Genetic aberrations in macroautophagy genes leading to diseases

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ABSTRACT

The catabolic process of macroautophagy, through the rapid degradation of unwanted cellular components, is involved in a multitude of cellular and organismal functions that are essential to maintain homeostasis. Those functions include adaptation to starvation, cell development and differentiation, innate and adaptive immunity, tumor suppression, autophagic cell death, and maintenance of stem cell stemness. Not surprisingly, an impairment or block of macroautophagy can lead to severe pathologies. A still increasing number of reports, in particular, have revealed that mutations in the autophagy-related (ATG) genes, encoding the key players of macroautophagy, are either the cause or represent a risk factor for the development of several illnesses. The aim of this review is to provide a comprehensive overview of the diseases and disorders currently known that are or could be caused by mutations in core ATG proteins but also in the so-called autophagy receptors, which provide specificity to the process of macroautophagy. Our compendium underlines the medical relevance of this pathway and underscores the importance of the eventual development of therapeutic approaches aimed at modulating macroautophagy.

1. Introduction

1.1. Autophagy, its effectors and their coding genes

Cells, tissues and organisms have to respond to a multitude of environmental, developmental and metabolic cues, and this is also achieved through the lysosomal turnover of intracellular components, via a set of pathways grouped under the term autophagy, from the Greek words for ‘self-eating’ (autos and phagein). Autophagic processes deliver long-lived, dysfunctional or aggregated proteins, damaged or superfluous organelles and invading pathogens to the lysosome for turnover, and the resulting degradation products are reused for anabolic processes or as a source of energy. There are three major types of autophagy: microautophagy, chaperone-mediated autophagy and macroautophagy [1]. During microautophagy, the lysosome directly engulfs and degrades parts of the cytoplasm through invagination and pinching off of its limiting membrane. Chaperone-mediated autophagy translocates proteins carrying a specific pentapeptide from the cytoplasm directly into the lysosome. Macroautophagy, in contrast, involves the sequestration of cytoplasmic components into a transient structure termed the phagophore that matures into a double-membrane vesicle called an autophagosome, which subsequently fuses with the lysosome to deliver its cargo (Fig. 1). The focus of this review is on macroautophagy, hereafter referred to as autophagy.

Autophagy is a tightly regulated process and the key players of this pathway are the Autophagy-related (ATG) proteins. Screens in yeast have lead so far to the isolation of 41 ATG genes, and several of them have one or more counterparts in mammalian cells, including the 16 that are part of the conserved ATG machinery that mediates the formation of autophagosomes in all eukaryotes [2]. Additional tissue- and organism-specific ATG genes have been identified in more complex eukaryotes [3]. The ATG core proteins have been classified in five functional groups (Table 1): I) The ULK kinase complex, composed of ULK1 or ULK2, ATG13, RB1CC1/FIP200 and ATG101; II) the class III...
phosphatidylinositol 3-kinase (PtdIns3K) complex, composed of BECN1/Beclin 1, ATG14, PIK3C3/VPS34 and PIK3R4/p150/VPS15; III) the ATG12 conjugation system, involving ATG7, ATG10, ATG12, ATG16L1 and ATG5; IV) the LC3 conjugation system, containing ATG4 proteases (ATG4A to ATG4D), ATG7, ATG3, WIPI2 and the members of the LC3 protein family, and V) the ATG9 trafficking system, which involves: ATG2A and ATG2B, WIPI4 and the transmembrane protein ATG9A [4].

The process of autophagy can be divided at least in 6 discrete steps: i) initiation, ii) nucleation of the phagophore, iii) expansion and closure of the phagophore, iv) autophagosome fusion with a lysosome, v) degradation of the autophagosomal cargo and vi) efflux of the resulting metabolites from the lysosomes [5] (Fig. 1). The initiation step of autophagosome biogenesis is marked by the association of certain ATG proteins [1]. This event can be triggered by different signalling cascades, which modulate the activity of the ULK complex, the PtdIns3K complex and/or the ATG9 trafficking system, and collectively mediate phagophore nucleation. One example of a signalling pathway regulating autophagy is the one centred on MTOR (mechanistic target of rapamycin kinase) (Fig. 1). Under normal growing conditions, in the presence on nutrients, MTOR phosphorylates ATG13 and ULK1 or ULK2, inhibiting the kinase activity of the ULK complex. Upon starvation, MTOR is inactivated and the ULK complex becomes dephosphorylated leading to its activation, which is required for the assembly of the ATG machinery [1]. In addition to undergoing autophosphorylation, the ULK complex also phosphorylates components of the PtdIns3K complex and ATG9A, two modifications that appear to be part of the initiation program of autophagosome formation [6,7]. The PtdIns3K complex synthesizes phosphatidylinositol 3-phosphate (PtdIns3P) on phagophore membranes, which acts as landmark to
recruit other ATG proteins to this precursor structure [8,9] (Fig. 1). ZFYVE1/DFCP1 is one of the early-acting proteins and it may play an important role in organizing the endoplasmic reticulum (ER) membrane around the phagophore in such a way that it forms a cradle-like structure, the omegasome (so named because of its Ω-structure) [1]. The ULK complex also appears to directly bind to ATG9A, possibly through RB1CC1/FIP200 [10]. ATG9A is the only transmembrane protein within the core ATG machinery, but it does not seem to integrate into the forming autophagosome, but rather has transient interactions with it, which are required for the elongation of the phagophore [11] (Fig. 1). The forming autophagosome gathers its membranes from several sites potentially including the ER, mitochondria, post-Golgi compartments, recycling endosomes and the plasma membrane, or contact sites between any of these [12-14].

The subsequent step, phagophore elongation, requires several factors including WIPI proteins. Although their exact function is unclear, WIPI2 can recruit the ATG16L1 complex to the autophagosome [15]. ATG16L1, as part of the ATG12–ATG5–ATG16L1 complex, is responsible for recruiting members of the LC3 protein family onto the phagophore membranes. LC3 proteins are ubiquitin-like proteins that conjugate LC3 proteins to the amino group of phosphatidyethanolamine (PE) present on the concave surface of phagophores is crucial for the recognition of autophagy receptors [18,19] (see below). Once the phagophore seals, LC3–PE is likely removed from the outer membrane of complete autophagosomes by the ATG4 proteases [20]. Complete autophagosomes require the CCZ1-MON1 guanosine exchange factor complex, its downstream effector RAB GTPase RAB7, the homotypic fusion and vacuole protein sorting tethering complex and SNAREs to fuse with lysosomes and form autolysosomes [21,22]. The hydrolases present in the lysosome then degrade the cargo carried by the autophagosomes, and permeases present on the limiting membrane of this organelle, transport these metabolites into the cytoplasm. Autolysosomes become lysosomes again through a process that has been termed “autophagic lysosome reformation” [23]. During this process, tubules emerge and extend from autolysosomes to become proto-lysosomes, which eventually mature into lysosomes by acquiring new lysosomal components.

