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Experimental Neurobiology

Review Article

ATP13A2 and Alpha-synuclein: a Metal Taste in Autophagy

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Parkinson's Disease (PD) is a complex and multifactorial disorder of both idiopathic and genetic origin. Thus far, more than 20 genes have been linked to familial forms of PD. Two of these genes encode for ATP13A2 and alpha-synuclein (asyn), proteins that seem to be members of a common network in both physiological and disease conditions. Thus, two different hypotheses have emerged supporting a role of ATP13A2 and asyn in metal homeostasis or in autophagy. Interestingly, an appealing theory might combine these two cellular pathways. Here we review the novel findings in the interaction between these two proteins and debate the exciting roads still ahead.

Key words: ATP13A2, alpha-synuclein, Parkinson's Disease, autophagy, metal homeostasis

PARKINSON'S DISEASE

PD is the second most common neurodegenerative disorder, after Alzheimer's disease, and is classically characterized by symptoms such as resting tremor, postural instability, bradykinesia and muscular rigidity [1]. Pathologically, PD is defined by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, and the presence of intracellular inclusions, known as Lewy Bodies, in surviving neurons [1]. Despite its initial classification as a motor disorder, PD is currently perceived as a whole-brain pathology since it affects multiple brain areas and presents a broad variety of symptoms in addition to the typical motor symptoms mentioned above. According to Braak's staging hypothesis, the pathological process is thought to initiate in either the lower brainstem or in the olfactory bulb. Then, as the disease evolves, Lewy body pathology occurs in other brain regions and circuits, including the cerebral cortex, leading to non-motor symptoms such as depression, cognitive decline and hallucination episodes [2-5]. The vast majority of PD cases are sporadic and only approximately 5-10% have been linked to genetic factors. Thus far, more than 20 genes have been associated with PD, and this number tends to increase as novel and more powerful studies are conducted [6]. The proteins encoded by these genes play a wide range of cellular roles but the precise functions of some of them are still not fully understood.

Understanding the interplay between different PD genes and, consequently, unravelling the molecular mechanisms underlying PD will bring new hope for the development of novel diagnostic and therapeutic tools.

ALPHA-SYNUCLEIN

Asyn is, by far, the most extensively studied protein in the

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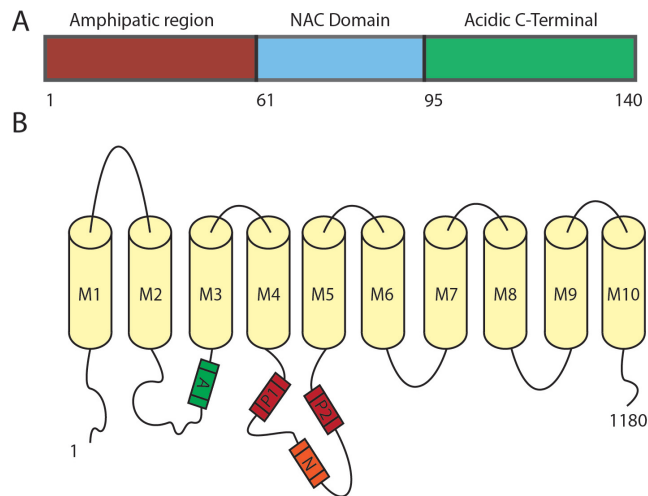
Fig. 1. Schematic representation of asyn and ATP13A2. (A) Asyn can be devided in three domains according to its amino acid composition: an N--terminal amphipathic domain where all 6 PD--associated point--mutations are located; a central region known as non--amyloid component (NAC) domain, responsible for the amyloidogenic properties of the protein; and the C--terminal region which is highly acidic. (B) ATP13A2 is an 1180 aa protein with 10 transmembrane domains and four functional domains: catalytic phosphorylation (P1), nucleotide binding (P2 and N) and actuator domain (A).

context of PD. Nevertheless, our understanding of its physiological function is still not entirely established. Three functional domains can be defined in the primary sequence of asyn (Fig. 1A). While the protein was initially thought to be natively unfolded and monomeric, recent studies proposed it may also adopt a tetrameric structure [7, 8]. Although these studies are controversial, they brought new perspectives into the process of asyn misfolding and aggregation, thought to be central in the context of PD [6]. In fact, aggregated asyn is the main component of Lewy Bodies [9]. Thus far, 6 point mutations have been associated with familial cases of PD. Interestingly, all 6 mutations are located in the N-terminal region of the protein [10-15]. In addition, multiplications of the SNCA gene, encoding for asyn, have also been associated with familial forms of PD [16, 17]. Furthermore, polymorphisms in the SNCA gene are known to increase the risk of PD [18-21].

The normal cellular function of asyn is still unclear, but distinct roles have been proposed, ranging from transcriptional regulation [22-27], mitochondrial homeostasis [28], vesicle trafficking [29], and neurotransmitter release [30-33]. Despite all these putative functions, mice lacking asyn reveal only minor physiological alterations [34] suggesting the existence of compensatory cellular mechanisms that can overcome the absence of the protein.

ATP13A2

ATP13A2 is a transmembrane protein of 1180 amino acids (aa) localized in the lysosomes and late endosomes [35] that belongs to a family with 5 members (ATP13A1-5) - the P5 type pump ATPases. ATP13A2 has 10 predicted transmembrane domains and three functional domains (Fig. 1B) [36]. The protein is highly expressed in the brain, especially in the *substantia nigra pars compacta* and is upregulated in the dopaminergic neurons of this region in the brains of PD patients [35, 37]. Mutations in ATP13A2 have been associated with different diseases including Kufor-Rakeb syndrome, PD [35, 38-46], and Neuroid Ceroid Lipofuscinosis (NCL) [47-49]. Interestingly, ATP13A2 knockout mice display characteristics of both NCL and PD, such as hippocampal accumulation of asyn, sensorimotor deficits and lipofuscinosis [50], suggesting that the phenotypes in these may not be solely gene dependent.

