events for the genes involved in the regulation of cell projection organization (GO:0031344). The *GBA1* (VIS2) mutant clonal line E10B is shown as an example. (B) Differentially Spliced Genes in Cell Projection Regulation: A table summarizing key cytoskeletal genes among the 148 differentially spliced genes in the regulation of cell projection organization (GO:0031344) category in the *GBA1* (VIS2) mutant.

compared to the EWT controls (Supplemental Fig. S13E and S13F). The differentially expressed genes identified through GO enrichment analysis are detailed in Supplemental Table S3. Focusing in on the GO term Cell Projection organization (GO:0031344), we find 322 altered splicing events (Fig, 6A). The 221 genes with altered splicing patterns include genes involved in

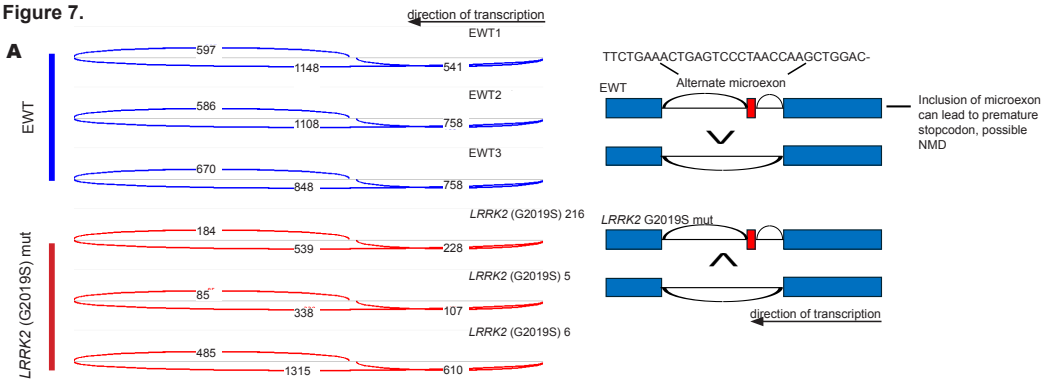
organelle movement, endosomes, lysosomes, autophagy and mitochondria (Fig. 6B). Again, these are genes encoding proteins linked to pathways that are disrupted in PD patients. Altered splicing patterns may contribute to defects in these pathways.

The ubiquitin ligase Ubr4 pre-mRNA splicing pattern is altered in the PD familial mutant hPSC cell lines and in PD patients

Following our analysis of individual familial PD lines, we conducted a cross-genotype comparison to determine if any specific genes were recurrently targeted by alternative splicing across the diverse genetic backgrounds of our platform. We screened the differential splicing profiles of the twelve PD mutant lines, comprising mutations in *PRKN, SNCA, LRRK2, PINK1, DNAJC6, FBXO7, SYNJ1, DJ1, VPS13C, ATP13A2* and *GBA1*, to identify commonalities. This systematic search revealed that *UBR4* (Ubiquitin Protein Ligase E3 Component N-Recognin 4) was differentially spliced in nine of the twelve mutant lines investigated. The high frequency of UBR4 splicing alterations across these distinct familial PD models suggests that it may serve as a convergent molecular node in the pathogenesis of both early- and late-onset Parkinson's disease.

UBR4 is a large ubiquitin ligase that as part of a protein complex linked to protein quality control and stress response signaling (Parsons et al. 2015; Kim et al. 2021a; Grabarczyk et al. 2025). The *UBR4* gene is large having over 100 exons and whose transcripts are subject to alternative splicing. Our JUM analysis shows that the normal transcript includes a microexon (exon #50 out of 108) that contains a stop codon, which we find to be more included in control EWT cells, whereas in *LRRK2* mutant cells the level of exon skipping (SE) is elevated (Fig. 7A). This would lead to more production of a protein-encoding transcript. We find that this particular exon (exon

Figure 7.



B

PD mutant	Splicing mode	Genomic Coordinates	ΔPSI (EWT-PD mut)
DJ1 **(X1_5DEL) 2860**	RI	chr1:19104666-19105046	0.18
DJ1 **(X1_5DEL) 2872**	RI	chr1:19110464-19110740	0.18
DJ1 **(X1_5DEL) 2876**	RI	chr1:19104666-19105046	0.28
DNAJC6 **(FS) G12_2**	SE	chr1:19148626-19150575	0.47
DNAJC6 **(FS) H10_2**	RI	chr1:19117910-19118870	-0.20
	SE	chr1:19148626-19150575	0.55
	composite	chr1:19139214-19143978	-0.09
FBXO7 **(R498X) A7_1**	RI	chr1:19185286-19186538	-0.32
	A3SS	chr1:19155075-19155439	-0.07
GBA1 **(IVS2) E10B**	SE	chr1:19148626-19150575	0.34
GBA1 **(IVS2) G2E**	SE	chr1:19148626-19150575	0.29
LRRK2 **(G2019S) 5**	RI	chr1:19160970-19161014	0.36
LRRK2 **(G2019S) 6**	SE	chr1:19148626-19150575	0.16
PINK1 **(Q129X) C4_1**	SE	chr1:19148626-19150575	0.45
PINK1 **(Q129X) E7_1**	A3SS	chr1:19145930-19146824	0.11
PRKN **(X3DEL) H2_2**	SE	chr1:19148626-19150575	-0.19
SNCA **(A30P) A2_3**	SE	chr1:19148626-19150575	-0.15
SNCA **(A30P) E1_3**	SE	chr1:19148626-19150575	-0.23
SNCA **(A53T) 2**	SE	chr1:19148626-19150575	0.36
	composite	chr1:19139214-19143978	-0.05
SNCA **(A53T) 3**	SE	chr1:19148626-19150575	0.31
SYNJ1 **(R258Q) A5_1**	SE	chr1:19148626-19150575	0.32
VPS13C **(W395C) C11_3**	RI	chr1:19104666-19105046	0.21
VPS13C **(W395C) C3_1**	RI	chr1:19104666-19105046	0.26
	RI	chr1:19110464-19110740	0.17
Patient Data	**Splicing mode**	**Genomic Coordinates**	**ΔPSI (control-PD patient)**
PD patients	A3SS	chr1:19155075-19155439	-0.10
	SE	chr1:19148626-19150575	0.27
DLB patients	A3SS	chr1:19101635-19104082	-0.06
	A3SS	chr1:19155075-19155439	-0.13
	RI	chr1:19093486-19093947	-0.24
	SE	chr1:19148626_-1915057	-0.33

Figure 7. The transcripts from the *UBR4* gene are differentially spliced in midbrain DA neuronal cells expressing familial PD mutations and in PD patient brain biopsies. (A) Sashimi plots of the differentially spliced regions from the UBR4 (SE: chr1:19,148,626-19,150,575) gene are shown with the number of splice junction reads indicated for the *LRRK2* (G2019S) mutant and EWT control. (B) Differential splicing events of *UBR4* gene in familial PD mutants are generated in this study and PD patient postmortem brains, including genomic coordinates and delta PSI.