1.2. Selective types of autophagy and autophagy receptors

Although autophagy can be a non-selective bulk process, in numerous circumstances this pathway leads to the exclusive degradation of targeted structures, which are specifically recognized and sequenced within autophagosomes through mechanisms that involve the so-called autophagy receptors (Fig. 2 and Table 2) [24-26]. There are several classes of selective types of autophagy that have been named after the cargo targeted for degradation, e.g. aggrephagy for protein aggregates, mitophagy for mitochondria, xenophagy for pathogens, ri- phagy for ribosomes, reticulophagy for ER, nucleophagy for the nuclear envelope, lipophagy for lipid droplets, lysophagy for lysosomes, pexophagy for peroxisomes, etc. [24,26]. Selective types of autophagy can be sub-divided into two categories, those that are ubiquitin-dependent and entail autophagy receptors binding to this posttranslational modification, and those that are ubiquitin-independent and involve the interaction of the autophagy receptors with other determinants (Fig. 2) [26]. Of note, specific types of selective autophagy such as mitophagy or xenophagy, have multiple autophagy receptors to ensure the disposal of the targeted structure [26].

The first mammalian autophagy receptor to have been characterized is SQSTM1/p62 [25]. This protein was initially found to simultaneously bind polyubiquitinated proteins present in protein aggregates and LC3 conjugated to phagophore membranes through the so-called LC3-interacting region motif. SQSTM1 has now been linked to other types of selective autophagy, such as mitophagy and xenophagy [24,26]. During the past decade, additional autophagy receptors for one or more types of selective autophagy have been identified, and include NBR1 (aggrephagy, pexophagy, lysophagy and midbody turnover), OPTN (optineurin; aggrephagy, mitophagy and xenophagy), CALCOOC2/NDP52 (xenophagy and nucleic acid) and RETREG1/FAM134B (reticulophagy) [24,26].

1.3. The physiological roles of autophagy and pathological consequences of autophagic dysfunction

Because of its ability to rapidly turn over unwanted structures, autophagy is integrally involved in a wide range of cellular and organismal functions that can be considered under the general concept of homeostasis. These include adaptation to various types of stress, participation in pathways of development and differentiation, contributing to certain aspects of innate and adaptive immunity, acting in tumor suppression, etc. Therefore, it is not surprising that mutations in genes that affect autophagic capacity can lead to severe illnesses. It must be noted that the autophagy defects are indirect in some of these pathologies, such as in lysosomal storage disorders including Fabry and Pompe diseases where lysosome malfunctioning causes impairment in the fusion of autophagosomes with lysosomes and/or autophagosomal cargo degradation [27,28]. Dysfunctional autophagy has also been observed in large number of other diseases, including cancer, muscular dystrophies and neurodegenerative disorders such as Alzheimer, Parkinson and Huntington diseases [29]. A still increasing number of reports, however, has revealed that numerous diseases are caused or appear to be caused by mutations in ATG genes and other genes involved in autophagy. This review focuses on these pathologies with the goal of giving a complete overview of congenital defects that could be directly linked to impairment in autophagy. Although autophagy plays an important role in numerous tumors, the exact contribution of this pathway and associated changes is influenced by, and depends upon, the specific tissue and what other mutations are present. Therefore, the discussion about cancer and its associated autophagy-related mutations deserve a separate review, because incorporating it would exceed the length limits of the current review. Several comprehensive reviews on cancer and autophagy have been published (e.g. [30,31]).

2. ATG and autophagy-associated genes as risk factors

2.1. Diseases caused by mutations in core ATG genes

2.1.1. Asthma

Asthma is a chronic inflammation of the airways that presents itself most commonly as episodes of wheezing, shortness of breath, chest tightness and coughing. Approximately 300 million people worldwide suffer from this disease [32]. Because autophagy is implicated in immune responses and inflammation, Martin and co-workers performed a study in 2012 linking several single nucleotide polymorphisms (SNPs) in ATG5 to different asthma patient cohorts, the most notable of these SNPs being ATG5 rs12201458, which localizes in the putative promoter region of ATG5 [32]. Interestingly, acute asthma patients have increased levels of ATGs in nasal mucosal cells, and stable asthma patients have levels in between healthy controls and acute patients [32]. Asthma patients can continue to experience exacerbations despite treatment, due to airway remodelling, or more specifically fibrosis, caused by collagen deposits in the airways. A more recent study associates ATG5 with these collagen deposits in airways, mainly those of
In particular, the authors suggested that via angiotensin II, autophagy could play a role in collagen deposition [33]. A study in Korean patients and healthy controls, however, found no association with asthma risk and $ATG5$ or $ATG7$ SNPs [34]. Nonetheless, the authors discovered that sequence changes in $ATG5$ at -335G $>$ A (rs12201458) and 769T $>$ C are associated with a higher sputum neutrophil count, and two SNPs in $ATG7$, at 100A $>$ G and 25108G $>$ A, lead to higher IL8 levels in the serum [34]. As both factors increase the severity of asthma, the main conclusion of this investigation is that alterations in autophagy might play a role in the disease course rather than its onset [34].

2.1.2. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a disease where there is an accelerated decline in lung function with periods of exacerbations [35]. The incidence of COPD is growing and is currently the fourth cause of death worldwide [36]. As autophagy has been associated with COPD pathogenesis, a 2015 study explored whether specific

![Principles underlying selective types of autophagy](image)

**Table 2**

| Protein       | Functional group | Disease                                      |
|---------------|------------------|----------------------------------------------|
| SQSTM1/p62    | Autophagy receptor | ALS [100], FTD [113], FTD-ALS [119], AAS [120], DMRV [126], SIBM [128], PDB [122], childhood neurodegeneration [121] |
| OPTN          | Autophagy receptor | ALS [109], FTD-ALS [119], PDB [124], NTG [141] |
| CALCOCO2/NPD52 | Autophagy receptor | CD [142]                                      |
| RETREG1/FAM134B | Autophagy receptor | HSANIB [138]                                  |
| TOLLIP        | Autophagy receptor | IPF [145], susceptibility to infectious diseases [149-152] |
| Tbk1          | Kinase            | ALS [173], NTG [141]                         |
| IRGM          | GTPase            | CD [64], NAFLD [144]                         |
| FA genes      | Mitophagy adaptors | FA [148]                                     |
genetic variants in ATG genes conferred susceptibility to smoking-related COPD [37]. The study found SNPs in ATG16L1 (rs 2,241,880, T300A) but also in EGR1, a transcription factor that can regulate LC3 expression [37]. EGR1 involvement is not totally surprising as this transcription factor is involved in the early response to oxidative stress, which is a causative factor for COPD, and modulates the expression of several genes involved in fibrosis-like reactions, such as matrix metalloproteinases. The risk mutation in ATG16L1 is curious, as it is at the same location as the one for Crohn disease (section 2.1.8), but the allele that confers risk to COPD is the A, the unchanged one and coding for T300, while that of Crohn disease is G (resulting in T300A) [37].

