Autophagy in Childhood Neurological Disorders

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Abstract

Autophagy is a tightly modulated lysosomal degradation pathway. Genetic disorders of autophagy during nervous system development may lead to developmental delay, neurodegeneration and other neurological signs in children. Here we aimed to summarize single gene disorders that perturb various steps of autophagy pathway and their roles in the causation of childhood neurological diseases. Numerous childhood-onset disorders are caused by mutations that impact the autophagy pathway. These can manifest with a range of features including ataxia, spastic paraplegia, and intellectual disability. Defective proteins causing such diseases can interfere with autophagy flux at different stages of the itinerary. Defective autophagy may be an important contributor to the pathological features of various childhood neurodegenerative disease and lead to the accumulation of aberrant protein and dysfunctional organelles. Insights into the relevant cell biological processes may help understand pathophysiological mechanisms and inspire autophagy-restoring therapeutic approaches.

What this paper adds:

Numerous childhood-onset disorders are caused by mutations that impact the autophagy pathway.

Defective autophagy is a feature of some mutations that cause ataxia, spastic paraplegia, and intellectual disability.

Defective autophagy may be an important contributor to the pathological features of various childhood neurodegenerative disease and lead to the accumulation of aberrant protein and dysfunctional organelles.

Introduction

Macroautophagy, henceforth autophagy, is a catabolic process that mediates the bulk degradation of cytosolic constituents by delivering them to lysosomes. While the process was originally identified as a key mechanism for maintaining cellular homeostasis, recent studies have shown that autophagy plays important roles in the degradation of damaged organelles, macromolecules, aggregate-prone proteins, bacteria and long-lived proteins inside the cell. While autophagy was largely considered as a non-selective bulk degradation process, recent studies have identified certain forms of autophagy which are selective for certain substrates including damaged organelles like mitochondria – mitophagy, peroxisomes – pexophagy; aggregate-prone proteins – aggrephagy; and pathogens – xenophagy.[1]

Autophagy involves the formation and maturation of a double-membraned autophagosome that engulfs the cargo to be degraded, which is then delivered to the lysosomes to form an 'autolysosome' (Figure 1). Autophagy involves several steps: initiation, nucleation, elongation, closure and fusion. Each step is tightly regulated by one or many protein(s)/protein complexes resulting in a pathway that is highly dynamic and responsive to various stimuli.[2]

The classic marker that differentiates autophagosomes from other vesicles is the presence of lipidated ATG8 (ATG = Autophagy related) ubiquitin-like family proteins on both sides of the vesicle. The ATG8 ubiquitin-like family of proteins in mammals consists of six members that are divided into two subfamilies viz. Microtubule-associated protein 1, light chain 3 alpha (LC3

601242) (refers to OMIM number) and GABA-A receptor-associated protein (GABARAP *605125).[2] A defining event in autophagy is the conjugation of ATG8s/LC3 to membranes that become autophagosomes. This involves two ubiquitin-like reactions. First, ATG12 (*609608) an ubiquitin-like molecule, is conjugated to ATG5 (*604261) with the help of the enzymes ATG7 (*608760) and ATG10 (*610800); the ATG5-12 complex then combines with ATG16L1 (*610767) to form an ATG5-ATG12-ATG16L1 complex. In order for ATG8s/LC3 to be accessible for the second set of conjugation reactions, pro-LC3 is converted into LC3-I by the cysteine protease ATG4A (*300663) exposing a free C-terminal glycine which then gets activated by ATG7. Next, the activated LC3-I is conjugated to phosphatidylethanolamine (PE) on autophagosome precursor membranes resulting in the formation of LC3-II, with the help of ATG3 (*609606) and the E3-like ligase activity of ATG5-ATG12-ATG16L1. The most common way of assessing autophagy therefore involves measuring the levels of LC3-II and counting the number of LC3-II puncta (autophagosomes) in cells.[3]