50) is skipped more often in many of the familial mutant cell lines, as well as in some PD patient biopsies (Fig. 7B; chr1: 19148626-19150575). However, in some mutants the level of exon inclusion of exon 50 is elevated, suggesting that levels if UBR4 would fluctuate in PD patients. Notably, the levels of exon skipping (SE) and/or intron retention (RI) for other splicing events in the UBR4 pre-mRNA are also elevated in many of the familial mutant cell lines tested (Fig. 7B). Intron retention events would yield transcripts that would not encode the full-length protein and should lower protein levels. These results indicate that splicing control would modulate the levels of active UBR4 protein and likely perturb the balance of UBR4 available to interact with its obligatory cofactors (Kim et al. 2018; Jeong et al. 2023; Grabarczyk et al. 2025) and thereby affect its ability to maintain proteostasis in PD patient neurons. Given the prevalence of these splicing changes and the link of *UBR4* to proteostasis and stress signaling, we speculate that variable mRNA isoforms of *UBR4* could affect its ability to function to maintain proteostasis in the context of dopaminergic neurons.

Shared alternative splicing events found in hPSC-derived mDA neurons and PD patient anterior cingulate cortex postmortem brain

We used a publicly available RNA-sequencing dataset from a study by Feleke et al. (2021) and reanalyzed it with our own software, JUM, for differential splicing analysis. The dataset includes postmortem brain cortex samples from 19 patients with different Lewy body diseases and 5 healthy controls, which we used for comparison with our own hPSC data. The Ryten group used Leafcutter (Li et al. 2018) for alternative splicing pattern analysis and determined that 305 PD-associated genes had splicing pattern changes (Feleke et al. 2021). Leafcutter uses an intron-centric view of splicing and therefore all detected splicing events are given as intron coordinates in split-read clusters. It defines AS events as clusters of overlapping introns which may involve multiple alternative 3’ and 5’ splice sites and skipped exons, making it hard to determine the actual patterns and modes of alternative splicing used by specific splicing junctions. Hence, we took the Ryten lab’s raw data (which was deeply sequenced at 150-250

million PE150 Illumina reads) and analyzed the data using JUM (Wang and Rio 2018), which allowed us to determine AS modes and patterns, while yielding a quantitative comparison of PSI values for usage of all detected splice junctions.

We compared the postmortem brain cortex data analyzed by either JUM or Leafcutter (Supplemental Fig. S15) to the RNA-seq data from the hPSC-derived mutant mDA neuron cultures (Supplemental Fig. S2D-S13D). Because JUM and Leafcutter use different algorithms for splicing pattern analysis, as well as different cutoffs for statistical analysis, there was some overlap but also differences in terms of the number of differentially spliced transcripts between the software programs. While there were slightly fewer splicing changes found by JUM in the PD patient data, the general trend was the same, in which the most differential splicing events identified in our cell models were observed in patients with DLB compared to PD or PDD (Supplemental Fig. S2D-S13D). This analysis shows that alternative splicing is an important mechanism in the development of Lewy body diseases, including PD and can be used to distinguish these three clinically distinct Lewy body diseases (Supplemental Fig. S15).

We next sought to pinpoint genes commonly affected by familial PD-associated mutations, focusing on those showing differential splicing or expression profiles. This approach identified 906 genes with significant splicing alterations and 172 genes with altered expression patterns (Supplemental Fig. S13). Comparative analysis of these two gene sets revealed a core group of six genes - *SLC38A10*, *CHL1*, *CRNDE*, *NPHP4*, *GALNTL6*, and *VGF* displayed changes in both splicing and expression across a broad spectrum of familial PD mutations. These genes participate in diverse neuronal processes relevant to PD: *SLC38A10* in glutamine metabolism (Hellsten et al. 2017; Tripathi et al. 2021), *CHL1* in dopamine neuron development (Nishimune et al. 2005; Bye et al. 2019; Fernandes et al. 2023), *NPHP4* in cilia function (Jauregui et al. 2008) and *VGF* as a potential PD biomarker (Quinn et al. 2021; Karayel et al. 2022; Filippini et al. 2023). While the direct role of some of these genes in PD requires further investigation,

future research into their involvement is warranted. Overall, however, it is apparent that in many cases there are many non-overlapping changes in splicing versus gene expression in these datasets, indicating that changes in protein isoforms due to alternative splicing would not be apparent from gene expression analysis (Supplemental Fig. S14).

Differential gene expression profiles of Day 35 hPSC-derived mDA cells carrying familial PD mutations

We also used the RNA-seq data from the *PRKN, SNCA, LRRK2, PINK1, DNAJC6, FBXO7, SYNJ1,* DJ1*, VPS13C, ATP13A2 and GBA1* mutant and edited wild type control cell lines to analyze overall gene expression (RNA transcript levels) using DESeq2 (Love et al. 2014). This analysis showed that there was differential gene expression of several hundred to several thousand genes in the *LRRK2*, *SNCA* (A53T), *PINK1*, DJ1*, VPS13C* and *SYNJ1* mutant cells (Supplemental Fig. S2-S13D and F and Supplemental Table 3). Mutations in the *SNCA (*A30P), *PRKN*, *FBXO7*, *ATP13A2*, *PINK1*, *DNAJC6*, DJ1, *VPS13C* and *SYNJ1* altered expression of genes linked to cell communication, synaptic transmission, membrane and ion transport, neuronal projections and protein trafficking to the membrane (Supplemental Fig. S2-S13E and F and Supplemental Table 3). We note that there is some variability in the extent of differentiation of each mutant or wild type cell clone in a given experiment, but these measurements were made in technical triplicate with 1-3 independent cell clones per mutation. We found that there was often variable overlap between gene expression versus pre-mRNA splicing changes in different mutant cells compared to wild type but that many of the splicing changes are distinct from the transcript level gene expression changes (Supplemental Fig. S14, Supplemental Table 4). This observation may not be surprising because it has been shown that alternative splicing in the nervous system can often result in mRNA levels whose overall expression does not change but alternative splicing generates mRNA isoforms encoding proteins with altered protein-protein interactions (Ellis et al. 2012; Gonatopoulos-Pournatzis and Blencowe 2020). These alterations in transcript levels or isoform changes could lead to different levels or

composition of protein complexes, altering proteostasis that would alter cellular phenotypes relevant to PD, including the cytoskeleton, GTPase regulation, organelle ad vesicle trafficking, cilia, neuronal projections, as well as altered splicing factor networks (Supplemental Fig. S2-S13; Supplemental Tables 2-3).