Mitochondrial impairment was observed in fibroblasts from patients carrying ATP13A2 mutations. This impairment was associated with reduced ATP production and increased maximum respiration capacity, due to an impairment of mitochondrial degradation that resulted in their accumulation. These phenotypes could be partially rescued upon ATP13A2 overexpression [51]. The process of autophagic mitochondria degradation, known as mitophagy, is a crucial quality control mechanism to ensure the proper function of the organelle [52] and has been associated to PD [53]. Interestingly, ATP13A2 has been directly linked to mitophagy in several studies [51, 54, 55] but, so far, little is known about the mechanisms involved. In addition to a role in mitophagy, ATP13A2 has been connected with protein autophagy [50, 56, 57] and metal/cation homeostasis [37, 55, 57-66]. These are also central cellular processes that have been associated with asyn biology, as discussed below.

Of the several disease-associated mutations identified in ATP13A2, only a few were investigated in detail thus far. In cells, ATP13A2 mutants exhibited loss of protein function, subcellular mislocalization in the endoplasmatic reticulum, increased cellular toxicity, and shorter protein half-life [67]. In a comprehensive study of the effects of ATP13A2 missense mutations associated with early-onset parkinsonism, several novel phenotypes were identified, including disruption of the protein vesicular localization, impairment of ATPase activity and of neurite outgrowth [68].

THE INTERPLAY BETWEEN ATP13A2 AND ALPHA-SYNUCLEIN

Most of the existing knowledge about the function of ATP13A2

has arisen from studies focusing on the interaction between this protein and asyn. Thus, in the sections below, we focus on our current understanding of the interplay between these two proteins in order to provide a framework for the challenges that lie ahead in this area of research.

METAL HOMEOSTASIS

Despite the lysosomal localization of ATP13A2, the first connection to asyn was not due to a putative role in protein degradation, but rather due to a role in metal homeostasis. ATP13A2 was shown to exert a protective effect in manganese (Mn^+)-mediated asyn toxicity, in both yeast and SH-SY5Y cells [60]. This connection with Mn^+ homeostasis was further explored in yeast and resulted in the identification of several genes being part of the network [62]. In addition, some ATP13A2 mutants were unable to rescue Mn^+ induced toxicity in mammalian cell culture [64]. Interestingly, two ATP13A2 polymorphisms enhance Mn^+ neurotoxic effect in patients [63].

Since Mn^+ has long been associated with asyn oligomerization and aggregation [69, 70], and with Parkinsonism itself [71], this metal was seen as an interesting culprit in the asyn:ATP13A2 interaction. However, a recent contradictory report concluded that lower levels of ATP13A2 did not affect Mn^+ sensitivity in SH-SY5Y cells [58].

In addition to Mn^+, ATP13A2 was shown to be protective against niquel-(Ni^{2+}), cadmium-(Cd^{2+}) and selenium-(Se^{2+}) induced toxicity in yeast and in mammalian cell culture [65, 66] but little is known about the role of these metals in the context of asyn toxicity and in PD.

ATP13A2 was also associated with zinc (Zn^{2+}) homeostasis in a study showing that mitochondrial impairment, due to high levels of Zn^{2+}, could be rescued by overexpression of ATP13A2 [55] (Fig. 2A).

It is unlikely that ATP13A2 is able to directly mediate the clearance of all these different metals. Thus, is seems more plausible that ATP13A2 might act as a general, intermediary player. Since both metals and asyn are thought to have a great impact in mitochondria [28, 72-75], it is possible that the protective effect of ATP13A2 could be related to mitochondrial function as it was also found to be upregulated under oxidative stress conditions [76]. Nevertheless, additional studies are required to test this hypothesis.

AUTOPHAGY

Autophagy-mediated protein degradation is an important component of the protein quality control system in the cell that

is mobilized upon the accumulation of misfolded, damaged, or unnecessary proteins. In PD, autophagy has assumed the central stage due to its involvement in the clearance of misfolded and aggregated asyn. Nevertheless, whether the interplay between autophagy and asyn is beneficial or deleterious to the cell is still controversial [77-85].

The finding that ATP13A2 is also present at the lysosomal membrane has further underscored the relevance of this proteolytic compartment in the context of PD. Strikingly, autophagy seems to directly connect asyn and ATP13A2 since knockdown [56] or knockout [50] of the latter resulted in impaired degradation of asyn (Fig. 2B).

In medaka fish, knockdown of ATP13A2 had a direct effect in the activity of the lysosomal aspartase cathepsin D [86], albeit no conclusive results were obtained regarding the intracellular content of asyn. In zebrafish, knockout of ATP13A2 led to embryonic lethality [87].

In other model systems, ATP13A2 deficiency also resulted in autophagy impairment, with alterations of lysosomal pH and in the levels of hydrolases, in the failure in autophagosome clearance, and in decreased proteolytic processing [56, 57, 59].

Besides its classical lysosomal localization, a recent report noted that ATP13A2 can be also found in multivesicular bodies (MVB) [58], which can have an important role in autophagy. Interestingly, this study reported exocytosis as the final outcome of MVB instead of autophagy. MVB are late endosomes that play a role in several intracellular trafficking mechanisms, including autophagy [88], but also in the clearance of exosomes [89]. Furthermore, considering that mitochondria can be degraded by autophagy (mitophagy) [90], it would be important to understand how asyn and ATP13A2 might affect this process (Fig. 2B). Previously, it was shown that accumulation of asyn at the mitochondria can enhance mitophagy [91, 92], so one possibility is that this process is mediated by ATP13A2 [58].

