Title

Rodent models based on endolysosomal genes involved in Parkinson's Disease

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Abstract

Genes associated with endolysosomal function have been recently associated with familial Parkinson's disease and described as risk factors for sporadic cases. This indicates that deficits in this pathway predispose to parkinsonism. In order to better understand the role of these genes in disease development, rodent models have been created by targeting genes playing a role in endolysosomal function, such as LRRK2, DNAJC6, SYNJ1, VPS35, GBA1, ATP13A2 and TMEM175. Here we review the latest findings describing parkinsonian features in these animal models secondary to endolysosomal dysfunction. Also, we provide suggestions for further development and application of these animal models to better understand the contribution of endolysosomal dysfunction in Parkinson's disease and provide novel models for testing therapeutic approaches.

Highlights

- Genes involved in the endolysosomal system confer risk for developing PD
- Animal models based on PD endolysosomal genes replicate relevant PD phenotypes
- Gene- and environment- interaction studies using these models are emerging
- New endolysosomal based models will help for a better understanding of PD pathology

INTRODUCTION

The pathogenesis of Parkinson's disease (PD) is multifactorial, with multiple pathways converging to cause a progressive and age-dependent loss of nigral dopaminergic (DA) neurons. The etiology is complex and involves an interplay between genetic and environmental factors that creates a context permissive for neurodegeneration [1]. Familial PD with Mendelian inheritance of genetic mutations represents 5-10% of cases, while genome-wide association studies (GWAS) have revealed multiple genetic risk factors that contribute to risk of sporadic PD [2]. These findings highlight genetic susceptibility as an important factor for the development of the disease.

Interestingly, many of the genes associated with risk for developing PD converge on the endolysosomal system. In particular, genes associated with familial cases of PD have been associated with various functions across the endolysosomal pathway, including clathrin-mediated trafficking and synaptic vesicle endocytosis, endolysosomal trafficking, and lysosomal function (Figure 1). In addition, recent meta-analysis of GWAS confirm that autophagy-lysosomal genes confer increased risk for sporadic PD [3]. Furthermore, a separate study found an important burden of lysosomal storage disorder gene variants in relation with risk of developing PD [4].

These findings suggest that endolysosomal dysfunction plays an important role in PD-related neurodegeneration. With this understanding, new animal models have been generated trying to replicate parkinsonism secondary to endolysosomal dysfunction. In this review, we will provide an overview of the genetic rodent models based on endolysosomal genes that are available till date (genes highlighted in Figure 1), focusing on the most recent findings. Also, we will discuss the successes and limitations of these approaches, as well critically remark directions that can be further undertaken to provide a better understanding of these genes in PD pathology.

CLATHRIN-MEDIATED ENDOCYTOSIS

Defects in clathrin-mediated trafficking and synaptic vesicle endocytosis have been associated with the pathogenesis of PD via mutations in the genes DNAJC6 and SYNJ1 (Figure 2).

DNAJC6 encodes auxilin, that functions in coordination with Hsc70 to mediate uncoating of clathrin-coated vesicles. Mutations in auxilin, including R927G and T741T, have been associated with early-onset autosomal recessive PD, and are predicted to contribute to disease pathogenesis via loss of function mechanisms [5]. Auxilin-dependent neurodegeneration may involve deficits in uncoating of clathrin-coated vesicles and the endocytic pathway at the soma or synaptic terminals. A recent preprint describes the phenotype of mice carrying a knock-in (KI) mutation in auxilin (R857G) at a site mimicking the human pathogenic R927G mutation. Interestingly, these mice develop age-dependent motor dysfunction in association with seizures, replicating the behavioral spectrum found in patients [6]. In addition, the mice develop lipofuscin-like accumulations in the nigrostriatal pathway and a decreased number of presynaptic synaptic vesicles, although no nigral DA cell loss nor loss of striatal DA terminals was present until 12 months of age.

Synaptojanin-1 (SYNJ1), similar to auxilin, plays a role in the uncoating of clathrin-coated vesicles. As is the case of auxilin mutations, synaptojanin-1 mutations (R258Q, R459P) have been associated with early-onset autosomal recessive PD [7]. Knock-in (KI) mice carrying the R258Q mutation in synaptojanin-1 at the corresponding position in the mouse protein, develop behavioral features reminiscent of synaptojanin-1 mutations in patients, including motor coordination defects and seizures [8]. The mice developed accumulation of clathrin-coated vesicles at synapses and reduction of synaptic

vesicles. While no DA neurodegeneration could be observed in the substantia nigra (SN) pars compacta (SNpc) at 8 months of age, the authors document the presence of dystrophic changes in DA-ergic nerve terminals in the dorsal striatum [8]. Although this model is interesting to study the impact of the R258Q mutation, its applicability is limited due to the lack of nigral cell loss and the high mortality which is possibly linked to the development of early seizures (only 60% of mice survived until adulthood). More recently, the phenotype of SYNJ1 +/- mice has been described as a model of synaptojanin-1 haploinsufficiency. These mice were found to develop PD-related changes including age-dependent loss of DA-ergic terminals, accumulation of α-synuclein phosphorylated at serine 129 (Ser129-P-α-synuclein) in aged (18 month old) mice in various regions of the brain, including SN, and development of age-dependent motor dysfunction [9]. These changes occurred again in the absence of nigral DA cell loss, as evaluated at 18 months of age.

Retromer Function

VPS35 is a subunit of the retromer complex, and as such regulates the recycling of cargo proteins from early endosomes to the trans-Golgi network or the plasma membrane (Figure 2). Several mutations in VPS35 have been associated with autosomal dominant familial forms of PD implicating retromer dysfunction in parkinsonism, with D620N being the most common identified mutation that has been confirmed as pathogenic [10].

