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Review

Regulation of vesicular trafficking by Parkinson's disease-associated genes

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Abstract: The regulatory mechanisms that control intracellular vesicular trafficking play important roles in cellular function and viability. Neurons have specific vesicular trafficking systems for synaptic vesicle formation, release and recycling. Synaptic vesicular trafficking impairments induce neuronal dysfunction and physiological and behavioral disorders. Parkinson's disease (PD) is an age-dependent neurodegenerative disorder characterized by dopamine depletion and loss of dopamine neurons in the midbrain. The molecular mechanism responsible for the neurodegeneration that occurs during PD is still not understood; however, recent functional analyses of familial PD causative genes suggest that a number of PD causative genes regulate intracellular vesicular trafficking, including synaptic vesicular dynamics. This review focuses on recent insights regarding the functions of PD causative genes, their relationship with vesicular trafficking and how mutations associated with PD affect vesicular dynamics and neuronal survival.

Keywords: Parkinson's disease; vesicular trafficking; synaptic vesicle dynamics; endosome; exocytosis; endocytosis; retromer; neurodegeneration

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder accompanied by motor symptoms, such as tremors, postural imbalance and rigidity, and non-motor symptoms, such as sleep disturbances, olfactory dysfunction and depression. Loss of dopamine neurons in the midbrain substantia nigra, which mainly causes the motor symptoms, is one of pathological features of PD. Accumulation of neuronal protein aggregations called Lewy bodies is often observed in the affected regions. Although

most PD cases are sporadic, some patients have an inherited form of PD. These patients provide researchers with an opportunity to assess the molecular mechanism of neurodegeneration. Over twenty PD causative genes have been identified to date. Studies of these PD genes have indicated that mitochondrial dysfunction is one of the major elements of PD pathogenesis. Recent studies have revealed that dysregulation of vesicular trafficking is also a considerable component. However, why midbrain dopamine neurons are relatively sensitive to PD gene mutations is still unknown. The isolated PD genes are expressed in a variety of tissues in addition to the dopaminergic neurons. Recent studies using neuronal cells and model animals addressed this challenging issue and provided some evidence that neuron-specific vesicular trafficking, such as synaptic release and recycling, is regulated by PD genes. The reduction of presynaptic functions and degeneration (dying-back) of axons from dopaminergic neurons are early events during PD pathogenesis, and these events support this idea. Here, we will review key studies showing the effects of PD genes on neuronal vesicular trafficking, discuss possible common mechanisms of PD and identify therapeutic molecular targets for PD.

2. Synaptic vesicle trafficking in neurons

Intracellular vesicle trafficking mechanisms that mediate protein and lipid transport are necessary for proper cellular function. Neurons have a specific trafficking system for synaptic vesicle (SV) dynamics (summarized in [1–3]). A variety of membrane proteins and lipids that compose SVs are properly transported from the cell bodies to presynaptic terminals via axonal transport. Mature SVs at the presynapse are equipped with SNARE proteins, such as Synaptobrevin, which facilitates synaptic vesicle release with Syntaxin-1 and SNAP-25, and neurotransmitter transporters, such as vesicular monoamine transporter (VMAT), to incorporate dopamine and other neurotransmitters into the SVs.

Mature SVs are classified into three types according to their condition in the synaptic terminals: reserve pools (RPs), recycling pools and readily releasable pools (RRPs). Approximately 80–90% of SVs reside as RPs, and these pools are released only during intense or high-frequency stimulation. Studies using neuromuscular junctions (NMJ) in *Drosophila* and frogs [4,5] suggest that release from RP is triggered after the depletion of recycling pools. RRPs account for less than 1–2% of the total number of SVs and are docked in the active zones for immediate release. The release of neurotransmitters into the synaptic cleft is regulated at least by the Ca^{2+} flux-sensor synaptotagmine, ATP-dependent N-ethylmaleimide-sensitive factor (NSF) and SNAP-25 [6]. This observation suggests that presynaptic mitochondria, which regulate Ca^{2+} flux and ATP production, have important roles in SV trafficking. Indeed, mitochondrial dysfunction reduces SV mobility in the presynaptic terminals of NMJs in *Drosophila* [7].

