TITLE:

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ROLE OF THE VPS35 D620N MUTATION IN PARKINSON'S DISEASE

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ABSTRACT:

Parkinson's disease (PD) is a neurodegenerative disorder involving the loss of dopaminergic neurons in the brain. Following the discovery of the PD-causing D620N mutation in the *VPS35* (Vacuolar sorting protein 35) gene, dysfunction in the subcellular retromer complex has been strongly implicated in pathogenesis of PD. Although the function and dysfunction of the retromer has been a focus of study for some time, the role of this complex in the development of PD is not fully understood. Investigating cellular alterations that occur when the retromer is rendered dysfunctional, such as when the D620N disease-causing mutation is introduced into various model systems, shows that endosomal processing defects are major contributors to the disease phenotype. Altered trafficking of retromer cargo molecules, reduced cellular survival and altered processing of alpha-synuclein have all been observed in the presence of the D620N mutation. In addition, interactions between the retromer and the protein products of other familial Parkinsonism-related genes, has made the retromer a prime target of research in PD. This review gives an overview of the changes in retromer function, identified thus far, that may contribute to the neurodegeneration observed in PD.

PARKINSON'S DISEASE:

Parkinson's disease (PD) is neurodegenerative disorder and was first described in the "Essay on shaking palsy" written by James Parkinson in 1817 [1]. It is the second most prevalent neurodegenerative disease after Alzheimer's disease [2] with a prevalence of 1-2% in a population of above 60yrs [3]. Some of the cardinal symptoms of the disease are motor related abnormalities such as rigidity, bradykinesia and tremors and non-motor related deficits such as neuropsychiatric disorders, sensory abnormalities such as olfactory dysfunction and pain [4]. Heterogeneous degeneration of the dopaminergic neurons in the *substantia nigra* region of the brain and the formation of intracellular protein aggregates called Lewy bodies in the soma, presynaptic terminals, neurites or extracellular spaces of the degenerating neurons are characteristic hallmarks of the disease [5, 6]. Between 50 and 90% of the dopaminergic neurons that control movement, mood and stress are lost in the post-mortem brain tissues of PD patients [7]. An interaction of multiple variables such as environmental exposures, genetic

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factors and increased age are all considered to alter the risk of the disease [8]. The diagnosis of PD during life is made clinically, and, whilst PD is a chronic progressive disease and there is no cure, the treatment strategies, both pharmacological and neurosurgical, do act to manage the symptoms of the disease and improve quality of life [9].

VPS35 D620N MUTATIONS IN PARKINSON'S DISEASE

In genetic terms, PD can be classified into sporadic (occurring with no family history) or familial (within a pedigree of multiple affected individuals). Early genetic linkage studies in rare pedigrees with inherited familial Parkinsonism, revealed mutations in the *SNCA* gene that codes for the alpha-synuclein protein present in Lewy bodies found in the brains of PD patients. [10, 11]. These findings not only confirmed the idea that PD could be inherited, but also highlighted that rare genetic findings could provide important clues as to what happens in the more common forms of the disease. An extensive hunt for other genes related to PD has revealed around 24 genetic risk loci associated with PD so far [12]. In 2011, using exome sequencing, a D620N mutation in the VPS35 subunit of the retromer complex was identified as rare causes of autosomal dominant familial PD [13, 14]. This discovery highlighted the involvement of the retromer complex in PD. Several other variants such as the p.P316S, p.Y507F, p.R524W, p.I560T, p.M571I, p.H599R, p.M607V, p.E787K and p.L774M have been subsequently reported; however proof that these are truly pathogenic remains debated [15, 16]. The frequency of the D620N mutation is currently estimated to be around 1.3% in familial PD and 0.3% in sporadic PD. The mutation is a rare cause of the disease in all populations that have been tested [17, 18]. The clinical phenotype of mutation carriers is similar to sporadic PD, with substantial variability in onset with incidences of both early and late age at onset being reported. The information available to date supports the idea that the mutation exhibits a relatively high but incomplete penetrance [16, 19-21]. Patients show a good response to L-dopa [17] and in one case enhanced motor functions were achieved in response to deep brain stimulation [17, 22]

THE RETROMER COMPLEX:

The retromer complex is comprised of a core tripartite cargo recognition complex consisting of VPS35, VPS26 and VPS29 subunits and a membrane deformation subunit, which contains SNX proteins [23] (Fig.1). Two orthologues of VPS26 (VPS26A and VPS26B) are found to form two different cargo selective complexes [24]. The retromer complex is localised on the endosomes with the help of Rab 7 and SNX3; the SNX binds to the endosome independent of the retromer complex [25-28]. The SNX proteins have a PX and BAR domain which binds to endosomal membranes through a phosphatidylinositol 3-monophosphate (PtdIns(3)P). After the cargo selective complex binds to the cytosolic tail of cargo molecules, the SNX proteins are thought to drive the formation of membrane tubule carriers that can transport the cargo [29]. The retromer proteins then dissociate from the membrane to undergo further rounds of cargo sorting while the carriers transport the cargo to its respective destinations within the cell.

RETROMER IN NEURODEGENERATIVE DISEASES:

A role for the retromer in neurodegeneration was first suggested in relation to Alzheimer's disease. Reduced expression of VPS35 and VPS26 protein levels were observed in the entorhinal cortex of AD patients when compared to healthy controls [30]. The retromer trafficks SORLA, a receptor for APP that is sequentially cleaved by BACE in the endosomal lumen to produce A β found in amyloid plaques in brains of AD patients. Variants in the *SORLA* gene have also been associated with AD [31]. Retromer defects cause mis-trafficking of SORLA causing APP retention in the endosomal lumen, which results in increased production of A β [32-34]. Elevated A β levels leads to hippocampal dependant memory loss, synaptic dysfunctions and neuronal loss in retromer deficient transgenic mouse and drosophila models rendered cognitively compromised due to heterozygous knockouts of retromer subunits [35]. Increased levels and activities of BACE1 in post mortem AD brain samples were first reported in 2002, and it is now known that BACE1 is a cargo molecule of the retromer [36]. Suppression of VPS35 causes altered distribution and increased activity of BACE1 leading to memory deficits, impaired postsynaptic glutamatergic neurotransmission and impaired neuronal morphogenesis in mouse models [37, 38]. It has also been suggested that genetic variants in several genes that encode retromer-related proteins, are genetically associated with increased risk for AD [39], although it is not clear how many of these associations will be consistently replicated. The retromer has been associated with a range of neurodegenerative disorders including: 1) Downs syndrome, hereditary spastic paraplegia (HSP) and neuronal ceroid lipofuscinosis (NCL) [40]. These findings demonstrate that retromer impairments can cause multiple neuron specific pathologies. This brings us to the question, how does retromer dysfunction impact the development and progression of the neurodegeneration observed in PD? To answer this question, this review seeks to summarise what is known about the general role of the retromer in maintaining cell physiology, the impact of the VPS35 D620N mutation on these functions and the retromer-related changes that are observed in sporadic PD.

CELLULAR FUNCTIONS OF THE RETROMER:

The retromer is ubiquitously found in all cells and various roles have been identified for the retromer within the cell (Table 1). It is a key player of endosomal sorting and trafficks a vast myriad of proteins from the endosomes to the trans Golgi network and from the endosome to the plasma membrane [28, 41]. It is also involved in the transport of proteins to and from the mitochondria and during autophagosome formation [42, 43]. A well-characterised role for the retromer is its recycling of cation independent mannose 6 phosphate receptor (CIMPR) in mammalian cells. The CIMPR is a receptor for the lysosomal acid hydrolase enzyme cathepsin D. Cathepsin D is an aspartic protease that mediates the lysosomal degradation of alpha-synuclein. Cathepsin D is synthesised at the ER as a precursor, recognised by the CIMPR and trafficked to the endosomes where it dissociates from the receptor and is transported to the lysosomes [44]. The retromer recycles the receptor from the maturing endosome to the trans Golgi network to aid in the repeated transport of newly synthesised