BRIDGING METAL HOMEOSTASIS AND AUTOPHAGY

The complex interaction between ATP13A2 and asyn has been studied primarily from one of two perspectives: metal dyshomeostasis or autophagy impairment. However, one possibility is that both pathways are connected in the biology of the two proteins. In fact, two recent studies investigated the interaction between asyn and ATP13A2 by looking at both metal homeostasis and autophagy regulation, and proposed a chain of deleterious events starting upon Zn^{2+} dyshomeostasis. The first study reported that alterations in Zn^{2+} intracellular levels and cellular sub-localization could promote lysosomal dysfunction and
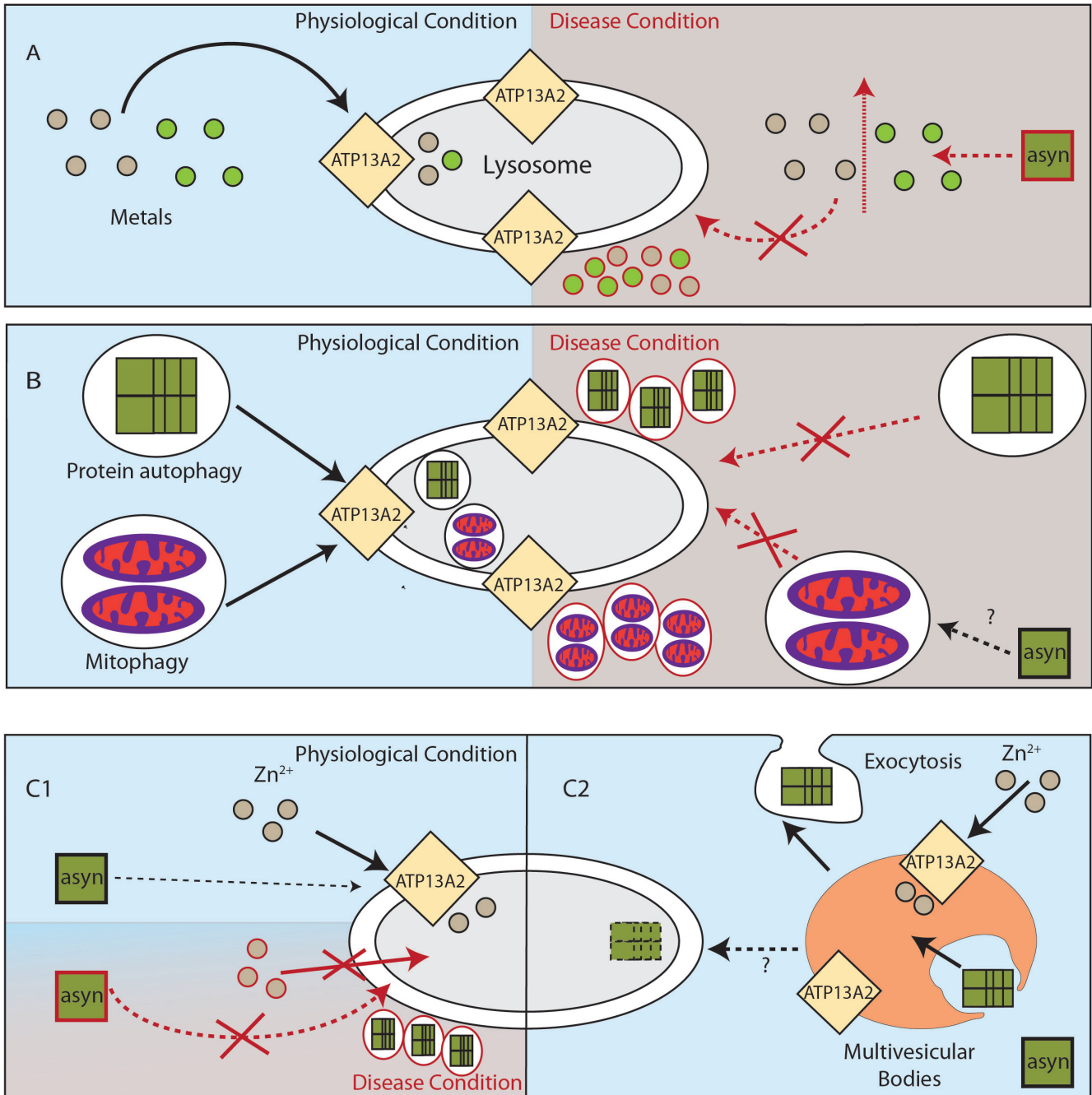
Fig. 2. Putative intracellular pathways connecting asyn and ATP13A2. (A) ATP13A2 may be responsible for metal clearance via the lysosome (left side). A failure in this process, caused by mutations or reduced activity of ATP13A2, would lead to the toxic accumulation of metals in the cytoplasm (right side). Furthermore in disease conditions, asyn may increase the intracellular levels of metals, exacerbating cytotoxic effects [55, 60, 62, 64]. (B) Protein and mitochondria degradation by autophagy and mitophagy, respectively, may be critically regulated by ATP13A2 (left side). Upon deficient ATP13A2 activity, accumulation of defective mitochondria or proteins (such as asyn) would contribute to cytotoxicity and disease (right side) [50, 54, 56, 86]. (C1) ATP13A2 may also impact on intracellular Zn^{2+} homeostasis. Under normal conditions, ATP13A2 may mediate Zn^{2+} transport across the lysosomal membrane, a process that it thought to influence lysosomal degradation of asyn. On the other hand, under pathological conditions, impaired Zn^{2+} clearance, caused by defective ATP13A2 activity at the lysosome, can trigger cytoplasmic accumulation and aggregation of asyn [59]. (C2) ATP13A2 may also play a role at the level of multivesicular bodies (MVBs). Thus, functional ATP13A2 might mediate the entrance of Zn^{2+} into MVB. MVBs may later fuse with autophagosomes containing asyn and be targeted to exocytosis [58].
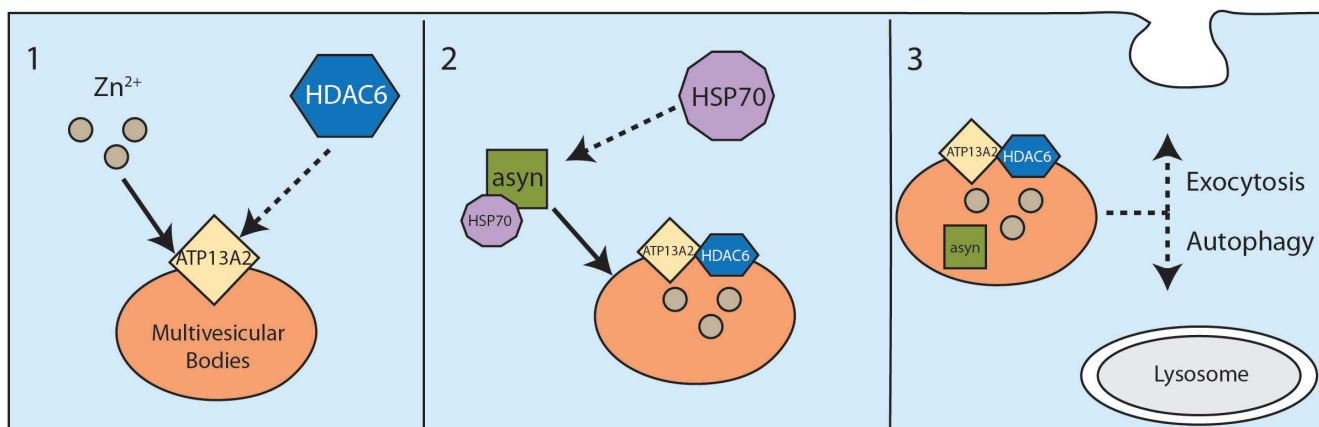
Fig. 3. Hypothetical model for the interplay between asyn and ATP13A2. (1) Delivery of Zn^{2+} to the MVB might be mediated by an ATP13A2/HDAC6 interaction, at the lysosomal membrane. (2) Afterwards, MVBs might engulf cellular contents, including asyn, in a process assisted by molecular chaperones, such as HSP70 (3) The final fate of MVBs (autophagy or exocytosis) might be mediated by these interactions and the cargo (including the proteins and/or organelles) that are being transported.