After exocytotic neurotransmitter release, SV membranes fused with the active zone are thought to be retrieved by three proposed recycling pathways: Clathrin-mediated endocytosis, the bulk endocytosis pathway and the very fast recycling pathway (Figure 1). Although the Clathrin-independent, very fast recycling pathway has been described as a “kiss-and-run” pathway, a recent study suggests that actin- and dynamin-dependent ultrafast endocytosis mediates very fast SV recycling instead of the “kiss-and-run” pathway, which occurs within 100 ms at the external sites of the active zone in a Clathrin-independent manner [8]. These recycled vesicles are filled with neurotransmitter to replenish SVs (5–20% of the total SVs) and are mixed with RRP and RP to maintain adequate amounts of SVs in each pool.

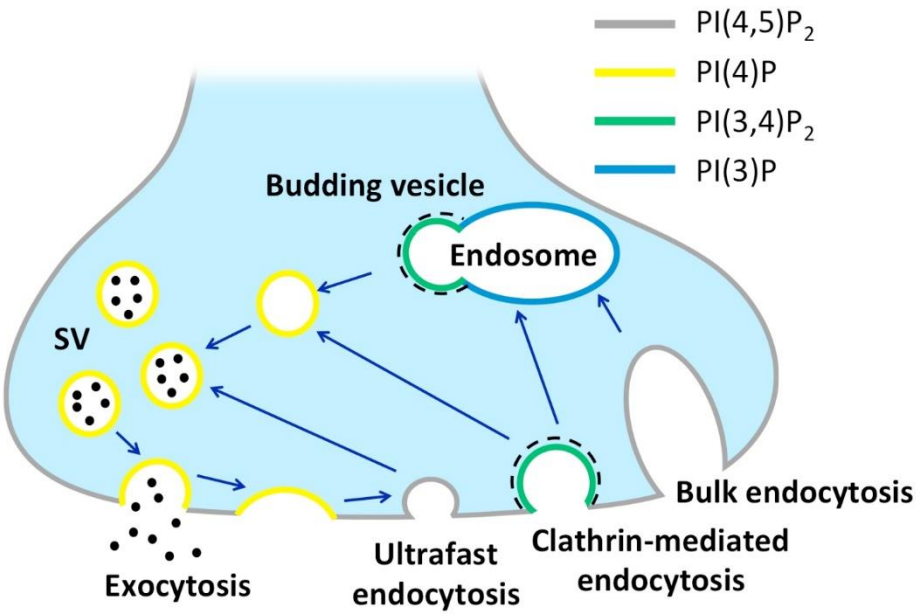

Figure 1. SV dynamics in the presynaptic terminal. After release of neurotransmitters (black dots), SV membranes fused with the plasma membrane are recycled by Clathrin-mediated, Clathrin-independent ultrafast or bulk endocytosis and are transported to the endosomes. SVs are replenished from the endosomes likely through the Clathrin-mediated budding process. The role of Clathrin-mediated endocytosis in the synaptic membrane is under debate [8]. Local production of specific phosphoinositides also regulates vesicle transport. Some PD-related genes affect SV dynamics and inositol phospholipid metabolism.

During Clathrin-mediated endocytosis, SV membranes fused with the plasma membrane of the presynapse are coated by pioneer proteins, such as Eps15. Clathrin is then recruited to the SV membranes [9] (Figure 2). Subsequently, Synaptojanin, Endophilin A (EndoA) and Dynamin are recruited to the scission sites to separate Clathrin-coated vesicles from the plasma membrane. In the presynaptic terminal, Clathrin-dependent or bulk endocytosis-mediated recycled SV membrane is transported to the endosomes to make new SVs [10]. The above study suggests that Clathrin plays a role in SV regeneration from the synaptic endosomes [8].