Complex signalling processes upstream of these conjugation system respond to various stimuli to modulate autophagy. One of the most upstream elements in the signalling is the ULK1/2 complex, which comprises UNC51-like kinase 1 (ULK1 *603168), or its homologue UNC51-like kinase 2 (ULK2 *608650) together with ATG13 (*615088), 200 KDa FAK Family Kinase-Interacting Protein (FIP200 *606837) and ATG101 (*615089). The ULK1-ATG13 interaction depends on the phosphorylation status of ATG13, which is regulated by kinases like mechanistic target of rapamycin (mTOR *601231) and AMP-activated protein kinase (AMPK *602740). The phosphorylation of ATG13 by mTOR under nutrient-rich conditions hinders the ULK1-ATG13 interaction, thereby inactivating the complex. However, during starvation, ATG13 is dephosphorylated and forms an active ULK1 complex. In addition, ULK1 activation and the ULK1-AMPK interaction also depend on mTOR-mediated ULK1 phosphorylation. The active ULK1 complex then phosphorylates FIP200, which, in turn, helps recruit the next complex in the cascade, the VPS34 complex, at the autophagosome formation site. The core of the VPS34 complex consists of phosphatidylinositol 3-kinase class 3 (VPS34 kinase *602609) Beclin 1 (BECN1 *604378) and phosphatidylinositol 3-kinase regulatory subunit 4 (VPS15 *602610), which can then associate with ATG14 (*0613515), UV radiation resistance-associated gene (UVRAG *602493), or run domain and cysteine rich domain-containing beclin1-interacting protein (Rubicon *613516), resulting in three distinct complexes. The VPS34 complex generates phosphatidylinositol 3-phosphate (PI3P) at the autophagosome initiation site, which helps recruit PI3P-binding proteins like the WD40 repeat protein

interacting with phosphoinositides 1 (WIPI) family proteins.[4] WIPI2 (*609225) then directs the localization of ATG5-ATG12-ATG16L1 complex, thereby dictating the site for LC3 membrane cojugation.[5] WIPI2 may also regulate the trafficking of the transmembrane mATG9 (ATG9,* 612204). While the function of mATG9 remains elusive, it has been speculated to assist membrane trafficking and delivery to the growing autophagosomes.[2, 4]

Autophagy is an important buffer against various late-onset neurodegenerative diseases, since it mediates the clearance of toxic, aggregate-prone proteins like huntingtin, tau and alpha-synuclein. Indeed, selective neuronal knockouts of key autophagy genes in mice lead to early lethality associated with the accumulation of aggregated proteins.[2]

This review focuses specifically on the role of autophagy in neurological diseases with early-onset (<20 years), which typically manifest in childhood (Table 1). Most of these disorders associated with defective autophagy follow autosomal-recessive inheritance, except BPAN, which is X-linked dominant.[6]

Hereditary childhood ataxias

Inherited ataxias comprise a genotypically and phenotypically diverse group of disorders that typically present with movement, balance and speech disturbance as a result of cerebellar dysfunction, which can be accompanied by a wide range of other signs, such as developmental delay, seizures or ocular abnormalities.[7]

Null mutations of sorting nexin 14 (SNX14 *616105) cause a form of autosomal recessive syndromic cerebellar atrophy. The distinct features of the affected patients include intellectual disability, dysmorphic facies, sensorineural hearing loss and hepatosplenomegaly, phenotypically resembling lysosomal storage diseases.[7] SNX14 binds phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P_2) and localizes to late endosomes/lysosomes containing PtdIns(3,5)P_2. Patient-derived neural progenitor cells carrying SNX14 mutations showed normal autophagosome formation, but slower autophagic flux after treatment with a proteasome inhibitor and excessive accumulation of autophagosomes after starvation. Taken together, the findings suggests that loss of SNX14 may disrupt autophagosome clearance by impairing lysosome function.[7]

A homozygous missense mutation (E122D) in *ATG5* (*604261) gene was identified in two siblings presenting with childhood ataxia associated with hypoplasia of the cerebellar vermis.[1, 8] Autophagic flux was attenuated in patient-derived lymphoblastoid cell lines compared to

controls. More interestingly, the levels of ATG5-ATG12 conjugate were severely reduced, although the expression levels of ATG5WT and ATG5E122D were similar. These data suggest that the reduction of autophagic flux in these patients might be due to defective lipidation of ATG8 family proteins.[1, 8] Recently, a polymorphism of *ATG5* gene (rs6568431) correlated with lower ATG5 expression was associated with childhood cerebral palsy (CP).[9] However, the detailed mechanism about how this polymorphism causes CP has not been reported.