asyn accumulation. Interestingly, this phenotype was exacerbated in ATP13A2 knock-down cells and in fibroblasts from patients carrying ATP13A2 mutations, and could be rescued upon ATP13A2 overexpression (Fig. 2C1) [59]. On the other hand, a separate study placed MVBs in the center of the interplay between asyn and ATP13A2. The authors found that MVBs are targeted to exocytosis, instead of autophagy, and constitute the main pathway underlying the decrease of asyn intracellular levels [58]. In this perspective, ATP13A2 was shown to modulate Zn^{2+} levels which, in turn, may influence the biogenesis of exosomes (Fig. 2C2). Ultimately, these two studies suggest that ATP13A2 can impact on asyn levels. Moreover, considering the converging players and organelles involved in autophagy and exocytosis pathways, one can hypothesize that the fate of asyn, as well as other accumulated proteins, can be determined by ATP13A2 levels, behaviour and its interactors at the membrane level of the MVB.

In a recent study investigating possible ATP13A2 interactors, histone deacetylase (HDAC) 6 was identified as an attractive candidate [93]. HDAC6 is a cytoplasmic HDAC that has been linked to (i) asyn clearance in a cell model [94], (ii) to its accumulation in Drosophila [95] and (iii) is present in Lewy Bodies from PD patients [96, 97]. Interestingly, HDAC6 has also been linked to mitophagy in PD [98]. Furthermore, this Zn^{2+} binding enzyme has been associated with key steps in the autophagy process, including aggresome formation and delivery to lysosomes [96], fusion between autophagosomes and the lysosomes, and was also found associated with MVBs [99]. Since a direct interaction between ATP13A2 and asyn is still to be proven, one can speculate that the effect of ATP13A2 on asyn clearance

and in the protection against asyn-induced toxicity might be, at least partially, mediated by HDAC6 (Fig. 3).

Interestingly, the same study also identified HSPA8 (also known as HSC70 and HSP73) as an interactor of ATP13A2 [93, 100]. HSP8A is an essential player in chaperone mediated autophagy, a process that was found to be involved in the clearance of soluble asyn [84, 101]. Thus, the interaction between ATP13A2 and HSPA8 might also play a role in the degradation of asyn.

CONCLUDING REMARKS

Currently, asyn and ATP13A2 are thought to be members of the same intracellular network, with the latter having a direct impact on the fate of asyn in the cell. Nevertheless, the precise biological function of ATP13A2 and whether it plays a direct or indirect role in the processing of asyn is still unknown. Although two main interacting networks have been proposed separately, recent studies tend to converge in a more appealing and comprehensive hypothesis that comprises a single process that includes both alterations in the levels of metals and in autophagy.

It will also be important to determine the effects of familial mutations in both ATP13A2 and in asyn on the interaction between the two proteins, and whether the occurrence of ATP13A2 in the lysosome can rescue deleterious effects of mutant asyn. In this context, since most studies focused on the effect of ATP13A2 knockdown on asyn, it will be important to assess the impact of asyn knockdown on ATP13A2. On the other hand, it seems likely that the function of ATP13A2 in the cell goes beyond its effects on asyn, suggesting that we need a broader understanding of its

role in both metal homeostasis and autophagy in order to better understand the biological function of ATP13A2 and, consequently, how it causes disease. With this knowledge at hand, it might then be possible to design novel strategies for therapeutic intervention in PD and other disorders associated with asyn and ATP13A2 dysfunction.

ACKNOWLEDGEMENTS

REFERENCES

1. Martí MJ, Tolosa E, Campdelacreu J (2003) Clinical overview of the synucleinopathies. Mov Disord 18:21-27.

2. Simuni T, Sethi K (2008) Nonmotor manifestations of Parkinson's disease. Ann Neurol 64 Suppl 2:S65-S80.

3. Park A, Stacy M (2009) Non-motor symptoms in Parkinson's disease. J Neurol 256 Suppl 3:293-298.

4. Gallagher DA, Lees AJ, Schrag A (2010) What are the most important nonmotor symptoms in patients with Parkinson's disease and are we missing them? Mov Disord 25:2493-2500.

5. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 24:197-211.

6. Wales P, Pinho R, Lázaro DF, Outeiro TF (2013) Limelight on alpha-synuclein: pathological and mechanistic implications in neurodegeneration. J Parkinsons Dis 3:415-459.

7. Bartels T, Choi JG, Selkoe DJ (2011) α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature 477:107-110.

8. Wang W, Perovic I, Chittuluru J, Kaganovich A, Nguyen LT, Liao J, Auclair JR, Johnson D, Landeru A, Simorellis AK, Ju S, Cookson MR, Asturias FJ, Agar JN, Webb BN, Kang C, Ringe D, Petsko GA, Pochapsky TC, Hoang QQ (2011) A soluble α-synuclein construct forms a dynamic tetramer. Proc Natl Acad Sci U S A 108:17797-17802.

9. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95:6469-6473.

10. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe

LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 276:2045-2047.

11. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schöls L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 18:106-108.

12. Zarranz JJ, Alegre J, Gómez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atarés B, Llorens V, Gomez Tortosa E, del Ser T, Muñoz DG, de Yebenes JG (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55:164-173.

13. Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, Weir D, Thompson C, Szu-Tu C, Trinh J, Aasly JO, Rajput A, Rajput AH, Jon Stoessl A, Farrer MJ (2013) Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. Mov Disord 28:811-813.

14. Lesage S, Anheim M, Letournel F, Bousset L, Honoré A, Rozas N, Pieri L, Madiona K, Dürr A, Melki R, Verny C, Brice A; French Parkinson's Disease Genetics Study Group (2013) G51D α-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. Ann Neurol 73:459-471.

15. Pasanen P, Myllykangas L, Siitonen M, Raunio A, Kaakkola S, Lyytinen J, Tienari PJ, Pöyhönen M, Paetau A (2014) A novel α-synuclein mutation A53E associated with atypical multiple system atrophy and Parkinson's disease-type pathology. Neurobiol Aging 35:2180.e1-2180.e5.

16. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawley A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blancato J, Hardy J, Gwinn-Hardy K (2003) alpha-Synuclein locus triplication causes Parkinson's disease. Science 302:841.

17. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Levecque C, Larvor L, Andrieux J, Hulihan M, Waucquier N, Defebvre L, Amouyel P, Farrer M, Destée A (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 364:1167-1169.

18. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, Kawaguchi T, Tsunoda T, Watanabe M, Takeda A, Tomiyama H, Nakashima K, Hasegawa K, Obata F, Yoshikawa T, Kawakami H, Sakoda S, Yamamoto M, Hattori N, Murata M, Nakamura Y, Toda T (2009) Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 41:1303-1307.

19. Simón-Sánchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, Lichtner P, Scholz SW, Hernandez DG,

Krüger R, Federoff M, Klein C, Goate A, Perlmutter J, Bonin M, Nalls MA, Illig T, Gieger C, Houlden H, Steffens M, Okun MS, Racette BA, Cookson MR, Foote KD, Fernandez HH, Traynor BJ, Schreiber S, Arepalli S, Zonozi R, Gwinn K, van der Brug M, Lopez G, Chanock SJ, Schatzkin A, Park Y, Hollenbeck A, Gao J, Huang X, Wood NW, Lorenz D, Deuschl G, Chen H, Riess O, Hardy JA, Singleton AB, Gasser T (2009) Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet 41:1308-1312.

20. Edwards TL, Scott WK, Almonte C, Burt A, Powell EH, Beecham GW, Wang L, Züchner S, Konidari I, Wang G, Singer C, Nahab F, Scott B, Stajich JM, Pericak-Vance M, Haines J, Vance JM, Martin ER (2010) Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. Ann Hum Genet 74:97-109.

21. Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M, Schulte C, Keller MF, Arepalli S, Letson C, Edsall C, Stefansson H, Liu X, Pliner H, Lee JH, Cheng R; International Parkinson's Disease Genomics Consortium; Parkinson's Study Group; Parkinson's Research: The Organized GENetics Initiative; 23andMe; GenePD; NeuroGenetics Research Consortium; Hussman Institute of Human Genomics; The Ashkenazi Jewish Dataset Investigator; Cohorts for Health and Aging Research in Genetic Epidemiology; North American Brain Expression Consortium; United Kingdom Brain Expression Consortium; Greek Parkinson's Disease Consortium; Alzheimer Genetic Analysis Group; Ikram MA, Ioannidis JP, Hadjigeorgiou GM, Bis JC, Martinez M, Perlmutter JS, Goate A, Marder K, Fiske B, Sutherland M, Xiromerisiou G, Myers RH, Clark LN, Stefansson K, Hardy JA, Heutink P, Chen H, Wood NW, Houlden H, Payami H, Brice A, Scott WK, Gasser T, Bertram L, Eriksson N, Foroud T, Singleton AB (2014) Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet 46:989-993.

22. Siddiqui A, Chinta SJ, Mallajosyula JK, Rajagopolan S, Hanson I, Rane A, Melov S, Andersen JK (2012) Selective binding of nuclear alpha-synuclein to the PGC1alpha promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: implications for Parkinson's disease. Free Radic Biol Med 53:993-1003.

23. Vasudevaraju P, Guerrero E, Hegde ML, Collen TB, Britton GB, Rao KS (2012) New evidence on α-synuclein and Tau binding to conformation and sequence specific GC* rich DNA: relevance to neurological disorders. J Pharm Bioallied

Sci 4:112-117.

24. Kontopoulos E, Parvin JD, Feany MB (2006) Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. Hum Mol Genet 15:3012-3023.

25. Zhou M, Xu S, Mi J, Uéda K, Chan P (2013) Nuclear translocation of alpha-synuclein increases susceptibility of MES23.5 cells to oxidative stress. Brain Res 1500:19-27.

26. Liu X, Lee YJ, Liou LC, Ren Q, Zhang Z, Wang S, Witt SN (2011) Alpha-synuclein functions in the nucleus to protect against hydroxyurea-induced replication stress in yeast. Hum Mol Genet 20:3401-3414.

27. Goers J, Manning-Bog AB, McCormack AL, Millett IS, Doniach S, Di Monte DA, Uversky VN, Fink AL (2003) Nuclear localization of alpha-synuclein and its interaction with histones. Biochemistry 42:8465-8471.

28. Nakamura K (2013) α-Synuclein and mitochondria: partners in crime? Neurotherapeutics 10:391-399.

29. Eisbach SE, Outeiro TF (2013) Alpha-synuclein and intracellular trafficking: impact on the spreading of Parkinson's disease pathology. J Mol Med (Berl) 91:693-703.