enzyme precursors. The retromer also recycles various other proteins in order to maintain homeostasis and polarity of various types of neuronal and non- neuronal cells. For example, the iron transporter DMT1-11 is recycled via the retromer [41]. So is the protein Wntless (wls), a transmembrane protein essential for the secretion of Wingless, a Drosophilla Wnt protein that is essential for morphogenesis and cell homeostasis [45-48]. The retromer also helps in the basolateral to apical transcytosis of pIgR (a polyimmunoglobulin receptor for IgA) in epithelial cells [49, 50] and mediates recycling of the apical determinant, crumbs (crb), that regulates apical- basal polarity and cell growth [51, 52]. The luminal protein, Serpentine, that regulates epithelial tube length in drosophila is also recycled by the retromer complex [53]. In neurons the retromer traffics neurotransmitter receptors and synapse-specific membrane proteins that help maintain synaptic transmissions. The retromer complex is also used by viral and bacterial pathogens to aid in their assembly, replication and movement within the cell and as a mechanism to evade destruction by the cells defence machinery. The retromer regulates retrieval of the of HIV and HPV proteins during infection of the cell and is downregulated by the herpesvirus saimiri (HVS) tyrosine kinase-interacting protein (Tip) to avoids destruction of the virus [54-57]. In addition, bacterial Shiga toxin (Stx) uses the retromer for its trafficking within the cell [58]. Considering the ubiquitous nature of the retromer, it is not surprising that a range of diseases other than neurodegenerative diseases are also associated with retromer dysfunctions. These include osteoporosis and gastric and colorectal cancers [59, 60]. Genome-wide association studies (GWAS) and reduced retromer levels in a hyperleucinemia mouse model have also linked the retromer complex to Type 1 and 2 Diabetes Mellitus and there is one report linking VPS35 haploinsufficiency to retinal ganglion neurodegeneration in mice, implicating a possible retromer dysfunctions in retinal degenerative diseases[61-63].

CONSEQUENCES OF THE D620N MUTATION AND RETROMER DYSFUNCTION IN PD:

Altered retromer trafficking of several proteins along different pathways in the presence of the D620N mutation have been identified (Fig.2, 3). Some of the studies included below show that the mutation causes disease in dominant gain of function mechanism while the others show disease as a result of a loss of function mechanism [64-67]. Although it remains to be determined exactly what the pathogenic nature of the mutant is, from studies so far it can be concluded that the VPS35 D620N mutation exhibits both a gain of function and loss of function mechanism depending on the context.

ENDOSOME TO TGN TRANSPORT DEFECTS:

To understand the effect of the Parkinson's disease-related mutation, D620N on the retromer's endosome to Golgi transport capabilities, several investigations have been performed in human cellular and animal models. These involve studies on CIMPR trafficking and the processing of cathepsin D and alpha-synuclein. The D620N mutation lies within the binding site between VPS35 and VPS29 but this amino acid change does not appear to interfere in the retromer's cargo recognition complex formation or folding [68]. The binding

site of the CIMPR, also lies in the region of the D620N mutation, but again, the presence of the mutation does not affect the binding capacity of the retromer to the CIMPR [69].

CIMPR trafficking and cathepsin D processing:

Human cellular models:

Down regulating VPS35 (and effectively the retromer) in HEK293, RPE1 and Hela cells displaces the natural distribution of CIMPR from its perinuclear location (association with the TGN) to a more dispersed pattern throughout the cell. This indicates a trafficking defect of the receptor by the retromer out of the endosomes, resulting in ineffective recycling of the receptor and increased degradation of the receptor [25, 70, 71]. In the RPE1 cell lines the observed phenotype was partially rescued by reintroducing the wild type VPS35 but not the VPS35 D620N variant which suggests the ability of the mutant to produce such a phenotype [71].

Neuronal models:

This loss of function activity has been observed in neuronal models as well. In the presence of the D620N mutation a similar kind of altered CIMPR distribution, where there is reduced association of the CIMPR with the Golgi apparatus has been observed in primary rat neuron cultures overexpressing the D620N variant[72].

Patient based cellular models:

PD patients carrying the D620N mutation also show similar distribution differences in the CIMPR and endosomal maturation defects as shown in fibroblasts obtained from the patients[69, 71]. An increased dispersal in CIMPR, which can be rescued, on suppression of the endogenous VPS35 and re expression of the wild type VPS35 was shown in these cells[71]. This further confirms an impaired retromer dependant endosome to TGN trafficking induced by the mutation.

To further characterise the observed trafficking defect several groups have studied cathepsin D processing in cells expressing the D620N mutation. Clear cathepsin D processing defects were observed in the presence of D620N in both HEK293 cells overexpressing the mutation and in fibroblasts from D620N mutation carriers [69]. In contrast to these observations two other studies, one investigating HeLa cells expressing the D620N mutant [68] and the other looking at fibroblasts from mutation carriers, did not show any altered association of the retromer with its cargo molecules or cathepsin D processing [64]. Further studies are required to address this discordance in literature, as there are likely many additional factors involved with these trafficking processes. It is likely that an endogenous D620N mutation would induce a subtler phenotype to that observed in crude overexpression or silencing experiments.

Cathepsin D and alpha-synuclein processing.

CIMPR and cathepsin D alterations in the presence of VPS35 mutations provide an important link between the retromer and the pathogenesis of PD, given the role of cathepsin D in alpha-

synuclein degradation via the lysosomal pathway. The degradation of alpha-synuclein is mediated by the proteosomal and the autophagy/lysosomal pathways; cathepsin D plays an important role in the processing of alpha-synuclein via the lysosomal pathway [73-75]. The importance of effective cathepsin D regulation in alpha-synuclein degradation has been highlighted in several studies [76-78].

Human cellular models:

Retromer impairment (through knockdown of the VPS35 subunit) causes lysosomal accumulation of alpha-synuclein in HEK293 cells due to the improper processing of cathepsin D. However, expression of the D620N mutation alone was unable to produce accumulation of alpha-synuclein [70].

Animal models:

Silencing VPS35 in drosophila expressing alpha-synuclein in the brain also led to increased alpha-synuclein accumulation, PD-like motor defects and eye disorganisation [70]. The processing of alpha-synuclein in the presence of the D620N mutant was also analysed in transgenic mice expressing alpha-synuclein. Expressing wildtype VPS35 in this disease model rescued the usual neurodegenerative phenotype; however, silencing VPS35 or expression of the mutant form, led to significant neuronal loss. It is also suggested that VPS35 may play a role in mitigating the intracellular seeding of pathogenic alpha-synuclein between neurons. Dhungel and co-workers found that expressing wildtype VPS35 reduced intracellular seed formation within rodent cortical neurons when compared to controls infected neurons. On the other hand, in neurons expressing the D620N mutations there was increased accumulation of synuclein seeds [79]. These findings highlight the importance of retromer-mediated trafficking of the CIMPR and other lysosomal enzyme associated receptors for the correct processing of alpha-synuclein within the cells to avoid toxic aggregate formation.

ENDOSOME TO PLASMA MEMBRANE DEFECTS:

Human cellular models

The WASH (Wiskott-Aldrich syndrome protein and scar homolog) complex, which is recruited to the endosome by the retromer, regulates endosome to plasma membrane recycling of a large number of molecules. The affinity with which VPS35 binds to the FAM21 tail LA motifs of the WASH complex was found to be reduced in HeLa cells expressing the D620N mutation [68]. The WASH complex binds to VPS35 using its FAM21 tail and the proteins SDCCAG3 and ANKRD5 and RME8 bind to the retromer via the FAM21 tail of the WASH complex. Therefore these proteins were also found to have reduced association with the retromer in the presence of the mutation. Surprisingly, the retromer complex is shown to displace the WASH complex from the endosomes, while silencing the FAM21 tail leads to the displacement of SDCCAG3 and ANKRD5, without affecting the binding of RME8 and FKBP15. This suggests that the latter two proteins are not totally dependent on the FAM21 tail to bind to the retromer complex [43, 71]. GLUT1 is another protein that is recycled to the

cell surface with the help of SNX27- retromer-WASH interactions and suppression of VPS35 in HeLa cells have shown to cause its mis-localisation [80].