Various rodent models have been developed to explore the pathogenicity and mechanisms of the VPS35 D620N mutation. Viral vector-mediated overexpression of human VPS35 D620N to the SN of rats was found to lead to nigrostriatal DA-ergic neurodegeneration [11]. Interestingly, in mice VPS35 D620N KI induced age-dependent loss of nigral DA neurons [12, 13], with degeneration of striatal DA-ergic terminals, appearance of progressive motor deficits and accumulation of α-synuclein [13], although none of these features were observed in a different VPS35 D620N KI model [12]. Furthermore, VPS35 +/- mice, as well as mice with a conditional homozygous deletion of VPS35 in DA neurons, showed loss of nigral DA neurons and axon terminals, and α-synuclein accumulation [14], suggesting that VPS35 haploinsufficiency can replicate features of PD pathology.

Mutations in VPS35 may also interact with α-synuclein-induced pathology. Using a viral vector mediated approach, Dhungel et al. found that overexpression of WT VPS35 was able to reduce α-synuclein pathology and neurodegeneration in the hippocampus of Thy1 α-synuclein transgenic mice, while overexpression of the D620N mutant exacerbated these effects [15]. On the other hand, Chen et al. failed to observe an aggravation of the lethal phenotype of A53T α-synuclein transgenic mice when crossed with VPS35 D620N KI mice [12]. Besides, preliminary findings suggest that the D620N mutation does not influence the initial progression of Ser129-P-α-synuclein pathology following intrastriatal injection of α-synuclein pre-formed fibrils (PFFs) [12]. Further studies are necessary to clarify the whether VPS35 pathogenic mutants interact with α-synuclein pathology in nigral DA neurons in vivo.

LRRK2

Leucine-rich repeat kinase 2 (LRRK2) is a large multidomain protein with multiple proposed functions, including synaptic vesicle recycling, retrograde trafficking, vesicle sorting via Golgi, and regulation of lysosomal function [16] (Figure 2). Mutations in LRRK2 are associated with autosomal dominant familial PD, and act to enhance its kinase activity [17], most likely disturbing its normal function within the endolysosomal system.

Various animal models have been generated in order to study the consequence of overexpression of the different LRRK2 pathogenic mutations, such as G2019S or R1441C. Most transgenic approaches in mice have been able to reproduce certain PD-related phenotypes, such as changes in DA transmission and behavioral deficits, although nigral cell loss and α-synuclein accumulation has been reported in a limited number of cases [18]. A recent successful approach in modeling LRRK2-induced neurotoxicity involved the expression of the LRRK2 G2019S mutant in a tetracycline-inducible conditional system using the TH promoter to control expression in catecholaminergic neurons. When expression of the transgene was triggered post-weaning, the mice developed age-dependent loss of nigral DA neurons and α-synuclein accumulation [19]. This model can be useful to further investigate the contribution of α-synuclein to LRRK2-induced pathology, as well as to study cell-autonomous mechanisms of LRRK2 toxicity.

On the other hand, expression of LRRK2 pathogenic mutants using more physiological expression systems, such as BAC transgenesis or KI mouse models was generally found to lead to more subtle effects: modifications of nigrostriatal DA-ergic neurotransmission and modest behavioral deficits, in absence of nigral cell loss or α-synuclein pathology [18]. This may be the consequence of lower expression levels of the LRRK2 mutants in nigral DA neurons, in comparison to the overexpression-based models.

In order to investigate the mechanisms of LRRK2-associated neurotoxicity, recent models have also been generated using large capacity viral vectors to deliver the pathogenic LRRK2 mutants to the striatum or SN of rodents. In these models, intrastriatal delivery of LRRK2 G2019S via herpes simplex virus (HSV) vectors or human adenoviral (Ad5) vectors leads to robust loss of nigral DA neurons [20] and presence of degenerative neuritic changes in the striatum, although in absence of α-synuclein pathology [21]. A caveat here is that these viral vector systems are known to induce considerable inflammatory responses, which can potentially complicate interpretation of the results.

Given the incomplete penetrance of the G2019S LRRK2 mutation in PD patients, animal models have been generated to investigate gene- or environment interactions. A recent study described increased susceptibility of mutant G2019S LRRK2 transgenic mice to sub-toxic concentrations of MPTP, providing an animal model suitable to study LRRK2 gene – MPTP interactions with robust nigral DA degeneration and motor impairment [22]. Interestingly, the expression of VPS35 was decreased in LRRK2 mutation carriers [23], while the VPS35 D620N mutant was found to enhance the LRRK2 kinase activity [24]. This suggests a possible role of retromer disruption in the pathogenic effects induced by the LRRK2 G2019S mutation..

LRRK2 knock-out (KO) models fail to replicate pathological changes in the nigrostriatal pathway [18]. On the other hand, LRRK2 deficient models have been instrumental to better define the contribution of LRRK2 to mechanisms associated with α-synuclein toxicity. The findings in this case have been contrasting, with some studies reporting protection from α-synuclein-related pathology, while others failing to establish this effect (reviewed in [25]). Utilizing an animal model based on adeno-associated viral vector (AAV)-mediated overexpression of human A53T α-synuclein in the SN, we recently uncovered a role for LRRK2 in modulating α-synuclein induced neuroinflammation. While LRRK2 KO rats challenged with α-synuclein vector showed a similar degree of nigral cell loss as WT littermates, we could show decreased microglial activation and immune cell infiltration in the SN [26]. This finding is in line with a possible role of LRRK2 in immune cells [27] and in modulating central and peripheral inflammation [28] which may be of interest to further investigate in animal models in the future.