Neurodegeneration with brain iron accumulation (NBIA)

Neurodegeneration with brain iron accumulation (NBIA) encompasses a clinically and genetically heterogeneous collection of diseases characterized by iron deposition in the basal ganglia.[6] The manifestations of NBIA include progressive dystonia, Parkinsonism, optic atrophy and neuropsychiatric abnormalities, with preservation of cognitive function in most cases. Interestingly, while iron accumulation remains a hallmark of NBIA, only two of the twelve causative genes are known to be directly involved in iron metabolism, with others having effects on a wide range of other cellular processes, including lipid metabolism or lysosome function.

Beta-propeller protein-associated neurodegeneration (BPAN)

Beta-propeller protein-associated neurodegeneration (BPAN) is a rare subtype of NBIA caused by X-linked dominant mutations of in the *WDR45* gene encoding WIPI4. Most described cases are females, with similar phenotypes seen in both sexes due to germline and somatic mosaicism. The disease was previously known as SENDA (static encephalopathy of childhood with neurodegeneration in adulthood). Patients manifest delay of motor and cognitive functions during early childhood, followed by rapid onset of progressive dystonia, Parkinsonism and dementia during adolescence or adulthood. BPAN patients exhibit prominent iron accumulation. As some *WDR45* mutation-carrying patients show neurodevelopmental abnormalities before iron accumulation is apparent by brain imaging, iron accumulation may be the consequence rather than the cause of neurodevelopmental abnormalities. WIPIs are a family of proteins that can bind to a variety of phosphoinositides with different affinities. In CNS-specific Wdr45 knockout mice, the lipidated LC3 levels were increased and the autophagy substrate p62 together with ubiquitin-positive protein aggregates accumulated in swollen axons. Cells from BPAN patients manifest abnormal accumulations of lipidated LC3, together with increased colocalisation of LC3 and mATG9, which is usually absent on mature autophagosomes.[6] The results suggest that WDR45 mutations might compromise

autophagosome maturation, potentially by regulating mATG9 localisation. The underlying mechanism of WIPI4 in BPAN needs to be studied further.

Mitochondrial-membrane Protein-Associated Neurodegeneration (MPAN)

MPAN, also called Hereditary spastic paraplegia type 43 (SPG43), is the third most common disease belonging to NBIA disorders. MPAN patients often suffer from cognitive decline, in addition to dystonia, gait and speech disturbance, or Parkinsonism.[10] The age-of-onset is highly variable, but most patients present in childhood to early adulthood with slow progression.[10] The only gene locus confirmed for MPAN to date is Chromosome 19 open reading frame 12 (*C19orf12* *614297), which encodes a mitochondrial membrane protein sharing structure similarity with N-terminal regulatory domain of the magnesium transporter MgtE. C19orf12 localizes at ER–mitochondria contact sites, which may be important for autophagosome formation and is translocated to the cytosol upon oxidative stress induction. In addition, overexpression of wild-type C19orf12 increased lipidated LC3 and reduced the amount of the autophagy substrate p62. However, overexpression of mislocalized mutants failed to promote autophagy and levels of basal autophagy remained unchanged during exposure to oxidative stress in HeLa cells.[10].

Hereditary spastic paraplegia (HSP)

Hereditary spastic paraplegias (HSP) include a diverse group of inherited neurological diseases characterized by retrograde neurodegeneration of the longest corticospinal motor neurons, manifesting as lower limb hypertonia and weakness, typically accompanied with decreased vibration sense.[11] Some autosomal recessive subtypes can present with additional complications ("complex HSP"), including varying degrees of ataxia, dementia or amyotrophy. Over 70 genetic loci have been mapped for HSPs (SPG1-72).[11] 70% of complex HSPs are caused by clinically indistinguishable mutations in SPG11 (*610844) and SPG15(*270700), manifesting as progressive leg spasticity, amyotrophy, learning difficulties and characteristic pigmentary retinopathy.[11] The SPG11 and SPG15 protein products, spatacsin and spastizin, are required for lysosome reformation and interact with the AP5 complex to regulate late endosome membrane sorting.[12] Spastizin also regulates autophagosome degradation through interaction with Beclin-UVRAG-Rubicon complex.[11]

