WDR45, one gene associated with multiple neurodevelopmental disorders

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ABSTRACT

The WDR45 gene is localized on the X-chromosome and variants in this gene are linked to six different neurodevelopmental disorders, i.e., β-propeller protein associated neurodegeneration, Rett-like syndrome, intellectual disability, and epileptic encephalopathies including developmental and epileptic encephalopathy, early-onset epileptic encephalopathy and West syndrome and potentially also specific malignancies. WDR45/WIPI4 is a WD-repeat β-propeller protein that belongs to the WIPI (WD repeat domain, phosphoinositide interacting) family. The precise cellular function of WDR45 is still largely unknown, but deletions or conventional variants in WDR45 can lead to macroautophagy/autophagy defects, malfunctioning mitochondria, endoplasmic reticulum stress and unbalanced iron homeostasis, suggesting that this protein functions in one or more pathways regulating directly or indirectly those processes. As a result, the underlying cause of the WDR45-associated disorders remains unknown. In this review, we summarize the current knowledge of the cellular and physiological functions of WDR45 and highlight how genetic variants in its encoding gene may contribute to the pathophysiology of the associated diseases. In particular, we connect clinical manifestations of the disorders with their potential cellular origin of malfunctioning and critically discuss whether it is possible that one of the most prominent shared features, i.e., brain iron accumulation, is the primary cause for those disorders.

Keywords: ATG/Atg: autophagy related; BPAN: β-propeller protein associated neurodegeneration; CNS: central nervous system; DEE: developmental and epileptic encephalopathy; EEG: electroencephalograph; EN02/neuron-specific enolase, enolase 2; EOEE: early-onset epileptic encephalopathy; ER: endoplasmic reticulum; ID: intellectual disability; IDR: intrinsically disordered region; MRI: magnetic resonance imaging; NBI: neurodegeneration with brain iron accumulation; NCOA4: nuclear receptor coactivator 4; PtdIns3P: phosphatidylinositol-3-phosphate; RLS: Rett-like syndrome; WDR45: WD repeat domain 45; WIPI: WD repeat domain, phosphoinositide interacting

WDR45/WIPI4

The human WDR45 gene is located on the X-chromosome [1] and is ubiquitously expressed in all tissues [2,3]. Spatial expression profiling in mice brains revealed that WDR45 is more abundantly expressed in the hippocampus, a region involved in learning and memory, compared to the rest of the brain [4]. Human WDR45 encodes for a protein of 360 amino acids, which is composed of a N-terminal β-propeller followed by a short intrinsically disordered region (IDR) (Figure S1A). β-propellers are structural motifs that are present in numerous proteins and are mostly involved in protein-protein interactions [5,6]. They are built of four to eight blades/β-sheets, consisting of a four-stranded anti-parallel β-strands. These blades are packed in a circular, ring-like array. β-propellers are often composed by the so-called WD-repeats, although not all of them contain this amino acid sequence motif [7,8]. The WD-repeat was originally defined as a core unit of approximately 40 amino acids, ending with tryptophan (W) and aspartic acid (D) residues [9]. WD-repeat-containing proteins form a large protein family that is present in all eukaryotes. They are involved in a variety of functions, including transcription regulation, signal transduction, cell cycle control, autophagy and apoptosis [7]. WDR45 is one the WD-repeat proteins [5,10]. The WD-repeats of WDR45 are bracketed by glycine-histidine and tryptophan-aspartic acid pairs, and are predicted to fold into a 7-bladed β-propeller (Figure S1A-B) [5]. Although the crystal structure of WDR45 is not resolved yet, based on the structure of some of its human and yeast homologs (see below) that have been solved [11–14], the overall conformation of WDR45 can be predicted with accuracy (Figure S1B).

WDR45 is highly conserved in mammals, and the amino acid sequences of the human, mouse, pig, bovine and horse protein have more than 97% identity [15]. Together with WIPI1, WIPI2 and WIPI3/WDR45B, it forms the WIPI (WD repeat domain, phosphoinositide interacting) protein family [9]). This is why
WDR45 is also known as WIPI4. Like WDR45, all WIPI proteins have 7 WD repeats and can be grouped into two clades according to their amino acid sequence identity. One clade contains WIPI1 and WIPI2, and the other WDR45B and WDR45 (Figure S1C) [15]. The first blade and the C-terminal IDR are the regions that are most divergent between the members from the two clades (Figure S1D). Consequently, the amino acid sequence identity and similarity between the members of the different clades are both less than 31%, while the sequence identity and similarity between human WDR45 and WDR45B is 46.0% and 59.8%, respectively, and between WIPI1 and WIPI2 is 53.8% and 68.1%, respectively. These characteristics also explain the redundant roles in autophagy between the members of the same clade. A common characteristic of all WIPI proteins is the F/LRRG motif that allows them to bind to phosphoinositides, and therefore WIPI proteins are also known as β-propellers that bind polyphosphoinositides (PROPPINs) [16]. This motif is localized in blade 6 (Figure S1D) and mediates their specific binding to phosphatidylinositol-3-phosphate (PtdIns3P) and phosphatidylinositol-3,5-biphosphate [3,15,17].

WIPI proteins have homologs in all eukaryotic species and their number varies in between organisms [3,18]. For example, yeast *Saccharomyces cerevisiae* possesses 3 WIPI proteins (Atg18, Atg21 and Hsv2), while *Caenorhabditis elegans* has 2 (APG-18 and EPG-6) [19–21]. Phylogenetic analyses have shown that the majority of the WIPI protein homologs fall in one of the two clades described above [3,18], probably reflecting different conserved molecular functions.