LYSOSOMAL FUNCTION

GBA1

GBA1 encodes the lysosomal sphingolipid degrading enzyme, glucocerebrosidase (GCase) (EC 3.2.1.45; glucosylceramidase beta) (Figure 2). Loss of function mutations in GBA1 cause Gaucher disease (GD), a lysosomal storage disorder. These mutations impair GCase activity leading to accumulation of its lipid substrates glucocerebroside (glucosylceramide, GlcCer) and glucosylsphingosine (GlcSph) in the cells [29] (Figure 2).

In the last years, GBA1 was discovered as the main genetic risk factor for idiopathic PD since around 10% of PD patients present severe and mild GBA1 mutations with decreased GCase activity [30]. Different genetic in vivo models were generated in order to understand the importance of GBA1 in PD pathology. However none of them have managed to fully mimic the main pathological hallmarks observed in PD patients: α-synuclein aggregation, nigrostriatal degeneration and motor function impairment. Furthermore, a subpopulation of GBA1 mutations carriers do not develop PD, suggesting that other genetic, epigenetic or non-genetic factors are required to generate a more reliable GBA1 related PD model (for more information [31]).

Recently, novel and more relevant GBA1 models have been developed in combination with α-synuclein overexpression. GBA1 L444P +/- KI mice, mimicking a severe GD mutation also found in PD patients, display 40% less GCase activity in the brain. Increased levels of proteinase K-resistant α-synuclein were observed in the striatum and SNpc of these mice, although this model failed to induce advanced-PD like phenotype [32]. Interestingly, injection of AAV encoding human α-synuclein in the SN of GBA1 L444P +/- mice significantly enhanced DA neuron loss in the SN [32]. In a more recent paper of the same group, GBA1 L444P +/- mice were inoculated with α-synuclein PFFs (aSyn-PFF) in the right striatum. Compared to WT α-synuclein-PFF injected mice, a higher density of Ser129-P-α-synuclein as well as LB- and LN-like aggregates were detected in different brain regions, including SNpc and striatum of GBA1 L444P+/- mice [33].

Homozygous or heterozygous GBA1 KO mice do not reproduce the full spectrum of a PD phenotype (for more information [31]). Interestingly, GBA1 +/- mice enhanced α-synuclein-induced pathology when crossed with α-synuclein BAC transgenic mice (SNCA tg/tg). Total Ser129-P-α-synuclein levels were increased compared to SNCA tg/tg mice and colocalized with the lysosomal marker LAMP2. TH positive cells in SNpc were decreased and GlcSph levels increased in GBA1 +/- SNCA tg/tg mice compared to wildtype [34].

These results in α-synuclein overexpression models and other studies point to a therapeutic potential of increasing GBA1 levels in PD. Indeed, AAV-mediated overexpression of hGBA1 in SN [35] and striatum [36] was able to prevent the pathological phenotype upon MPTP administration, or in Thy-SNCA mice respectively. Remarkably, PD patients without GBA1 mutations also present decreased GBA1 mRNA levels and enzyme activity [37] which makes this therapeutic strategy potentially beneficial for sporadic PD as well.

ATP13A2 AND ATP10B

ATP13A2 (PARK9) is a P-type ATPase recently described as a lysosomal exporter of polyamines (Figure 2) [38], and has been genetically associated with neurodegenerative disorders including early onset PD [39] and Kufor-Rakeb syndrome [40].

ATP13A2 KO cells present a decrease in lysosomal degradation together with an impairment in lysosomal membrane integrity [38]. Two mouse models of full-body ATP13A2 KO were generated independently. Both models show lipofuscinosis in the hippocampus [41, 42], cerebellum and cortex [42] at 18 months old. Besides, lysosomal markers accumulation (LAMP1/2) together with defects in autophagy function were detected [42]. In addition, specific motor deficits were observed in the absence of DA-ergic neuron loss. However, reports on α-synuclein pathology were contradictory and inconsistent. While one model displayed increased insoluble α-synuclein in the hippocampus [41], in the second model no changes in α-synuclein accumulation were detected [42].

ATP13A2 overexpression has also been combined with α-synuclein overexpression models using viral vector technology. While AAV-ATP13A2 WT overexpression in SN in rats was not able to rescue α-synuclein-induced dopaminergic neurodegeneration, AAV-ATP13A2 D513N, an ATPase deficient mutation, caused on its own significant dopaminergic neuron loss in the SNpc at 4 weeks [43]. Strikingly, autophagy pathway and lysosomal activity were not affected [43]. It appears that further and more extensive research is needed to get a better comprehension of the relation between ATP13A2 and α-synuclein pathology in vivo, since in cell culture several interactions between ATP13A2 have been reported [44-46]. In particular, assessment of polyamines levels in these models and of ATP13A2 polyamine transporter function will help to define if these substrates modulate the observed effects and/or can be used as a biomarker for therapeutic approach. As previous studies mention, polyamines have a cell-protective role [47] and are significantly downregulated in the plasma of PD patients [48].

A recent paper highlights the relevance of another P-type ATPase protein in PD pathology, ATP10B. Mutations in this gene have been found in families with PD [49] and in a cohort of early onset PD patients [50]. Moreover, mRNA levels were reduced in the SN and medulla oblongata of idiopathic PD patients compared to controls [50]. ATP10B was found to function as a late-endolysosomal lipid flippase that translocates GluCer and phosphatidylcholine to the cytoplasm (Figure 2). Knock-down of ATP10B in isolated mouse cortical neurons sensitized to cell death in basal conditions and decreased the degradation activity of lysosomes [50]. Although currently unexplored, the generation of an in vivo model will help to better understand how this ATPase influences PD pathology as well as to uncover the importance of its lysosomal function in α-synuclein dynamics. As both ATP10B and GBA1 modulate lysosomal GluCer homeostasis, a common pathway between these two proteins could open a highly interesting research line to explore in the future.