A rare form of HSP that develops in infancy or teenagers is caused by an autosomal-recessive single base deletion in the tectonin beta-propeller repeat-containing protein 2 gene (*TECPR2* *615000), resulting in formation of premature stop codon.[13] In addition to spastic paraparesis,

patients suffered from mental disability, episodic central hypoventilation and gastroesophageal reflux disease. TECPR2 is a WD repeat protein that positively regulates autophagosome formation. It binds to the COPII coat protein SEC24-related gene family, member D (SEC24D *607186) to maintain export from ER exit site (ERES) and its function was shown to be dependent on its interaction with lipidated LC3C.[13]

Adaptor protein-4 (AP-4) deficiency syndrome is a subtype of complex HSP caused by autosomal recessive mutations of any subunit of AP-4 (SPG47 and 52 *614066, *614067).[14] Patients exhibit a complex phenotype, including early hypotonia progressing to hypertonia, intellectual disability, and microcephaly. As a coat protein, AP-4 is recruited onto membranes with their selected cargo to sort them into trafficking vesicles. AP4 sorts mATG9 from the trans-Golgi network to maintain the axonal delivery of mATG9.[15]

A clinically distinct autosomal recessive form of HSP, presenting as mutilating sensory neuropathy with spastic paraplegia, with evidence of severe posterior column atrophy, can be caused by autosomal recessive mutations of CCT5, the epsilon subunit of the cytosolic chaperonin-containing t-complex-1 (*610150).[16] The CCT complex prevented accumulation various autophagy substrates primarily by mediating autophagy rather than through its direct chaperone activity preventing aggregation of such proteins. CCT5 promotes autophagosome degradation by regulating lysosome function, as it was shown that CCT5 disruption reduced the trafficking of lysosomal enzymes and the v-ATPase to lysosomes, possibly via interactions with cytoskeleton proteins.[17]

Huntington's disease

Huntington's disease is an inherited neurodegenerative proteinopathy caused by N-terminal polyglutamine tract expansions in huntingtin, resulting in chorea, dementia and psychiatric symptoms. The 5' end of the huntingtin gene has a block of repeated cytosine-adenine-guanine (CAG) trinucleotides. Normally, the CAG segment repeats itself about 10 to 35 times, while people with or more than 36 CAG trinucleotide repeats almost always develop Huntington disease. The average age of onset is 40 and is inversely correlated with the size of the CAG repeat.[18] Juvenile forms of HD (onset <20 y), comprises 7% of HD cases, and is caused by high numbers of CAG gene repeats (60 or more).[18] The clinical signs in pediatric HD differs from that of classical HD – chorea is often absent, with developmental delay, cerebellar ataxia, rigidity or seizures being more prominent features.[19]

Wild-type huntingtin protein positively regulates selective autophagy in stress conditions by binding and releasing ULK1 from mTOR, and facilitating the interaction of p62 adaptor with LC3.[19] Mutant huntingtin, which is aggregate-prone and toxic, can be cleared by autophagy.[19] In addition, the soluble huntingtin mutant protein is also toxic and competes with protective deubiquitinating enzyme Ataxin 3 (*607047) for binding with Beclin-1, thereby inhibiting autophagy. In the brain samples from Huntington's disease patients, Beclin-1 levels and LC3-II levels decreased.[19]

Vici syndrome

Vici syndrome is a severe, multisystem disorder resulting from an autosomal recessive mutations in EPG5 (P-granules autophagy protein 5 *615068), resulting in corpus callosum agenesis, cardiomyopathy, cataracts, oculocutaneous albinism and combined immunodeficiency. The presence of the additional three features of microcephaly, developmental delay and failure to thrive increases the likelihood of an EPG5 mutation to 89%.[20] The median survival for the disorder is 24 months, with recurrent sepsis and cardiorespiratory failure being the most common causes of premature mortality.[20] EPG5 encodes ectopic P-granules autophagy protein 5, a RAS-associated protein (Rab7 *602298) effector which is important for autophagosome-lysosome fusion. Loss of EPG5 activity caused elevated assembly of STX17-SNAP25-VAMP8 complexes, leading to abnormal fusion of autophagosomes with various endocytic vesicles.[20]

Leukoencephalopathy (LE)