**The role of WDR45 and the other WIPI proteins in autophagy**

WIPI proteins are PtdIns3P-binding effectors that are part of the ATG machinery [10]. Autophagy is an evolutionary conserved process essential to maintain cellular homeostasis, in which cell components such as dysfunctional and/or aggregated proteins, damaged or redundant organelles, and other cytoplasmic material, are degraded in lysosomes for the subsequent recycling of the resulting metabolites (Figure 1) [22,23]. Autophagy is maintained at a basal level in most cells and is induced by nutrient deprivation and other stresses [22,23]. This process is characterized by the maturation of a transient membranous structure known as the phagophore, into a double-membraned vesicle, called the autophagosome, which then fuses with lysosomes to release its cytoplasmic cargo into the hydrolytic interior of this organelle [22,23]. This process is regulated by ATG (autophagy-related) proteins, which are organized in functional complexes [22,23]. One of them, is the class III phosphatidylinositol 3-kinase complex, which comprises BECN1, ATG14, PIK3R4/VPS15, PIK3C3/VPS34 and NRB2F2, and catalyzes the local synthesis of PtdIns3P on the phagophore membrane [23–25]. PtdIns3P has an important function in the formation of autophagosomes as it acts as a scaffold for the recruitment of specific components of the ATG machinery [23,24]. The expansion and eventual closure of the phagophore to form an autophagosome depends on conjugation of members of the MAP1LC3/LC3 (microtubule associated protein 1 light chain 3) protein family to phosphatidylethanolamine, a process that involves two ubiquitin-like conjugation systems [23]. First, the E1 enzyme-like ATG7 and the E2 enzyme-like ATG10 lead to the formation of the ATG12–ATG5–ATG16L1 complex. Second, the E1 enzyme-like ATG7 and the E2 enzyme-like ATG3, guided by the ATG12–ATG5–ATG16L1 complex, promote the formation of the conjugates between LC3 proteins and phosphatidylethanolamine on the phagophore membrane [23,26].

WIPI proteins participate to both the biogenesis and maturation of autophagosomes (Figure 1) [10]. The members of the two different evolutionary clades, i.e., WIPI1/WIPI2 and WDR45B/WDR45, function differently in these two steps of autophagy [15,21,27]. WIPI2 has been shown to connect PtdIns3P production with the recruitment of the ATG12–ATG5–ATG16L1 complex for subsequent LC3 lipidation at

![Figure 1. The autophagy pathway and the role of the WIPI proteins.](image-url)
the phagophore [15], with ATG16L1 directly binding to WIPI2 [28]. In addition, WIPI2 interacts with RAB11A at early stages of autophagosome formation [29]. WIPI1 also acts at early steps of autophagy [30] and interacts with WIPI2, possibly supporting WIPI2 in ATG16L1 recruitment for LC3 protein lipidation [27,31]. The notion that WIPI1 and WIPI2 have a function different from WDR45 (and WDR45B), i.e., act at a different step of autophagosome formation, is supported by the observation that depletion of WIPI1 or WIPI2 suppresses the formation of LC3 puncta, while downregulation of WDR45B or WDR45 enhances their number. This suggests that WIPI1 and WIPI2 are mainly involved in the formation of phagophores while WDR45B and WDR45 are principally participating in phagophore expansion and/or closure, or even autophagosome maturation [15,21,27]. Thus, WDR45 and WDR45B, mainly function downstream of WIPI1 and WIPI2, and possibly control the size and maturation of nascent autophagosomes [27]. In C. elegans, mutants of atg-18, the WIPI1 and WIPI2 homolog, and epg-6, the WDR45B and WDR45 counterpart, show distinct phenotypes in autophagy with ATG-18 functions upstream of EPG-6 [21], confirming a different function of those proteins in autophagy.

Genetic studies in mice have revealed that the central nervous system (CNS)-specific wdr45−/− deficient mice display an impaired autophagy flux with accumulation of SQSTM1/p62- and ubiquitin-positive protein aggregates in neurons (Figure 2A) [2]. The autophagy defect in wdr45−/− mice, however, is weaker compared to mice lacking core Atg genes, which may indicate that the autophagy impairment is restricted to neurons [2]. Similar to the conditional wdr45−/− animals, full body wdr45b−/− mice also show an autophagy defect [4], however, the affected brain regions are not overlapping, i.e., defects were most obvious in thalamus, cortex and hippocampus for wdr45−/− animals while the cerebellum was mostly affected in the wdr45b−/− mice, indicating that these genes are required for autophagy in different brain regions. Interestingly, wdr45b−/− wdr45−/− double deficient mice exhibit a more severe autophagy defect in the brain than single knockout animals and moreover, die within a day after birth similar to mice lacking core Arg genes [4]. These observations also support the notion that WDR45B and WDR45 function redundantly, and that these two proteins are critical for neural homeostasis.

Although there are not too many functional lines of evidence to explain the involvement of WDR45 in autophagy, it has been very well established that this protein interacts with ATG2A and ATG2B [27,32–36], stronger than do other WIPI proteins [32]. ATG2A and ATG2B have redundant functions that are essential for autophagy progression [37]. Binding between ATG2A and WDR45 has been suggested to be mediated by Asp1376, Glu1378 and Lys1539 of ATG2A, and Lys89 and Lys134 of WDR45 [32,34] and via a WIPI-interacting-region (WIR)-motif present in ATG2A [38]. Mechanistically, this latter motif unwinds and wraps around WDR45 (or other WIPI proteins) and binds to three sites on three different propeller blades [38]. Two other residues in WDR45, Asn15 and Asp17, may also mediate WDR45 binding to ATG2A [27]. Crucially, it has been shown that ATG2 proteins are able to transfer phospholipids between adjacent liposomes [35,36,39] and binding of WDR45 to ATG2A or ATG2B accelerate this lipid transfer activity [36,40]. Since the ATG2A-WDR45 complex tethers membranes in vitro and probably in vivo [34,41], it might well be that it is involved in the transport of lipids from the ER to one or more subcellular compartments, including nascent autophagosomes.

The functional relevance in autophagy of the ATG2A-WDR45 and ATG2B-WDR45 complexes remains to be fully established, especially because WDR45 appears not to play a similar major role in autophagy as the ATG2 proteins [42]. Although, ATG2A and ATG2B are required for phagophore formation during autophagy [37], their interaction with WDR45 is dispensable for phagophore formation and therefore not required for early autophagy events [42]. However, mutations in the WIR-motif in ATG2A could not rescue the autophagy defect phenotype of ATG2A-depleted cells, indicating that this complex is required for autophagy [38].

WDR45 also forms a larger complex that contains ATG2A, ULK1 and S’ AMP-activated protein kinase (AMPK) under nutrient-rich conditions. When autophagy is induced or AMPK is specifically activated, WDR45 and ATG2A dissociate from this complex and more WDR45-positive punctate structures are formed [27,31]. Thus, autophagy induction leads to the activation and translocation of ATG2A-WDR45 possibly onto phagophore membranes, contributing to autophagosome formation [31]. Importantly, under autophagy-induced conditions, WDR45 localizes with WDR45B, which is also found on lysosomal structures, indicating that WDR45-ATG2A might also have a role in autophagosome maturation [27]. It remains unclear whether the ATG2B-WDR45 is regulated in a similar manner.