2.1.5. Behçet disease

Behçet disease (BD) is a rare and complex illness that is suspected to be a systemic autoimmune disorder. It occurs mostly among people in the Mediterranean and the Far East. Symptoms include aphthous ulcers (i.e., in the mouth or on the tongue), ulcers in the genital mucosa, eye inflammation with possible vision loss, gastrointestinal inflammation, vascular and articular inflammation and neurological inflammations [38]. The risk factor most strongly associated with this disease is the human leukocyte antigen (HLA)-B51 allele [38]. One study, however, also linked ATG5 to BD in a Chinese patient cohort [39]. The TT SNP at the ATG5 rs573777S is less common in BD patients and thus seems to have a protective effect. The TT genotype results in higher levels of mRNA in response to lipopolysaccharide stimulation, and the authors suggested that this could prevent a strong inflammatory reaction. Curiously enough, BD patients have higher ATG5 mRNA levels compared to healthy controls [39]. This study, however, did not provide further experimental insights into the role ATG5 might play in BD inflammatory responses, and research into this is needed.

2.1.6. Rheumatoid arthritis

Rheumatoid arthritis (RA) is chronic inflammatory disease that affects the joints [42]. The HLA antigen D related beta chain paralog (HLA-DRB1), in particular the HLA-DRB1*0401 haplotype, is the highest risk factor for RA, but many others have been identified and they are mostly involved in T-cell activation [42,43]. One of these is the rs548234 SNPs at the intergenic region between the PRDM1 and ATG5 genes, but this is only valid in Caucasians and it cannot be found in the Chinese Han population [42,44]. As a result, the genetic connection between RA and autophagy is still not conclusive and it could be that some of the ATG genes have a peculiar role in this disease.

2.1.7. Systemic sclerosis

Systemic sclerosis is another autoimmune disease characterized by autoantibody generation. Characteristic symptoms are fibrosis of the skin and non-inflammatory vasculopathy [54]. Antibodies produced include anti-centromere and anti-topoisomerase antibodies [54]. Patients’ small blood vessels and other connective tissues are attacked and damaged, and collagen is deposited in affected areas [55]. The disease can be fatal if heart, kidneys and/or lungs are targeted [55]. The main genomic risk factor for this disease is associated with specific MHC class II alleles, but many other risk loci have been identified. An attempt to discover previously overlooked causals factors for susceptibility to systemic sclerosis using microchip analysis identified ATG5 [54]. The variants in ATG5 are at 6q21 but they are not exonic and consequently they do not lead to mutations in the protein, indicating that all variants may alter binding sites for DNA-binding proteins [54]. Thus, while the association seems to be present, the exact role that ATG5 variants have in the development of systemic sclerosis has to be investigated further.

2.1.8. Crohn disease

Crohn disease (CD) is an inflammatory bowel disease (IBD) that is characterized by relapsing inflammation of the gastrointestinal tract mucosa. In patients, this leads to abdominal pain, diarrhoea and fatigue during relapses, and complications such as strictures, abscesses and...
fistulas [56,57]. Remission is possible with medical therapies, but this is often not indefinite and many patients require surgical procedures [56].

CD is a multifactorial pathology and the exact causes remain unclear. A factor involved in the disease appears to be an inappropriate immune response to antigens in the intestinal lumen, specifically antigens belonging to the commensal flora [57]. Approximately 100 loci have been associated with IBD and the functions of the corresponding genes range from bacterial recognition to lymphocyte activation and epithelial protection [58]. Environmental factors are also involved, because disease often develops later in life, while predisposing genes are present from birth [59].

In 2007, two studies comparing SNPs in CD patients with healthy controls identified a risk factor at rs2241880 in the gene encoding ATG16L1, i.e. corresponding to ATG16L1T300A [60,61]. It turns out that ATG16L1 modulates SQSTM1/p62 ubiquitination via neddylation of TMEM59 through its WD40 domain, and the cellular response to autophagy activity, it decreases both ATG16L1 interaction with phylococcus aureus infection, and it increases IL1B signalling [62,63].

Often not indefinitely and many patients require surgical procedures [56].

In 2015, Kyöstilä and colleagues published a study linking a progressive neurological disorder in Lagotto Romagnolo dogs to a missense mutation in the animals’ ATG4D gene [71]. The dogs suffer from progressive cerebellar ataxia with, in some cases, involuntary eye movement and behavioral changes. Histological analysis revealed vacuolization of several tissues both inside and outside the brain, and axonal swellings. Electron microscopy showed that the vacuoles have a single membrane and are mostly empty in control samples, whereas they contain degenerated mitochondria in axonal swellings, where autophagosomes and cytoplasmic aggregations are also occasionally observed [71].

Vertical transmission of canine ataxia highlights the role of ATG4D [68]. Many risk variants associated with IBD were found not to be in genes but in DNA regulatory elements, including the one of ATG9A [68].

Many autophagy proteins thus appear to play a role in the development of CD. It remains to be firmly established whether mutations in the ATG genes lead to CD by altering autophagy activity or other pathways, such as the ones modulating IL1B signalling.

2.1.9. Celiac disease

Celiac disease (CeD) is a chronic autoimmune disease of the small intestine, where ingesting dietary gluten leads to damage of this organ [69]. A genome-wide and refined mapping study of several autoimmune diseases associated ULK3 rs1378938 with CeD [70]. The authors confirmed this connection by showing that biopsies from patients carrying the risk T allele have increased expression of ATG proteins, and increased expression of IL6 in response to lipopolysaccharides [70]. This observation indicated that one of the functions of autophagy—i.e., in immunity—could be involved in CeD [70]. Another study found that autophagy could indeed play a role in CeD, and that ATG7, BECN1 and their two regulating microRNAs, i.e. miR-17 and miR-30a, could serve as biomarkers for CeD [69]. In particular, CeD onset can be detected through the measurement of an autophagy decline in intestinal epithelial cells by monitoring a decrease in ATG7 and BECN1 expression, which is then followed by a reduction in MIR17 and MIR30 as part of a putative compensatory mechanism [69]. Future studies will need to identify what causes the differential expression of ATG proteins and their microRNAs.

2.1.10. Vogt-Koyanagi-Harada syndrome

Vogt-Koyanagi-Harada syndrome (VKHS) is a systemic autoimmune disease that principally causes an inflammation of the eye, but also targets many other pigmented tissues in the body [39]. A SNPs analysis aimed at exploring the possible involvement of ATG genes in 1061 VKHS patients from the Chinese Han population, determined that having two A nucleotides at rs4703863 of ATG10 is associated with a lower VKHS risk, whereas the C allele appears to confer a higher susceptibility to the disease [39]. This study, however, could not explain the association between ATG10 and VKHS as there was no difference in mRNA levels between these genotypes, nor there was a difference between patients [39]. Further research is needed to test whether ATG10 is indeed involved in VKHS syndrome, and, if so, how.

2.1.11. Ataxias

ATG4D is responsible for lipidating LC3-I and de-lipidating LC3-II. In 2015, Kyöstilä and colleagues published a study linking a progressive neurological disorder in Lagotto Romagnolo dogs to a missense mutation in the animals’ ATG4D gene [71]. The dogs suffer from progressive cerebellar ataxia with, in some cases, involuntary eye movement and behavioral changes. Histological analysis revealed vacuolization of several tissues both inside and outside the brain, and axonal swellings. Electron microscopy showed that the vacuoles have a single membrane and are mostly empty in control samples, whereas they contain degenerated mitochondria in axonal swellings, where autophagosomes and cytoplasmic aggregations are also occasionally observed [71].