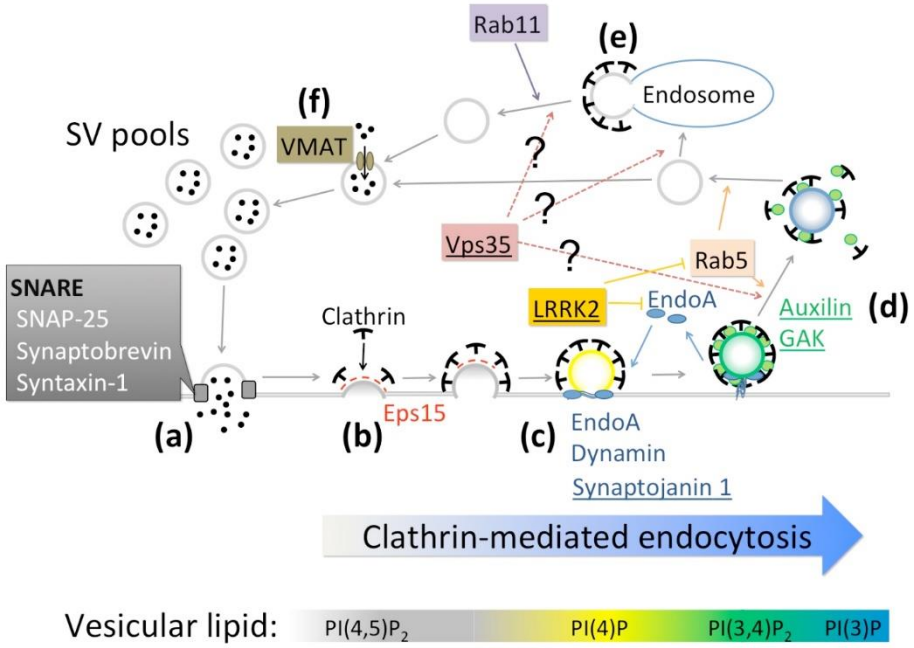

Figure 2. Working hypothesis of PD gene involvement in Clathrin-mediated endocytosis at the presynapse. (a) Neurotransmitters are released from SVs with the assistance of SNARE proteins, including Synaptobrevin, Syntaxin-1 and SNAP-25, through exocytosis. (b) Eps15 binds to the SV membrane fused with the plasma membrane and promotes Clathrin-coated pit assembly. (c) The Clathrin-coated vesicle is separated from the plasma membrane by EndoA, Synaptojanin and Dynamin. LRRK2 negatively regulates EndoA-mediated vesicle separation. (d) Rab5 regulates vesicle endocytosis. Auxilin and GAK play a role in the removal of Clathrin from the endocytosed vesicles. (e) Recycling vesicles are supplied by the budding of endosomes (f) and are filled with neurotransmitters again to replenish SVs. The names of PD-associated gene products are underlined. Transition of the inositol phospholipid composition in the vesicle membrane is also depicted.

Several Rab small GTPases are involved in SV dynamics [11–13]. Rab5 regulates endocytosis and vesicular trafficking to the early endosomes, and Rab11 participates in membrane-associated protein sorting at the recycling endosomes and in the SV recycling pathway. Rab7 is involved in the maturation of late endosomes and in the autophagy-lysosomal pathway that controls the breakdown of unnecessary proteins and lipids to maintain cellular signaling and metabolism. Recent advances in PD gene research have shown that some PD gene products, including α-Synuclein, LRRK2 and VPS35, interact with Rab GTPases and regulate vesicular dynamics (Figure 3). Newly identified PD genes may also be involved in this pathway. Here, we discuss new insights into the possible roles of PD genes in terms of vesicular trafficking, especially SV trafficking, in the following sections.