Taken together, these experiments provide a comprehensive list of genes whose expression levels change in cells carrying 12 distinct familial PD mutations when the hPSCs are differentiated to mDA neurons *in vitro*. Many of the genes whose expression levels are affected, like Rab family members (Bonet-Ponce and Cookson 2019; Bellucci et al. 2022; Alessi and Pfeffer 2024) and SNCA itself (Polymeropoulos et al. 1997; Dauer and Przedborski 2003; Singleton et al. 2003) are linked to PD. In other neurodegenerative diseases, RNA binding proteins, such as TARDBP/TDP-43 and FUS (Neumann et al. 2006; Hewitt et al. 2010; Mackenzie et al. 2010; Moens et al. 2025) are altered and which have been linked to ALS (Supplemental Table 3). The discovery of widespread splicing alterations across this diverse panel of 12 familial PD mutations suggests that aberrant pre-mRNA processing is a common molecular signature of Parkinson's disease, transcending individual genetic etiologies to drive neuronal dysfunction.

Discussion

Parkinson's disease (PD) lacks a cure, and L-DOPA, its main treatment, loses efficacy and causes side effects over time (Kwon et al. 2022; Dias et al. 2023). Early detection is challenging due to limited biomarkers (Concha-Marambio et al. 2023; Fernandes Gomes et al. 2023). Intensive fundamental research is vital to uncover underlying mechanisms for developing early diagnostics and new therapies (Schekman and Riley 2019). Defects in protein homeostasis, aggregation, RNA binding proteins and RNA processing lead to pathologic physiological changes in PD, as is the case for other neurodegenerative diseases, like amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) (Brinegar and Cooper

2016; Conlon and Manley 2017; Mann and Donnelly 2021). Identifying RNA processing events and pre-mRNA splicing isoforms linked to these disease states has led to identification of target disease-relevant transcripts for therapeutic interventions using antisense oligonucleotides (ASOs) (Bennett et al. 2019; Meyer et al. 2020), small molecules (Meyer et al. 2020), and gene therapy (Li et al. 2023). Given the advances in the delivery of nucleic acid therapeutics (Damase et al. 2021) and messenger RNA (Huang et al. 2022), it is likely that once key RNA level targets are identified for PD, therapeutic interventions can be developed. The platform developed and outlined here utilizing hPSCs with PD relevant mutations has the potential to aid in the development of PD therapeutics.

It is clear that for other diseases, such as a variety of cancers, including blood cancers like myelodysplastic syndrome (MDS), and neurodegenerative diseases like SMA, Huntington disease (HD) and ALS, that mutations in RNA binding proteins and known pre-mRNA splicing factors and specific splicing events can play causative roles in the disease states and are now successful therapeutic targets (Kapeli et al. 2017; Gebauer et al. 2021; Rogalska et al. 2023). Many neurodegenerative disease states involve changes in proteostasis, mitochondrial, lysosomal and other metabolic and cell biological pathways (Jagtap et al. 2023; Wilson et al. 2023). In many cases, protein aggregation, for instance the TDP-43 protein in ALS and in PD α-synuclein aggregates are key indicators of disease. In the case of ALS, two key RNA processing events involving the *stathmin 2* (*STMN2*) (Baughn et al. 2023) and *UNC13A* genes (Brown et al. 2022; Lipstein 2022; Ma et al. 2022) are targets for therapeutic interventions. In addition, splicing alterations in *STMN-2* and *UNC13A* have recently been found in Alzheimer's disease patients (Agra Almeida Quadros et al. 2024).

Here, we have developed a system to investigate RNA-level changes that are linked to 11 of the 20 most common familial PD hereditary genes, including *PRKN, SNCA, LRRK2, PINK1,*

DNAJC6, FBXO7, SYNJ1, DJ1*, VPS13C, ATP13A2 and GBA1* (Poewe et al. 2017). Our results linking splicing pattern alterations to defective PD pathways are consistent with uncovered defects in global pre-mRNA splicing patterns discovered in other neurodegenerative diseases such as ALS (Wang et al. 2020; Li et al. 2021), SMA (Singh and Singh 2018) and various cancers (Zhang and Manley 2013; El Marabti and Abdel-Wahab 2021; Stanley and Abdel-Wahab 2022; Bradley and Anczukow 2023; Choi et al. 2023). It is also clear that mutations in RNA binding protein genes or altered expression levels of RNA binding proteins, such as TDP-43 (Kapeli et al. 2017; Nussbacher et al. 2019), splicing factors and spliceosome components, such as U2AF1 and SF3B1 (Choi et al., 2023, Bradley and Anczukow, 2023, Stanley and Abdel-Wahab, 2022, El Marabti and Abdel-Wahab, 2021) play roles in neurodegeneration and cancer, respectively.

One important finding from this work is that there are many RNA splicing changes, as well as gene expression level changes, in the PD mutant hPSC-derived mDA cells. The PD relevance of these data is supported by the splicing pattern changes in genes linked to defective organelle trafficking and function. Further, analysis of RNA-seq data from PD patient postmortem brain samples (Feleke et al. 2021), which overlaps with some of the splicing pattern changes found in our RNA-seq analysis of hPSC-derived mDA cells carrying familial PD mutations, provide connections to pathways affected in PD. In our initial studies with the familial *PRKN, SNCA, LRRK2, PINK1, DNAJC6, FBX7, SYNJ1,* DJ1*, VPS13C, ATP13A2 and GBA1* mutants, we have identified common splicing pattern changes for the well-known ubiquitin ligase UBR4, which is part of a ubiquitylation complex, linked to stress signaling pathways (Parsons et al. 2015; Kim et al. 2021a; Grabarczyk et al. 2025). Alterations in *UBR4* splicing can lead to different mRNA isoforms that could affect the function of UBR4 in proteostasis in mDA and PD patient neurons. Thus, the platform we have developed enabled the identification of common defects in RNA processing patterns in mDA cells derived from hPSCs engineered with other PD familial mutations (Busquets et al., 2025).