Leukoencephalopathies comprise of a broad group of heterogeneous disorders of multiple aetiologies, characterized by white matter abnormalities in the central nervous system. A form of LE results from a missense vacuolar protein sorting 11 (VPS11 * 608549) mutation causing severe hypotonia, microcephaly, developmental delay and seizures. The C846G VPS11 mutation caused aberrant ubiquitination and accelerated its turnover, which impaired autophagy in human cells. VPS11 is a core subunit of endosomal tethering complexes, which are recruited onto autophagosomes and interact with SNAREs to mediate membrane fusion. As a component of HOPS complex, loss of VPS11 function caused accumulation of p62 and lipidated LC3, indicating that it is required for autophagosome clearance.[21]

Lafora disease

Lafora disease is a rare condition belonging to a group of progressive myoclonic epilepsies, resulting from autosomal recessive mutation in either EPM2A (*607566) or EPM2B (*608072), encoding laforin and malin, respectively. Typically, the disease onset is in late childhood, when first symptoms of myoclonus and occipital seizures appear, which can present as transient blindness or prominent visual hallucinations. This is followed by rapid dementia and worsening of intractable tonic-clonic seizures, eventually leading to death within 10 years of symptom onset. Patients manifest an accumulation of abnormally branched glycogen in the brain, liver and sweat glands in form of Lafora Bodies. Laforin, a dual-specificity protein phosphatase, has been shown to be involved in dephosphorylation of glycogen, ensuring its appropriate branching and solubility. Malin, which forms a complex with laforin, is an E3 ubiquitin ligase and might play a role in activation of glycogen synthase as well as ubiquitin-dependent proteasomal degradation of proteins involved in glycogen biosynthesis. While evidence suggests that aberrant glycogen accumulation might be crucial for the development of the disease, defects of autophagosome have also been demonstrated in this condition.[22]

Tuberous sclerosis complex

Tuberous sclerosis is an autosomal-dominant neurocutaneous disorder seen in 1:6000 births, characterised by benign tumour growth in multiple locations, including the brain, kidneys, lungs and heart. Affected patients tend to present with neurological symptoms, such as seizures, developmental delay, or autism. The disease-causing mutations for tuberous sclerosis have been mapped to TSC1 (*605284) and TSC2 (*191092), also known as hamartin and tuberin. Together with stabilising protein TBC1 domain family member 7 (TBC1D7 *612655), TSC1 and TSC2 form a heterotrimeric complex, which negatively regulates Rheb GTPase, and in turn, the mTORC1 pathway, which is usually tightly controlled by signals including energy levels, nutritional status or growth factors. TSC1 or TSC2 mutations cause mTORC1 hyperactivation, which reduces autophagosome formation, among other effects. Knowledge of this pathway has spawned multiple clinical trials utilising pharmacological mTORC1 inhibitors, such as rapamycin and everolimus, which appear to reduce the size of the tumours in the brain and kidneys. However, the tumours tend to regrow after withdrawal of treatment, necessitating a lifelong therapy with mTORC1 inhibitors.[23] .

Charcot-Marie-Tooth disease

Charcot-Marie-Tooth (CMT) disease, also called hereditary motor and sensory neuropathy (HSMN), has a prevalence of 1:2500 individuals. Classically, CMT patients present with difficulty walking, high stepping gait, pes cavus, leg weakness and wasting of distal muscles of the upper and lower limb. Nerve conduction studies show either a symmetrical demyelinating, axonal or mixed pathology. To date, more than 70 genes and risk loci have been identified for the disease.[24]

A mutation in dynein cytoplasmic 1 heavy chain 1 (DYNC1H1 *600112) coding for a cytoplasmic dynein causes hereditary childhood-onset spinal muscular atrophy with predominant involvement of the lower limbs, hence phenotypically resembling CMT.[24] Disruption of dynein function decreases autophagic clearance of aggregate-prone protein and increases lipidated LC3 levels, possibly through impairment of microtubule-dependent autophagosome-lysosome fusion.[2]

Dynamin 2 (*602378) mutations can also cause CMT associated with neutropenia and early-onset cataracts, as well as dominant centronuclear myopathy, presenting as distal muscle weakness, pes cavus, facial weakness and ptosis. Loss of Dynamin 2 function causes the accumulation of immature autophagosomes, probably due to defective lysosome acidification.[24]