Linkage analysis and subsequent Sanger sequencing of the affected animals led to the association with the ATG4D gene (c.1288G > A; p. Ala430Thr) [71], with 22 out of 25 dogs suffering from the disease carrying this mutation. Because the residue is not in a known functional part of the protein, but rather between the peptidase domain and the BH3 motif near the carboxy terminus, it is unclear how this mutation affects the protein function [71]. Knocking out atg4a in zebrafish, however, results in neurological symptoms, providing further evidence that the gene itself could cause the effect. Affected dogs have significantly higher LC3-II levels and a higher number of LC3-positive spots under nutrient-rich conditions than healthy controls [72]. Using baflomycin A1 to inhibit lysosomal degradation results in an increase of LC3-II vesicles in both healthy controls and affected cells, showing that only basal autophagy is affected by the ATG4D mutation. One could speculate that LC3-II is still generated, but the second function of ATG4D, i.e. de-lipidating LC3-II, is hampered by the mutation and this delays autophagosome fusion with lysosomes. Evidence to support this hypothesis will require further research, but what is clear is that a final autophagosome maturation step appears to be delayed in this disease, which probably causes the alteration seen in the nerve cells. Recently, two young Turkish patients that had ataxia and developmental delay since childhood were found to have a mutation in the ATG5 gene by whole exome sequencing [73].

Overall, autophagy or ATG proteins appear to play major roles in the development of ataxias.
sequencing by Haack et al. and Saito et al. identified heterozygous mutations in WDR45/WIPI4 at Xp11.23 as a causative factor of BPAN, which is inherited dominantly [74,78]. The mutations were all de novo mutations, and only two out of 20 subjects had the same mutation [74]. Sequencing of a heterogeneous NBIA cohort led to the identification of one patient with a missense mutation in WDR45, c.626CNA p.Ala209Asp, and the clinical manifestation matched the pattern of early developmental delay with further deterioration at a later age [79].

2.1.13. Developmental and epileptic encephalopathy

Patients with developmental and epileptic encephalopathy (DEE) suffer from refractory seizures and epilepsy-like activity, which lead to a developmental delay and even regression from an early age (as opposed to BPAN where the delay and degeneration onset is in adulthood) [80]. A recent study aiming to establish whether DEE could also be caused by mutations in WDR45, found 7 de novo mutations: 1 truncation, 5 indels leading to a frame-shift and one missense mutation (Gly205Asp) [80]. Why these mutations result in DEE and not NBIA is still unknown.

2.1.14. RETT-like syndrome

RETT syndrome is a neurodevelopmental disorder characterized by a seemingly normal development during the first years of life, which is then followed by degeneration and loss of language and motor skills. The disease mainly affects girls [81]. RETT-like syndrome or alternative Rett, is diagnosed when the patients meet a number, but not all, of the criteria of Rett syndrome such as loss of hand skills, spoken language, gait abnormality, breathing disturbances and growth retardation [82,83]. WDR45 was linked to Rett-like syndrome through sequencing studies in single patients. For instance, Hoffjan and colleagues described an Argentinean patient with the splicing site mutation c.440-2A > G in WDR45 [81]. In another case, however, the patient appeared to have Rett-like syndrome, but was later diagnosed with BPAN [84]. Like with DEE, it would be interesting to determine which WDR45 functions are disturbed to cause Rett-like syndrome and which others lead to BPAN.

2.1.15. Early-onset epileptic encephalopathy

Whereas most changes in WDR45 sequence appear to affect girls and it is thought that the mutation of this gene might be lethal for males, there are cases of males with a mutation in WDR45. In these cases, the effects manifest as early-onset epileptic encephalopathies (EOEE), or least as epileptic seizures in combination with other neurological issues [85,86]. WDR45 was linked to BPAN where the delay and degeneration onset is in adulthood) [80]. A recent study aiming to establish whether DEE could also be caused by mutations in WDR45, found 7 de novo mutations: 1 truncation, 5 indels leading to a frame-shift and one missense mutation (Gly205Asp) [80]. Why these mutations result in DEE and not NBIA is still unknown.

2.1.16. Parkinson disease

Parkinson disease (PD) is a neurodegenerative disorder, with tremors as its hallmark symptom. PD is incurable and it affects approximately 2% of the population over 50 years, resulting in approximately 6 million cases worldwide [87]. PD can be both familial and sporadic [87]. The disease is characterized by the loss of dopaminergic neurons in the substantia nigra, which causes progressive motor deficits [87]. In the affected neurons, proteins aggregate in inclusions called Lewy bodies, and they are mainly composed of SNCA/α-synuclein [87]. The overexpression or aggregation of SNCA/α-synuclein causes dysfunction of the mitochondrial respiratory chain leading to a decrease in ATP and an increase in reactive oxygen species (ROS) [87]. Some risk factors of PD, such as mutations in PRKN/PARKIN and PINK1, are associated with proteins that are involved in mitophagy (paragraph 2.2.5). In agreement with this notion, mutations in core ATG genes have also been linked to PD. Specifically, several variations in the ATG12–ATG5 conjugation system and LC3B, but not ATG16L1, have been associated with PD [88–92]. Mutations in the promoter of ATG5 (106,774,459T > A), ATG7 (11313449G > A, 11313811T > C, 11313913G > A and 11314041G > A) and ATG12 (115842507G > T, 115842394C > T and 115841817_18del) lead to higher expression levels of the corresponding protein, something that correlates with observations in PD patients’ brain tissues [88,90,92]. Some variations in the LC3B promoter region have also been discovered, but only one patient had a 21-base pair insert that causes increased expression, while the other changes that increase expression are only found in healthy controls or in both PD patients and controls [91]. How all these variations affect the disease progression, and whether they are the cause or consequence of the pathology, remains unclear. As the investigated patient group and the number of patients with these variants affecting expression levels are low, it is unlikely that these variants are driving factors in PD etiology. Nonetheless, further research could be relevant to establish their role and that of autophagy in PD.

2.1.17. Paget disease of bone

In Paget disease of bone (PDB), osteoclasts induce osteolytic lesions, which can lead to bone sclerosis and deformations over time [93]. PDB is a chronic disease and the second most common bone disease worldwide [94]. In this disease, osteoclasts appear to have cytoplasmic inclusions and those could represent protein aggregates [95]. A study in a cohort of Spanish patients found that polymorphisms in several ATG genes is associated with PDB. These genetic changes included a C allele in ATG16L1 rs241880 and a G allele in ATG5 rs2245214, which increase the risk of developing PDB, and a T allele in ATG10 rs1864183 that decreases the risk [95]. How these polymorphisms influence the disease etiology remains to be established.

2.1.18. Kashin-Beck disease

Kashin-Beck disease (KB) is an osteoarthropathy that is endemic to China. KB causes degeneration of cartilage and cartilage matrix, and chondrocyte necrosis and apoptosis in growth plate and articular cartilage [96]. Approximately 40% of the cases have been linked to genetic factors [96]. Because diseased chondrocytes show dysfunctional autophagy, a 2017 study explored whether autophagy, and more specifically mutations in ATG4C, could confer susceptibility to KB [96]. The authors found significant associations with ATG4C at rs11208030, rs4409699, rs12097658 and rs6587988, and confirmed that ATG4C mRNA and protein levels are lower in KB chondrocytes [96]. This investigation, however, did not provide a functional explanation for the association between ATG4C and KB, and solving this interrelationship might offer valuable insights into the specific function of ATG4C and the role of autophagy in KB etiology.