TMEM175

One of the most recently described PD in vivo models was generated by modulation of the lysosomal gene TMEM175. TMEM175 is a pore-forming protein that, together with AKT, is part of a K+ channel in lysosomes. Two uncommon loss of function variants of this protein (M390T and Q65P) are associated with an increased risk of PD. Homozygous KO TMEM175 mice displayed significant dopaminergic loss in SNpc at 18-22 months of age [51]. Remarkably, heterozygous mice also showed a small but significant reduction in the number and size of dopaminergic neurons in SNpc as well as a reduction in the density of axons that are projecting from SN to the striatum. Cultured hippocampal neurons of the TMEM175 KO mice also displayed enhanced aSYN pathology after seeding with PFFs. In addition, these mice exhibited a significant impairment in the rotarod and wire-hang test [51]. Further studies are needed to further characterize this promising mouse model and test its interaction with PD related pathways.

Conclusion and Perspectives

In conclusion, endolysosomal impairment is emerging as an important contributor to PD pathogenesis and animal models have been recently created in order to study nigrostriatal degeneration and other PD-related phenotypic changes. As mentioned in this review, some of the models have succeeded to recapitulate relevant PD hallmarks, but further research is needed to better define the contribution of endolysosomal dysfunction in PD.

From our perspective, several outstanding questions remain unsolved (Table 1). In addition, future studies are necessary to investigate the relevance of other endolysosomal genes that have been implicated in PD, but not yet modeled in vivo. Since overall the absence of robust dopaminergic cell loss remains a limitation of these animal models, it will be important in the future to better characterize the neurotoxicity associated with endolysosomal dysfunction in PD.

Conflict of Interest Statement

Nothing declared.

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Figure Legends

Figure 1. Genes conferring increased risk for PD associated with distinct functions across the endolysosomal system. Depicted in bold are the genes which have been modeled in rodents leading to PD-related phenotypes, and which are covered in the current review.

Figure 2. Genes associated with PD phenotypes in animal models play distinct roles within the endolysosomal system. These include (1) modulation of clathrin-mediated endocytosis and synaptic vesicle endocytosis/recycling (insert), (2) endolysosomal trafficking and sorting, and recycling of cargo to the trans-Golgi network and plasma membrane via the retromer complex , and (3) regulation of lysosomal function and homeostasis, including regulation of lipid, polyamine and potassium concentration and export. Disturbance of these pathways leads to defects in the endocytic pathway, synaptic vesicle recycling, trafficking through the endolysosomal system, retromer function, and lysosomal degradation, and predisposes to parkinsonism in patients and animal models.

FIGURES

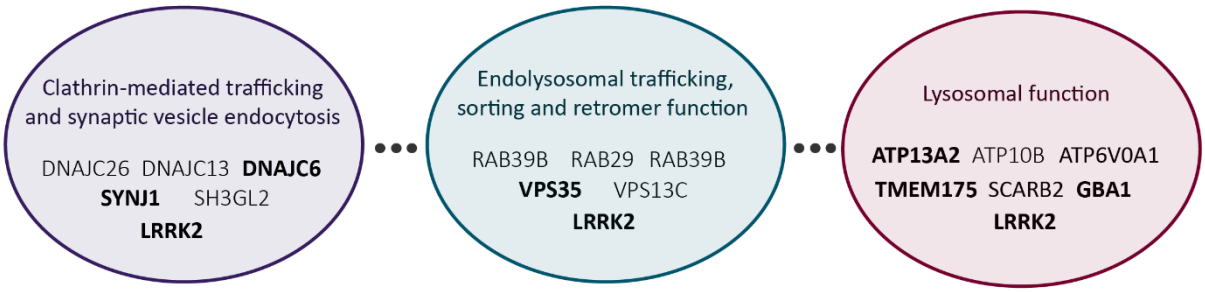

FIGURE 1.