**WDR45 functions in other cellular pathways**

Increasing evidence shows that WDR45 also functions in other important cellular pathways, such as endoplasmic reticulum (ER) homeostasis or mitochondria organization (Figure 2A). In full body wdr45−/− mice, large amounts of ER proteins significantly accumulate in the brain. This accumulation is caused by a reduced ability in degrading ER proteins via the proteasome and lysosome, resulting in ER expansion, elevated ER stress and eventually cell death [43]. By treating wdr45−/− neurons or WDR45-depleted HeLa cells with the autophagy inducer rapamycin or by reducing ER stress, those cells were rescued from undergoing apoptosis, indicating that autophagy induction can compensate to a certain degree the cellular insults caused by WDR45 depletion. Although, this result suggests that WDR45 is involved in ER quality control, the ER stress in wdr45−/− mice was only observed in old animals, whereas the autophagy defect was already detected in young animals, indicating that impaired autophagy might also contribute to the enhanced ER stress.

Loss of WDR45 leads to a variety of morphological alterations in mitochondria as well. An abnormal accumulation of mitochondrial proteins and enlarged mitochondria are observed in wdr45−/− deficient mice brain and WDR45−/− HeLa cells, respectively [43,44]. These data highlight that WDR45 loss in neuronal and non-neuronal cells cause mitochondrial damage and oxidative stress, pointing to a possible autophagy-independent function of WDR45. Notably, two mass spectrometry analyses have uncovered interactors of WDR45 that have not been connected to autophagy yet. This observation supports the notion of a possible autophagy-independent function of WDR45.
**WDR45-associated disorders**

Gene mutations in WDR45 are associated with several neurodevelopmental and neurodegenerative disorders and have been shown to can occur sporadically. The phenotypic spectrum of patients with WDR45 variants is broad, ranging from severe early-onset epileptic encephalopathies in males to mild cognitive impairment in females. The differences in severity between male and female patients is very likely because WDR45 is X-chromosome linked. The course of WDR45-associated disorders is mostly progressive and patients with mild and relative static cognitive issues in childhood, can develop dystonia and dementia later in adulthood. Six different, albeit overlapping disorders, i.e., β-propeller protein associated neurodegeneration (BPAN), Rett-like syndrome (RLS), intellectual disability (ID), developmental and epileptic encephalopathy (DEE), early-onset epileptic encephalopathy (EOEE) and West syndrome, have been associated with variants in WDR45. The vast majority of the WDR45 variants (81%) are de novo nucleotide changes, but there are 9 cases, including BPAN and West syndrome clinical manifestations, where the variant has been inherited (Table 1). The origin of the variants has not been determined in 12% of the cases (Table 1).

To get an overview of all the documented patients that harbor a WDR45 variant (independently of the origin of the
variant), we performed a systematic literature search. This search revealed that to date (November 2020), 140 WDR45 variants have been identified and associated to a pathology (Table 1). In a recent report, limited to 123 patients [45] 17 new cases were described, but not conclusively categorized into any diseases. We therefore, listed those cases as “unclassified” in our analysis (Table 1, Figures 2B and 5). Altogether, our analysis showed that WDR45 variants have led to a BPAN, a RLS, an ID a DEE a EOEE and a West syndrome diagnosis in 66.4%, 2.1%, 1.4%, 12.1%, 2.9% and 2.1% of the cases respectively (Figure 2B). 12.9% of the cases are unclassified (Figure 2B). This analysis further revealed that nine different WDR45 variants can lead to different clinical manifestations thereby causing different disorders (Table 2).

β-propeller protein associated neurodegeneration (BPAN)

Variants in WDR45 have been identified in numerous patients suffering from neurodegeneration with brain iron accumulation (NBIA) (Figure 2A). NBIA is characterized by iron accumulation generally observed in the globus pallidus and substantia nigra, and occasionally in the cortex and cerebellum [44,46,47]. So far 10 different genes have been identified and associated with the different NBIA subtypes [48].

In 2012, two laboratories discovered that variants in WDR45 were responsible a particular subset of idiopathic NBIA patients [46,49,50]. These patients were characterized by a global developmental delay in early childhood, intellectual disability, impairment of motor skills, speech and seizures. The difference between these patients and the ones suffering from other NBIA pathologies, was that symptoms of the disease remained non-progressive until early adulthood or even later before they started to exhibit signs of parkinsonism, dystonia and dementia [49]. This type of NBIA was originally described as static encephalopathy of childhood with neurodegeneration in adulthood (SENDA) [50], but subsequently replaced with BPAN [46,49]. It is estimated that BPAN accounts for 35–40% of all cases of NBIA [51].

The pathognomonic sign of BPAN is the presence of a hyperintense ‘halo’ on T1-weighted MRI images at the level of substantia nigra and the cerebral peduncle, but less in globus pallidus. This is a unique imaging characteristic, in addition to the diagnosis by gene sequencing, that permits to distinguish BPAN from other forms of NBIA [44,46]. Currently, there are no specific biomarkers for BPAN, making clinical diagnosis difficult especially in early infancy. In one case report, however, a persistent elevation of the ENO2/NSE (enolase 2) in both serum and cerebrospinal fluid was found in a patient still in childhood age. ENO2 is an enzyme that is exclusively expressed in neuronal cells and has been shown to be a reliable marker protein for neural injury [52]. This finding therefore indicates that neuronal damage might already start in infancy but also that ENO2 might be a suitable biomarker for an early diagnosis of BPAN [53]. Another retrospective study that summarized clinical characteristics of ten BPAN children found that a calculated ratio of plasma:serum concentration of soluble (s) TFRC (transferrin receptor) divided by logarithm (log) of plasma:serum concentration of FT (ferritin; sTFRC/logFT) might be another possible biomarker to detect BPAN. Other biomarkers connected to iron metabolism, including iron, transferrin, ferritin levels in the serum, however, were normal. Interestingly, 5 out of 6 patients that were tested for ENO2 also showed an increased serum level of ENO2 [54]. Despite these encouraging observation, analyses of additional patients are necessary to confirm whether ENO2 levels or sTFRC/logFT in the serum could indeed be used as a biomarker for an early diagnosis [46,55].