2.2. Diseases caused by mutations in genes coding for autophagy receptors and related proteins

2.2.1. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), or Lou Gehrig’s disease, is a fatal neurodegenerative disorder that presents itself in adulthood. The disease is characterized by cytoplasmic protein aggregates in the large motor neurons of the brain and the spinal cord [97,98]. The motor neurons progressively lose their connection with their target muscles, leading to their atrophy. The disease is fatal within several years, when the respiratory muscles fail [99]. Approximately 3–5 people per 100,000 suffer from the disease in the United States and Europe, with 10% of these being familial ALS, where there is a usually a dominant heritable genetic factor [99]. In 1993, mutations in SOD1 were found to be a risk factor for ALS, and many others have subsequently been identified and confirmed in patients. Mice expressing mutant SOD1 variants such as SOD1G93A, display neurodegeneration and are used as models for the disease. A few proteins connected to autophagy have also been identified as risk factors for ALS, including SQSTM1/p62, OPTN and TBK1 [100,101]. The SQSTM1/p62 autophagy receptor has been found in ubiquitin-positive inclusions in ALS patients [102].
protein also accumulates in the spinal cord of the SOD1
ALS mouse model, together with ubiquitin and ALS variants of SOD1, but not wild-type SOD1 [98]. Removing the ubiquitin-binding domain from SQSTM1/p62 reduces these aggregates [98]. Because SQSTM1/p62 is involved in both proteasome function and autophagy, it is not surprising that mutations in this protein aggravate diseases characterized by protein aggregates. Conversely, overexpression of SQSTM1/p62 reduces aggregates formed by TARDBP/TDP-43 (TAR DNA binding protein), another risk factor for ALS [103,104]. As an autophagy receptor, SQSTM1/p62 is involved in selective types of autophagy, including mitophagy, which appears to play a critical role in the pathogenesis of ALS, as e.g. SOD1G93A mice show increased numbers of autophagosomes, but not mitophagosomes, and an increased number of damaged mitochondria [105]. Mitochondrial dysfunction plays a prominent role in ALS, and SOD1G93A displaces HK1 (hexokinase 1) from its receptor in the mitochondrial membrane, inhibiting its function in metabolism [106,107]. The affected motor neurons also show reduced levels of SQSTM1/p62 and of other proteins involved in mitophagy, such as BNIP3, PINK1, and PRKN [105]. Other ALS risk factors such as OPTN and TBK1 are involved in this process as well; TBK1 regulates OPTN and SQSTM1/p62 by phosphorylating their ubiquitin- and LC3-binding sites during mitophagy [100]. Whereas it appears that SQSTM1/p62 is mainly involved in ALS caused by mutated SOD1, OPTN function is more prominent in sporadic ALS and SOD1-independent familial ALS [108]. OPTN associates with damaged mitochondria extremities independently from SQSTM1/p62, triggering the recruitment of ZFYVE1/DFCP1 and LC3 essential for subsequent mitophagy [109,110]. Depletion of OPTN hampers mitophagy, and the ALS-associated OPTN C.976G mutant cannot rescue this phenotype [109,110]. Altogether, several genes involved in providing specificity in selective types of autophagy are mutated in ALS, and experimental evidence points to dysfunctional mitophagy being part of the pathophysiology of this disorder. However, it cannot be excluded that concomitant impairment of other autophagic pathways, such as aggrephagy, contribute to ALS as they would promote the accumulation of intraneuronal aggregates when impaired. Interestingly, one of the main risk factors for ALS, C9orf72, has recently been found to bind the ULK1 complex and thus regulate autophagy [111].

2.2.2. Frontotemporal dementia

Frontotemporal dementia (FTD), frontal lobe dementia, or Pick disease, is a group of disorders that are caused by frontal or temporal lobe atrophy, which hampers the cognitive abilities or the processing of sensory inputs, respectively. Patients with FTD can present behavioral changes, loss of language and dementia [112]. A study aimed to determine whether common neurodegenerative diseases share mutations in the promoter region of the SQSTM1/p62 gene, reached the conclusion that many of them, including FTD, display an oxidative damage in this chromosomal region that causes decreased transcription in the affected tissues [113]. An earlier study also found SQSTM1/p62 accumulating together with ubiquitin in cytoplasmic inclusions in neurons and glia of FTD patients [114]. Consequently, a likely mechanism whereby SQSTM1/p62 could be involved in preventing FTD development is also through mitophagy. Interestingly, SQSTM1/p62 knockdown decreases mitochondrial membrane potential to approximately 80% compared to controls by causing a drop in the levels of NADH, which is required to generate an H+ gradient across the mitochondrial inner membrane [115]. Low NAD(P)H and FAD levels are due to a non-functional complex I in the mitochondrial respiratory chain, which has the consequence of leading to higher ROS production in these SQSTM1/p62-depleted cells, something that is also observed in other FTD neurodegenerative disorders [115]. Mitochondrial dysfunction is likely an important factor in FTD pathogenesis, and SQSTM1/p62 could play a role in the disease by being part of related and non-related autophagy pathways. The ALS risk factor C9orf72, which was found to regulate autophagy, is also a risk factor for ALS-FTD [111]. Moreover, CHMP2B, a component of the ESCRT-III complex, which is important for autophagosomal maturation [116], has also been found to be mutated in FTD [117].

2.2.3. FTD-ALS

ALS can also present itself with FTD symptoms, and this subtype of the disease, which is observed in approximately 5% of ALS cases, is known as ALS-FTD [118]. With increasing research and probably similar mechanisms underlying ALS and FTD pathologies, ALS and FTD are often seen as an ALS to FTD continuum [119]. OPTN and SQSTM1/p62, as well as other factors that are involved in ALS, are also mutated in ALS-FTD [118,119]. Whereas SQSTM1/p62 has not been shown to have a direct role in mitochondrial respiration in either ALS or FTD, mitochondrial dysfunction plays a role in both diseases, and a mitophagy defect would exacerbate both pathological phenotypes. With SQSTM1/p62 and OPTN also found to be mutated in other diseases, such as PDB, it would be interesting to determine how these pathways are distinct and what intrinsic and extrinsic factors determine the development of one particular disease rather than another.

2.2.4. Atypical apraxia of speech

Mutations in SQSTM1/p62 (p.Lys238del) that are seen in FTD can also cause similar but distinctive phenotypes according to a study on the possible relevance of mutations in SQSTM1/p62 in atypical apraxia of speech (AAS) in one family [120]. The patients within this family suffer from dysexecutive syndrome (i.e., lack of ability to perform executive functions such as planning and abstract thought), visuo-constructural disabilities and apraxia of speech (i.e., an oral motor speech disorder, rendering the translation of ideas to spoken words impossible) [120]. Whereas the p.Lys238del mutation is seen in FTD and ALS pathologies, the AAS patients have no motor neuron symptoms and do not meet all FTD symptoms [120]. The authors of this study therefore argued that SQSTM1/p62 mutations could result in heterogeneous phenotypes and pointed out that patients with AAS or other behavioral disorders associated with a visuo-constructive deficit could also be caused by SQSTM1/p62 dysfunction [120].