Neuronal models:

Interestingly, Lewy bodies, the pathological inclusions observed in PD, stain positive for RME8 and the recent discovery of a *DNAJC13* mutation that partially segregates with PD in a large family with autosomal dominant PD is an additional piece of evidence to emphasise the importance of the retromer in endosome to plasma membrane sorting [81]. VPS35 colocalises with DNAJC13 in mouse primary cortical neuronal cultures and *DNAJC13* mutations cause endosomal accumulation of the transferrin receptor in COS7 cells[81]. Down regulation of RME8 expression causes accumulation of endosomal tubules containing retromer cargo [82]. Though the presence of the DNAJC13 mutation in the family did give additional evidence of the involvement of the retromer in PD recently it was identified that the cause for disease in this family may be due to a p.Arg141Leu mutation in the *TMEM230* gene. *TMEM230* codes for a transmembrane protein of synaptic vesicles and was shown to co-localise with VPS35 and the endosomes in Neuro2A cells and mouse primary neurons [83]. It is suggested that dysregulation of synaptic vesicle trafficking or recycling may be the underlying effect of the mutation and this again indicates a shared pathway with the retromer complex in vesicle and endosomal trafficking. These findings suggest that such endosome to plasma membrane trafficking defects can contribute significantly to the development of Parkinson's disease

MITOCHONDRIAL DEFECTS:

Human cellular models:

Mitochondrial fission and fusion defects contribute to neurodegeneration in PD and AD and several genes implicated in PD code for proteins that regulate mitochondrial dynamics [84-86]. Mitochondrial dynamics are also disturbed by perturbations of the retromer complex. The work of Braschi and colleagues has shown that the retromer participates in the formation of MDVs (Mitochondria- derived vesicles) and transport of MAPL (mitochondrial-anchored protein ligase) from the mitochondria to the peroxisomes in MCF7 cells. On silencing the retromer in these cells there is a reduction in MAPL transport to the peroxisomes [42]. Researchers have now also reported that VPS35 interacts with DLP1 (dynamin like protein 1), that regulates mitochondrial fission. In the presence of the VPS35 D620N mutation in M17 cells an increased interaction and lysosomal turnover of DLP1 via the MDV trafficking was observed. In addition to this increased mitochondrial fragmentation increased mitochondrial dysfunction (in the context of increased ROS formation and decreased membrane potential) and neuronal loss was observed in fibroblasts carrying the VPS35 D620N mutation and in neurons expressing the D620N mutation. This correlated with increased fission events in the presence of the mutant [87].

Animal models:

More recently, VPS35 mutations and deficiencies in transgenic mice caused loss of a mitofusin 2 protein (MFN2) which resulted in impaired mitochondrial fusion, dopaminergic

loss and increase in alpha synuclein levels [88]. Interestingly the D620N mutation also increased sensitivity to mitochondrial stressors such as MPP+ and rotenone in transgenic neuronal cells and drosophila models [65, 89]. Mitochondrial dysfunctions have been one of the pathogenic hallmarks for PD and VPS35 (thereby the retromer) now adds to the list of the various PD related genes such as *PINK1*, *PARK2* (Parkin), *PARK 7*(DJ-1), *PARK8* (LRRK2) that are involved in regulation of mitochondrial dynamics. In addition to VPS35 more recently two other loci, *PARK22* (CHCHD2) and *PARK 23* (VPS13C) have been added to the PARK genes that affect mitochondrial dynamics in PD.

AUTOPHAGY DEFECTS:

The retromer has also been implicated in the autophagy-mediated pathway, which is critical for degradation of the PD-related protein, alpha-synuclein. The ATG9 protein is involved in the formation of the autophagosomes and localises to VPS35 and the WASH complex. In the presence of the VPS35 D620N mutation in HeLa cells, defective trafficking of ATG9 was observed, which could lead to impaired auto phagosome formation [68]. Compromised autophagic fusion was also observed on impaired recycling of the SNARE (N-ethylmaleimide-sensitive factor attachment protein receptor) complex by the retromer from the endosome to TGN [90]. In addition to this, the retromer has recently been implicated in the chaperone mediated autophagy pathway through its interactions with the LAMP2a receptor. The LAMP2a receptor regulates the CMA mediated protein degradation pathway. The retromer complex mediates trafficking of LAMP2a and is necessary for the morphogenesis and function of the lysosomes. The VPS35 D620N mutation causes impaired endosome to Golgi retrieval of LAMP2a and causes a decrease in LAMP2a levels within neuronal cells. This, in turn, causes increased alpha-synuclein build up within these cells [66]. Alpha-synuclein accumulation is considered a fundamental step in PD neurodegeneration and the involvement of the retromer in its degradation via all the different degradative pathways makes the retromer an important therapeutic target in PD. Reduced levels of retromer has been reported in AD and PD brains and increasing retromer levels seems to enhance the degradation of protein aggregates in AD models. It has been shown in APP transgenic mice that, increasing abundance of the retromer complex facilitated the degradation of A β plaques by enhanced phagocytosis and the recycling of phagocytosis receptors by the retromer [91]. Therefore increasing the abundance of retromer may be a possible therapeutic strategy in PD also.

NEURON SPECIFIC DEFECTS:

Studies using animal models and neuronal cultures help to replicate the possible events happening in the living PD brain; something that is not currently possible to do in a human patient.

Animal models:

The retromer regulates a vast myriad of proteins within the cell and therefore a knockdown of the VPS35 has shown to be lethal in animal models [37]. Expression of the D620N mutation in the *substantia nigra* of a rat produces axonal pathology and a dopaminergic neuron

degeneration phenotype [64]. Similarly in mice, the expression of VPS35 mutations in regions of the brain usually affected in PD results in an accumulation of alpha-synuclein, loss of DA transmitters and impairment of locomotor activity [66]. Reduced surface expression and impaired glutamatergic neurotransmission and synaptic spine maturation defects were also observed in a VPS35 heterozygous mouse model [92]. Selective knockdown of VPS35 in drosophila also results in neuronal loss, reduced life span and locomotor defects [70, 93, 94].

Neuronal models:

The retromer is important to the normal functioning of neurons with neurotransmitter receptors being cargo molecules of the retromer. A recent study showed the importance of the retromer for the correct expression of various signalling receptors (including like β 2AR, AMPAR and NMDAR) at the surface of dendrites and synapses in rat spiny neurons [95]. GluR1 AMPARs subunits interact with the retromer and are recruited to the synapse to aid in excitatory synaptic transmission and synaptic plasticity. In the presence of the D620N mutation in both mouse cortical neurons and dopaminergic neurons induced from pluripotent cells from D620N carriers there was increased co-localisation of the retromer with the receptor which lead to altered receptor recycling and synaptic transmissions [96]. Such perturbations may be the cause for progressive neuronal loss especially in the presence of other stressors in PD. Taken together; these studies demonstrate that the neuronal pathology, seen in PD patients, can be brought about by a dysfunctional retromer complex

INTERACTION BETWEEN RETROMER AND PARK GENE PROTEINS:

Recent investigations into the interacting partners of the retromer with other *PARK* gene products have identified some potential new leads as to how the retromer may contribute to the pathogenesis of the PD (Fig.3). A physical interaction between LRRK2 and VPS35 proteins has been reported in rat primary neuronal cultures and drosophila models. Phenotypes induced by the *LRRK2* mutations (G2019S, R1441C or I2020T) could be rescued by WT VPS35. Both *LRRK2* and *VPS35* mutations produced similar phenotypes (reduced neurite length, lysosomal swelling, MPR mislocalisation and locomotor defects) suggesting a similar mode of function for these proteins [93, 94]. LRRK2 is also implicated in synaptic vesicle trafficking, like VPS35 and SNCA. *DNAJC6* and *SYNJ1* are two other genes that contain mutations leading to autosomal recessive juvenile Parkinsonism; their protein products are also involved in synaptic vesicle recycling [97]. A definitive interaction between the retromer and these genes has not yet been identified although they may work along similar pathways. *Parkin* and *PINK1* mutations are pathogenic causes of recessive PD and these gene products help in regulating mitochondrial dynamics. In one study, overexpression of VPS35 rescued parkin mutant phenotypes [67]. In addition to this the recently identified mutations in CHCH2 and VPS13C proteins also seem to contribute to regulating mitochondrial dynamics and a possible vesicular trafficking by VPS13C in a similar pathway as the retromer complex. The CHCH2 gene mutations are associated with autosomal dominant PD. It is present on the intermembrane space of the mitochondria and any dysfunctions associated with the gene are suggested to contribute to mitochondrial respiration defects in PD [98]. Similarly mutations in the VPS13C are associated with autosomal recessive early onset Parkinsonism. Down

regulation of the VPS13C was found to cause several mitochondrial dysfunctions including lowered membrane potential and fragmentation [99]. Although VPS13C is associated with endosomal to Golgi transport in yeast it remains to be identified what its role with respect to the retromer and mitochondrial function in PD could be. Hits from genetic screens also support a link between VPS35 and EIF4G1 [79]. *EIF4G1* mutations segregate with autosomal dominant PD and upregulation of EIF4G1 caused toxicity associated with alpha-synuclein aggregation; this could be suppressed by the expression of VPS35 [79, 100]. These interactions suggest that the retromer is part of a common pathway that, if perturbed can contribute to the pathogenesis of PD.