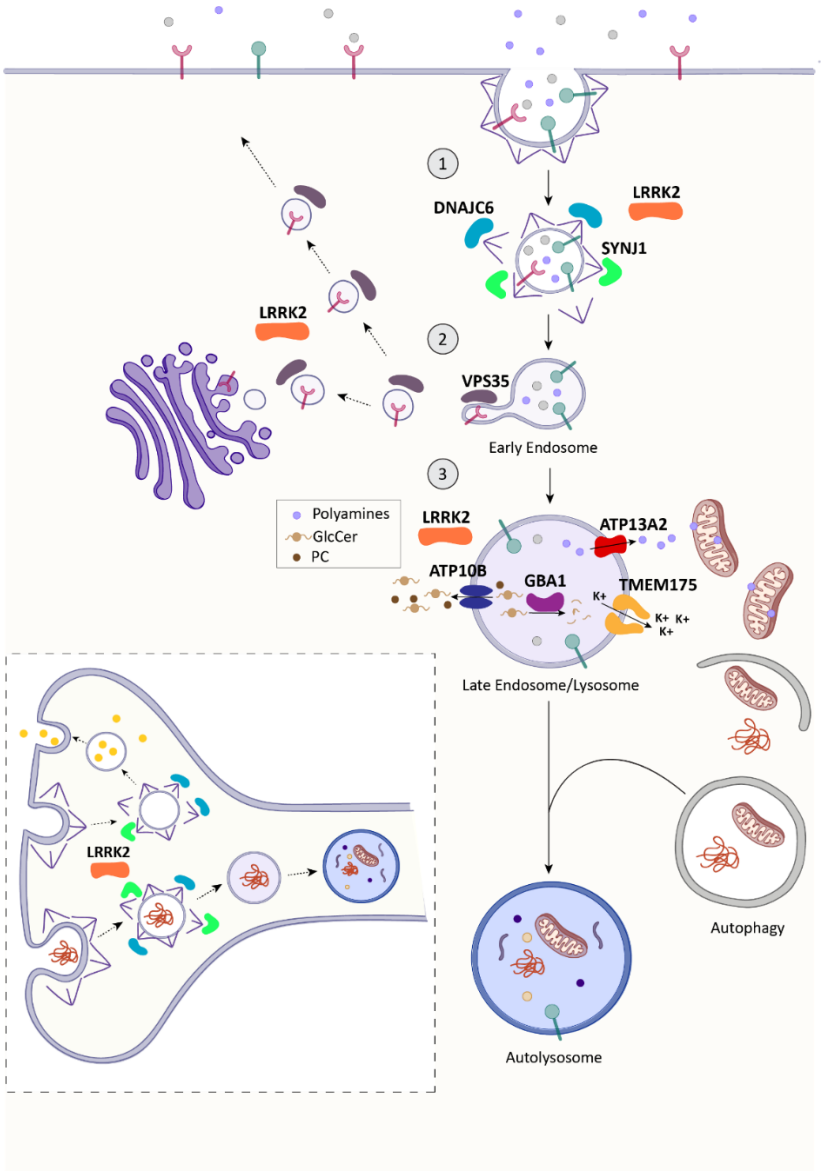

FIGURE 2.

TABLES

Table 1. Key questions to address in rodent models based on PD endolysosomal genes

EXPLORE EFFECTS IN DIFFERENT CELL TYPES AND BRAIN CIRCUITS
What is the role played by the PD endolysosomal genes in different cell types?
What are the mechanisms of neurotoxicity (cell-autonomous and/or non-cell-autonomous)?
What is the role played by PD endolysosomal genes in other synaptic circuits or regions of the brain?
What is the impact of PD endolysosomal genes in non-motor behavior?
EXPLORE INTERACTIONS WITH PD PATHOGENIC PATHWAYS
What is the role of PD endolysosomal genes in α-synuclein aggregation and pathology spread?
Is there a requirement for α-synuclein in the neurotoxic effects of mutations in PD endolysosomal genes?
What is the interaction between PD endolysosomal genes and mitochondrial health/function?
EXPLORE GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS
Is there an interaction between the different PD endolysosomal genes/ pathways?
What are the effects of modeling gene-environment interactions in the context of aging?

Annotated References

(*) 5. Roosen, D.A., et al., Mutations in Auxilin cause parkinsonism via impaired clathrin-mediated trafficking at the Golgi apparatus and synapse. bioRxiv, 2019.

This study provided the first animal model replicating the R927G mutation in DNAJC6 in PD. KI mice develop motor dysfunction with seizures, in association with decreased number of presynaptic synaptic vesicles in the striatum and intracellular lipofuscin-like accumulation, but did not develop loss of nigral DA neurons or striatal DA terminals at 12 months of age.

(*) 9. Pan, P.Y., et al., Synj1 haploinsufficiency causes dopamine neuron vulnerability and alpha-synuclein accumulation in mice. Hum Mol Genet, 2020. 29(14): p. 2300-2312.

SYNJ1 haploinsufficiency animal model replicating mechanisms of SYNJ1 loss of function in PD. The SYNJ1 +/- mice develop age-dependent motor function abnormalities, as well as Ser129-P-α-synuclein accumulation and loss of striatal DA terminals, however in absence of nigral DA cell loss at 18 months of age.

(*) 12. Chen, X., et al., Parkinson's disease-linked D620N VPS35 knockin mice manifest tau neuropathology and dopaminergic neurodegeneration. Proc Natl Acad Sci U S A, 2019. 116(12): p. 5765-5774.

This study characterizes the phenotype of VPS35 D620N KI mice as a model of this mutation in PD. The KI mice show age-dependent nigral DA neurodegeneration with presence of tau, but not α-synuclein, pathology throughout the brain, and in absence of detectable loss of striatal DA terminals or motor deficits. In addition, the VPS35 D620N KI mutation is unable to modify the lethal neurodegenerative phenotype in human A53T-α-synuclein transgenic mice or α-synuclein pathology in an intrastriatal α-synuclein PFF model.

(**) 13. Niu, M., et al., VPS35 D620N knockin mice recapitulate cardinal features of Parkinson's disease. Aging Cell, 2021: p. e13347

This study provides a description of a new VPS35 D620N KI model replicating the main features of PD. The KI mice develop age-dependent progressive motor deficits, degeneration of nigral DA neurons, loss of DA terminals in the striatum, decreases in striatal DA levels and accumulation and aggregation of α-synuclein. In addition, in a combination model, adult VPS35 D620N KI mice showed increased susceptibility to MPTP-induced loss of nigral DA neurons and striatal DA fibers compared to WT littermates.

(**) 22. Arbez, N., et al., G2019S-LRRK2 mutation enhances MPTP-linked Parkinsonism in mice. Hum Mol Genet, 2020. 29(4): p. 580-590.

LRRK2-MPTP combination model that reflects gene-environment interactions in the context of the LRRK2 G2019S mutation. Exposure of G2019S LRRK2 mice to a sub-toxic protocol of MPTP leads to motor impairment, robust nigral DA neurodegeneration and loss of striatal DA fibers. The effect in WT LRRK2 mice challenged with MPTP is also significant, but milder than with the G2019S LRRK2 mutation, whereas non transgenic animals were unaffected. In addition, administration of an LRRK2 kinase inhibitor reduced nigral DA cell loss and motor deficits in this combination model.

(*) 26. Van der Perren, A., et al., LRRK2 Ablation Attenuates Alphalpha-Synuclein-Induced Neuroinflammation Without Affecting Neurodegeneration or Neuropathology In Vivo. Neurotherapeutics, 2021.

This study reveals that LRRK2 KO or LRRK2 kinase inhibition is unable to protect against α-synuclein-induced motor deficits, nigral DA neurodegeneration or α-synuclein pathology in an α-synuclein viral vector based rat model that shows robust α-synuclein -induced neurodegeneration and α-synuclein aggregation. LRRK2 KO rats injected with the α-synuclein viral vector presented with less reactive microglia and infiltrating T cells in the SN compared to wild-type rats, indicating a role of LRRK2 in α-synuclein-induced neuroinflammation.