2.2.5. Childhood-onset neurodegeneration with ataxia, dystonia, and gaze palsy

In addition to the study of AAS, another investigation found that loss of SQSTM1/p62 causes a largely undefined group of neurodegenerative disorders that share childhood-onset neurodegeneration with ataxia, dystonia, and gaze palsy as symptoms [121]. Three different bi-allelic loss-of-function variants of SQSTM1/p62 (c.286C > T (p.Arg96*), c.311_312del (p.Glu104Valfs*48) and c.2T > A) were found in four different families [121]. This study thus also underlines that pathologies related to SQSTM1/p62 mutations are heterogeneous in their manifestation.

2.2.6. Paget disease of bone

As previously mentioned in section 2.1.17, PDB is characterized by osteolytic lesions in osteoclasts, which can lead to sclerosis in the bone and bone deformations over time [93]. Three mutations in the gene encoding SQSTM1/p62 are found in familial PDB, and one of these also in sporadic PDB. All these genetic changes affect the ubiquitin-binding domain of SQSTM1/p62 [122], but it remains unknown why they lead to PDB. Further studies into PDB without SQSTM1/p62 mutations identified rs1561570 in OPTN as a risk factor [123]. Another study found that rs2234968, or rather two loci in linkage disequilibrium with this SNP, cause alternative splicing of OPTN, resulting in a premature stop codon and loss of protein function [124]. The same group also found that OPTN levels in the rs3829923 variant can be upregulated by TARDBP/TDP-43, and one of these also observed in other FTD neuromuscular disorders that share childhood-onset neurodegeneration with ataxia, dystonia, and gaze palsy as symptoms [121]. Three different bi-allelic loss-of-function variants of SQSTM1/p62 (c.286C > T (p.Arg96*), c.311_312del (p.Glu104Valfs*48) and c.2T > A) were found in four different families [121]. This study thus also underlines that pathologies related to SQSTM1/p62 mutations are heterogeneous in their manifestation.

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autophagy receptors play an important role in the development of PDB, although the exact etiology remains to be established.

2.2.7. Distal myopathies with rimmed vacuoles

Distal myopathies are a heterogeneous group of muscle disorders with approximately 20 different forms, which are characterized by weakness and atrophy of muscles in hands and/or feet. The different myopathy types can be traced to mutations in different genes. Some are autosomal recessive whereas others are inherited in a dominant fashion [125]. Analyses of muscle biopsies of most distal myopathies show disorganization of myofibrils [126]. In some cases, the so-called rimmed vacuoles, which appear to be accumulations of protein and membrane debris with a possible autophagic origin, are detected in microfibers [127]. Distal myopathies with rimmed vacuoles (DMRV) are also called inclusion body myopathies and often occur in conjunction with PDB or FTD [127]. One study of a family with autosomal dominant DMRV, identified the c.1165 + 1 G > A splice donor variant in the SQSTM1/p62 gene in the affected individuals but also in one non-family related patient [126]. Interestingly, DMRV patients also have SQSTM1/p62 inclusions [126]. Apart from the wild type protein, which is still produced in patients, the generated cryptic splicing site leads to two additional versions of SQSTM1/p62, one deletion variant lacking the PEST domain (i.e., SQSTM1ΔPEST2), and one truncated protein missing the C-terminal UBA domain (i.e., SQSTM1ΔUBA-DK). These truncated versions of SQSTM1/p62 result in a lower molecular weight protein that is present in patient fibroblasts and skeletal muscle cells [126]. SQSTM1ΔPEST2-expressing cells display large ubiquitin- and LC3-positive cytoplasmatic and sarcoplasmatic inclusions, which are very similar to the inclusions present in DMRV patient cells. SQSTM1ΔUBA-DK, in contrast, is homogeneously distributed throughout the cell cytoplasm and on striations in mouse skeletal muscle [126]. Moreover, it does not colocalize with ubiquitin and LC3 [126]. DMRV can thus also be caused by a defect in SQSTM1/p62. As rimmed vacuoles can be sporadically seen in cells of people affected by ALS, PDB and FTD, it would be interesting to identify which other genetic and/or environmental factors determine the eventual disease outcome.

2.2.8. Sporadic inclusion body myositis

Sporadic inclusion body myositis (SIBM) is the most common myopathy in people over 45 years of age [128]. SIBM presents itself as a characteristic pattern of progressive muscle weakness and atrophy in proximal and distal muscles, especially the wrist and finger flexors and knee extensors [128]. While the exact nature of the disease, i.e. degenerative or inflammatory, and its causes are unknown, there are some key traits that serve as hallmarks, among which is the existence of rimmed vacuoles, sarcoplasmatic inclusions and deposits of degenerative proteins [128]. One of these deposited proteins is SQSTM1/p62 [128]. Some missense mutations in SQSTM1/p62 (i.e. A117V, G194R, K238E and P392L) have been found in SIBM patients. Because some of these mutations are also associated with ALS, FTD or PDB, SIBM patients have been screened to exclude these other pathologies. Patients carrying these mutations do not have increased expression of SQSTM1/p62, but show increased expression of immune-related genes, such as those corresponding to MHC class I and II [128]. The authors proposed that the SQSTM1/p62 aggregates could be caused by increased protein stability [128]. Although they do not provide insights into SIBM development, SQSTM1/p62 variants could play a direct role other than just accumulating in inclusions. Further research into their function and prevalence is required to draw any conclusions. Interestingly, mutations in FYCO1, a RAB7 effector that directs autophagosome transport on microtubules [129], also lead to SIBM [130].

2.2.9. Parkinson disease

PD is a neurodegenerative disorder described in section 2.1.15, that is characterized by the loss of dopaminergic neurons in the substantia nigra, causing progressive motor deficits [87]. When overexpressed or aggregated, SNCA/a-synuclein causes dysfunction of the mitochondrial respiratory chain leading to a decrease in ATP and an increase in ROS in the affected neurons [87]. Not surprisingly, mitophagy appears to be an important factor in PD. PINK1 and PRKN are proteins that are commonly found mutated in autosomal recessive PD cases. The PINK1 kinase is stabilized upon mitochondrial damage, promoting its phosphorylation of PRKN, necessary for the recruitment and activation of this E3 ubiquitin ligase onto the mitochondrial outer membrane [87]. PRKN has a number of downstream targets that are recognized by autophagy receptors and enable the formation of LC3-positive phagophores adjacent to the damaged mitochondria to promote mitophagy [87]. There is redundancy within the autophagy receptors that are recruited, i.e. SQSTM1/p62, OPTN, NBR1, CALCOCO2/NDP52 and TAX1BP1, and this could explain why mutations in these proteins are not found to cause PD, although SQSTM1/p62 and NBR1 do localize to PD inclusions [131,132]. A factor regulating these autophagy receptors is their phosphorylation by TBK1 [100]. Yet, intriguingly enough, whereas PINK1 is required for TBK1 function, and thus OPTN function, TBK1 and OPTN mutations are only associated with ALS and not with PD, while PINK1 is associated only with PD [100]. PRKN and PINK1 also interact with the kinesin adaptor protein RHTO1/Miro1 to trigger its proteasomal turnover, which leads to the dissociation of damaged mitochondria from the cytoskeleton promoting their engulfment by phagophores [133]. A third protein associated with PD is LRRK2, which under normal circumstances forms a complex with RHTO1/Miro1 essential for its proteasomal degradation, but not when carrying a PD-associated mutation [134]. LRRK2 downregulation increases autophagy, and causes accumulation of autophagic structures [135]. Therefore, LRRK2 could also genetically link PD development with impairment in mitophagy. Yet, while LRRK2 is found on mitochondria, and the G2019S variant alters mitochondrial morphology, ROS levels and mitochondrial membrane potential, there is no direct link between this protein and mitophagy [135]. While the roles of PINK1, PRKN and LRRK2 in preventing PD can be linked to mitophagy, it remains unclear why other proteins involved in this pathway are not found mutated in PD patients and why related mutations lead to ALS rather than PD.