Methods

WIBR3-hPSCs derived DA neuron differentiation:

WIBR3 hPSCs (passage 25-30) were maintained and grown on iMatrix-511 (Takara, T303) coated 6-well plates in mTESR plus media (Stem Cell Technologies, 100-0276). For DA neuron differentiation, the hPSCs were dissociated into single cells and seeded on matrigel (Corning, 356231) coated plates with Rock inhibitor (Chemdea CD0141) at 10 μM to initiate the differentiation. We followed the recently published protocol (Kim et al. 2021b) with slight modifications. Briefly, single cell suspension of hPSCs were maintained with Neurobasal N2/B27 (ThermoFisher, 21103049) media containing 2 mM glutamine (Sigma, G8450) with 200 ng/ml SHH C25II (R&D Systems, 1845-SH-100) 250 nM LDN (Stemgent, 04-0074),10 μM SB431542 (SelleckChem, S1067), 0.7 μM CHIR99021 (Tocris, 4423) on day 1 of differentiation. On day 4, CHIR concentration was increased to 7.5 μM and on day 7 LDN, SB, SHH were removed, and media was supplemented with only CHIR. On day 10, media was changed to Neurobasal B27 with 2 mM glutamine supplemented with 20 ng/ml BDNF (Peprotech, 450-02), 20 ng/ml GDNF (Peprotech, 450-10), 200 μM ascorbic acid (AA) (Sigma, A40-34) 500 μM dbcAMP (SelleckChem, S7858) and 1 ng/ml TGFb3 (R&D Systems, 8420-B3-005). On Day 11, cells were dissociated with Accutase (ThermoFisher, 00-4555-56) and split into a 1:2 or 1:4 ratio and replated on Poly L-Ornithine (PLO; 30 μg/ml; Sigma, P3655), Cultrex laminin (1 ug/ml; R&D Systems, 3400-010-02) coated plates and maintained in maturation media containing BDNF, GDNF, TGFb3, AA, DAPT from day 12. On day 16, cells were dissociated with Accutase (ThermoFisher, 00-4555-56) and replated at desired high density (1-2x10^6 cells per well of 12 well plate) depending on cell growth and maintained in maturation medium (at this stage cells were cryopreserved in serum free cryopreservation media cell banker 2 (Amsbio, 11914) and thawed as per the experiment). On day 25, cells were dissociated with Accutase

(ThermoFisher, 00-4555-56) and replated at higher density (>1x10^6 cells per of 12 well plate until the experiment. On day 35, cells were lysed in the RLT lysis buffer using Qiagen kit (RNeasy, 74104) and RNA was isolated for bulk RNA sequencing.

RT-PCR validation for splicing analysis

To validate several changes in alternative pre-mRNA splicing patterns found in the RNA-seq data we used reverse-transcription polymerase chain reaction (RT-PCR). In these experiments, we used analysis of *GAPDH* as a loading control. We found alterations in the levels of spliced mRNAs (Supplemental Fig. S8). 0.5 microgram of total RNA was reverse-transcribed according to the manufacturer's instructions (Bio-Rad, 1708891) and subjected to RT–PCR with the following conditions: 30 sec at 98°C (one cycle); 10 sec at 98°C, 30 sec at 60°C, and 30 sec at 72°C (35 cycles); and 5 min at 72°C (one cycle). The primer sets used in the RT-PCR reaction is listed in Supplement Figure S10B. RT-PCR products were resolved, visualized, and quantitated by use of an 2% agarose gels. Differential splicing events for *UBR4*, *DOCK3*, and *CAMPTA2* were shown as examples in *GBA1* (FS) mut compared to EWT control cells (Supplemental Fig. S10).

RNA-seq library preparation and sequencing

RNA isolation was performed using the RNeasy Minikit (Qiagen, 74104), followed by 30 min. of DNase treatment (Ambion, AM2238) at 37°C, poly(A)$^+$ RNA transcripts were isolated [NEBNext poly(A) mRNA magnetic isolation module; New England Biolabs, E7490] from 0.5 µg of total RNA for RNA library preparation and sequencing using NEBNext Ultra Directional RNA Library Preparation Kit for Illumina (New England Biolabs, E7420S) according to the manufacturer's instruction. The samples were sequenced on an Illumina S1 or S4 with 150-bp paired-end reads at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California at Berkeley. Typical samples gave ~200-300M PE150 reads.

Analysis of pre-mRNA splicing patterns using JUM and differential expression analysis using DESeq2

Pre-mRNA splicing analysis using JUM.2.0.2 software to detect pre-mRNA splicing patterns was performed as described before (Lee et al. 2018; Wang and Rio 2018). An adjusted *p*-value (FDR) of 0.1 was used as the statistical cutoff for differentially spliced AS events with at least 10 junction reads.

For Differential expression analysis, uniquely mapped reads were mapped to hg38 using STAR_2.5.3a (Dobin et al. 2013) modified version using --outFilterMultimapNmax 1. Read counts were calculated using HTSeq script htseq-count (Anders et al. 2015) with the following specifications: -s yes -r pos -f. Differential gene expression analysis was performed using DESeq2 (Love et al. 2014), only genes with at least 10 reads across all samples were considered for further analysis.

Competing Interest Statement

There are no competing interests.

Data deposition

The data reported will be deposited in the Gene Expression Omnibus (GEO) database upon the acceptance of the manuscript.

Publicly available RNA-seq data for WIBR3 hPSCs were downloaded (GEO accession numbers: GSM3069316, GSM3069317, GSM3069324, and GSM3069325) for comparison with differentiated EWT and familial PD mutants.

Acknowledgments:

This work was funded by Aligning Science Across Parkinson's (ASAP-024409, ASAP-000486) through the Michael J. Fox Foundation for Parkinson's Research (MJFF) and the NIH (R35GM118121; DR). For the purpose of open access, the authors have applied a CC-BY public copyright license to the Author Accepted Manuscript version arising from this submission. Illumina RNA sequencing was carried out at the U.C., Berkeley QB3 Coates Genome Sequencing Laboratory (GSL), supported by National Institutes of Health (NIH) S10 Instrumentation grants S10RR025622, S10RR029668, and S10RR027303.

Author Contributions:

D.H., F.S., H.S.B and D.C.R. supervised the study; Y.J.L. performed RNA isolation and RNA-seq library preparation, quality controls, RT-PCR validation, the bioinformatic and statistical analyses of alternative splicing and gene expression profiling, generated all the figures and tables. K.M.S performed dopaminergic differentiations from genome-edited cell lines and contributed Fig. 1A. H.L, O.B, J.D established the PD familial mutant cell lines. Y.J.L. and D.C.R. wrote the initial draft of the manuscript. All authors contributed to and edited the final manuscript.

References:

Aarsland D, Batzu L, Halliday GM, Geurtsen GJ, Ballard C, Ray Chaudhuri K, Weintraub D. 2021. Parkinson disease-associated cognitive impairment. *Nat Rev Dis Primers* **7**: 47.

Agra Almeida Quadros AR, Li Z, Wang X, Ndayambaje IS, Aryal S, Ramesh N, Nolan M, Jayakumar R, Han Y, Stillman H et al. 2024. Cryptic splicing of stathmin-2 and UNC13A mRNAs is a pathological hallmark of TDP-43-associated Alzheimer's disease. *Acta Neuropathol* **147**: 9.