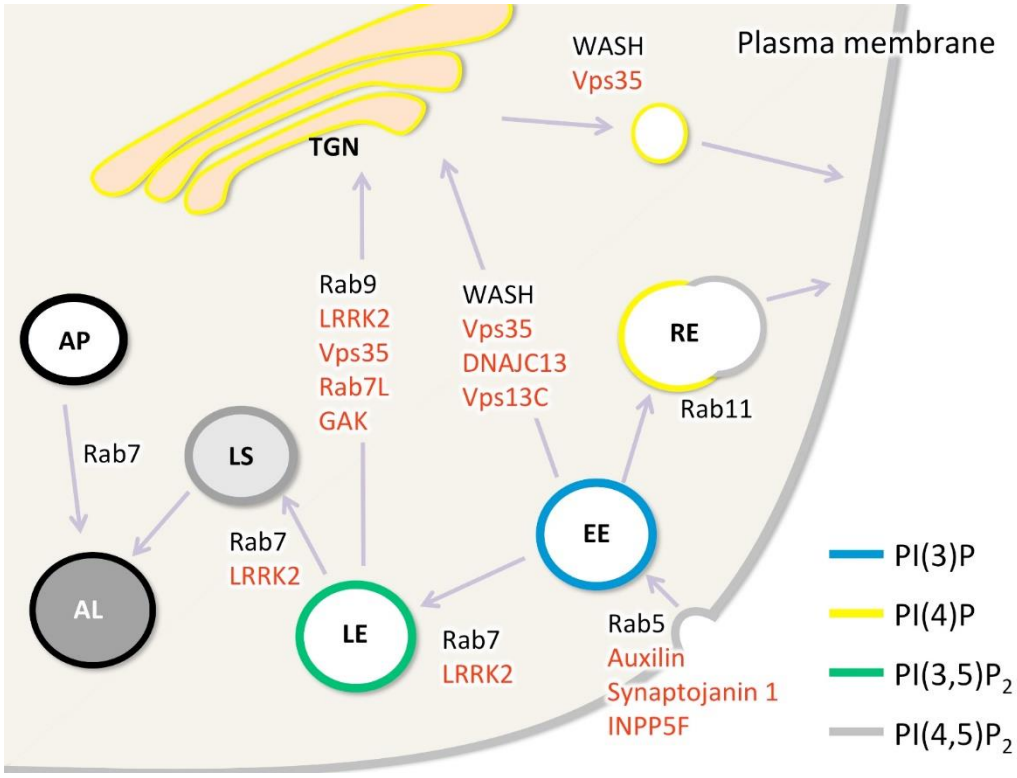

Figure 3. Possible roles of PD-associated genes in vesicular trafficking. Endosomal vesicular trafficking is regulated by Rab GTPases, including Rab5, Rab7, Rab9 and Rab11, in conjunction with the transition of inositol phospholipids. LRRK2 regulates endosome maturation, autophagy-lysosomal trafficking and trafficking from the late endosome to the trans-Golgi network (TGN). The Vps35-containing retromer complex transports cargo proteins from early or late endosomes to the TGN and also controls transport from the endosome to the cell surface. Synaptojanin 1 and INPP5F are involved in inositol phospholipid metabolism. Rab7L and Vps13C may regulate endosomal trafficking. Auxilin and GAK function as co-chaperones for the Clathrin uncoating of Clathrin-coated vesicles. The names of PD-associated gene products are highlighted in red. AL, autolysosome; AP, autophagosome, LS, lysosome; EE, early endosome; LE, late endosome; RE, recycling endosome.

3. *SNCA* (*PARK1/PARK4*)

SNCA is linked to an autosomal dominant form of PD and encodes α-Synuclein, which is a main component of Lewy bodies, a hallmark of PD pathology. Two independent missense mutations at position 53 (Ala to Thr (A53T)) and position 30 (Ala to Pro (A30P)) cause autosomal dominant familial early-onset PD. These mutations have been extensively characterized [14,15].

α-Synuclein, which is well conserved among humans, birds and frogs (but not in yeast, *C. elegans* or *Drosophila*), is widely expressed in the central nervous system [16,17]. Excessive amounts of α-Synuclein, even wild-type form, produce adverse effects in neurons and promote deleterious aggregations, which are thought to be a precursor of Lewy bodies. This idea is supported by the fact that *PARK4* results from *SNCA* triplication [18]. Because α-Synuclein exhibits a high affinity for phospholipids, the dysregulation of α-Synuclein expression may compromise membrane dynamics [19,20]. Indeed, the

overexpression of α-Synuclein disturbs ER-to-Golgi vesicular transport in yeast, leads to neuron loss in *Drosophila* and *C. elegans* models [21–23], and negatively affects the axonal transport system [24].

The precise physiological and pathogenic roles of α-Synuclein remain unclear. α-Synuclein is abundant in the presynaptic terminals of the adult brain [25] and is implicated in the regulation of dopamine release [26]. An electrophysiological study indicated that α-Synuclein plays a role in SV dynamics, especially in the recycling pathway [27]. Excess amounts of α-Synuclein or its A53T mutant reduce SV recycling during high-frequency stimulation and increase the presence of large cisternal structures that are often observed during reductions of SV recycling [28]. Similarly, oligomers of α-Synuclein induce SV clustering and decrease the motility of SVs in the synapses [29]. In *Drosophila* models, the expression of α-Synuclein in neurons affected spontaneous and stimulation-induced neural activity and SV size, which was accompanied by a reduction in survival rate, locomotion and climbing behaviors and dopamine neuron survival [30]. Interestingly, these abnormalities were rescued by the expression of Rab11. Thus, the enhancement of the Rab11-dependent vesicle recycling pathway could alleviate α-Synuclein-induced neurotoxicity [30].