(*) 33. Migdalska-Richards, A., et al., L444P Gba1 mutation increases formation and spread of alpha-synuclein deposits in mice injected with mouse alpha-synuclein pre-formed fibrils. PLoS One, 2020. 15(8): p. e0238075.

In this study for the first time the authors perform α-synuclein PFF injection in the striatum of GBA L444P +/- knock in mice. The study revealed an increase of Ser129-P-α-synuclein density in the cortex and striatum, suggesting that a decrease in GCase activity boosts the formation of pathological Ser129-P-α-synuclein following PFF injection.

(*) 34. Ikuno, M., et al., GBA haploinsufficiency accelerates alpha-synuclein pathology with altered lipid metabolism in a prodromal model of Parkinson's disease. Hum Mol Genet, 2019. 28(11): p. 1894-1904.

Heterozygous GBA1 KO mice enhance different aspects of α-synculein pathology when crossed with α-synuclein transgenic mice. TH positive cells in SN were decreased in this model although this was not observed in the single α-synuclein transgenic mice. In addition, this model presents a further decrease of GCase activity and an increase in glucosylsphingosine without any noticeable accumulation of glucosylceramide.

(**) 50. Martin, S., et al., Mutated ATP10B increases Parkinson's disease risk by compromising lysosomal glucosylceramide export. Acta Neuropathol, 2020. 139(6): p. 1001-1024.

In this study ATP10B is described as a late-endolysosomal lipid flippase that translocates GluCer and phosphatidylcholine to the cytoplasm. Loss of function mutations are found in early onset PD patients. Besides, ATP10B is an important protein for lysosomal functionality and membrane integrity and knock-down in cells sensitizes to environmental risk factors for PD.

(**) 51. Wie, J., et al., A growth-factor-activated lysosomal K(+) channel regulates Parkinson's pathology. Nature, 2021. 591(7850): p. 431-437.

In vivo model of TMEM175 is developed for the first time to study PD pathology. KO homozygous mice show a significant loss of TH positive cells in SNpc. The authors confirm these results in KO heterozygous mice which also exhibit a significant reduction in the size and number of dopaminergic neuron in SNpc as well as a dopaminergic terminal loss in the striatum. Impairments in rotarod and wire-hang tests are detected in the KO mice.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Pang SY, Ho PW, Liu HF, Leung CT, Li L, Chang EES, Ramsden DB, Ho SL: ***The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease***. *Transl Neurodegener* 2019, **8**: p. 23.
2. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, Tan M, Kia DA, Noyce AJ, Xue A*, et al.*: ***Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies***. *Lancet Neurol* 2019, **18**: p. 1091-1102.
3. Chang D, Nalls MA, Hallgrimsdottir IB, Hunkapiller J, van der Brug M, Cai F, International Parkinson's Disease Genomics C, andMe Research T, Kerchner GA, Ayalon G*, et al.*: ***A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci***. *Nat Genet* 2017, **49**: p. 1511-1516.
4. Robak LA, Jansen IE, van Rooij J, Uitterlinden AG, Kraaij R, Jankovic J, International Parkinson's Disease Genomics C, Heutink P, Shulman JM: ***Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease***. *Brain* 2017, **140**: p. 3191-3203.
5. Roosen DA, Blauwendraat C, Cookson MR, Lewis PA: ***DNAJC proteins and pathways to parkinsonism***. *FEBS J* 2019, **286**: p. 3080-3094.
6. Roosen DA, Landeck N, Conti M, Smith N, Saez-Atienzar S, Ding J, Beilina A, Kumaran R, Kaganovich A, du Hoffmann J*, et al.*: ***Mutations in Auxilin cause parkinsonism via impaired clathrin-mediated trafficking at the Golgi apparatus and synapse***. *bioRxiv* 2019.
7. Cunningham LA, Moore DJ: ***Endosomal sorting pathways in the pathogenesis of Parkinson's disease***. *Prog Brain Res* 2020, **252**: p. 271-306.
8. Cao M, Wu Y, Ashrafi G, McCartney AJ, Wheeler H, Bushong EA, Boassa D, Ellisman MH, Ryan TA, De Camilli P: ***Parkinson Sac Domain Mutation in Synaptojanin 1 Impairs Clathrin Uncoating at Synapses and Triggers Dystrophic Changes in Dopaminergic Axons***. *Neuron* 2017, **93**: p. 882-896 e5.
9. Pan PY, Sheehan P, Wang Q, Zhu X, Zhang Y, Choi I, Li X, Saenz J, Zhu J, Wang J*, et al.*: ***Synj1 haploinsufficiency causes dopamine neuron vulnerability and alpha-synuclein accumulation in mice***. *Hum Mol Genet* 2020, **29**: p. 2300-2312.
10. Sassone J, Reale C, Dati G, Regoni M, Pellecchia MT, Garavaglia B: ***The Role of VPS35 in the Pathobiology of Parkinson's Disease***. *Cell Mol Neurobiol* 2021, **41**: p. 199-227.
11. Tsika E, Glauser L, Moser R, Fiser A, Daniel G, Sheerin UM, Lees A, Troncoso JC, Lewis PA, Bandopadhyay R*, et al.*: ***Parkinson's disease-linked mutations in VPS35 induce dopaminergic neurodegeneration***. *Hum Mol Genet* 2014, **23**: p. 4621-38.
12. Chen X, Kordich JK, Williams ET, Levine N, Cole-Strauss A, Marshall L, Labrie V, Ma J, Lipton JW, Moore DJ: ***Parkinson's disease-linked D620N VPS35 knockin mice manifest tau neuropathology and dopaminergic neurodegeneration***. *Proc Natl Acad Sci U S A* 2019, **116**: p. 5765-5774.
13. Niu M, Zhao F, Bondelid K, Siedlak SL, Torres S, Fujioka H, Wang W, Liu J, Zhu X: ***VPS35 D620N knockin mice recapitulate cardinal features of Parkinson's disease***. *Aging Cell* 2021: p. e13347.
14. Tang FL, Liu W, Hu JX, Erion JR, Ye J, Mei L, Xiong WC: ***VPS35 Deficiency or Mutation Causes Dopaminergic Neuronal Loss by Impairing Mitochondrial Fusion and Function***. *Cell Rep* 2015, **12**: p. 1631-43.