Alessi DR, Pfeffer SR. 2024. Leucine-Rich Repeat Kinases. *Annu Rev Biochem* **93**: 261-287.

Anders S, Pyl PT, Huber W. 2015. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**: 166-169.

Anthony K. 2022. RNA-based therapeutics for neurological diseases. *RNA Biol* **19**: 176-190.

Baughn MW, Melamed Z, Lopez-Erauskin J, Beccari MS, Ling K, Zuberi A, Presa M, Gonzalo-Gil E, Maimon R, Vazquez-Sanchez S et al. 2023. Mechanism of STMN2 cryptic splice-polyadenylation and its correction for TDP-43 proteinopathies. *Science* **379**: 1140-1149.

Beilina A, Van Der Brug M, Ahmad R, Kesavapany S, Miller DW, Petsko GA, Cookson MR. 2005. Mutations in PTEN-induced putative kinase 1 associated with recessive parkinsonism have differential effects on protein stability. *Proc Natl Acad Sci U S A* **102**: 5703-5708.

Bellucci A, Longhena F, Spillantini MG. 2022. The Role of Rab Proteins in Parkinson's Disease Synaptopathy. *Biomedicines* **10**.

Bennett CF, Krainer AR, Cleveland DW. 2019. Antisense Oligonucleotide Therapies for Neurodegenerative Diseases. *Annu Rev Neurosci* **42**: 385-406.

Boecker CA, Goldsmith J, Dou D, Cajka GG, Holzbaur ELF. 2021. Increased LRRK2 kinase activity alters neuronal autophagy by disrupting the axonal transport of autophagosomes. *Curr Biol* **31**: 2140-2154 e2146.

Bonet-Ponce L, Cookson MR. 2019. The role of Rab GTPases in the pathobiology of Parkinson' disease. *Curr Opin Cell Biol* **59**: 73-80.

Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, Brice A, van Duijn CM, Oostra B, Meco G et al. 2003a. DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurol Sci* **24**: 159-160.

Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, Dekker MC, Squitieri F, Ibanez P, Joosse M et al. 2003b. Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* **299**: 256-259.

Bradley RK, Anczukow O. 2023. RNA splicing dysregulation and the hallmarks of cancer. *Nat Rev Cancer* **23**: 135-155.

Brinegar AE, Cooper TA. 2016. Roles for RNA-binding proteins in development and disease. *Brain Res* **1647**: 1-8.

Brown AL, Wilkins OG, Keuss MJ, Hill SE, Zanovello M, Lee WC, Bampton A, Lee FCY, Masino L, Qi YA et al. 2022. TDP-43 loss and ALS-risk SNPs drive mis-splicing and depletion of UNC13A. *Nature* **603**: 131-137.

Busquets O, Li H, Syed KM, Jerez PA, Dunnack J, Bu RL, Verma Y, Pangilinan GR, Martin A, Straub J et al. 2025. iSCORE-PD: an isogenic stem cell collection to research Parkinson's Disease. *bioRxiv*.

Bye CR, Rytova V, Alsanie WF, Parish CL, Thompson LH. 2019. Axonal Growth of Midbrain Dopamine Neurons is Modulated by the Cell Adhesion Molecule ALCAM Through Trans-Heterophilic Interactions with L1cam, Chl1, and Semaphorins. *J Neurosci* **39**: 6656-6667.

Canet-Aviles RM, Wilson MA, Miller DW, Ahmad R, McLendon C, Bandyopadhyay S, Baptista MJ, Ringe D, Petsko GA, Cookson MR. 2004. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. *Proc Natl Acad Sci U S A* **101**: 9103-9108.

Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, Larvor L, Andrieux J, Hulihan M et al. 2004. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* **364**: 1167-1169.
Chen Z, Li G, Liu J. 2020. Autonomic dysfunction in Parkinson's disease: Implications for pathophysiology, diagnosis, and treatment. *Neurobiol Dis* **134**: 104700.
Choi S, Cho N, Kim EM, Kim KK. 2023. The role of alternative pre-mRNA splicing in cancer progression. *Cancer Cell Int* **23**: 249.
Choudhry H, Aggarwal M, Pan PY. 2021. Mini-review: Synaptojanin 1 and its implications in membrane trafficking. *Neurosci Lett* **765**: 136288.
Concha-Marambio L, Pritzkow S, Shahnawaz M, Farris CM, Soto C. 2023. Seed amplification assay for the detection of pathologic alpha-synuclein aggregates in cerebrospinal fluid. *Nat Protoc* **18**: 1179-1196.
Conlon EG, Manley JL. 2017. RNA-binding proteins in neurodegeneration: mechanisms in aggregate. *Genes & development* **31**: 1509-1528.
Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP. 2021. The Limitless Future of RNA Therapeutics. *Front Bioeng Biotechnol* **9**: 628137.
Dauer W, Przedborski S. 2003. Parkinson's disease: mechanisms and models. *Neuron* **39**: 889-909.
Dehay B, Ramirez A, Martinez-Vicente M, Perier C, Canron MH, Doudnikoff E, Vital A, Vila M, Klein C, Bezard E. 2012. Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. *Proc Natl Acad Sci U S A* **109**: 9611-9616.
Di Fonzo A, Dekker MC, Montagna P, Baruzzi A, Yonova EH, Correia Guedes L, Szczerbinska A, Zhao T, Dubbel-Hulsman LO, Wouters CH et al. 2009. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology* **72**: 240-245.
Dias CMV, Leal DAB, Brys I. 2023. Levodopa-induced dyskinesia is preceded by increased levels of anxiety and motor impairment in Parkinson's disease patients. *Int J Neurosci* **133**: 1319-1325.
Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**: 15-21.
Dodson MW, Guo M. 2007. Pink1, Parkin, DJ-1 and mitochondrial dysfunction in Parkinson's disease. *Curr Opin Neurobiol* **17**: 331-337.
Drouet V, Lesage S. 2014. Synaptojanin 1 mutation in Parkinson's disease brings further insight into the neuropathological mechanisms. *Biomed Res Int* **2014**: 289728.
Edvardson S, Cinnamon Y, Ta-Shma A, Shaag A, Yim YI, Zenvirt S, Jalas C, Lesage S, Brice A, Taraboulos A et al. 2012. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. *PLoS One* **7**: e36458.
El Marabti E, Abdel-Wahab O. 2021. Therapeutic Modulation of RNA Splicing in Malignant and Non-Malignant Disease. *Trends Mol Med* **27**: 643-659.
Ellis JD, Barrios-Rodiles M, Colak R, Irimia M, Kim T, Calarco JA, Wang X, Pan Q, O'Hanlon D, Kim PM et al. 2012. Tissue-specific alternative splicing remodels protein-protein interaction networks. *Mol Cell* **46**: 884-892.
Feleke R, Reynolds RH, Smith AM, Tilley B, Taliun SAG, Hardy J, Matthews PM, Gentleman S, Owen DR, Johnson MR et al. 2021. Cross-platform transcriptional profiling identifies common and distinct molecular pathologies in Lewy body diseases. *Acta Neuropathol* **142**: 449-474.
Fellner L, Gabassi E, Haybaeck J, Edenhofer F. 2021. Autophagy in alpha-Synucleinopathies-An Overstrained System. *Cells* **10**.
Fernandes Gomes B, Farris CM, Ma Y, Concha-Marambio L, Lebovitz R, Nellgard B, Dalla K, Constantinescu J, Constantinescu R, Gobom J et al. 2023. alpha-Synuclein seed