CONCLUSIONS:

PD-related mutations in VPS35 lead to phenotypes of retromer dysfunction. Conversely, PD specific phenotypes can be generated by modulating the retromer in the different cellular, animal, fly and yeast models. Of all the retromer functions that have been identified, retromer-mediated processing of alpha-synuclein by cathepsin D provides the closest link between the retromer and the pathogenesis of PD so far. In addition to this, functional interactions between the retromer and other PD related proteins such as LRRK2, DNAJC13, EIF4G1 and alpha-synuclein support the idea that all these proteins converge upon endosomal/vesicle trafficking defects as a common pathway leading to protein aggregation contributing to neurodegenerative pathology. With this in view, enhancing retromer stability and function using molecular chaperones has been identified as a possible way of altering disease phenotypes in AD and this may be the way forward in targeting neuronal death in PD as well [101, 102]. Nevertheless, it must be remembered that the retromer is a modulator for many pathways and altering its activity would mean modifying many pathways which could be detrimental. It remains difficult to say with certainty how central retromer dysfunction is in the process of neurodegeneration considering its numerous roles in the normal functioning of cells. Clearly, further research into the functional aspects of retromer function and dysfunction is merited to provide critical new binding partners and potential novel leads in the search for a cure to Parkinson's disease.

COLOUR ON WEB

Fig 1. The retromer complex assembly on the endosome: The different retromer complex (VPS35, VPS26 and VPS29) assemblies that carry cargo from the endosome to the plasma membrane and the TGN are shown here. The retromer-SNX-BAR complex and retromer-SNX3 complex mediate endosome to trans-Golgi transport of receptors. The Retromer-SNX27 complex in association with the WASH complex mediates the endosome to plasma membrane recycling of the receptor. The arrows indicate the direction of transport of the carrier vesicles that carry the cargo.

Figure 2: Altered protein trafficking in presence of the *vps35 d620n* mutation: Cargo proteins of the retromer complex whose trafficking is affected in the presence of the D620N mutation. The different trafficking pathways are highlighted in blue with the arrows indicating an increased function or decreased function or impaired trafficking in the presence of the mutant. The different pathways in which the retromer complex participates are from endosomes to trans Golgi network, endosomes to plasma membrane and mitochondria to peroxisomes/ lysosomes).

Fig 3: Consequences of the VPS35 D620N mutation: The different cellular functions that have been affected by the altered trafficking of cargo molecules by the VPS35 D620N retromer complex are indicated by the different colours. The other PARK gene proteins that the retromer interacts or is associated with are represented on the outer circle.

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REFERENCES:

- [1] J. Parkinson, An essay on the shaking palsy. 1817, *J Neuropsychiatry Clin Neurosci* 14(2) (2002) pp. 223-236; discussion 222.
- [2] J. Massano, K.P. Bhatia, Clinical approach to Parkinson's disease: features, diagnosis, and principles of management, *Cold Spring Harb Perspect Med* 2(6) (2012) pp. a008870.
- [3] A.E. Lang, A.M. Lozano, Parkinson's disease. First of two parts, *N Engl J Med* 339(15) (1998) pp. 1044-1053.
- [4] D.J. Gelb, E. Oliver, S. Gilman, Diagnostic criteria for Parkinson disease, *Arch Neurol* 56(1) (1999) pp. 33-39.
- [5] H. Braak, K. Del Tredici, U. Rub, R.A. de Vos, E.N. Jansen Steur, E. Braak, Staging of brain pathology related to sporadic Parkinson's disease, *Neurobiol Aging* 24(2) (2003) pp. 197-211.
- [6] P. Damier, E.C. Hirsch, Y. Agid, A.M. Graybiel, The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease, *Brain* 122 (Pt 8) (1999) pp. 1437-1448.
- [7] J.H. Kordower, C.W. Olanow, H.B. Dodiya, Y. Chu, T.G. Beach, C.H. Adler, G.M. Halliday, R.T. Bartus, Disease duration and the integrity of the nigrostriatal system in Parkinson's disease, *Brain* 136(Pt 8) (2013) pp. 2419-2431.
- [8] L.M. de Lau, M.M. Breteler, Epidemiology of Parkinson's disease, *Lancet Neurol* 5(6) (2006) pp. 525-535.
- [9] V.S. Fung, New and emerging treatments for Parkinson disease, *Med J Aust* 202(6) (2015) pp. 283-284.
- [10] M.H. Polymeropoulos, J.J. Higgins, L.I. Golbe, W.G. Johnson, S.E. Ide, G. Di Iorio, G. Sanges, E.S. Stenroos, L.T. Pho, A.A. Schaffer, A.M. Lazzarini, R.L. Nussbaum, R.C. Duvoisin, Mapping of a gene for Parkinson's disease to chromosome 4q21-q23, *Science* 274(5290) (1996) pp. 1197-1199.
- [11] R. Kruger, W. Kuhn, T. Muller, D. Woitalla, M. Graeber, S. Kosel, H. Przuntek, J.T. Epplen, L. Schols, O. Riess, Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease, *Nat Genet* 18(2) (1998) pp. 106-108.
- [12] M.A. Nalls, N. Pankratz, C.M. Lill, C.B. Do, D.G. Hernandez, M. Saad, A.L. DeStefano, E. Kara, J. Bras, M. Sharma, C. Schulte, M.F. Keller, S. Arepalli, C. Letson, C. Edsall, H. Stefansson, X. Liu, H. Pliner, J.H. Lee, R. Cheng, C. International Parkinson's Disease Genomics, G.I. Parkinson's Study Group Parkinson's Research: The Organized, andMe, GenePd, C. NeuroGenetics Research, G. Hussman Institute of Human, I. Ashkenazi Jewish Dataset, H. Cohorts for, E. Aging Research in Genetic, C. North American Brain Expression, C. United Kingdom Brain Expression, C. Greek Parkinson's Disease, G. Alzheimer Genetic Analysis, M.A. Ikram, J.P. Ioannidis, G.M. Hadjigeorgiou, J.C. Bis, M. Martinez, J.S. Perlmutter, A. Goate, K. Marder, B. Fiske, M. Sutherland, G. Xiomerisiou, R.H. Myers, L.N. Clark, K. Stefansson, J.A. Hardy, P. Heutink, H. Chen, N.W. Wood, H. Houlden, H. Payami, A. Brice, W.K. Scott, T. Gasser, L. Bertram, N. Eriksson, T. Foroud, A.B. Singleton, Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease, *Nat Genet* 46(9) (2014) pp. 989-993.
- [13] C. Vilarino-Guell, C. Wider, O.A. Ross, J.C. Daxsel, J.M. Kachergus, S.J. Lincoln, A.I. Soto-Ortolaza, S.A. Cobb, G.J. Wilhoite, J.A. Bacon, B. Behrouz, H.L. Melrose, E. Hentati, A. Puschmann, D.M. Evans, E. Conibear, W.W. Wasserman, J.O. Aasly, P.R. Burkhard, R. Djaldetti, J. Ghika, F. Hentati, A. Krygowska-Wajs, T. Lynch, E. Melamed, A. Rajput, A.H. Rajput, A. Solida, R.M. Wu, R.J. Uitti, Z.K.