2.2.10. Hereditary sensory and autonomic neuropathy II B

Hereditary neuropathies are a heterogeneous group of diseases that are classified by their alterations of motor, sensory or autonomic neurons [136]. Hereditary sensory and autonomic neuropathy II (HSANIIIB) is a rare sensory and autonomic neuropathy leading to reduced sensation to touch, cold and pain [137]. This often results in infections, osteomyelitis and mutilations, with the possible requirement of limb amputation in patients, similar to leprosy [138]. In 2009, mutations in RETREG1/FAM134B were identified as a causative factor for one of the subtypes of HSANIIIB [138,139]. Initially, it was thought that RETREG1 was a Golgi protein, as its depletion results in a disorganization of this organelle [139]. Khaminets, et al., however, showed that RETREG1 serves as an autophagy receptor for reticulophagy and the mutations found in HSANIIIB patients result in a protein unable to carry out its receptor function [140]. This defect has the ultimate consequence of causing an expansion of the ER due to an inhibition in its turnover, which in turn sensitizes the cells to apoptosis, leading to the sensory neuron loss seen in HSANIIIB [140].

2.2.11. Normal tension glaucoma

Glaucomas are a set of neurodegenerative disease affecting retinal ganglion cells, which leads to degeneration of the optic nerve fibers and ultimately to blindness [141]. Glaucomas are subdivided into different types, and one of them, the normal tension glaucoma (NTG), is associated with mutations in OPTN [141]. The OPTN*G962* variant, in particular, is a risk factor for NTG [141]. This variant provokes an increase in TFRC (transferrin receptor) uptake by phagophores, leading to an imbalance in the cells’ iron metabolism and consequent cell death [141]. TBK1 is required to functionally link OPTN to LC3, and TBK1
mutations can similarly cause NTG [141]. If modulation of autophagy ameliorates NTG and possibly other neurodegenerative diseases, this is certainly worth deeper investigation.

2.2.12. CROHN'S disease

As explained in section 2.1.7, CD is an IBD that is characterized by relapsing inflammation of the gastrointestinal tract mucosa. A 2013 whole exome sequencing study with single nucleotide variant filtering found that SNP rs2303015 (Val248Ala) of CALCOCCO2/NDP52 also confers susceptibility to CD [142]. CALCOCCO2/NDP52 is a selective autophagy receptor for xenophagy [142], and it also plays an immunosuppressive role in NFKB/NF-κB signalling through toll-like receptors (TLRs), including TLR3. The Val248Ala mutant, however, is unable to negatively regulate NFKB signalling. As CALCOCCO2/NDP52 but not the Val248Ala variant is degraded by autophagy and the pro-teasome after being ubiquitinated when TLR3 is activated, the authors propose that one or more post-translational modifications of CALCOCCO2/NDP52 could be affected by the mutation, making the protein more stable and thus increasing NFKB signalling [142]. Nonetheless, this rare variant in CALCOCCO2/NDP52 could strengthen the notion of a role of autophagy in CD.

2.2.13. Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is characterized by the progressive accumulation of fat in the liver. Because autophagy can regulate fat metabolism through lipophagy, it seemed reasonable to test whether mutations in ATG genes are associated with NAFLD [143]. Thus, IRGM has been linked with NAFLD susceptibility [144]. In particular, five SNPs in the IRGM gene were found to be risk factors for NAFLD, and especially the rs10065172 TT genotype increases the odds of NAFLD by 2.059 [144]. The siRNA-based knockdown of IRGM results in lower autophagic flux and doubles the number of lipid droplets in HepG2 and PLC/PRF/5 cells, a phenotype that can be rescued by induction of autophagy with rapamycin, and phenocopied by inhibiting this pathway with wortmannin [144]. Accordingly, cells overexpressing a GFP-tagged version of IRGM appear to show a decrease in the number and size of lipid droplets. The authors thus proposed that IRGM is required for lipophagy in hepatocytes and that the rs10065172 TT genotype puts people at risk for NAFLD [144].

2.2.14. Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a rare but severe disease that is often fatal, when excessive fibrous tissue is formed in the lungs [145]. The only treatment option is a lung transplant [145]. A GWAS identified three SNPs in TOLLIP, i.e., rs11521887, rs5743890 and rs5743894, which all lead to a reduced expression of TOLLIP, ranging from 20 to 50% of the normal levels [145]. Curiously, the G allele at rs5743890 in TOLLIP is more often seen in healthy people than in patients, suggesting that it could confer a protective effect but it increases the mortality risk when the disease develops [145]. In addition to being involved in selective types of autophagy [146], TOLLIP participate in TLR signalling, and in intracellular trafficking and the degradation of TGFβ (transforming growth factor beta) type 1 receptors [145]. A focus for further research into IPF should be on the effect of these SNPs at the cellular level, and how this could explain the disease development and severity.

2.2.15. FANCONI anemia

Fanconi anemia (FA) is a rare and complex disorder caused by mutations in one or more of the 16 proteins that function together in repairing DNA damage caused by crosslinking [147]. Patients with mutations in any of the FA genes are often diagnosed when they start to suffer from bone marrow failure in adulthood. Other symptoms are in line with outcomes expected with a dysfunction in a DNA damage repair pathway; that is, acute myeloid leukaemia, reduction in mature blood cells, hearing failure, endocrine and gastrointestinal abnormalities, congenital limb deformities, skin hyper-pigmentation, low bone density and high risks of developing solid tumors [147]. Two FA genes are infamous for their involvement in familial breast cancer and ovarian cancer development: BRCA1/FANCS and BRCA2/FANCD1 [148]. Some of the proteins in the FA pathway have functions other than DNA damage repair. A recent study found that FANCC is involved in virophagy and mitophagy, and that FANCA, FANCF, FANCL, FANCD2, BRCA1 and BRCA2 are all also required for mitophagy [148]. Moreover FANCC interacts with PRKN [148]. FANCC mutants are known for a hypersensitive inflammatory cytokine response, which is independent from the role of this protein in DNA-damage repair [148]. Interestingly, a mutation in FANCC that produces a shorter protein, i.e. c.67delG, results in a milder disease phenotype [148]. Cells expressing c.67delG protein cannot repair DNA damage but have normal clearance of dysfunctional mitochondria through mitophagy and are also not sensitive to inflammatory cytokine cell death [148]. The role in hypersensitivity thus appears to segregate with its role in mitophagy. This observation suggests that impairment of one or more functions connected with autophagy could be part of the pathophysiology of FA, in particular when FANCC is mutated [148].