amplification assay as a diagnostic tool for parkinsonian disorders. *Parkinsonism Relat Disord* **117**: 105807.
Fernandes L, Kleene R, Congiu L, Freitag S, Kneussel M, Loers G, Schachner M. 2023. CHL1 depletion affects dopamine receptor D2-dependent modulation of mouse behavior. *Front Behav Neurosci* **17**: 1288509.
Filippini F, Nola S, Zahraoui A, Roger K, Esmaili M, Sun J, Wojnacki J, Vlieghe A, Bun P, Blanchon S et al. 2023. Secretion of VGF relies on the interplay between LRRK2 and post-Golgi v-SNAREs. *Cell Rep* **42**: 112221.
Fujii T, Nagamori S, Wiriyasermkul P, Zheng S, Yago A, Shimizu T, Tabuchi Y, Okumura T, Fujii T, Takeshima H et al. 2023. Parkinson's disease-associated ATP13A2/PARK9 functions as a lysosomal H(+),K(+)-ATPase. *Nat Commun* **14**: 2174.
Gebauer F, Schwarzl T, Valcarcel J, Hentze MW. 2021. RNA-binding proteins in human genetic disease. *Nat Rev Genet* **22**: 185-198.
Gonatopoulos-Pournatzis T, Blencowe BJ. 2020. Microexons: at the nexus of nervous system development, behaviour and autism spectrum disorder. *Curr Opin Genet Dev* **65**: 22-33.
Grabarczyk DB, Ehrmann JF, Murphy P, Yang WS, Kurzbauer R, Bell LE, Deszcz L, Neuhold J, Schleiffer A, Shulkina A et al. 2025. Architecture of the UBR4 complex, a giant E4 ligase central to eukaryotic protein quality control. *Science* **389**: 909-914.
Greggio E, Cookson MR. 2009. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro* **1**.
Hellsten SV, Hagglund MG, Eriksson MM, Fredriksson R. 2017. The neuronal and astrocytic protein SLC38A10 transports glutamine, glutamate, and aspartate, suggesting a role in neurotransmission. *FEBS Open Bio* **7**: 730-746.
Hewitt C, Kirby J, Highley JR, Hartley JA, Hibberd R, Hollinger HC, Williams TL, Ince PG, McDermott CJ, Shaw PJ. 2010. Novel FUS/TLS mutations and pathology in familial and sporadic amyotrophic lateral sclerosis. *Arch Neurol* **67**: 455-461.
Huang X, Kong N, Zhang X, Cao Y, Langer R, Tao W. 2022. The landscape of mRNA nanomedicine. *Nat Med* **28**: 2273-2287.
Huh YE, Usnich T, Scherzer CR, Klein C, Chung SJ. 2023. GBA1 Variants and Parkinson's Disease: Paving the Way for Targeted Therapy. *J Mov Disord* **16**: 261-278.
Ibanez P, Lesage S, Lohmann E, Thobois S, De Michele G, Borg M, Agid Y, Durr A, Brice A, French Parkinson's Disease Genetics Study G. 2006. Mutational analysis of the PINK1 gene in early-onset parkinsonism in Europe and North Africa. *Brain* **129**: 686-694.
Isaacs D, Claassen D, Bowman AB, Hedera P. 2017. Phenotypic Discordance in Siblings with Identical Compound Heterozygous PARK2 Mutations. *Brain Sci* **7**.
Ishihara-Paul L, Hulihan MM, Kachergus J, Upmanyu R, Warren L, Amouri R, Elango R, Prinjha RK, Soto A, Kefi M et al. 2008. PINK1 mutations and parkinsonism. *Neurology* **71**: 896-902.
Jagtap YA, Kumar P, Kinger S, Dubey AR, Choudhary A, Gutti RK, Singh S, Jha HC, Poluri KM, Mishra A. 2023. Disturb mitochondrial associated proteostasis: Neurodegeneration and imperfect ageing. *Front Cell Dev Biol* **11**: 1146564.
Jaleel M, Nichols RJ, Deak M, Campbell DG, Gillardon F, Knebel A, Alessi DR. 2007. LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem J* **405**: 307-317.
Jauregui AR, Nguyen KC, Hall DH, Barr MM. 2008. The Caenorhabditis elegans nephrocystins act as global modifiers of cilium structure. *J Cell Biol* **180**: 973-988.
Jeong DE, Lee HS, Ku B, Kim CH, Kim SJ, Shin HC. 2023. Insights into the recognition mechanism in the UBR box of UBR4 for its specific substrates. *Commun Biol* **6**: 1214.
Kapeli K, Martinez FJ, Yeo GW. 2017. Genetic mutations in RNA-binding proteins and their roles in ALS. *Hum Genet* **136**: 1193-1214.
Karayel O, Virreira Winter S, Padmanabhan S, Kuras YI, Vu DT, Tuncali I, Merchant K, Wills AM, Scherzer CR, Mann M. 2022. Proteome profiling of cerebrospinal fluid reveals biomarker candidates for Parkinson's disease. *Cell Rep Med* **3**: 100661.