Lysosome storage diseases

Lysosome storage diseases are caused by genetic deficiencies of lysosomal proteins, including enzymes required for degradation in lysosomes, leading to the inappropriate storage of material in various parts of the body, including the nervous system. While a detailed discussion of this group of diseases is beyond the scope of this review, these do impact on autophagic flux, as lysosome storage diseases are associated with defective autophagosome-lysosome fusion.[25]

Conclusions

This review highlights that the autophagic process can be disrupted at multiple stages of its itinerary in different childhood-onset neurological diseases (Fig 1). This will likely lead to the accumulation of aggregation-prone proteins and an increased vulnerability to different stresses. While it is possible that pathology in many of these diseases may also be due to autophagy-independent processes, the identification and characterisation of the autophagy defect may provide opportunities for therapeutic strategies.

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Abbreviations: TSC – Tuberous Sclerosis Complex, HSP – Hereditary Spastic Paraplegia, HD – Huntington's disease, LD – Lafora disease, MPAN – Mitochondrial-membrane Protein-Associated Neurodegeneration, CMT – Charcot Marie Tooth disease, LSD – Lysosomal Storage Disorders, SMA – Spinal Muscular Atrophy

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Affected stage of autophagy pathway	Gene	Disease
Initiation and signalling	*ATG5*	Ataxia
	EPM2A/EPM2B	Lafora disease
	TSC1/TSC2	Tuberous sclerosis
Precursor formation	*AP4*	A rare form of hereditary spastic paraplegia
	SPG43	Mitochondrial-membrane Protein-Associated Neurodegeneration (MPAN)
	SPG49/TECPR2	A rare form of hereditary spastic paraplegia
Adaptors	*P62*	Childhood onset neurodegeneration with Ataxia
	HTT	Huntington's disease
Maturation	*WIPI4*	Beta-propeller Protein-associated Neurodegeneration (BPAN)
	EPG5	Vici Syndrome
Autolysosome formation	*VPS11*	Leukoencephalopathy
Lysosome function	*CCT5*	Mutilating sensory neuropathy, a rare form of hereditary spastic paraplegia
	SPG11/SPG15	Hereditary spastic paraplegia
	SNX14	Cerebellar ataxia syndrome (SCAR17)
Lysosome storage diseases (LSDs) that are neurological and childhood onset	*NPC1*	Niemann-Pick Type C (NP-C) disease
	GBA	Gaucher's disease
	GALC	Krabbe disease
	IDUA	Mucopolysaccharidosis
	β-hexosaminidase	GM2 Gangliosidoses
	TFEB	LSDs including Gaucher's disease
	SMUF1	Multiple sulfatase deficiency
	Iduronate-2-sulfatase (*I2S*)	Hunter syndrome
Trafficking	Dynamin 2	Charcot-Marie-Tooth and Centronuclear Myopathy
	DYNC1H1	Charcot-Marie-Tooth and Spinal muscular atrophy