4. LRRK2 (*PARK8*)

LRRK2 is a protein kinase with multiple domains containing a leucine-rich repeat motif, ROC (Ras of complex proteins domain), COR domain (C-terminal of ROC) and WD40 domain [31,32]. Missense mutations of *LRRK2*, which are found throughout these domains [33], are linked to autosomal dominant forms of late onset PD. Two independent genome-wide association studies **(**GWAS) have identified *LRRK2* as a risk gene for sporadic PD implying that altered LRRK2 signaling is an intrinsic cause of general PD [34,35]. Although it remains unclear how these pathogenic mutations of LRRK2 affect its protein function, including its protein kinase activity, overexpression of pathogenic forms of LRRK2 reduce cell viability [36–38]. Cultured chromaffin cells from knock-in mice with the R1441C mutation in the LRRK2 ROC domain have reduced catecholamine release [39].

LRRK2 and its *Drosophila* homologue dLRRK are localized to endosomes and promote endocytosis and endosomal recycling [40–42]. In an electrophysiological study, evoked excitatory junctional currents (EJCs) were reduced in *dLRRK* knockout flies, while spontaneous miniature EJCs were increased in both *dLRRK* knockouts and transgenic flies expressing human LRRK2 with the G2019S mutation in the kinase domain suggesting that the kinase activity of LRRK2 regulates SV dynamics [43]. Consistent with the above study, endocytic SV recycling is impaired in *dLRRK* knockout flies and is rescued by a reduction of EndoA activity [44]. EndoA regulates synaptic vesicle endocytosis by promoting membrane tubulation. dLRRK-mediated phosphorylation of EndoA stimulates the dissociation of EndoA from the synaptic membrane, which inhibits its function. However, both phospho-mimetic and phospho-deficient forms of EndoA cause defects in SV endocytosis and reduce SV number and the appearance of cisternal structures. Thus, this study suggests that the EndoA-dependent recycling of SVs is regulated by the LRRK2/dLRRK phosphorylation cycle. A similar molecular mechanism has been demonstrated in *LRRK2* knockout mice [45]. In contrast, a combination study with electrophysiological and imaging analyses in cortical neuron cultures revealed that SV motility and recycling is enhanced by the reduction of LRRK2 activity [46]. Another study reported that LRRK2 phosphorylates Snapin, a SNAP-25 interacting protein, which suppresses the interaction of Synaptotagmin 1 and SNAP-25-containing SNARE complexes. Thus, LRRK2-mediated phosphorylation of Snapin decreases the number of RRPs and the extent of exocytotic release in cultured rat primary neurons [47].

The role of LRRK2 in endosomes has been studied in mammalian cultured cells and *Drosophila*. LRRK2 interacts with Rab5b and negatively regulates Rab5b-mediated endocytosis through phosphorylation [48,49]. Overexpression of wild-type or pathogenic LRRK2 caused defects in synaptic vesicle endocytosis (but not exocytosis) in cultured rat primary neurons, which was suppressed by the overexpression of Rab5b [48]. In the same experimental model, LRRK2 knockdown also decreased the rate of synaptic endocytosis [48]. Such phenomena were often observed in regulators of SV recycling [50–52]. LRRK2/dLRRK is also implicated in the regulation of the lysosomal pathway through binding to Rab7 and Rab9 [53–55]. Upon endocytosis of EGF, pathogenic forms of LRRK2 impair the transition of EGF from Rab5-positive endosomes to Rab7-positive late endosomes through the inhibition of Rab7 activity [56]. Accumulations of ubiquitinated proteins and aggregated α-Synuclein are observed in *LRRK2*-deficient mice [57]. These results imply that LRRK2/dLRRK regulates not only the endocytosis but also the autophagy-lysosomal pathway, although the loss of LRRK2 alters autophagy activity differently with age [58,59].

5. Vps35 (*PARK17*) and DNAJC13 (*PARK21*)

Vps35 is a component of the retromer complex that regulates vesicular trafficking in the endosome-to-Golgi pathway and the endosome-to-cell surface pathway. Vps35 has been identified as an autosomal dominant form of a late-onset PD gene [60,61]. Vps35, Vps26 and Vps29 form the retromer complex, and Vps35 associates with cargo proteins for endosomal protein sorting [62]. An RNAi screening of genes involved in endocytosis using macrophage-like Schneider's 2 (S2) cells of *Drosophila* identified Vps35 as a protein involved in endocytosis [63]. Vps35 directly binds to Rab7 for retromer-mediated protein sorting in late endosomes, and Vps35 also localizes in Rab5-positive early endosomes in *Drosophila* [63].