30. Scott DA, Tabarean I, Tang Y, Cartier A, Masliah E, Roy S (2010) A pathologic cascade leading to synaptic dysfunction in alpha-synuclein-induced neurodegeneration. J Neurosci 30:8083-8095.

31. Yavich L, Tanila H, Vepsäläinen S, Jäkälä P (2004) Role of alpha-synuclein in presynaptic dopamine recruitment. J Neurosci 24:11165-11170.

32. Gaugler MN, Genc O, Bobela W, Mohanna S, Ardah MT, El-Agnaf OM, Cantoni M, Bensadoun JC, Schneggenburger R, Knott GW, Aebischer P, Schneider BL (2012) Nigrostriatal overabundance of α-synuclein leads to decreased vesicle density and deficits in dopamine release that correlate with reduced motor activity. Acta Neuropathol 123:653-669.

33. Lundblad M, Decressac M, Mattsson B, Björklund A (2012) Impaired neurotransmission caused by overexpression of α-synuclein in nigral dopamine neurons. Proc Natl Acad Sci U S A 109:3213-3219.

34. Abeliovich A, Schmitz Y, Fariñas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25:239-252.

35. Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, Goebel I, Mubaidin AF, Wriekat AL, Roeper J, Al-Din A, Hillmer AM, Karsak M, Liss B, Woods CG, Behrens MI, Kubisch C (2006) Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a

lysosomal type 5 P-type ATPase. Nat Genet 38:1184-1191.

36. Schultheis PJ, Hagen TT, O'Toole KK, Tachibana A, Burke CR, McGill DL, Okunade GW, Shull GE (2004) Characterization of the P5 subfamily of P-type transport ATPases in mice. Biochem Biophys Res Commun 323:731-738.

37. Ramonet D, Podhajska A, Stafa K, Sonnay S, Trancikova A, Tsika E, Pletnikova O, Troncoso JC, Glauser L, Moore DJ (2012) PARK9-associated ATP13A2 localizes to intracellular acidic vesicles and regulates cation homeostasis and neuronal integrity. Hum Mol Genet 21:1725-1743.

38. Di Fonzo A, Chien HF, Socal M, Giraudo S, Tassorelli C, Iliceto G, Fabbrini G, Marconi R, Fincati E, Abbruzzese G, Marini P, Squitieri F, Horstink MW, Montagna P, Libera AD, Stocchi F, Goldwurm S, Ferreira JJ, Meco G, Martignoni E, Lopiano L, Jardim LB, Oostra BA, Barbosa ER; Italian Parkinson Genetics Network, Bonifati V (2007) ATP13A2 missense mutations in juvenile parkinsonism and young onset Parkinson disease. Neurology 68:1557-1562.

39. Ning YP, Kanai K, Tomiyama H, Li Y, Funayama M, Yoshino H, Sato S, Asahina M, Kuwabara S, Takeda A, Hattori T, Mizuno Y, Hattori N (2008) PARK9-linked parkinsonism in eastern Asia: mutation detection in ATP13A2 and clinical phenotype. Neurology 70:1491-1493.

40. Santoro L, Breedveld GJ, Manganelli F, Iodice R, Pisciotta C, Nolano M, Punzo F, Quarantelli M, Pappatà S, Di Fonzo A, Oostra BA, Bonifati V (2011) Novel ATP13A2 (PARK9) homozygous mutation in a family with marked phenotype variability. Neurogenetics 12:33-39.

41. Lin CH, Tan EK, Chen ML, Tan LC, Lim HQ, Chen GS, Wu RM (2008) Novel ATP13A2 variant associated with Parkinson disease in Taiwan and Singapore. Neurology 71:1727-1732.

42. Paisán-Ruiz C, Guevara R, Federoff M, Hanagasi H, Sina F, Elahi E, Schneider SA, Schwingenschuh P, Bajaj N, Emre M, Singleton AB, Hardy J, Bhatia KP, Brandner S, Lees AJ, Houlden H (2010) Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and spatacsin mutations. Mov Disord 25:1791-1800.

43. Fong CY, Rolfs A, Schwarzbraun T, Klein C, O'Callaghan FJ (2011) Juvenile parkinsonism associated with heterozygous frameshift ATP13A2 gene mutation. Eur J Paediatr Neurol 15:271-275.

44. Malakouti-Nejad M, Shahidi GA, Rohani M, Shojaee SM, Hashemi M, Klotzle B, Fan JB, Elahi E (2014) Identification of p.Gln858* in ATP13A2 in two EOPD patients and presentation of their clinical features. Neurosci Lett 577:106-111.

45. Djarmati A, Hagenah J, Reetz K, Winkler S, Behrens MI, Pawlack H, Lohmann K, Ramirez A, Tadić V, Brüggemann N, Berg D, Siebner HR, Lang AE, Pramstaller PP, Binkofski F, Kostić VS, Volkmann J, Gasser T, Klein C (2009) ATP13A2 variants in early-onset Parkinson's disease patients and controls. Mov Disord 24:2104-2111.

46. Eiberg H, Hansen L, Korbo L, Nielsen IM, Svenstrup K, Bech S, Pinborg LH, Friberg L, Hjermind LE, Olsen OR, Nielsen JE (2012) Novel mutation in ATP13A2 widens the spectrum of Kufor-Rakeb syndrome (PARK9). Clin Genet 82:256-263.

47. Bras J, Verloes A, Schneider SA, Mole SE, Guerreiro RJ (2012) Mutation of the parkinsonism gene ATP13A2 causes neuronal ceroid-lipofuscinosis. Hum Mol Genet 21:2646-2650.

48. Farias FH, Zeng R, Johnson GS, Wininger FA, Taylor JF, Schnabel RD, McKay SD, Sanders DN, Lohi H, Seppälä EH, Wade CM, Lindblad-Toh K, O'Brien DP, Katz ML (2011) A truncating mutation in ATP13A2 is responsible for adult-onset neuronal ceroid lipofuscinosis in Tibetan terriers. Neurobiol Dis 42:468-474.

49. Wöhlke A, Philipp U, Bock P, Beineke A, Lichtner P, Meitinger T, Distl O (2011) A one base pair deletion in the canine ATP13A2 gene causes exon skipping and late-onset neuronal ceroid lipofuscinosis in the Tibetan terrier. PLoS Genet 7:e1002304.