BPAN-associated genetic changes are quite different in nature, ranging from missense to nonsense variants, frame shifts, splicing defects and deletions (Table 1). Moreover, mapping all BPAN-associated variants over the entire WDR45 sequence, did not results in specific domains that could be associated to BPAN (Figure 3). So far, 93 patients carrying a WDR45 variant have been conclusively associated to BPAN, and 84 of them are females while only 9 are male (Table 1). Notably, male cases that have somatic mosaic variants or germline variants, albeit rare, in WDR45 can survive but are associated with a more severe phenotype than those observed in heterozygous females [45,56–58]. This gives rise to the assumption that the majority of germline WDR45 variants are likely lethal for males probably because this gene is located on the X chromosome.

Rett-like syndrome (RLS)

RLS is characterized by seizures, motor abnormalities, abnormal hand movements, problems with language, features of autism and the tendency to lose skills throughout adulthood [59]. These symptoms start between 6 and 18 months of age [59]. The most common genetic cause for RLS has been attributed to MECP2 variants, but in recent years several other genes, including WDR45, CDKL5 and FOXG1, have been linked to RLS as well [59]. We found in total seven diagnosed RLS cases with WDR45 variants that are all female (Table 1). As for BPAN, the variants associated with RLS are distributed over the entire WDR45 amino acid sequence (Figure 4). Overlapping clinical features exist between early stages of BPAN and RLS, e.g., developmental delay, stereotypical hand movements, seizures, sleep disorder and spasticity. Therefore, in four out of the seven cases (Table 1), patients that were initially diagnosed with RLS or displayed RLS-like symptoms, were later reevaluated to have BPAN, based on iron accumulation that only manifested at an older age [60–63]. Although careful examination of brain MRI scans in retrospect might reveal signs of iron accumulation [62,63], it is often very difficult to detect this at early stages of BPAN [46]. Therefore, to avoid misdiagnosis and as proposed already in 2013 by Hayflick and colleagues [46], genetic testing for WDR45 variants of putative RLS patients, especially with no variants in common RLS-associated genes, should be performed or even included into the gene testing panel for RLS (https://www.ncbi.nlm.nih.gov/igr/tests/500160/).

Although cases are diagnosed with RLS [55,64,65], longitudinal follow-up of those patients will show whether they will evolve BPAN pathology (e.g., signs of iron accumulation in the brain). Since the overall frequency of WDR45 variants in RLS cases is rather low compared to the overall numbers of RLS patients, more studies/patient follow-ups are required to confirm whether a RLS diagnosis could reflect an early symptom of BPAN in patients with WDR45 variations.

Intellectual disability (ID)

ID is defined by significant impairment of cognitive and adaptive functions with the disease onset occurring before 18 years
Table 1. Reported WDR45 variants associated with different diseases.

| Diagnosis | WDR45 variant | Inheritance | Variant type | Sex | Age | Reference |
|-----------|---------------|-------------|--------------|-----|-----|-----------|
| BPAN      | c.1A>G        | de novo     | nonsense     | F   | 31  | 46        |
| BPAN      | c.15del       | de novo     | nonsense     | F   | 30  | 49        |
| BPAN      | c.1362del     | unknown     | nonsense     | F   | 10  | 54        |
| BPAN      | c.19C>T       | de novo     | nonsense     | F   | 34  | 49        |
| BPAN      | c.19dup       | de novo     | frame shift  | M   | 31  | 49        |
| BPAN      | c.127dup(hemi) | inherited  | frame shift  | F   | 34  | 112       |
| BPAN      | c.39G>C       | de novo     | missense     | F   | 44  | 49        |
| BPAN      | c.55+1G>C     | de novo     | splicing defect | F  | 43  | 49        |
| BPAN      | c.55+1G>C     | unknown     | splicing defect | M  | 10mo| 54        |
| BPAN      | c.56G>A       | de novo     | splicing defect | F  | 22  | 49        |
| BPAN      | c.64del       | de novo     | frameshift   | F   | 36  | 113, 114  |
| BPAN      | c.135dup      | unknown     | frameshift   | F   | 2   | 115, 116  |
| BPAN      | c.1312A>G     | de novo     | splicing defect | F  | 15  | 54        |
| BPAN      | c.161_163del  | inherited   | deletion     | M   | 20  | 57        |
| BPAN      | c.161_163del  | inherited   | deletion     | F   | 14  | 57        |
| BPAN      | c.183G>A      | de novo     | missense     | F   | 17  | 49        |
| BPAN      | c.196del      | de novo     | frameshift   | F   | 16  | 46        |
| BPAN      | c.224C>A      | unknown     | nonsense     | F   | 1   | 116       |
| BPAN      | c.228-229del  | de novo     | frameshift   | M   | 37  | 49        |
| BPAN      | c.235+1G>A    | de novo     | splicing defect | F  | 35  | 49        |
| BPAN      | c.236-18A>G   | de novo     | splicing defect | F  | 6   | 117       |
| BPAN      | c.240G>A      | de novo     | nonsense     | F   | 29  | 118       |
| BPAN      | c.251A>G      | de novo     | missense     | F   | 18  | 119       |
| BPAN      | c.293T>C      | de novo     | missense     | F   | 29  | 49        |
| BPAN      | c.293T>C      | de novo     | missense     | F   | 41  | 120       |
| BPAN      | c.299T>C      | de novo     | missense     | F   | 6   | 121       |
| BPAN      | c.305dup      | de novo     | frame shift  | M   | 44  | 122       |
| BPAN      | c.322del      | de novo     | frameshift   | F   | 39  | 122       |
| BPAN      | c.342-2A>C    | de novo     | splicing defect | F  | 15  | 123       |
| BPAN      | c.344-44A>C   | de novo     | splicing defect | M  | 10  | 54        |
| BPAN      | c.344+1G>A    | de novo     | splicing defect | F  | 124  | 124       |
| BPAN      | c.34S+4G>A    | de novo     | splicing defect | F  | N.A. | 125       |
| BPAN      | c.359dup      | de novo     | frameshift   | F   | 38  | 125       |
| BPAN      | c.400C>T      | de novo     | nonsense     | F   | 45  | 120       |
| BPAN      | c.400C>T      | de novo     | nonsense     | F   | 49  | 120       |
| BPAN      | c.400C>T      | de novo     | nonsense     | F   | 5   | 54        |
| BPAN      | c.405C>T      | de novo     | nonsense     | F   | 5   | 54        |
| BPAN      | c.405_409del  | de novo     | frameshift   | F   | 40  | 49        |
| BPAN      | c.411dup      | unknown     | frameshift   | F   | 34  | 122       |
| BPAN      | c.412insT     | de novo     | frameshift   | F   | 39  | 122       |
| BPAN      | c.414_419del  | de novo     | deletion     | F   | 36  | 120       |
| BPAN      | c.437dup      | unknown     | splicing defect | F  | 40  | 50        |
| BPAN      | c.439+1G>T    | de novo     | splicing defect | F  | 33  | 50        |
| BPAN      | c.439+1T>G    | de novo     | splicing defect | F  | 24  | 128       |
| BPAN      | c.447T           | de novo     | nonsense     | F   | 0   | 8         |
| BPAN      | c.447_448del  | de novo     | nonsense     | F   | 8   | 129       |
| BPAN      | c.476del      | de novo     | frameshift   | F   | 43  | 49        |
| BPAN      | c.487del      | de novo     | frameshift   | F   | 25  | 130       |
| BPAN      | c.511C>T      | de novo     | nonsense     | F   | 5   | 54        |
| BPAN      | c.516G>C      | de novo     | frameshift   | F   | 28  | 50        |
| BPAN      | c.519+1_519+3del | de novo*  | splicing defect | F  | 31  | 131       |
| BPAN      | c.519+1G>A    | de novo     | splicing defect | F  | 1   | 132       |
| BPAN      | c.551del      | de novo     | frameshift   | F   | 14  | 133       |
| BPAN      | c.587_588del  | de novo     | frameshift   | F   | 33  | 120       |
| BPAN      | c.587_588del  | de novo     | frameshift   | F   | 37  | 120       |
| BPAN      | c.596del      | de novo     | frameshift   | F   | 34  | 134       |
| BPAN      | c.597_599del  | de novo     | frameshift   | M   | 1   | 54        |
| BPAN      | c.606C>G      | de novo     | missense     | F   | 37  | 135       |
| BPAN      | c.626C>A      | de novo     | frameshift   | F   | 30  | 136       |
| BPAN      | c.628T>C      | de novo     | missense     | F   | 33  | 120       |
| BPAN      | c.637C>T      | unknown     | nonsense     | F   | 51  | 50        |
| BPAN      | c.654del      | de novo     | frameshift   | F   | 13  | 54        |
| BPAN      | c.662_663del  | de novo     | nonsense     | F   | 33  | 137       |
| BPAN      | c.694_703del  | de novo     | frameshift   | F   | 44  | 49        |
| BPAN      | c.700C>T      | de novo     | nonsense     | F   | 39  | 49        |
| BPAN      | c.700C>T      | de novo     | nonsense     | F   | 40  | 138       |
| BPAN      | c.729_72C>G   | de novo     | splicing defect | F  | 22  | 128       |
| BPAN      | c.752_754del  | unknown     | deletion     | F   | 52  | 137       |
| BPAN      | c.830_11G>A   | de novo     | nonsense     | F   | 5   | 60        |
| BPAN      | c.830_11G>A   | de novo     | nonsense     | F   | 6   | 60        |
| BPAN      | c.830_11G>A   | de novo     | nonsense     | F   | 11  | 139       |
| BPAN      | c.830_11G>A   | de novo     | nonsense     | F   | 37  | 49        |
| BPAN      | c.830+2T>C    | de novo     | splicing defect | F  | 27  | 46        |
| BPAN      | c.831-1G>C    | de novo     | splicing defect | F  | 13  | 54        |
| BPAN      | c.835C>T      | unknown     | nonsense     | F   | 12  | 140       |
| BPAN      | c.873C>G      | de novo     | nonsense     | M   | 34  | 58        |
| BPAN      | c.921del      | de novo     | frameshift   | F   | 6   | 78        |
| BPAN      | c.969_970insT | de novo     | frameshift   | F   | 30  | 78, 120   |