Although mutations of Vps35 are a rare cause of PD, the D620N mutation is found in different populations and is well characterized [64,65]. The Vps35 D620N mutation minimally affects the formation of the retromer complex with Vps29 and Vps26 [66]. However, this mutation impairs the binding of Vps35 to the FAM21-containing WASH complex, which mediates the production of branched actin networks on the surface of endosomes [67,68]. The retromer complex together with sorting nexin 27 (SNX27) and the WASH complex cooperate in endosome-to-cell surface recycling of proteins, such as the β2-adrenergic receptor and $\alpha_5\beta_1$ integrin [69,70]. PD mutations could affect the above recycling pathway in conjunction with the trafficking of the autophagy protein ATG9 to autophagosomes [67]. The newly identified PD gene *DNAJC13* is also involved in retromer-mediated protein sorting [71]. DNAJC13 interacts with the WASH complex subunit FAM21 along with SNX1 and regulates endosomal tubulation and the retrograde sorting pathway [72,73]. The above RNAi screening in *Drosophila* S2 cells determined that DNAJC13, Auxilin (described below) and Vps35 are involved in endocytosis [63].

Although Vps35 is expressed ubiquitously, emerging evidence suggests neuron-specific functions for Vps35. Vps35 is localized throughout neurons, namely in cell bodies and in both axons and dendrites of mammalian cultured neurons [74]. A reduction of Vps35 induced abnormal synaptogenesis, and the expression of pathogenic mutant forms of Vps35 induced locomotor defects and dopaminergic neurodegeneration in *Drosophila* [63,75]. Reduced Vps35 expression impedes the endosomal recycling of a membrane-residing protease BACE1, which increases the BACE1-mediated cleavage of APP and resultant Aβ production. As a result of Aβ accumulation, AMPA and NMDA receptor-mediated glutamatergic synaptic transmission and synaptic plasticity are attenuated in

hippocampal neurons suggesting that Vps35 contributes to the neuropathology of Alzheimer's disease as well as PD [76]. Vps35 also regulates the recycling of the AMPA receptor, which is partially affected by the Vps35 D620N mutant and impairs excitatory synaptic transmission.

Pathogenic mutant forms of LRRK2-mediated neurotoxicity are suppressed by the overexpression of Vps35 and Rab7L/Rab29 in *Drosophila* through their direct interaction. However, a different study failed to detect the colocalization of LRRK2 and Vps35 [77–79]. Rab7L could be a risk gene for sporadic PD within the *PARK16* locus. Rab7L is involved in vesicular sorting at the Golgi apparatus [34,35] suggesting that disturbed vesicular sorting due to LRRK2 mutations is suppressed by the enhancement of retromer functions in the endosome-to-Golgi pathway. LRRK2 forms a complex with Cyclin G-associated kinase (GAK; described in detail below) and Rab7L. This complex promotes the clearance of trans-Golgi—derived vesicles through the autophagy—lysosomal pathway, and LRRK2 pathogenic mutants potentiate Golgi clearance [78].