50. Schultheis PJ, Fleming SM, Clippinger AK, Lewis J, Tsunemi T, Giasson B, Dickson DW, Mazzulli JR, Bardgett ME, Haik KL, Ekhator O, Chava AK, Howard J, Gannon M, Hoffman E, Chen Y, Prasad V, Linn SC, Tamargo RJ, Westbroek W, Sidransky E, Krainc D, Shull GE (2013) Atp13a2-deficient mice exhibit neuronal ceroid lipofuscinosis, limited α-synuclein accumulation and age-dependent sensorimotor deficits. Hum Mol Genet 22:2067-2082.

51. Grünewald A, Arns B, Seibler P, Rakovic A, Münchau A, Ramirez A, Sue CM, Klein C (2012) ATP13A2 mutations impair mitochondrial function in fibroblasts from patients with Kufor-Rakeb syndrome. Neurobiol Aging 33:1843.e1-1843.e7.

52. Ashrafi G, Schwarz TL (2013) The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ 20:31-42.

53. de Vries RL, Przedborski S (2013) Mitophagy and Parkinson's disease: be eaten to stay healthy. Mol Cell Neurosci 55:37-43.

54. Gusdon AM, Zhu J, Van Houten B, Chu CT (2012) ATP13A2 regulates mitochondrial bioenergetics through macroautophagy. Neurobiol Dis 45:962-972.

55. Park JS, Koentjoro B, Veivers D, Mackay-Sim A, Sue CM (2014) Parkinson's disease-associated human ATP13A2 (PARK9) deficiency causes zinc dyshomeostasis and mitochondrial dysfunction. Hum Mol Genet 23:2802-2815.

56. Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D (2012) Deficiency of ATP13A2 leads to lysosomal dysfunction, α-synuclein accumulation, and neurotoxicity. J Neurosci 32:4240-4246.

57. Dehay B, Ramirez A, Martinez-Vicente M, Perier C, Canron MH, Doudnikoff E, Vital A, Vila M, Klein C, Bezard E (2012) Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. Proc Natl Acad Sci U S A 109:9611-9616.

58. Kong SM, Chan BK, Park JS, Hill KJ, Aitken JB, Cottle L, Farghaian H, Cole AR, Lay PA, Sue CM, Cooper AA (2014) Parkinson's disease-linked human PARK9/ATP13A2 maintains zinc homeostasis and promotes α-Synuclein externalization via exosomes. Hum Mol Genet 23:2816-2833.

59. Tsunemi T, Krainc D (2014) Zn(2)(+) dyshomeostasis caused by loss of ATP13A2/PARK9 leads to lysosomal dysfunction and alpha-synuclein accumulation. Hum Mol Genet 23:2791-2801.

60. Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, Caldwell KA, Caldwell GA, Cooper AA, Rochet JC, Lindquist S (2009) Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nat Genet 41:308-315.

61. Lambie EJ, Tieu PJ, Lebedeva N, Church DL, Conradt B (2013) CATP-6, a C. elegans ortholog of ATP13A2 PARK9, positively regulates GEM-1, an SLC16A transporter. PLoS One 8:e77202.

62. Chesi A, Kilaru A, Fang X, Cooper AA, Gitler AD (2012) The role of the Parkinson's disease gene PARK9 in essential cellular pathways and the manganese homeostasis network in yeast. PLoS One 7:e34178.

63. Rentschler G, Covolo L, Haddad AA, Lucchini RG, Zoni S, Broberg K (2012) ATP13A2 (PARK9) polymorphisms influence the neurotoxic effects of manganese. Neurotoxicology 33:697-702.

64. Tan J, Zhang T, Jiang L, Chi J, Hu D, Pan Q, Wang D, Zhang Z (2011) Regulation of intracellular manganese homeostasis by Kufor-Rakeb syndrome-associated ATP13A2 protein. J Biol Chem 286:29654-29662.

65. Schmidt K, Wolfe DM, Stiller B, Pearce DA (2009) Cd2+, Mn2+, Ni2+ and Se2+ toxicity to Saccharomyces cerevisiae lacking YPK9p the orthologue of human ATP13A2. Biochem Biophys Res Commun 383:198-202.

66. Covy JP, Waxman EA, Giasson BI (2012) Characterization of cellular protective effects of ATP13A2/PARK9 expression and alterations resulting from pathogenic mutants. J Neurosci Res 90:2306-2316.

67. Ugolino J, Fang S, Kubisch C, Monteiro MJ (2011) Mutant Atp13a2 proteins involved in parkinsonism are degraded by ER-associated degradation and sensitize cells to ER-stress induced cell death. Hum Mol Genet 20:3565-3577.

68. Podhajska A, Musso A, Trancikova A, Stafa K, Moser R, Sonnay S, Glauser L, Moore DJ (2012) Common pathogenic effects of missense mutations in the P-type ATPase ATP13A2 (PARK9) associated with early-onset parkinsonism. PLoS One 7:e39942.

69. Xu B, Jin CH, Deng Y, Liu W, Yang TY, Feng S, Xu ZF (2014) Alpha-synuclein oligomerization in manganese-induced nerve cell injury in brain slices: a role of NO-mediated S-nitrosylation of protein disulfide isomerase. Mol Neurobiol (in press).

70. Verina T, Schneider JS, Guilarte TR (2013) Manganese exposure induces α-synuclein aggregation in the frontal cortex of non-human primates. Toxicol Lett 217:177-183.

71. Lucchini RG, Albini E, Benedetti L, Borghesi S, Coccaglio R, Malara EC, Parrinello G, Garattini S, Resola S, Alessio L (2007) High prevalence of Parkinsonian disorders associated to manganese exposure in the vicinities of ferroalloy industries. Am J Ind Med 50:788-800.

72. Martinez-Finley EJ, Gavin CE, Aschner M, Gunter TE (2013) Manganese neurotoxicity and the role of reactive oxygen species. Free Radic Biol Med 62:65-75.

73. Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12:1161-1208.