(Continued)
Table 1. (Continued).

| BPAN | c.970_971del | de novo | frameshift | F | 4 | 54, 120 |
| BPAN | c.1007_1008del | de novo | nonsense | F | 23 | 49, 54 |
| BPAN | c.1007_1008del | de novo | frameshift | F | 24 | 49 |
| BPAN | c.1007_1008del | de novo | frameshift | F | 31 | 49, 51 |
| BPAN | c.1007_1008del | de novo | frameshift | M | 31 | 49, 51 |
| BPAN | c.1030del | de novo | frameshift | F | 42 | 49, 137 |
| BPAN | c.1033_1034dupAA | de novo | frameshift | F | 33 | 50, 137 |
| BPAN | c.1040_1041del | de novo | frameshift | F | 3 | 50, 141 |
| BPAN | c.1056C>G | unknown | nonsense | F | 9 | 60, 72 |
| BPAN (DEE) | c.814G>A | unknown | nonsense | F | 3 | 60, 72 |
| BPAN (DEE) | c.831T>G | de novo | splicing defect | F | 3 | 53 |
| BPAN (RLS) | c.761_762insAG | unknown | nonsense | F | 16 | 60 |
| BPAN (RLS) | c.830_1G-A | de novo | nonsense | F | 14 | 63 |
| BPAN (RLS) | c.830_1T>C | unknown | splicing defect | F | 42 | 61 |
| BPAN (RLS) | c.868C>T | de novo | nonsense | F | 6 | 62 |
| DEE | c.19C>T | de novo | nonsense | F | 3 | 143 |
| DEE | c.197T>A | unknown | missense | M | 10 | 74 |
| DEE | c.400C>T | de novo | nonsense | F | 6 | 141 |
| DEE | c.454del | de novo | splicing defect | F | 7 | 72 |
| DEE | c.503G>A | de novo | missense | F | 5 | 143 |
| DEE | c.629del | de novo | frameshift | F | 2 | 143 |
| DEE | c.660del | de novo | frameshift | F | 2 | 72 |
| DEE | c.700C>T | de novo | nonsense | F | 2 | 143 |
| DEE | c.700C>T | de novo | nonsense | F | 11 | 72 |
| DEE | c.726C>G | de novo | nonsense | F | 4 | 72 |
| DEE | c.732_745del | de novo | deletion | M | 17 | 143 |
| DEE | c.730_755del | de novo | nonsense | F | 7 | 146 |
| DEE | c.830_1G>A | de novo | nonsense | M | 4 | 147 |
| DEE | c.912del | de novo | frameshift | F | 1 | 143 |
| DEE | c.1007_1008del | de novo | nonsense | M | 2 | 148 |
| DEE | c.1007_1008del | de novo | nonsense | F | 7 | 72 |
| EOE | chrX:48,809,279-48,829,265del | de novo | deletion | M | 3 mo | 56 |
| EOE | c.251del | de novo | missense | F | 8 | 78 |
| EOE | c.344_1G>A | de novo | splicing defect | F | 2 | 78 |
| EOE | c.439T>G | de novo | missense | F | 13 | 78 |
| ID | c.19C>T | de novo | nonsense | F | 3 | 67 |
| RLS | c.777del | de novo | frameshift | F | 29 | 71 |
| RLS | c.235+1G>T | de novo | splicing defect | F | 22 | 65 |
| RLS | c.319_320del | unknown | frameshift | F | 6 | 64 |
| RLS | c.440_2A>G | de novo | splicing defect | F | 5 | 55 |