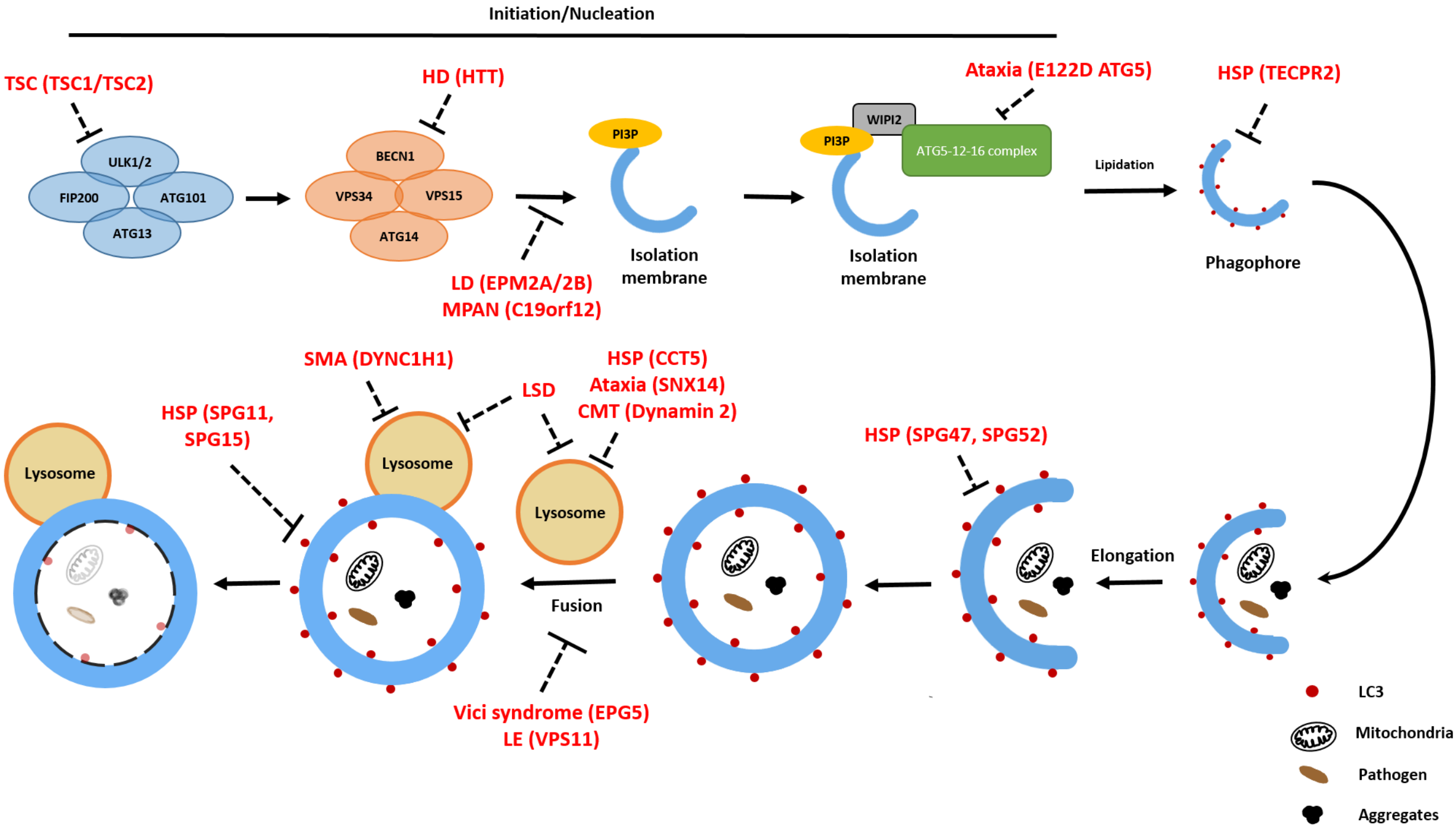
Table 1: List of genes and diseases associated with impaired autophagy, showing where the defect may be in the autophagy pathway.