15. Dhungel N, Eleuteri S, Li LB, Kramer NJ, Chartron JW, Spencer B, Kosberg K, Fields JA, Stafa K, Adame A*, et al.*: ***Parkinson's disease genes VPS35 and EIF4G1 interact genetically and converge on alpha-synuclein****. Neuron* 2015, **85**: p. 76-87.
16. Erb ML, Moore DJ: ***LRRK2 and the Endolysosomal System in Parkinson's Disease****. J Parkinsons Dis* 2020, **10**: p. 1271-1291.
17. Taylor M, Alessi DR: ***Advances in elucidating the function of leucine-rich repeat protein kinase-2 in normal cells and Parkinson's disease****. Curr Opin Cell Biol* 2020, **63**: p. 102-113.
18. Seegobin SP, Heaton GR, Liang D, Choi I, Blanca Ramirez M, Tang B, Yue Z: ***Progress in LRRK2-Associated Parkinson's Disease Animal Models****. Front Neurosci* 2020, **14**: p. 674.
19. Xiong Y, Neifert S, Karuppagounder SS, Liu Q, Stankowski JN, Lee BD, Ko HS, Lee Y, Grima JC, Mao X*, et al.*: ***Robust kinase- and age-dependent dopaminergic and norepinephrine neurodegeneration in LRRK2 G2019S transgenic mice****. Proc Natl Acad Sci U S A* 2018, **115**: p. 1635-1640.
20. Lee BD, Shin JH, VanKampen J, Petrucelli L, West AB, Ko HS, Lee YI, Maguire-Zeiss KA, Bowers WJ, Federoff HJ*, et al.*: ***Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease****. Nat Med* 2010, **16**: p. 998-1000.
21. Nguyen APT, Tsika E, Kelly K, Levine N, Chen X, West AB, Boularand S, Barneoud P, Moore DJ: ***Dopaminergic neurodegeneration induced by Parkinson's disease-linked G2019S LRRK2 is dependent on kinase and GTPase activity****. Proc Natl Acad Sci U S A* 2020, **117**: p. 17296-17307.
22. Arbez N, He X, Huang Y, Ren M, Liang Y, Nucifora FC, Wang X, Pei Z, Tessarolo L, Smith WW*, et al.*: ***G2019S-LRRK2 mutation enhances MPTP-linked Parkinsonism in mice****. Hum Mol Genet* 2020, **29**: p. 580-590.
23. Zhao Y, Perera G, Takahashi-Fujigasaki J, Mash DC, Vonsattel JPG, Uchino A, Hasegawa K, Jeremy Nichols R, Holton JL, Murayama S*, et al.*: ***Reduced LRRK2 in association with retromer dysfunction in post-mortem brain tissue from LRRK2 mutation carriers****. Brain* 2018, **141**: p. 486-495.
24. Mir R, Tonelli F, Lis P, Macartney T, Polinski NK, Martinez TN, Chou MY, Howden AJM, Konig T, Hotzy C*, et al.*: ***The Parkinson's disease VPS35[D620N] mutation enhances LRRK2-mediated Rab protein phosphorylation in mouse and human****. Biochem J* 2018, **475**: p. 1861-1883.
25. Dues DJ, Moore DJ: ***LRRK2 and Protein Aggregation in Parkinson's Disease: Insights From Animal Models****. Front Neurosci* 2020, **14**: p. 719.
26. Van der Perren A, Cabezudo D, Gelders G, Peralta Ramos JM, Van den Haute C, Baekelandt V, Lobbestael E: ***LRRK2 Ablation Attenuates Alphalpha-Synuclein-Induced Neuroinflammation Without Affecting Neurodegeneration or Neuropathology In Vivo****. Neurotherapeutics* 2021.
27. Cabezudo D, Baekelandt V, Lobbestael E: ***Multiple-Hit Hypothesis in Parkinson's Disease: LRRK2 and Inflammation****. Front Neurosci* 2020, **14**: p. 376.
28. Kozina E, Sadasivan S, Jiao Y, Dou Y, Ma Z, Tan H, Kodali K, Shaw T, Peng J, Smeyne RJ: ***Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration in vivo****. Brain* 2018, **141**: p. 1753-1769.
29. Grabowski GA, Gatt S, Horowitz M: ***Acid beta-glucosidase: enzymology and molecular biology of Gaucher disease****. Crit Rev Biochem Mol Biol* 1990, **25**: p. 385-414.
30. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A*, et al.*: ***Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease****. N Engl J Med* 2009, **361**: p. 1651-61.
31. Farfel-Becker T, Do J, Tayebi N, Sidransky E: ***Can GBA1-Associated Parkinson Disease Be Modeled in the Mouse?*** *Trends Neurosci* 2019, **42**: p. 631-643.
32. Migdalska-Richards A, Wegrzynowicz M, Rusconi R, Deangeli G, Di Monte DA, Spillantini MG, Schapira AHV: ***The L444P Gba1 mutation enhances alpha-synuclein induced loss of nigral dopaminergic neurons in mice****. Brain* 2017, **140**: p. 2706-2721.
33. Migdalska-Richards A, Wegrzynowicz M, Harrison IF, Verona G, Bellotti V, Spillantini MG, Schapira AHV: ***L444P Gba1 mutation increases formation and spread of alpha-synuclein***

deposits in mice injected with mouse alpha-synuclein pre-formed fibrils. *PLoS One* 2020, **15**: p. e0238075.