6. Auxilin (*PARK19*), Synaptojanin 1 (*PARK20*), GAK and Vps13C

Another two genes that regulate SV dynamics have been recently identified as PD causative genes. *DNAJC6* is responsible for a recessive form of early-onset PD and encodes a J domain family protein called Auxilin. Auxilin is neuronally expressed and regulates the Clathrin-mediated endocytosis pathway [80–82]. Auxilin acts as a co-chaperone of Hsc70 that mediates Clathrin uncoating of Clathrin-coated vesicles. GAK is a ubiquitously expressed protein that is closely homologous to Auxilin except for an additional N-terminal kinase domain [83]. The *GAK* locus was also identified as a risk allele (by GWAS) in familial PD [84]. The loss of Auxilin leads to a reduction in synaptic endocytosis and results in the accumulation of Clathrin-coated vesicles and Clathrin-cages at synapses. These effects can be partially suppressed by compensating with upregulation of GAK [85]. GAK is also reported to regulate α-Synuclein-mediated toxicity (likely controlling its protein turnover) [86]. *SYNJ1* mutations are linked to an autosomal recessive early-onset PD and codes for the presynaptic protein Synaptojanin 1 [87]. Synaptojanin 1 is highly conserved from yeast to humans and comprises two inositol phosphatase domains—an N-terminal Sac1 inositol phosphatase domain and a central inositol 5-phosphatase domain. Another gene for the Sac domain-containing protein, INPP5F/Sac2, has also been identified as a new risk locus for PD according to a meta-analysis of the PD GWAS dataset [88]. A genetic modifier screening for endosome-Golgi trafficking identified *Synaptojanin*, *Vps35* and *Vps13* in yeast [89]. In yeast, Synaptojanin plays a role in trafficking between the endosome and Golgi [89]. Mouse *SYNJ1*-deficient neurons exhibit accumulations of Clathrin-coated vesicles at synapses that are very similar to *DNAJC6*-deficient neurons. Thus, Synaptojanin 1 in cooperation with Auxilin regulates Clathrin-mediated synaptic endocytosis in neurons [85,90–92]. Interestingly, the human *Vps13* homolog *Vps13C* was identified as a risk locus for PD in the aforementioned meta-analysis [88].

7. Conclusions

The discovery of genes linked to late-onset PD has highlighted an important fact that the disturbance of vesicular dynamics, including SV exo-/endocytosis, the endosome-Golgi, endosomal recycling and autophagy-lysosomal pathways, is one of the major causes of PD etiology (Table 1). While the phenotypes of these gene mutations mostly resemble sporadic PD with Lewy body pathologies, an issue arises in which protein, lipid and/or organelle transport affects neuronal survival

and contributes to Lewy body formation. Along with the aforementioned study of late-onset PD, the characterization of early-onset PD genes, including Parkin (*PARK2*), PINK1 (*PARK6*) and DJ-1 (*PARK7*) revealed that dysregulation of mitochondrial maintenance is another component of PD etiology (Table 1). Mitochondrial dysfunction directly affects cell viability and also impairs SV release and recycling through the reduction of ATP synthesis and dysregulation of Ca^{2+} flux [93–97]. Recent studies suggest that Vps35 regulates mitochondrial vesicle transport, which controls mitochondria dynamics [98,99]. Many studies have indicated a pathogenic relationship between α-Synuclein and mitochondria [100–103]. Because molecular deficits in these processes could produce the phenotypic spectrum of PD ranging from mitochondrial dysfunction without inclusions to typical synucleinopathy and tauopathy, a comprehensive understanding of these pathogenic pathways is essential to establish a preventable procedure to overcome this disease.

Table 1. PD-related genes featured in this review.

	Gene symbol	Gene name	Possible functions	Hereditary form	Onset age	Lewy body pathology
PD causative genes	PARK1/PARK4	α-Synuclein (SNCA)	Regulation of synapse vesicle trafficking	AD	early	+
	PARK2	Parkin	Ubiquitin ligase	AR	early	−
	PARK6	PINK1	Mitochondrial kinase	AR	early	−
	PARK7	DJ-1	Antioxidant protein	AR	early	−
	PARK8	LRRK2	Protein kinase	AD	late	+/−
	PARK13	HtrA2	Mitochondrial serine protease	AS	early/late	+
	PARK14	iPLA2 ß	Phospholipase A2	AR	early	+
	PARK15	FBOX7	Ubiquitin ligases	AR	early	+
	PARK17	Vps35	Retromer component	AD	late	−
	PARK19	Auxilin	Cochaperone	AR	early	+
	PARK20	Synaptojanin 1	Polyphosphoinositide phosphatase	AR	early	+
	PARK21	DNAJC13	Endosomal transport	AD	late	+
	CHCHD2	CHCHD2	Mitochondrial protein	AD	late	+
Risk loci	RAB7L1/Rab29	Rab7L	Rab family protein			
	GAK	GAK	Cochaperone with a kinase domain			
	INPP5F/Sac2	INPP5F	Phosphoinositide 4-phosphatase			
	VPS13C	Vps13C	Endosomal transport			

Proteins for membrane dynamics and for mitochondrial functions are highlighted in blue and green, respectively. AD, autosomal dominant form; AR, autosomal recessive form; AS, Autosomal dominant, susceptibility gene.

Conflicts of Interest

The authors declare no conflicts of interest.

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