Khan SS, Sobu Y, Dhekne HS, Tonelli F, Berndsen K, Alessi DR, Pfeffer SR. 2021. Pathogenic LRRK2 control of primary cilia and Hedgehog signaling in neurons and astrocytes of mouse brain. *Elife* **10**.
Kim JG, Shin HC, Seo T, Nawale L, Han G, Kim BY, Kim SJ, Cha-Molstad H. 2021a. Signaling Pathways Regulated by UBR Box-Containing E3 Ligases. *Int J Mol Sci* **22**.
Kim ST, Lee YJ, Tasaki T, Hwang J, Kang MJ, Yi EC, Kim BY, Kwon YT. 2018. The N-recognin UBR4 of the N-end rule pathway is required for neurogenesis and homeostasis of cell surface proteins. *PLoS One* **13**: e0202260.
Kim TW, Piao J, Koo SY, Kriks S, Chung SY, Betel D, Socci ND, Choi SJ, Zabierowski S, Dubose BN et al. 2021b. Biphasic Activation of WNT Signaling Facilitates the Derivation of Midbrain Dopamine Neurons from hESCs for Translational Use. *Cell Stem Cell* **28**: 343-355 e345.
Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, Shimizu N. 1998. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392**: 605-608.
Komori T, Kuwahara T. 2023. An Update on the Interplay between LRRK2, Rab GTPases and Parkinson's Disease. *Biomolecules* **13**.
Kouli A, Torsney KM, Kuan WL. 2018. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. in *Parkinson's Disease: Pathogenesis and Clinical Aspects* (eds. TB Stoker, JC Greenland), Brisbane (AU).
Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, Przuntek H, Epplen JT, Schols L, Riess O. 1998. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* **18**: 106-108.
Kwon DK, Kwatra M, Wang J, Ko HS. 2022. Levodopa-Induced Dyskinesia in Parkinson's Disease: Pathogenesis and Emerging Treatment Strategies. *Cells* **11**.
Lee YJ, Wang QQ, Rio DC. 2018. Coordinate regulation of alternative pre-mRNA splicing events by the human RNA chaperone proteins hnRNPA1 and DDX5. *Gene Dev* **32**: 1060-1074.
Lehtonen S, Sonninen TM, Wojciechowski S, Goldsteins G, Koistinaho J. 2019. Dysfunction of Cellular Proteostasis in Parkinson's Disease. *Front Neurosci* **13**: 457.
Lesage S, Brice A. 2009. Parkinson's disease: from monogenic forms to genetic susceptibility factors. *Hum Mol Genet* **18**: R48-59.
Lesage S, Drouet V, Majounie E, Deramecourt V, Jacoupy M, Nicolas A, Cormier-Dequaire F, Hassoun SM, Pujol C, Ciura S et al. 2016. Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and Increases PINK1/Parkin-Dependent Mitophagy. *Am J Hum Genet* **98**: 500-513.
Li D, Mastaglia FL, Yau WY, Chen S, Wilton SD, Akkari PA. 2022a. Targeted Molecular Therapeutics for Parkinson's Disease: A Role for Antisense Oligonucleotides? *Mov Disord* **37**: 2184-2190.
Li D, McIntosh CS, Mastaglia FL, Wilton SD, Aung-Htut MT. 2021. Neurodegenerative diseases: a hotbed for splicing defects and the potential therapies. *Transl Neurodegener* **10**: 16.
Li H, Busquets O, Verma Y, Syed KM, Kutnowski N, Pangilinan GR, Gilbert LA, Bateup HS, Rio DC, Hockemeyer D et al. 2022b. Highly efficient generation of isogenic pluripotent stem cell models using prime editing. *Elife* **11**.
Li Y, Zeng H, Wei Y, Ma X, He Z. 2023. An Overview of the Therapeutic Strategies for the Treatment of Spinal Muscular Atrophy. *Hum Gene Ther* **34**: 180-191.
Li YI, Knowles DA, Humphrey J, Barbeira AN, Dickinson SP, Im HK, Pritchard JK. 2018. Annotation-free quantification of RNA splicing using LeafCutter. *Nat Genet* **50**: 151-158.
Lipstein N. 2022. Mechanism underlying a risk gene in neurodegeneration. *Nature* **603**: 33-34.
Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**: 550.

Ma XR, Prudencio M, Koike Y, Vatsavayai SC, Kim G, Harbinski F, Briner A, Rodriguez CM, Guo C, Akiyama T et al. 2022. TDP-43 represses cryptic exon inclusion in the FTD-ALS gene UNC13A. *Nature* **603**: 124-130.
Mackenzie IR, Rademakers R, Neumann M. 2010. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet Neurol* **9**: 995-1007.
Mann JR, Donnelly CJ. 2021. RNA modulates physiological and neuropathological protein phase transitions. *Neuron* **109**: 2663-2681.
Mendoza-Velasquez JJ, Flores-Vazquez JF, Barron-Velazquez E, Sosa-Ortiz AL, Illigens BW, Siepmann T. 2019. Autonomic Dysfunction in alpha-Synucleinopathies. *Front Neurol* **10**: 363.
Meyer SM, Williams CC, Akahori Y, Tanaka T, Aikawa H, Tong Y, Childs-Disney JL, Disney MD. 2020. Small molecule recognition of disease-relevant RNA structures. *Chem Soc Rev* **49**: 7167-7199.
Moens TG, Da Cruz S, Neumann M, Shelkovnikova TA, Shneider NA, Van Den Bosch L. 2025. Amyotrophic lateral sclerosis caused by FUS mutations: advances with broad implications. *Lancet Neurol* **24**: 166-178.
Navarro-Romero A, Montpeyo M, Martinez-Vicente M. 2020. The Emerging Role of the Lysosome in Parkinson's Disease. *Cells* **9**.
Neumann J, Bras J, Deas E, O'Sullivan SS, Parkkinen L, Lachmann RH, Li A, Holton J, Guerreiro R, Paudel R et al. 2009. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain* **132**: 1783-1794.
Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM et al. 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**: 130-133.
Nikom D, Zheng S. 2023. Alternative splicing in neurodegenerative disease and the promise of RNA therapies. *Nat Rev Neurosci* **24**: 457-473.
Nishimune H, Bernreuther C, Carroll P, Chen S, Schachner M, Henderson CE. 2005. Neural adhesion molecules L1 and CHL1 are survival factors for motoneurons. *J Neurosci Res* **80**: 593-599.
Nussbacher JK, Tabet R, Yeo GW, Lagier-Tourenne C. 2019. Disruption of RNA Metabolism in Neurological Diseases and Emerging Therapeutic Interventions. *Neuron* **102**: 294-320.
O'Hara DM, Pawar G, Kalia SK, Kalia LV. 2020. LRRK2 and alpha-Synuclein: Distinct or Synergistic Players in Parkinson's Disease? *Front Neurosci* **14**: 577.
Olgiati S, Quadri M, Fang M, Rood JP, Saute JA, Chien HF, Bouwkamp CG, Graafland J, Minneboo M, Breedveld GJ et al. 2016. DNAJC6 Mutations Associated With Early-Onset Parkinson's Disease. *Ann Neurol* **79**: 244-256.
Onal G, Yalcin-Cakmakli G, Ozcelik CE, Boussaad I, Seker UOS, Fernandes HJR, Demir H, Kruger R, Elibol B, Dokmeci S et al. 2024. Variant-specific effects of GBA1 mutations on dopaminergic neuron proteostasis. *J Neurochem* **168**: 2543-2560.
Pankratz N, Pauciulo MW, Elsaesser VE, Marek DK, Halter CA, Wojcieszek J, Rudolph A, Shults CW, Foroud T, Nichols WC et al. 2006. Mutations in DJ-1 are rare in familial Parkinson disease. *Neurosci Lett* **408**: 209-213.
Park JS, Blair NF, Sue CM. 2015. The role of ATP13A2 in Parkinson's disease: Clinical phenotypes and molecular mechanisms. *Mov Disord* **30**: 770-779.
Parsons K, Nakatani Y, Nguyen MD. 2015. p600/UBR4 in the central nervous system. *Cell Mol Life Sci* **72**: 1149-1160.
Pfeffer SR. 2023. LRRK2 phosphorylation of Rab GTPases in Parkinson's disease. *FEBS Lett* **597**: 811-818.
Plotegher N, Duchen MR. 2017. Crosstalk between Lysosomes and Mitochondria in Parkinson's Disease. *Front Cell Dev Biol* **5**: 110.
Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE. 2017. Parkinson disease. *Nat Rev Dis Primers* **3**: 17013.

Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R et al. 1997. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**: 2045-2047.
Quinn JP, Kandigian SE, Trombetta BA, Arnold SE, Carlyle BC. 2021. VGF as a biomarker and therapeutic target in neurodegenerative and psychiatric diseases. *Brain Commun* **3**: fcab261.
Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J et al. 2006. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. *Nat Genet* **38**: 1184-1191.
Rogalska ME, Vivori C, Valcarcel J. 2023. Regulation of pre-mRNA splicing: roles in physiology and disease, and therapeutic prospects. *Nat Rev Genet* **24**: 251-269.
Sasaki S, Shirata A, Yamane K, Iwata M. 2004. Parkin-positive autosomal recessive juvenile Parkinsonism with alpha-synuclein-positive inclusions. *Neurology* **63**: 678-682.
Schekman R, Riley EAU. 2019. Coordinating a new approach to basic research into Parkinson's disease. *eLife* **8**: e51167.
Schormair B, Kemlink D, Mollenhauer B, Fiala O, Machetanz G, Roth J, Berutti R, Strom TM, Haslinger B, Trenkwalder C et al. 2018. Diagnostic exome sequencing in early-onset Parkinson's disease confirms VPS13C as a rare cause of autosomal-recessive Parkinson's disease. *Clin Genet* **93**: 603-612.
Schröder LF, Peng W, Gao G, Wong YC, Schwake M, Krainc D. 2024. VPS13C regulates phospho-Rab10-mediated lysosomal function in human dopaminergic neurons. *J Cell Biol* **223**.
Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A et al. 2009. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med* **361**: 1651-1661.
Singh RN, Singh NN. 2018. Mechanism of Splicing Regulation of Spinal Muscular Atrophy Genes. *Adv Neurobiol* **20**: 31-61.
Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R et al. 2003. alpha-Synuclein locus triplication causes Parkinson's disease. *Science* **302**: 841.
Smolders S, Philtjens S, Crosiers D, Sieben A, Hens E, Heeman B, Van Mossevelde S, Pals P, Asselbergh B, Dos Santos Dias R et al. 2021. Contribution of rare homozygous and compound heterozygous VPS13C missense mutations to dementia with Lewy bodies and Parkinson's disease. *Acta Neuropathol Commun* **9**: 25.
Soukup SF, Vanhauwaert R, Verstreken P. 2018. Parkinson's disease: convergence on synaptic homeostasis. *EMBO J* **37**.
Stanley RF, Abdel-Wahab O. 2022. Dysregulation and therapeutic targeting of RNA splicing in cancer. *Nat Cancer* **3**: 536-546.
Su CH, D D, Tarn WY. 2018. Alternative Splicing in Neurogenesis and Brain Development. *Front Mol Biosci* **5**: 12.
Taymans JM, Greggio E. 2016. LRRK2 Kinase Inhibition as a Therapeutic Strategy for Parkinson's Disease, Where Do We Stand? *Curr Neuropharmacol* **14**: 214-225.
Thomas KJ, McCoy MK, Blackinton J, Beilina A, van der Brug M, Sandebring A, Miller D, Maric D, Cedazo-Minguez A, Cookson MR. 2011. DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Hum Mol Genet* **20**: 40-50.
Tian Z, Zhang Y, Xu J, Yang Q, Hu D, Feng J, Gai C. 2024. Primary cilia in Parkinson's disease: summative roles in signaling pathways, genes, defective mitochondrial function, and substantia nigra dopaminergic neurons. *Front Aging Neurosci* **16**: 1451655.
Tripathi R, Aggarwal T, Fredriksson R. 2021. SLC38A10 Transporter Plays a Role in Cell Survival Under Oxidative Stress and Glutamate Toxicity. *Front Mol Biosci* **8**: 671865.

Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG et al. 2004. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304**: 1158-1160.

Wang Q, Conlon EG, Manley JL, Rio DC. 2020. Widespread intron retention impairs protein homeostasis in C9orf72 ALS brains. *Genome Res* **30**: 1705-1715.

Wang Q, Rio DC. 2018. JUM is a computational method for comprehensive annotation-free analysis of alternative pre-mRNA splicing patterns. *Proceedings of the National Academy of Sciences of the United States of America* **115**: E8181-E8190.

Wang X, Xu P, Bentley-DeSousa A, Hancock-Cerutti W, Cai S, Johnson BT, Tonelli F, Shao L, Talaia G, Alessi DR et al. 2025. The bridge-like lipid transport protein VPS13C/PARK23 mediates ER-lysosome contacts following lysosome damage. *Nat Cell Biol*.

West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, Biskup S, Zhang Z, Lim KL, Dawson VL et al. 2007. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum Mol Genet* **16**: 223-232.

Wilson DM, 3rd, Cookson MR, Van Den Bosch L, Zetterberg H, Holtzman DM, Dewachter I. 2023. Hallmarks of neurodegenerative diseases. *Cell* **186**: 693-714.

Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, Lichtenberg M, Menzies FM, Ravikumar B, Imarisio S et al. 2010. alpha-Synuclein impairs macroautophagy: implications for Parkinson's disease. *J Cell Biol* **190**: 1023-1037.

Winslow AR, Rubinsztein DC. 2011. The Parkinson disease protein alpha-synuclein inhibits autophagy. *Autophagy* **7**: 429-431.

Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atares B et al. 2004. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol* **55**: 164-173.

Zhang J, Manley JL. 2013. Misregulation of pre-mRNA alternative splicing in cancer. *Cancer Discov* **3**: 1228-1237.

Zhou ZD, Lee JCT, Tan EK. 2018. Pathophysiological mechanisms linking F-box only protein 7 (FBXO7) and Parkinson's disease (PD). *Mutat Res Rev Mutat Res* **778**: 72-78.