- Wszolek, F. Vingerhoets, M.J. Farrer, VPS35 mutations in Parkinson disease, *Am J Hum Genet* 89(1) (2011) pp. 162-167.
- [14] A. Zimprich, A. Benet-Pages, W. Struhal, E. Graf, S.H. Eck, M.N. Offman, D. Haubenberger, S. Spielberger, E.C. Schulte, P. Lichtner, S.C. Rossle, N. Klopp, E. Wolf, K. Seppi, W. Pirker, S. Presslauer, B. Mollenhauer, R. Katzenschlager, T. Foki, C. Hotzy, E. Reinthaler, A. Harutyunyan, R. Kralovics, A. Peters, F. Zimprich, T. Brucke, W. Poewe, E. Auff, C. Trenkwalder, B. Rost, G. Ransmayr, J. Winkelmann, T. Meitinger, T.M. Strom, A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease, *Am J Hum Genet* 89(1) (2011) pp. 168-175.
- [15] H. Deng, K. Gao, J. Jankovic, The VPS35 gene and Parkinson's disease, *Mov Disord* 28(5) (2013) pp. 569-575.
- [16] M. Sharma, J.P. Ioannidis, J.O. Aasly, G. Annesi, A. Brice, L. Bertram, M. Bozi, M. Barcikowska, D. Crosiers, C.E. Clarke, M.F. Facheris, M. Farrer, G. Garraux, S. Gispert, G. Auburger, C. Vilarino-Guell, G.M. Hadjigeorgiou, A.A. Hicks, N. Hattori, B.S. Jeon, Z. Jamrozik, A. Krygowska-Wajs, S. Lesage, C.M. Lill, J.J. Lin, T. Lynch, P. Lichtner, A.E. Lang, C. Libioulle, M. Murata, V. Mok, B. Jasinska-Myga, G.D. Mellick, K.E. Morrison, T. Meitinger, A. Zimprich, G. Opala, P.P. Pramstaller, I. Pichler, S.S. Park, A. Quattrone, E. Rogaeva, O.A. Ross, L. Stefanis, J.D. Stockton, W. Satake, P.A. Silburn, T.M. Strom, J. Theuns, E.K. Tan, T. Toda, H. Tomiyama, R.J. Uitti, C. Van Broeckhoven, K. Wirdefeldt, Z. Wszolek, G. Xiomerisiou, H.S. Yomono, K.C. Yueh, Y. Zhao, T. Gasser, D. Maraganore, R. Kruger, G. Consortium, A multi-centre clinico-genetic analysis of the VPS35 gene in Parkinson disease indicates reduced penetrance for disease-associated variants (vol 49, pg 721, 2012), *J Med Genet* 50(3) (2013) pp. 721-726.
- [17] S. Fujioka, K. Ogaki, P.M. Tacik, R.J. Uitti, O.A. Ross, Z.K. Wszolek, Update on novel familial forms of Parkinson's disease and multiple system atrophy, *Parkinsonism Relat Disord* 20 Suppl 1 (2014) pp. S29-34.
- [18] M. Ando, M. Funayama, Y. Li, K. Kashihara, Y. Murakami, N. Ishizu, C. Toyoda, K. Noguchi, T. Hashimoto, N. Nakano, R. Sasaki, Y. Kokubo, S. Kuzuhara, K. Ogaki, C. Yamashita, H. Yoshino, T. Hatano, H. Tomiyama, N. Hattori, VPS35 mutation in Japanese patients with typical Parkinson's disease, *Mov Disord* 27(11) (2012) pp. 1413-1417.
- [19] W. Struhal, S. Presslauer, S. Spielberger, A. Zimprich, E. Auff, T. Bruecke, W. Poewe, G. Ransmayr, V.P.S.I.T. Austrian, VPS35 Parkinson's disease phenotype resembles the sporadic disease, *J Neural Transm* 121(7) (2014) pp. 755-759.
- [20] A. Zimprich, A. Benet-Pages, W. Struhal, E. Graf, S.H. Eck, M.N. Offman, D. Haubenberger, S. Spielberger, E.C. Schulte, P. Lichtner, S.C. Rossle, N. Klopp, E. Wolf, K. Seppi, W. Pirker, S. Presslauer, B. Mollenhauer, R. Katzenschlager, T. Foki, C. Hotzy, E. Reinthaler, A. Harutyunyan, R. Kralovics, A. Peters, F. Zimprich, T. Brucke, W. Poewe, E. Auff, C. Trenkwalder, B. Rost, G. Ransmayr, J. Winkelmann, T. Meitinger, T.M. Strom, A Mutation in VPS35, Encoding a Subunit of the Retromer Complex, Causes Late-Onset Parkinson Disease, *Am J Hum Genet* 89(1) (2011) pp. 168-175.
- [21] C. Vilarino-Guell, C. Wider, O.A. Ross, J.C. Dachselt, J.M. Kachergus, Sarah J. Lincoln, A.I. Soto-Ortolaza, S.A. Cobb, G.J. Wilhoite, J.A. Bacon, B. Behrouz, H.L. Melrose, E. Hentati, G.J. Wilhoite, J.A. Bacon, B. Behrouz, H.L. Melrose, E. Hentati, A. Puschmann, D.M. Evans, E. Conibear, W.W. Wasserman, J.O. Aasly, P.R. Burkhard, R. Djaldetti, J. Ghika, F. Hentati, A. Krygowska-Wajs, T. Lynch, E. Melamed, A. Rajput, A.H. Rajput, A. Solida, R.-M. Wu, R.J. Uitti, Zbigniew K. Wszolek, F.o. Vingerhoets, M.J. Farrer, VPS35 Mutations in Parkinson Disease, *Am J Hum Genet* 89 (2011) pp. 162-167.
- [22] V. Fleury, C. Wider, J. Horvath, A. Zacharia, J. Bally, P. Pollak, C. Pollo, F.J. Vingerhoets, P.R. Burkhard, Successful long-term bilateral subthalamic nucleus deep brain stimulation in VPS35 Parkinson's disease, *Parkinsonism Relat Disord* 19(7) (2013) pp. 707-708.
- [23] M.N. Seaman, J.M. McCaffery, S.D. Emr, A membrane coat complex essential for endosome-to-Golgi retrograde transport in yeast, *J Cell Biol* 142(3) (1998) pp. 665-681.
- [24] A. Bugarcic, Y. Zhe, M.C. Kerr, J. Griffin, B.M. Collins, R.D. Teasdale, Vps26A and Vps26B subunits define distinct retromer complexes, *Traffic* 12(12) (2011) pp. 1759-1773.

- [25] R. Rojas, T. van Vlijmen, G.A. Mardones, Y. Prabhu, A.L. Rojas, S. Mohammed, A.J.R. Heck, G. Raposo, P. van der Sluijs, J.S. Bonifacino, Regulation of retromer recruitment to endosomes by sequential action of Rab5 and Rab7, *Journal of Cell Biology* 183(3) (2008) pp. 513-526.
- [26] M. Harterink, F. Port, M.J. Lorenowicz, I.J. McGough, M. Silhankova, M.C. Betist, J.R. van Weering, R.G. van Heesbeen, T.C. Middelkoop, K. Basler, P.J. Cullen, H.C. Korswagen, A SNX3-dependent retromer pathway mediates retrograde transport of the Wnt sorting receptor Wntless and is required for Wnt secretion, *Nat Cell Biol* 13(8) (2011) pp. 914-923.
- [27] M.N. Seaman, Cargo-selective endosomal sorting for retrieval to the Golgi requires retromer, *J Cell Biol* 165(1) (2004) pp. 111-122.
- [28] C.N. Arighi, L.M. Hartnell, R.C. Aguilar, C.R. Haft, J.S. Bonifacino, Role of the mammalian retromer in sorting of the cation-independent mannose 6-phosphate receptor, *J Cell Biol* 165(1) (2004) pp. 123-133.
- [29] P.J. Cullen, H.C. Korswagen, Sorting nexins provide diversity for retromer-dependent trafficking events, *Nat Cell Biol* 14(1) (2012) pp. 29-37.
- [30] S.A. Small, K. Kent, A. Pierce, C. Leung, M.S. Kang, H. Okada, L. Honig, J.P. Vonsattel, T.W. Kim, Model-guided microarray implicates the retromer complex in Alzheimer's disease, *Ann Neurol* 58(6) (2005) pp. 909-919.
- [31] E. Rogaeva, Y. Meng, J.H. Lee, Y. Gu, T. Kawarai, F. Zou, T. Katayama, C.T. Baldwin, R. Cheng, H. Hasegawa, F. Chen, N. Shibata, K.L. Lunetta, R. Pardossi-Piquard, C. Bohm, Y. Wakutani, L.A. Cupples, K.T. Cuenco, R.C. Green, L. Pinessi, I. Rainero, S. Sorbi, A. Bruni, R. Duara, R.P. Friedland, R. Inzelberg, W. Hampe, H. Bujo, Y.Q. Song, O.M. Andersen, T.E. Willnow, N. Graff-Radford, R.C. Petersen, D. Dickson, S.D. Der, P.E. Fraser, G. Schmitt-Ulms, S. Younkin, R. Mayeux, L.A. Farrer, P. St George-Hyslop, The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease, *Nat Genet* 39(2) (2007) pp. 168-177.
- [32] S.I. Vieira, S. Rebelo, H. Esselmann, J. Wiltfang, J. Lah, R. Lane, S.A. Small, S. Gandy, E.S.E.F. da Cruz, E.S.O.A. da Cruz, Retrieval of the Alzheimer's amyloid precursor protein from the endosome to the TGN is S655 phosphorylation state-dependent and retromer-mediated, *Mol Neurodegener* 5 (2010) pp. 40.
- [33] A. Bhalla, C.P. Vetanovetz, E. Morel, Z. Chamoun, G. Di Paolo, S.A. Small, The location and trafficking routes of the neuronal retromer and its role in amyloid precursor protein transport, *Neurobiol Dis* 47(1) (2012) pp. 126-134.
- [34] A.W. Fjorback, M. Seaman, C. Gustafsen, A. Mehmedbasic, S. Gokool, C. Wu, D. Militz, V. Schmidt, P. Madsen, J.R. Nyengaard, T.E. Willnow, E.I. Christensen, W.B. Mobley, A. Nykjaer, O.M. Andersen, Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing, *J Neurosci* 32(4) (2012) pp. 1467-1480.
- [35] A. Muhammad, I. Flores, H. Zhang, R. Yu, A. Staniszewski, E. Planel, M. Herman, L. Ho, R. Kreber, L.S. Honig, B. Ganetzky, K. Duff, O. Arancio, S.A. Small, Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration and Abeta accumulation, *Proc Natl Acad Sci U S A* 105(20) (2008) pp. 7327-7332.
- [36] H. Fukumoto, B.S. Cheung, B.T. Hyman, M.C. Irizarry, Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease, *Arch Neurol* 59(9) (2002) pp. 1381-1389.
- [37] L. Wen, F.L. Tang, Y. Hong, S.W. Luo, C.L. Wang, W. He, C. Shen, J.U. Jung, F. Xiong, D.H. Lee, Q.G. Zhang, D. Brann, T.W. Kim, R. Yan, L. Mei, W.C. Xiong, VPS35 haploinsufficiency increases Alzheimer's disease neuropathology, *J Cell Biol* 195(5) (2011) pp. 765-779.
- [38] C.-L. Wang, F.-L. Tang, Y. Peng, C.-Y. Shen, L. Mei, W.-C. Xiong, VPS35 regulates developing mouse hippocampal neuronal morphogenesis by promoting retrograde trafficking of BACE1, *Biol Open* 0 (2012) pp. 1-10.
- [39] B.N. Vardarajan, S.Y. Bruesegem, M.E. Harbour, R. Inzelberg, R. Friedland, P. St George-Hyslop, M.N. Seaman, L.A. Farrer, Identification of Alzheimer disease-associated variants in genes that regulate retromer function, *Neurobiol Aging* 33(9) (2012) pp. 2231 e2215-2231 e2230.
- [40] S.A. Small, G.A. Petsko, Retromer in Alzheimer disease, Parkinson disease and other neurological disorders, *Nat Rev Neurosci* 16(3) (2015) pp. 126-132.