For West syndrome cases, as reported in the literature and based on the diagnostic criteria, the genetic alterations are inferred.

| West syndrome | c.131_1G>A | de novo | splicing defect | M | 1 | 85 |
| West syndrome | c.2480G>A | de novo | nonsense | M | 2 | 85 |
| West syndrome | c.400T>C | inherited | nonsense | M | 7 | 85 |

| unclassified | c.19C>T | inherited | nonsense | M | N.A. | 45 |
| unclassified | c.522C>T | de novo | nonsense | F | N.A. | 45 |
| unclassified | c.493G>A | de novo | missense | F | N.A. | 45 |
| unclassified | c.412G>T | de novo | nonsense | F | N.A. | 45 |
| unclassified | c.442_446del | inherited | nonsense | M | N.A. | 45 |
| unclassified | c.519_2T>C | de novo | splicing defect | F | N.A. | 45 |
| unclassified | c.587_588del | de novo | frameshift | F | 11 | 149 |
| unclassified | c.700G>T | de novo | nonsense | F | N.A. | 45 |
| unclassified | c.718dupA | de novo | nonsense | F | N.A. | 45 |
| unclassified | c.728_1T>A | de novo | splicing defect | F | N.A. | 45 |
| unclassified | c.729_1G>A | de novo | splicing defect | F | N.A. | 45 |
| unclassified | c.830_1G>A | inherited | nonsense | M | N.A. | 45 |
| unclassified | c.830_1G>A | de novo | nonsense | F | N.A. | 45 |
| unclassified | c.944_953del | de novo | nonsense | F | N.A. | 45 |
| unclassified | c.1020_1023del | de novo | frameshift | F | N.A. | 45 |

The disease within brackets indicates the first diagnosed pathology, before classifying the individual as a BPAN patient. For the cases categorized as "unclassified", no conclusive disease diagnostic was mentioned in the published case studies. F, female; M, male; N.A., not available. *only the mother was sequenced.

of age. ID has an estimated worldwide prevalence of 1–3%, affecting up to 0.5% of the population in Western countries with moderate or severe forms (IQ <50) [66]. Disease-associated variants have been so far associated with multiple genes, including WDR45B and WDR45 [67,68]. Genetic alterations in ID ranges from cytogenetic abnormalities to single nucleotide variants, in rare cases even epigenetic defects in methylations has been identified [69]. Yet the genetic cause of ID remains elusive in over 50% of the cases [70].

So far, only two reported cases, both female (Table 1) with ID are caused by a variant in WDR45 (Figure 4) [67,71]. One 3-year-old ID patient with a nonsense variant in the first WD-repeat of WDR45, and one 29-year-old ID patient with a frameshift variant in the sixth WD repeat (Figure 4)
showing that these ID patients do not carry a similar type of variant in a specific domain of WDR45. Moreover, no iron accumulation has been reported in the brain of these two patients. In light of the knowledge acquired from WDR45-associated RLS diagnosed patients, it would be interesting to determine on a long term whether these two patients will develop a brain iron accumulation later in life.

**Epileptic encephalopathies**

a) **DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY (DEE)**

DEE is characterized by refractory seizures, electroencephalographic (EEG) abnormalities and frequent epileptiform activity that contribute to developmental slowing and regression in the childhood that can lead to an early death [72,73].

There are 17 reported DEE patients carrying a variant in WDR45 (Table 1, Figure 2B). As depicted in Figure 3, the DEE-associated WDR45 variants are distributed over the entire protein, without concentrating to a specific region. So far, there are four male cases within DEE patients (Table 1). Interestingly, Khoury and coworkers described a 10-year-old boy with profound developmental delay, spastic quadriaparesis, and intractable epilepsy with tonic and atypical absence seizures [74]. This patient was first noted to have a development delay at 9 months, showed diffusely decreased tone at 17 months and seizures started at the age of two [74]. Only at the age of six, MRI brain scans showed iron accumulation in globus pallidus interna and externa, and signs of iron accumulation in substantia nigra [74]. This late onset of brain iron accumulation suggests that iron deposition is not one of the first symptoms of WDR45-associated DEE, similar to what is observed in BPAN.

b) **EARLY-ONSET EPILEPTIC ENCEPHALOPATHIES (EOEE)**

Another disorder that is linked with WDR45 is EOEE which is characterized by severe epilepsies and neurodevelopmental disorders [56]. Further clinical signs of EOEE include stormy seizures associated with an altered interictal EEG pattern. The disease-related epileptic disorders are highly heterogeneous in terms of etiology as they can have symptomatic causes (e.g., cortical malformation or strokes), or can have metabolic or genetic origin [75]. In fact, variants in over 100 genes, including KCNQ2, STXB1, SCN2A, PNPO, PIGA, SEPP1, and WDR45, were shown or suggested to cause EOEE [76]. Based on the interictal/ictal EEG pattern, type of seizures and age at onset, EOEE is further sub-classified into Ohtahara syndrome, early myoclonic encephalopathy, Dravet syndrome and Lennox-Gastaut syndrome, Landau-Kleffner syndrome and West syndrome [77]. In contrast to DEE where first symptoms occur later, symptoms of EOEE, such as severe epilepsies, are already occurring in the first 3 months of life [56,74].
Figure 3. Schematic distribution of the documented WDR45 variants causing BPAN over the entire protein sequence. 93 BPAN-associated WDR45 variants have been reported. Of the patients, 84 are women, 9 are males. Those variants also found in patients affected by another disorder are highlighted in red. N.A., not available.