74. Cannino G, Ferruggia E, Luparello C, Rinaldi AM (2009) Cadmium and mitochondria. Mitochondrion 9:377-384.

75. Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ, Nawrot T, Vangronsveld J, Smeets K (2010) Cadmium stress: an oxidative challenge. Biometals 23:927-940.

76. Xu Q, Guo H, Zhang X, Tang B, Cai F, Zhou W, Song W (2012) Hypoxia regulation of ATP13A2 (PARK9) gene transcription. J Neurochem 122:251-259.

77. Winslow AR, Rubinsztein DC (2011) The Parkinson disease protein α-synuclein inhibits autophagy. Autophagy 7:429-431.

78. Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, Lichtenberg M, Menzies FM, Ravikumar B, Imarisio S, Brown S, O'Kane CJ, Rubinsztein DC (2010) α-Synuclein impairs macroautophagy: implications for Parkinson's disease. J Cell Biol 190:1023-

1037.

79. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC (2003) Alpha-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem 278:25009-25013.

80. Colasanti T, Vomero M, Alessandri C, Barbati C, Maselli A, Camperio C, Conti F, Tinari A, Carlo-Stella C, Tuosto L, Benincasa D, Valesini G, Malorni W, Pierdominici M, Ortona E (2014) Role of alpha-synuclein in autophagy modulation of primary human T lymphocytes. Cell Death Dis 5:e1265.

81. Xilouri M, Vogiatzi T, Vekrellis K, Park D, Stefanis L (2009) Abberant alpha-synuclein confers toxicity to neurons in part through inhibition of chaperone-mediated autophagy. PLoS One 4:e5515.

82. Mak SK, McCormack AL, Manning-Bog AB, Cuervo AM, Di Monte DA (2010) Lysosomal degradation of alpha-synuclein in vivo. J Biol Chem 285:13621-13629.

83. Martinez-Vicente M, Talloczy Z, Kaushik S, Massey AC, Mazzulli J, Mosharov EV, Hodara R, Fredenburg R, Wu DC, Follenzi A, Dauer W, Przedborski S, Ischiropoulos H, Lansbury PT, Sulzer D, Cuervo AM (2008) Dopamine-modified alpha-synuclein blocks chaperone-mediated autophagy. J Clin Invest 118:777-788.

84. Vogiatzi T, Xilouri M, Vekrellis K, Stefanis L (2008) Wild type alpha-synuclein is degraded by chaperone-mediated autophagy and macroautophagy in neuronal cells. J Biol Chem 283:23542-23556.

85. Sridhar S, Botbol Y, Macian F, Cuervo AM (2012) Autophagy and disease: always two sides to a problem. J Pathol 226:255-273.

86. Matsui H, Sato F, Sato S, Koike M, Taruno Y, Saiki S, Funayama M, Ito H, Taniguchi Y, Uemura N, Toyoda A, Sakaki Y, Takeda S, Uchiyama Y, Hattori N, Takahashi R (2013) ATP13A2 deficiency induces a decrease in cathepsin D activity, fingerprint-like inclusion body formation, and selective degeneration of dopaminergic neurons. FEBS Lett 587:1316-1325.

87. Lopes da Fonseca T, Correia A, Hasselaar W, van der Linde HC, Willemsen R, Outeiro TF (2013) The zebrafish homologue of Parkinson's disease ATP13A2 is essential for embryonic survival. Brain Res Bull 90:118-126.

88. Fader CM, Colombo MI (2009) Autophagy and multivesicular bodies: two closely related partners. Cell Death Differ 16:70-78.

89. Simons M, Raposo G (2009) Exosomes--vesicular carriers for intercellular communication. Curr Opin Cell Biol 21:575-581.

90. Lemasters JJ (2014) Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). Redox Biol 2:749-754.

91. Chinta SJ, Mallajosyula JK, Rane A, Andersen JK (2010) Mitochondrial α-synuclein accumulation impairs complex I function in dopaminergic neurons and results in increased mitophagy in vivo. Neurosci Lett 486:235-239.

92. Sampaio-Marques B, Felgueiras C, Silva A, Rodrigues M, Tenreiro S, Franssens V, Reichert AS, Outeiro TF, Winderickx J, Ludovico P (2012) SNCA (α-synuclein)-induced toxicity in yeast cells is dependent on sirtuin 2 (Sir2)-mediated mitophagy. Autophagy 8:1494-1509.

93. Usenovic M, Knight AL, Ray A, Wong V, Brown KR, Caldwell GA, Caldwell KA, Stagljar I, Krainc D (2012) Identification of novel ATP13A2 interactors and their role in α-synuclein misfolding and toxicity. Hum Mol Genet 21:3785-3794.

94. Su M, Shi JJ, Yang YP, Li J, Zhang YL, Chen J, Hu LF, Liu CF (2011) HDAC6 regulates aggresome-autophagy degradation pathway of α-synuclein in response to MPP+-induced stress. J Neurochem 117:112-120.

95. Du G, Liu X, Chen X, Song M, Yan Y, Jiao R, Wang CC (2010) Drosophila histone deacetylase 6 protects dopaminergic neurons against {alpha}-synuclein toxicity by promoting inclusion formation. Mol Biol Cell 21:2128-2137.

96. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP (2003) The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell 115:727-738.

97. Miki Y, Mori F, Tanji K, Kakita A, Takahashi H, Wakabayashi K (2011) Accumulation of histone deacetylase 6, an aggresome-related protein, is specific to Lewy bodies and glial cytoplasmic inclusions. Neuropathology 31:561-568.

98. Lee JY, Nagano Y, Taylor JP, Lim KL, Yao TP (2010) Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. J Cell Biol 189:671-679.

99. Gao YS, Hubbert CC, Yao TP (2010) The microtubule-associated histone deacetylase 6 (HDAC6) regulates epidermal growth factor receptor (EGFR) endocytic trafficking and degradation. J Biol Chem 285:11219-11226.

100. Stricher F, Macri C, Ruff M, Muller S (2013) HSPA8/HSC70 chaperone protein: structure, function, and chemical targeting. Autophagy 9:1937-1954.

101. Cuervo AM, Wong E (2014) Chaperone-mediated autophagy: roles in disease and aging. Cell Res 24:92-104.