34. Ikuno M, Yamakado H, Akiyama H, Parajuli LK, Taguchi K, Hara J, Uemura N, Hatanaka Y, Higaki K, Ohno K*, et al.*: ***GBA haploinsufficiency accelerates alpha-synuclein pathology with altered lipid metabolism in a prodromal model of Parkinson's disease***. *Hum Mol Genet* 2019, **28**: p. 1894-1904.
35. Yun SP, Kim D, Kim S, Kim S, Karuppagounder SS, Kwon SH, Lee S, Kam TI, Lee S, Ham S*, et al.*: ***alpha-Synuclein accumulation and GBA deficiency due to L444P GBA mutation contributes to MPTP-induced parkinsonism***. *Mol Neurodegener* 2018, **13**: p. 1.
36. Rockenstein E, Clarke J, Viel C, Panarello N, Treleaven CM, Kim C, Spencer B, Adame A, Park H, Dodge JC*, et al.*: ***Glucocerebrosidase modulates cognitive and motor activities in murine models of Parkinson's disease***. *Hum Mol Genet* 2016, **25**: p. 2645-2660.
37. Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, Cooper A, Garner B, Halliday GM: ***Reduced glucocerebrosidase is associated with increased alpha-synuclein in sporadic Parkinson's disease***. *Brain* 2014, **137**: p. 834-48.
38. van Veen S, Martin S, Van den Haute C, Benoy V, Lyons J, Vanhoutte R, Kahler JP, Decuypere JP, Gelders G, Lambie E*, et al.*: ***ATP13A2 deficiency disrupts lysosomal polyamine export***. *Nature* 2020, **578**: p. 419-424.
39. Shachar T, Lo Bianco C, Recchia A, Wiessner C, Raas-Rothschild A, Futerman AH: ***Lysosomal storage disorders and Parkinson's disease: Gaucher disease and beyond***. *Mov Disord* 2011, **26**: p. 1593-604.
40. Ramirez A, Heimbach A, Grundemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J*, et al.*: ***Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase***. *Nat Genet* 2006, **38**: p. 1184-91.
41. Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, Dickson DW, Mazzulli JR, Bardgett ME, Haik KL*, et al.*: ***Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited alpha-synuclein accumulation and age-dependent sensorimotor deficits***. *Hum Mol Genet* 2013, **22**: p. 2067-82.
42. Kett LR, Stiller B, Bernath MM, Tasset I, Blesa J, Jackson-Lewis V, Chan RB, Zhou B, Di Paolo G, Przedborski S*, et al.*: ***alpha-Synuclein-independent histopathological and motor deficits in mice lacking the endolysosomal Parkinsonism protein Atp13a2***. *J Neurosci* 2015, **35**: p. 5724-42.
43. Daniel G, Musso A, Tsika E, Fiser A, Glauser L, Pletnikova O, Schneider BL, Moore DJ: ***alpha-Synuclein-induced dopaminergic neurodegeneration in a rat model of Parkinson's disease occurs independent of ATP13A2 (PARK9)***. *Neurobiol Dis* 2015, **73**: p. 229-43.
44. Kong SM, Chan BK, Park JS, Hill KJ, Aitken JB, Cottle L, Farghaian H, Cole AR, Lay PA, Sue CM*, et al.*: ***Parkinson's disease-linked human PARK9/ATP13A2 maintains zinc homeostasis and promotes alpha-Synuclein externalization via exosomes***. *Hum Mol Genet* 2014, **23**: p. 2816-33.
45. Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D: ***Deficiency of ATP13A2 leads to lysosomal dysfunction, alpha-synuclein accumulation, and neurotoxicity***. *J Neurosci* 2012, **32**: p. 4240-6.
46. Si J, Van den Haute C, Lobbestael E, Martin S, van Veen S, Vangheluwe P, Baekelandt V: ***ATP13A2 Regulates Cellular alpha-Synuclein Multimerization, Membrane Association, and Externalization***. *Int J Mol Sci* 2021, **22**.
47. Handa AK, Fatima T, Mattoo AK: ***Polyamines: Bio-Molecules with Diverse Functions in Plant and Human Health and Disease***. *Front Chem* 2018, **6**: p. 10.
48. Saiki S, Sasazawa Y, Fujimaki M, Kamagata K, Kaga N, Taka H, Li Y, Souma S, Hatano T, Imamichi Y*, et al.*: ***A metabolic profile of polyamines in parkinson disease: A promising biomarker***. *Ann Neurol* 2019, **86**: p. 251-263.
49. Tesson C, Lohmann E, Devos D, Bertrand H, Lesage S, Brice A: ***Segregation of ATP10B variants in families with autosomal recessive parkinsonism***. *Acta Neuropathol* 2020, **140**: p. 783-785.

50. Martin S, Smolders S, Van den Haute C, Heeman B, van Veen S, Crosiers D, Beletchi I, Verstraeten A, Gossye H, Gelders G*, et al.*: ***Mutated ATP10B increases Parkinson's disease risk by compromising lysosomal glucosylceramide export****. Acta Neuropathol* 2020, **139**: p. 1001-1024.
51. Wie J, Liu Z, Song H, Tropea TF, Yang L, Wang H, Liang Y, Cang C, Aranda K, Lohmann J*, et al.*: ***A growth-factor-activated lysosomal K(+) channel regulates Parkinson's pathology****. Nature* 2021, **591**: p. 431-437.