There are only four cases of EOEE patients, one of them a male, associated with a variant in WDR45 (Table 1). These variants include a deletion, a splicing defect and a missense variant in WDR45 and, as for the other disorders, those variants are not concentrated at a specific region of the protein (Table 1, Figure 4). As for RLS, none of the four
EOEE patients display signs of brain iron accumulation, at the time when they were tested which was at the early stage of EOEE [56,78].

c) WEST SYNDROME

West syndrome is the most common subtype of EOEE with an incidence of 2–5 per 10,000 births [79], and it principally manifests at 3–12 months of age, predominantly affecting males, i.e., in 60–70% of the cases [80]. This disease represents as a specific epileptic encephalopathy characterized by unique type of attacks, called infantile spasms, hypsarrhythmia and delays in the psychomotoric development [81,82]. West syndrome can also be part of more complex disorders, where
some may manifest with a developmental delay, like for example in DEE [83]. Neurodevelopmental delay and/or regression have interchangeably been part of the definition of West syndrome, and are currently considered as part of the triad of symptoms characterizing this disease, i.e., infantile spasms, hyspsarrhythmia, and retardation.

So far only 3 cases of West syndrome patients carrying a variant in WDR45 have been documented (Table 1), making the West syndrome frequency with WDR45-associated diseases as low as the one of ID or EOEE (Figure 2B). As for all WDR45-associated diseases, the documented WDR45 variants causing West syndrome are also distributed throughout the whole protein. The 3 patients are all male, which might indicate a gender-specificity for WDR45-associated West syndrome cases. These individuals showed early-onset intractable seizures, profound intellectual disability and developmental delay, and the MRI brain scans showed cerebral atrophy [84,85]. Interestingly, like DEE and EOEE, West syndrome patients showed no brain iron accumulation and altogether, these three WDR45-associated disorders are also the ones that show most frequently male patients. This could imply that diseases progressions that eventually lead to brain iron accumulation cannot or hardly be tolerated by male.

Cancer

Almost all of the documented WDR45-related diseases as of today fall into the neurodevelopmental and neurodegenerative disorders category described above, however, in a metadata analysis study by Lebovitz and colleagues, WDR45 was identified as a potential target for specific cancers. In particular, they analyzed DNA sequences and RNA expression levels of ATG genes from cancer patients using datasets from The Cancer Genome Atlas consortium (http://cancergenome.nih.gov/). They found that WDR45 is one of the ATG genes that is frequently genetically altered in uterine corpus endometrial carcinoma patients [86], indicating that WDR45 variants might also contribute to cancer development. A further indication that WDR45 may play a role in cancer comes from a bioinformatics study that showed that WDR45 expression is affected by OTUD5, a deubiquitinase. Reduced expression of OTUD5 correlates with a poor prognosis in cervical cancer and when OTUD5 is depleted, WDR45 expression is reduced, suggesting a possible connection between WDR45 function and cervical cancer [87].

Potential connections between WDR45 molecular functions and the pathogenicity associated with its variants

Mouse models are often key to relate consequences of a gene mutation to the pathophysiology of the disease it is causing. So far, three different wdr45−/− mouse models have been described [2,4,43]. The first was a CNS-specific wdr45−/− mouse [2]. On a cellular level, these mouse brains show axonal swelling, and accumulation of ubiquitin- and SQSTM1/p62-positive aggregates, but little neuronal damage [2]. These mice also display deficits in cognitive functions such as learning and memory, however, only older animals show motor dysfunction [2]. The others mouse models are constitutive, full-body wdr45−/− mice [4,43] and display similar phenotypes as the CNS-specific wdr45−/− animals with older mice display neuronal loss and the appearance of seizures [43]. However, none of the mice models accumulated iron in the brain. Notably, all wdr45−/− mice are born normally and survive the post-natal starvation period, which is in contrast to mice lacking essential ATG proteins [43]. Therefore, although a mild autophagy defect was observed in brain cells of those animals and some cognitive dysfunction might be connected with this alteration, it seems that Wdr45 is not an essential ATG gene, in agreement with most of the cellular studies (see section The role of WDR45 and the other WIPI proteins in autophagy).

Another approach to connect molecular dysfunctions with disease pathophysiology is by using patient material. The first study that investigated WDR45-associated disease patient material, in particular BPAN patient material, showed that WDR45 levels were significantly reduced in lymphoblastoid cells derived from 5 BPAN patients [50]. Moreover, compared to cells from healthy family members, those cells displayed a reduction in autophagic flux and a augmentation of lipitated LC3 and LC3-positive labeled structures, which is indicative of an accumulation of autophagosomes and/or autophagosomal intermediates [50]. More recently, Seibler and colleagues investigated fibroblasts derived from one BPAN patient in more detail [88] and also observed a defect in autophagy as others did [2,24,43,50]. The examined cells also showed drastic alterations in mitochondrial membrane potential and network organization, resulting in elevated mitochondrial stress [88]. Mitochondrial dysfunction is a reoccurring phenotype in WDR45−/− cell lines, including HeLa [43] and SH-SY5Y [88] cells, but it is also observed in swollen axons of the wdr45−/− mice [2]. The altered mitochondria morphology and function is of particular interest since this phenotype is associated with many other neurodegenerative diseases, e.g., Huntington and Parkinson diseases, Rett syndrome, etc. [89] but also other NIBA disorders, including PKAN, PLAN, CoPAN and MPAN [90]. Thus, mitochondrial dysfunction might be a crucial trait underlying the neurodegeneration that characterizes WDR45-associated diseases.

The most striking brain phenotype of WDR45-related patients is, however, an iron deposition in the basal ganglia region. Interestingly and like mitochondrial dysfunction, brain iron accumulation is not only observed in WDR45-associated patients, but also in individuals affected many other neurodegenerative diseases, including Huntington’s, Parkinson’s and Alzheimer’s diseases. Those patients exhibit a dysfunction in iron metabolism, elevated free iron and iron deposition at the sites of neurodegeneration [91]. As iron reacts with hydrogen peroxide and catalyzes the generation of highly reactive hydroxyl radicals, high levels of iron can increase the cellular oxidative stress and even trigger an iron-dependent form of cell death, known as ferroptosis [92,93]. Consistently, increased levels in oxidative stress-related marker proteins and an elevated susceptibility to iron-induced stress has been observed in BPAN patient derived fibroblasts [88]. Interestingly, two studies could
show that BPAN patient-derived cells, i.e., fibroblast and dopaminergic neurons, display a defect in iron homeostasis and an increase in iron level, which are accompanied by lower amounts of ferritin [88,94]. The observed iron overload and the reduced ferritin levels could be restored by inducing autophagy in BPAN patient-derived dopaminergic neurons [88], indicating that some phenotypical alterations in WDR45-depleted cells can be overcome by enhancing autophagy. Mechanistically, iron overload in the cells could be due to an overexpression of the SLC11A2/DMT1 (solute carrier family 11 member 2), which is upregulated in BPAN patient cells [94] as well as in cells lacking PLA2G6, the gene than when mutated causes another NBIA disorder, PLAN [95]. In contrast, another iron cell importer, the transferrin receptor, is downregulated in BPAN patient-derived cells [94]. Transferrin receptor is required for both the endocytosis of extracellular transferrin-bound iron and the storage of iron in ferritin cages and its subsequent controlled release [96,97]. Therefore, misregulation of this receptor likely also contribute to the observed cellular iron overload.