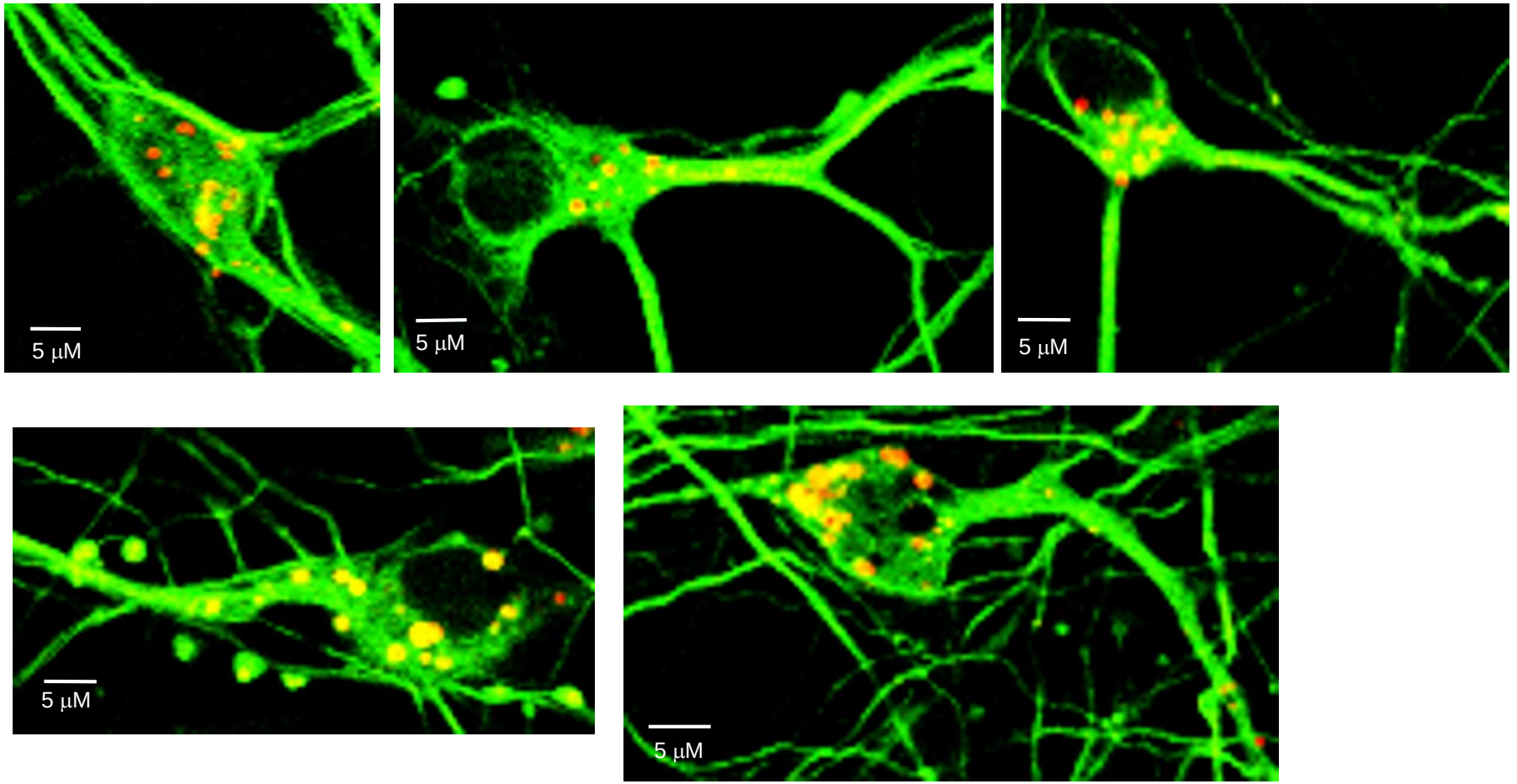
Figure 1: The autophagic pathway, with known childhood neurological disorders affecting the process highlighted in red.

Upstream autophagy initiation signals converge at ULK1/2 signalling pathway, which activates VPS34 complex by phosphorylation of FIP200, a member of VPS34 complex. VPS34 complex stimulates formation of PI3P, recruiting WIPI proteins and ATG5-12-16 complex, which direct lipidation of the isolation membrane aiding the formation of a cup-shaped phagophore. ATG5-12-16 complex then orchestrates expansion and maturation of the phagophore into a LC3-II containing autophagosome via series of conjugation processes, while substrates for autophagy are engulfed by the extending autophagosomal membrane. In the last step, the autophagosomes fuse with lysosomes, facilitating cargo degradation and recycling.

Figure 2: Autophagosomes (yellow) and autolysosomes (red) in primary neurons.

The image shows primary neurons from a transgenic mouse model expressing mRFP-GFP-LC3 (ref. 17). This model allows one to distinguish non-acidified autophagosomes (red and green = yellow) from acidified autolysosomes (red only), as the GFP fluorescence is more rapidly quenched by the low lysosomal pH.


Initiation/Nucleation
TSC (TSC1/TSC2)
ULK1/2
FIP200
ATG101
ATG13
HD (HTT)
BECN1
VPS34
VPS15
ATG14
PI3P
LD (EPM2A/2B)
MPAN (C19orf12)
Isolation membrane
WIPI2
PI3P
ATG5-12-16 complex
Ataxia (E122D ATG5)
Isolation membrane
Lipidation
HSP (TECPR2)
Phagophore
Elongation
HSP (SPG47, SPG52)
Lysosome
Fusion
SMA (DYNC1H1)
LSD
HSP (CCT5)
Ataxia (SNX14)
CMT (Dynamin 2)
HSP (SPG11, SPG15)
Vici syndrome (EPG5)
LE (VPS11)
LC3
Mitochondria
Pathogen
Aggregates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