- [41] M. Tabuchi, I. Yanatori, Y. Kawai, F. Kishi, Retromer-mediated direct sorting is required for proper endosomal recycling of the mammalian iron transporter DMT1., *J Cell Sci* (2010) pp. 756-766.
- [42] E. Braschi, V. Goyon, R. Zunino, A. Mohanty, L. Xu, H.M. McBride, Vps35 Mediates Vesicle Transport between the Mitochondria and Peroxisomes, *Curr Biol* 20 (2010) pp. 1310-1315.
- [43] E. Zavodszky, M.N. Seaman, D.C. Rubinsztein, VPS35 Parkinson mutation impairs autophagy via WASH, *Cell Cycle* 13(14) (2014) pp. 2155-2156.
- [44] P. Benes, V. Vetvicka, M. Fusek, Cathepsin D--many functions of one aspartic protease, *Crit Rev Oncol Hematol* 68(1) (2008) pp. 12-28.
- [45] F. Port, M. Kuster, P. Herr, E. Furger, C. Banziger, G. Hausmann, K. Basler, Wingless secretion promotes and requires retromer-dependent cycling of Wntless, *Nat Cell Biol* 10(2) (2008) pp. 178-185.
- [46] X. Franch-Marro, F. Wendler, S. Guidato, J. Griffith, A. Baena-Lopez, N. Itasaki, M.M. Maurice, J.P. Vincent, Wingless secretion requires endosome-to-Golgi retrieval of Wntless/Evi/Sprinter by the retromer complex, *Nat Cell Biol* 10(2) (2008) pp. 170-177.
- [47] P. Zhang, Y. Wu, T.Y. Belenkaya, X. Lin, SNX3 controls Wingless/Wnt secretion through regulating retromer-dependent recycling of Wntless, *Cell Res* 21(12) (2011) pp. 1677-1690.
- [48] R.E. de Groot, H.F. Farin, M. Macurkova, J.H. van Es, H.C. Clevers, H.C. Korswagen, Retromer dependent recycling of the Wnt secretion factor Wls is dispensable for stem cell maintenance in the mammalian intestinal epithelium, *PLoS One* 8(10) (2013) pp. e76971.
- [49] M. Verges, F. Luton, C. Gruber, F. Tiemann, L.G. Reinders, L. Huang, A.L. Burlingame, C.R. Haft, K.E. Mostov, The mammalian retromer regulates transcytosis of the polymeric immunoglobulin receptor, *Nat Cell Biol* 6(8) (2004) pp. 763-769.
- [50] M. Mellado, Y. Cuartero, R. Brugada, M. Verges, Subcellular localisation of retromer in post-endocytic pathways of polarised Madin-Darby canine kidney cells, *Biol Cell* 106(11) (2014) pp. 377-393.
- [51] B. Zhou, Y. Wu, X. Lin, Retromer regulates apical-basal polarity through recycling Crumbs, *Dev Biol* 360(1) (2011) pp. 87-95.
- [52] S.M. Pocha, T. Wassmer, C. Niehage, B. Hoflack, E. Knust, Retromer controls epithelial cell polarity by trafficking the apical determinant Crumbs, *Curr Biol* 21(13) (2011) pp. 1111-1117.
- [53] B. Dong, K. Kakiyama, T. Otani, H. Wada, S. Hayashi, Rab9 and retromer regulate retrograde trafficking of luminal protein required for epithelial tube length control, *Nat Commun* 4 (2013) pp. 1358.
- [54] E. Groppelli, A.C. Len, L.A. Granger, C. Jolly, Retromer regulates HIV-1 envelope glycoprotein trafficking and incorporation into virions, *PLoS Pathog* 10(10) (2014) pp. e1004518.
- [55] A. Lipovsky, A. Popa, G. Pimienta, M. Wyler, A. Bhan, L. Kuruvilla, M.A. Guie, A.C. Poffenberger, C.D. Nelson, W.J. Atwood, D. DiMaio, Genome-wide siRNA screen identifies the retromer as a cellular entry factor for human papillomavirus, *Proc Natl Acad Sci U S A* 110(18) (2013) pp. 7452-7457.
- [56] A. Popa, W. Zhang, M.S. Harrison, K. Goodner, T. Kazakov, E.C. Goodwin, A. Lipovsky, C.G. Burd, D. DiMaio, Direct binding of retromer to human papillomavirus type 16 minor capsid protein L2 mediates endosome exit during viral infection, *PLoS Pathog* 11(2) (2015) pp. e1004699.
- [57] D. Kingston, H. Chang, A. Ensser, H.R. Lee, J. Lee, S.H. Lee, J.U. Jung, N.H. Cho, Inhibition of retromer activity by herpesvirus saimiri tip leads to CD4 downregulation and efficient T cell transformation, *J Virol* 85(20) (2011) pp. 10627-10638.
- [58] M.V. Bujny, V. Popoff, L. Johannes, P.J. Cullen, The retromer component sorting nexin-1 is required for efficient retrograde transport of Shiga toxin from early endosome to the trans Golgi network, *J Cell Sci* 120(Pt 12) (2007) pp. 2010-2021.
- [59] W.F. Xia, F.L. Tang, L. Xiong, S. Xiong, J.U. Jung, D.H. Lee, X.S. Li, X. Feng, L. Mei, W.C. Xiong, Vps35 loss promotes hyperresorptive osteoclastogenesis and osteoporosis via sustained RANKL signaling, *J Cell Biol* 200(6) (2013) pp. 821-837.
- [60] C.H. An, Y.R. Kim, H.S. Kim, S.S. Kim, N.J. Yoo, S.H. Lee, Frameshift mutations of vacuolar protein sorting genes in gastric and colorectal cancers with microsatellite instability, *Hum Pathol* 43(1) (2012) pp. 40-47.