Although the number of studies that have investigated the WDR45 dysfunction and the resulting pathology are limited, they concurrently show that WDR45 mutations leads to an alteration of autophagy progression, mitochondrial morphology and function, ER stress and iron homeostasis (Figure 2A). However, it remains to be determined whether WDR45 dysfunction affects multiple cellular processes simultaneously, or whether the inhibition of a single pathway has pleiotropic negative effects on several other cellular processes.

**Conclusions**

Six different WDR45-associated neurodegenerative disorders have been identified so far. Amongst those, BPAN is the only one that appears to be exclusively caused by variants in WDR45 while the other disorders can also be caused by other gene variants or environmental factors. Because of WDR45’s role in autophagy (Figure 1), most of the investigations have tried to connect autophagy dysfunction with the onset and pathophysiology of those disorders. WDR45 depletion, however, also causes cellular alterations that are unrelated to autophagy because of its probable function in other cellular pathways (Figure 2A), similar to other ATG genes [98–100]. The best characterized molecular function of WDR45 is promoting lipid transfer together with ATG2 proteins between adjacent membranes. This function at the phagophore-ER membrane contact sites is important for autophagy [34–41,101]. It cannot be excluded a priori that these proteins also participate in transferring lipids at other membrane contact sites and that a disruption caused by WDR45 mutations could contribute to the pathogenicity of WDR45-associated diseases. In particular, misregulated ER-mitochondria membrane contact sites could explain the morphological and functional alterations of mitochondria and ER in cells lacking WDR45 [102,103]. The most studied cellular function of WDR45 is the one in autophagy, however, the very partial autophagy inhibition [2,4,27,50,88] and the absence of developmental defects in mice lacking Wdr45 [2,4,43], suggest that WDR45 is not a core ATG gene. Interestingly, when depleted together with WDR45B, the severity of the autophagy and developmental defects in mice drastically increase [4], similarly to animals lacking core ATG genes [104,105]. This notion indicates that WDR45 and WDR45B might function redundantly at least in the brain. Therefore, it is tempting to speculate that WDR45B expression levels might influence the severity of WDR45-associated diseases, i.e., lower WDR45B expression levels lead to a severer pathology. This notion and a reciprocal causative connection between these two genes are also evoked by the fact that variants in WDR45B can lead to ID [66,67].

Besides general bulk autophagy, it might be that WDR45 is required for selective autophagy that target for example mitochondria (i.e. mitophagy) or ferritin (i.e. ferritinophagy) [106]. The role of WDR45 in those pathways has not been studied, yet it could provide important insights why WDR45 depletion is causing the observed phenotypical and functional alterations; i.e., ferritinophagy plays a crucial role in iron homeostasis, something that is misregulated in many WDR45-associated disorders. During ferritinophagy, the NCOA4 (nuclear receptor coactivator 4) interacts with ferritin and is required for the delivery of iron complexed with ferritin to lysosomes via autophagosomes [107,108]. Interestingly, mice lacking NCOA4 show increased levels of ferritin and iron in several tissues [109,110], something reminiscent with BPAN patients. Thus, the potential connection between WDR45 and ferritinophagy could be an explanation why WDR45-associated disorders are often accompanied with iron accumulation.

It is still unknown which cellular alterations, present in patient material, are the primary cause of these pathologies, and which are consequences. In this context, it is important to note that some BPAN patients did not show iron accumulation at their first diagnosis, but were detected in their brains with age [55]. Moreover, patients suffering of other specific WDR45-associated disorders, i.e, ID, EOEE and West syndrome, do not show iron accumulation at all. Interestingly, in those disorders the frequency of male patients is much higher than in those that develop iron accumulation (Table 1). This may indicate that iron accumulation is a consequence of one or more WDR45-associated cellular dysregulations and not a primary feature.

Despite the variety of clinical manifestations in patients that harbor WDR45 variants, it appears that there is not a phenotype-genotype correlation (Figures 3 and 4) [45]. Genetic variants are diverse and are distributed over the entire amino acids sequence of WDR45 (Figure 3, Table 1) and in nine cases the variants are even causing different diseases (Table 2). Thus, it is possible that because of the complex and compact structure of WDR45, variants in different parts all lead to the disruption of the -propeller structure (Figure S1B), resulting in a complete loss-of-function [50]. It cannot be excluded, however, that some variants are leading to gain-of-function proteins.

Although there is overlap in the spectrum of clinical symptoms and genetic and environmental factors are likely
playing roles in the clinical manifestations of WDR45-associated disorders, it remains still unclear, why the disease severity varies so much. Fundamentally, it is crucial to determine the primary cellular defects caused by WDR45 variants and try to correlate the consequences with the appearance of biomarkers in patients that may appear at different disease stages. This knowledge will be vital to design effective therapies that specifically counteract the causes of WDR45-associated disorders rather than the consequences.

Added in proof
A manuscript providing additional mechanistic insights on the function of WDR45 has very recently been published [111]. This study reveals the redundant function of WDR45 and WDR45B, in particular in the maturation of autophagosomes into autolysosomes in neurons. Mechanistically, WDR45 and WDR45B interact with the tether protein EPG5 and facilitate its localization to late endosomes and lysosomes. Thus, in the absence of WDR45 and WDR45B, the correct assembly of the EPG5-dependent SNARE complexes that are required for autophagosome-lysosome fusion is compromised. An additional important observation in this study is that although autophagosomes can still be formed in the absence of WDR45 and WDR45B, their size is greatly reduced. This might be due to insufficient lipid transfer during autophagosome formation because it could be partially rescued by ATG2A overexpression. Of note, the autophagosome maturation defects were not observed in other non-neuronal cells or when any of the genes was deleted singularly in neurons.

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