- [61] C. Reitz, The role of the retromer complex in aging-related neurodegeneration: a molecular and genomic review, *Mol Genet Genomics* 290(2) (2015) pp. 413-427.
- [62] M.V. Morabito, D.E. Berman, R.T. Schneider, Y. Zhang, R.L. Leibel, S.A. Small, Hyperleucinemia causes hippocampal retromer deficiency linking diabetes to Alzheimer's disease, *Neurobiol Dis* 65 (2014) pp. 188-192.
- [63] W. Liu, F.L. Tang, J. Erion, H. Xiao, J. Ye, W.C. Xiong, Vps35 haploinsufficiency results in degenerative-like deficit in mouse retinal ganglion neurons and impairment of optic nerve injury-induced gliosis, *Mol Brain* 7 (2014) pp. 10.
- [64] E. Tsika, L. Glauser, R. Moser, A. Fiser, G. Daniel, U.-M. Sheerin, A. Lees, J.C. Troncoso, P.A. Lewis, R. Bandopadhyay, B.L. Schneider, D.J. Moore, Parkinson's disease-linked mutations in VPS35 induce dopaminergic neurodegeneration, *Hum Mol Genet* 23(17) (2014) pp. 4621-4638.
- [65] H.S. Wang, J. Toh, P. Ho, M. Tio, Y. Zhao, E.K. Tan, In vivo evidence of pathogenicity of VPS35 mutations in the *Drosophila*, *Mol Brain* 7(1) (2014) pp. 73.
- [66] F.L. Tang, J.R. Erion, Y. Tian, W. Liu, D.M. Yin, J. Ye, B. Tang, L. Mei, W.C. Xiong, VPS35 in Dopamine Neurons Is Required for Endosome-to-Golgi Retrieval of Lamp2a, a Receptor of Chaperone-Mediated Autophagy That Is Critical for alpha-Synuclein Degradation and Prevention of Pathogenesis of Parkinson's Disease, *J Neurosci* 35(29) (2015) pp. 10613-10628.
- [67] B.R. Malik, V.K. Godena, A.J. Whitworth, VPS35 pathogenic mutations confer no dominant toxicity but partial loss of function in *Drosophila* and genetically interact with parkin, *Hum Mol Genet* 24(21) (2015) pp. 6106-6117.
- [68] E. Zavodszky, M.N. Seaman, K. Moreau, M. Jimenez-Sanchez, S.Y. Breusegem, M.E. Harbour, D.C. Rubinsztein, Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy, *Nat Commun* 5 (2014) pp. 3828.
- [69] J. Follett, S.J. Norwood, N.A. Hamilton, M. Mohan, O. Kovtun, S. Tay, Y. Zhe, S.A. Wood, G.D. Mellick, P.A. Silburn, B.M. Collins, A. Bugarcic, R.D. Teasdale, The Vps35 D620N Mutation Linked to Parkinson's Disease Disrupts the Cargo Sorting Function of Retromer, *Traffic* 15(2) (2014) pp. 230-244.
- [70] E. Miura, T. Hasegawa, M. Konno, M. Suzuki, N. Sugeno, N. Fujikake, S. Geisler, M. Tabuchi, R. Oshima, A. Kikuchi, T. Baba, K. Wada, Y. Nagai, A. Takeda, M. Aoki, VPS35 dysfunction impairs lysosomal degradation of alpha-synuclein and exacerbates neurotoxicity in a *Drosophila* model of Parkinson's disease, *Neurobiol Dis* 71 (2014) pp. 1-13.
- [71] I.J. McGough, F. Steinberg, D. Jia, P.A. Barbuti, K.J. McMillan, K.J. Heesom, A.L. Whone, M.A. Caldwell, D.D. Billadeau, M.K. Rosen, P.J. Cullen, Retromer binding to FAM21 and the WASH complex is perturbed by the Parkinson disease-linked VPS35(D620N) mutation, *Curr Biol* 24(14) (2014) pp. 1670-1676.
- [72] D.A. MacLeod, H. Rhinn, T. Kuwahara, A. Zolin, G. Di Paolo, B.D. MacCabe, K.S. Marder, L.S. Honig, L.N. Clark, S.A. Small, A. Abeliovich, RAB7L1 Interacts with LRRK2 to Modify Intraneuronal Protein Sorting and Parkinson's Disease Risk, *Neuron* 77(3) (2013) pp. 425-439.
- [73] J.L. Webb, B. Ravikumar, J. Atkins, J.N. Skepper, D.C. Rubinsztein, alpha-synuclein is degraded by both autophagy and the proteasome, *Journal of Biological Chemistry* 278(27) (2003) pp. 25009-25013.
- [74] L. Qiao, S. Hamamichi, K.A. Caldwell, G.A. Caldwell, T.A. Yacoubian, S. Wilson, Z.L. Xie, L.D. Speake, R. Parks, D. Crabtree, Q. Liang, S. Crimmins, L. Schneider, Y. Uchiyama, T. Iwatsubo, Y. Zhou, L. Peng, Y. Lu, D.G. Standaert, K.C. Walls, J.J. Shacka, K.A. Roth, J. Zhang, Lysosomal enzyme cathepsin D protects against alpha-synuclein aggregation and toxicity, *Mol Brain* 1 (2008) pp. 17.
- [75] D. Ebrahimi-Fakhari, I. Cantuti-Castelvetri, Z. Fan, E. Rockenstein, E. Masliah, B.T. Hyman, P.J. McLean, V.K. Unni, Distinct roles in vivo for the ubiquitin-proteasome system and the autophagy-lysosomal pathway in the degradation of alpha-synuclein, *J Neurosci* 31(41) (2011) pp. 14508-14520.
- [76] D. Crabtree, M. Dodson, X. Ouyang, M. Boyer-Guittaut, Q. Liang, M.E. Ballestas, N. Fineberg, J. Zhang, Over-expression of an inactive mutant cathepsin D increases endogenous alpha-synuclein and cathepsin B activity in SH-SY5Y cells, *J Neurochem* 128(6) (2014) pp. 950-961.

- [77] V. Cullen, M. Lindfors, J. Ng, A. Paetau, E. Swinton, P. Kolodziej, H. Boston, P. Saftig, J. Woulfe, M.B. Feany, L. Myllykangas, M.G. Schlossmacher, J. Tynnela, Cathepsin D expression level affects alpha-synuclein processing, aggregation, and toxicity in vivo, *Mol Brain* 2 (2009) pp. 5.
- [78] D. Sevlever, P.Z. Jiang, S.H.C. Yen, Cathepsin D is the main lysosomal enzyme involved in the degradation of alpha-synuclein and generation of its carboxy-terminally truncated species, *Biochemistry* 47(36) (2008) pp. 9678-9687.
- [79] N. Dhungel, S. Eleuteri, L.B. Li, N.J. Kramer, J.W. Chartron, B. Spencer, K. Kosberg, J.A. Fields, K. Stafa, A. Adame, H. Lashuel, J. Frydman, K. Shen, E. Masliah, A.D. Gitler, Parkinson's Disease Genes VPS35 and EIF4G1 Interact Genetically and Converge on alpha-Synuclein, *Neuron* 85(1) (2015) pp. 76-87.
- [80] F. Steinberg, M. Gallon, M. Winfield, E.C. Thomas, A.J. Bell, K.J. Heesom, J.M. Tavaré, P.J. Cullen, A global analysis of SNX27-retromer assembly and cargo specificity reveals a function in glucose and metal ion transport, *Nat Cell Biol* 15(5) (2013) pp. 461-471.
- [81] C. Vilarino-Guell, A. Rajput, A.J. Milnerwood, B. Shah, C. Szu-Tu, J. Trinh, I. Yu, M. Encarnacion, L.N. Munsie, L. Tapia, E.K. Gustavsson, P. Chou, I. Tatarnikov, D.M. Evans, F.T. Pishotta, M. Volta, D. Beccano-Kelly, C. Thompson, M.K. Lin, H.E. Sherman, H.J. Han, B.L. Guenther, W.W. Wasserman, V. Bernard, C.J. Ross, S. Appel-Cresswell, A.J. Stoessl, C.A. Robinson, D.W. Dickson, O.A. Ross, Z.K. Wszolek, J.O. Aasly, R.M. Wu, F. Hentati, R.A. Gibson, P.S. McPherson, M. Girard, M. Rajput, A.H. Rajput, M.J. Farrer, DNAJC13 mutations in Parkinson disease, *Hum Mol Genet* 23(7) (2014) pp. 1794-1801.
- [82] C.L. Freeman, G. Hesketh, M.N. Seaman, RME-8 coordinates the activity of the WASH complex with the function of the retromer SNX dimer to control endosomal tubulation, *J Cell Sci* 127(Pt 9) (2014) pp. 2053-2070.
- [83] H.X. Deng, Y. Shi, Y. Yang, K.B. Ahmeti, N. Miller, C. Huang, L. Cheng, H. Zhai, S. Deng, K. Nuytemans, N.J. Corbett, M.J. Kim, H. Deng, B. Tang, Z. Yang, Y. Xu, P. Chan, B. Huang, X.P. Gao, Z. Song, Z. Liu, F. Fecto, N. Siddique, T. Foroud, J. Jankovic, B. Ghetti, D.A. Nicholson, D. Krainc, O. Melen, J.M. Vance, M.A. Pericak-Vance, Y.C. Ma, A.H. Rajput, T. Siddique, Identification of TMEM230 mutations in familial Parkinson's disease, *Nat Genet* 48(7) (2016) pp. 733-739.
- [84] A.B. Knott, G. Perkins, R. Schwarzenbacher, E. Bossy-Wetzel, Mitochondrial fragmentation in neurodegeneration, *Nat Rev Neurosci* 9(7) (2008) pp. 505-518.
- [85] K.F. Winklhofer, C. Haass, Mitochondrial dysfunction in Parkinson's disease, *Biochim Biophys Acta* 1802(1) (2010) pp. 29-44.
- [86] N. Exner, A.K. Lutz, C. Haass, K.F. Winklhofer, Mitochondrial dysfunction in Parkinson's disease: molecular mechanisms and pathophysiological consequences, *EMBO J* 31(14) (2012) pp. 3038-3062.
- [87] W. Wang, X. Wang, H. Fujioka, C. Hoppel, A.L. Whone, M.A. Caldwell, P.J. Cullen, J. Liu, X. Zhu, Parkinson's disease-associated mutant VPS35 causes mitochondrial dysfunction by recycling DLP1 complexes, *Nat Med* 22(1) (2016) pp. 54-63.
- [88] F.L. Tang, W. Liu, J.X. Hu, J.R. Erion, J. Ye, L. Mei, W.C. Xiong, VPS35 Deficiency or Mutation Causes Dopaminergic Neuronal Loss by Impairing Mitochondrial Fusion and Function, *Cell Rep* 12(10) (2015) pp. 1631-1643.
- [89] F. Bi, F. Li, C. Huang, H. Zhou, Pathogenic mutation in VPS35 impairs its protection against MPP(+) cytotoxicity, *Int J Biol Sci* 9(2) (2013) pp. 149-155.
- [90] P.Y. Pan, Z. Yue, Genetic causes of Parkinson's disease and their links to autophagy regulation, *Parkinsonism Relat Disord* 20 Suppl 1 (2014) pp. S154-157.
- [91] K.M. Lucin, C.E. O'Brien, G. Bieri, E. Czirr, K.I. Mosher, R.J. Abbey, D.F. Mastroeni, J. Rogers, B. Spencer, E. Masliah, T. Wyss-Coray, Microglial beclin 1 regulates retromer trafficking and phagocytosis and is impaired in Alzheimer's disease, *Neuron* 79(5) (2013) pp. 873-886.
- [92] Y. Tian, F.L. Tang, X. Sun, L. Wen, L. Mei, B.S. Tang, W.C. Xiong, VPS35-deficiency results in an impaired AMPA receptor trafficking and decreased dendritic spine maturation, *Mol Brain* 8(1) (2015) pp. 70.
- [93] R. Linhart, S.A. Wong, J. Cao, M. Tran, A. Huynh, C. Ardrey, J.M. Park, C. Hsu, S. Taha, R. Peterson, S. Shea, J. Kurian, K. Venderova, Vacuolar protein sorting 35 (Vps35) rescues locomotor

- deficits and shortened lifespan in *Drosophila* expressing a Parkinson's disease mutant of Leucine-Rich Repeat Kinase 2 (LRRK2), *Mol Neurodegener* 9 (2014) pp. 23.
- [94] D.A. MacLeod, H. Rhinn, T. Kuwahara, A. Zolin, G. Di Paolo, B.D. McCabe, K.S. Marder, L.S. Honig, L.N. Clark, S.A. Small, A. Abeliovich, RAB7L1 interacts with LRRK2 to modify intraneuronal protein sorting and Parkinson's disease risk, *Neuron* 77(3) (2013) pp. 425-439.
- [95] R.W. Choy, M. Park, P. Temkin, B.E. Herring, A. Marley, R.A. Nicoll, M. von Zastrow, Retromer mediates a discrete route of local membrane delivery to dendrites, *Neuron* 82(1) (2014) pp. 55-62.
- [96] L.N. Munsie, A.J. Milnerwood, P. Seibler, D.A. Beccano-Kelly, I. Tatarnikov, J. Khinda, M. Volta, C. Kadgien, L.P. Cao, L. Tapia, C. Klein, M.J. Farrer, Retromer-dependent neurotransmitter receptor trafficking to synapses is altered by the Parkinson's Disease VPS35 mutation p.D620N, *Hum Mol Genet* (2014).
- [97] V. Bonifati, Genetics of Parkinson's disease--state of the art, 2013, *Parkinsonism Relat Disord* 20 Suppl 1 (2014) pp. S23-28.
- [98] M. Funayama, K. Ohe, T. Amo, N. Furuya, J. Yamaguchi, S. Saiki, Y. Li, K. Ogaki, M. Ando, H. Yoshino, H. Tomiyama, K. Nishioka, K. Hasegawa, H. Saiki, W. Satake, K. Mogushi, R. Sasaki, Y. Kokubo, S. Kuzuhara, T. Toda, Y. Mizuno, Y. Uchiyama, K. Ohno, N. Hattori, CHCHD2 mutations in autosomal dominant late-onset Parkinson's disease: a genome-wide linkage and sequencing study, *Lancet Neurol* 14(3) (2015) pp. 274-282.
- [99] S. Lesage, V. Drouet, E. Majounie, V. Deramecourt, M. Jacoupy, A. Nicolas, F. Cormier-Dequaire, S.M. Hassoun, C. Pujol, S. Ciura, Z. Erpapazoglou, T. Usenko, C.A. Maurage, M. Sahbatou, S. Liebau, J. Ding, B. Bilgic, M. Emre, N. Erginel-Unaltuna, G. Guven, F. Tison, C. Tranchant, M. Vidailhet, J.C. Corvol, P. Krack, A.L. Leutenegger, M.A. Nalls, D.G. Hernandez, P. Heutink, J.R. Gibbs, J. Hardy, N.W. Wood, T. Gasser, A. Durr, J.F. Deleuze, M. Tazir, A. Destee, E. Lohmann, E. Kabashi, A. Singleton, O. Corti, A. Brice, S. French Parkinson's Disease Genetics, C. International Parkinson's Disease Genomics, Loss of VPS13C Function in Autosomal-Recessive Parkinsonism Causes Mitochondrial Dysfunction and Increases PINK1/Parkin-Dependent Mitophagy, *Am J Hum Genet* 98(3) (2016) pp. 500-513.
- [100] M.C. Chartier-Harlin, J.C. Dachsel, C. Vilarino-Guell, S.J. Lincoln, F. Lepretre, M.M. Hulihan, J. Kachergus, A.J. Milnerwood, L. Tapia, M.S. Song, E. Le Rhun, E. Mutez, L. Larvor, A. Duflot, C. Vanbesien-Mailliot, A. Kreisler, O.A. Ross, K. Nishioka, A.I. Soto-Ortolaza, S.A. Cobb, H.L. Melrose, B. Behrouz, B.H. Keeling, J.A. Bacon, E. Hentati, L. Williams, A. Yanagiya, N. Sonenberg, P.J. Lockhart, A.C. Zubair, R.J. Uitti, J.O. Aasly, A. Krygowska-Wajs, G. Opala, Z.K. Wszolek, R. Frigerio, D.M. Maraganore, D. Gosal, T. Lynch, M. Hutchinson, A.R. Bentivoglio, E.M. Valente, W.C. Nichols, N. Pankratz, T. Foroud, R.A. Gibson, F. Hentati, D.W. Dickson, A. Destee, M.J. Farrer, Translation initiator EIF4G1 mutations in familial Parkinson disease, *Am J Hum Genet* 89(3) (2011) pp. 398-406.
- [101] V.J. Mecozzi, D.E. Berman, S. Simoes, C. Vetanovetz, M.R. Awal, V.M. Patel, R.T. Schneider, G.A. Petsko, D. Ringe, S.A. Small, Pharmacological chaperones stabilize retromer to limit APP processing, *Nat Chem Biol* 10(6) (2014) pp. 443-449.
- [102] D.E. Berman, D. Ringe, G.A. Petsko, S.A. Small, The Use of Pharmacological Retromer Chaperones in Alzheimer's Disease and other Endosomal-related Disorders, *Neurotherapeutics* 12(1) (2015) pp. 12-18.