2.2.16. Infectious diseases

Whereas infectious diseases are not directly caused by gene mutations, there are mutations that can confer susceptibility to infection. Mutations that affect the autophagy receptor TOLLIP are associated with susceptibility to several infectious diseases: leprosy, leishmaniasis, tuberculosis and sepsis [149-152]. TOLLIP also negatively regulates TLR signalling via IL6 (interleukin 6), TNF/TNF-α and IL10 [150]. For leprosy, the rs3793964 TT variant confers susceptibility to Mycobacterium leprae and is linked to increased expression of TOLLIP and IL1R1/IL-1Ra in the skin [149]. The rs5743899 and rs3750920 SNPs, in contrast, are associated with leishmaniasis and tuberculosis [150,151], whereas the rs5743867 SNP confers susceptibility to sepsis by increasing TOLLIP mRNA and decreasing IL6 and TNF levels [152]. Of note, UK1 variants causing susceptibility to Mycobacterium tuberculosis have been identified [153]. Whereas TOLLIP can act as an autophagy receptor, whether the major role of TOLLIP in preventing infectious diseases is linked to this function or to its modulation of TLRs, or both, remains to be elucidated.

3. Conclusions

In this review, we have tried to provide a complete list of those congenital pathologies that are caused by or could be caused by mutations in genes that encode proteins that are part of the core autophagy machinery, that function as autophagy receptors or that are otherwise related to this process (Tables 1 and 2). It must be noted that this list is not exhaustive due to space limitations; we did not cover, for example, those diseases that are due to alterations in genes known to be involved in autophagy but not part of the core ATG machinery. One such example is the Charcot Marie Tooth 2B (CMT2B) disease, which is an inherited peripheral neuropathy characterized by the loss of peripheral sensory and motor functions rather than sensory and autonomous functions, and is caused by genetic alterations in RAB7 that lead to high levels of the active form of this RAB GTPase [136,154]. CMT2B symptoms can be explained by major lysosomal and autophagic activities leading to a premature breakdown of the nerve growth factor (NGF) receptor NTRK1/tropomysin receptor kinase A, resulting in impaired NGF binding and signalling [155].

Another example is Vici syndrome, a recessively inherited multisystem disorder that is invariably fatal [156]. Children with this disease have a poorly developed corpus callosum (callosal agenesis), cataracts, cardiomyopathy, hypopigmentation, progressive microcephaly, failure to thrive, a global development delay and immunodeficiency [156]. EPG5 is the causative locus for Vici syndrome [157] and the encoded protein also appears to be a RAB7 effector involved in
carried out by autophagosome maturation [158], but that is also be responsible for transporting the TLR9 ligand CpG to the late endosomal-lysosomal compartments [156].

Finally, hereditary spastic paraparesis or paraplegia (HSP) is characterized by axonal degradation of the corticospinal motor- and sensory neurons, which leads to partial (i.e., paraparesis) or complete (i.e., paraplegia) loss of limb motor control causing spasticity, progressive weakness and hyperreflexia of the lower limbs [159]. Three autosomal recessive variants of HSP are associated with loci indirectly involved in autophagy: SPG11/SPATACIN, ZFYVE26/SPG15/SPASTIZIN and CYP2U1/SPG49 [160,161]. ZFYVE26 interacts with the PtdIns3K complex, which is responsible for autophagosome maturation as it is involved in endosomal trafficking. Patient gene variants lead to truncated proteins or to an inability of ZFYVE26 to bind this PtdIns3K complex. ZFYVE26 interacts with SPG11, and when one of these two proteins is depleted, affected cells show increased numbers of enlarged autophagosomes and decreased numbers of lysosomes [160]. CYP2U1 is a more recently discovered variant of HSP, caused by mutations in TECPR2 (tectonin beta-propeller repeat containing 2) [161]. TECPR2 regulates COP11-dependent ER export, and as ER-exit sites are important for early autophagosome formation, it may be that mutations in TECPR2 affect this ER subdomain and indirectly impair autophagy progression [162].

It is interesting to note that most diseases due to a genetic alteration of autophagy can be classified as either neurological disorders or inflammatory pathologies (Tables 3 and 4). This is not an entirely surprising observation. A process such as autophagy that is vital for cellular homeostasis is naturally more important in post-mitotic neurons than in cell types capable of regeneration. Several autophagy receptors, which direct the selective breakdown of compromised proteins or organelles, lead to neurodegeneration when mutated. For example, mutated OPTN is associated with ALS, FTD-ALS and NTG, whereas mutated SQSTM1/p62 is associated with ALS, PD, FTD, FTD-ALS and AAS (Table 2). Not surprisingly, many of these diseases are characterized by the accumulation of cytoplasmic aggregates and dysfunctional organelles, including mitochondria. OPTN and SQSTM1/p62 are risk factors of another illness, PDB, which is not neurological but presents cytoplasmic inclusions. Interestingly, the affected cells are osteoblasts that, similar to neurons, do not divide and therefore could rely greatly on autophagy to eliminate damage.

The link between inflammatory diseases and autophagy dysfunction is also not surprising. In addition to being relevant for the turnover of intracellular pathogens through xenophagy, autophagy regulates numerous aspects of immunity, including control of inflammation through various mechanisms, secretion of immunomediators and adaptive immunity, development of different subtypes of immune cells, immunotolerance, etc. [163]. Consequently, it is easy to imagine that autophagy alterations could have a negative multisystemic effect on the immune system, thus promoting predominantly the development of inflammatory diseases. In this regard, it is interesting to highlight that null and missense mutations are only found in autophagy receptors and related proteins, but not in the core ATG machinery, where most of the genetic changes do not have a major impact on the function of the protein. Because of the multitude of physiological roles of autophagy, it is expected that the absence of a core ATG gene is prenatally lethal. In contrast, loss of an autophagy receptor would only affect a subset of selective types of autophagy, and as a result the onset of the disease can also be postnatal. This consideration is in keeping with the finding that knockout mice lacking core Atg genes die during embryonic development or shortly after birth, whereas mice lacking SQSTM1/p62 or NBR1 live longer [164–166]. An exception appears to be WDR45/WIPI4, but in most of the cases the manifestations of the diseases associated with this protein are sporadic and, when genetically inherited, the loss of function is probably compensated by WDR45B/WIPI3 as shown in cell-line studies [167].

Although it was thought for a long time that ATG proteins solely regulate autophagy, we now know that they are also involved individually or in groups in other pathways through so-called non-canonical functions [1,4]. As a result, some of the described pathologies could be due to the alteration of one of the non-canonical functions or a combinatorial effect together with an impairment of autophagy. For example, the ATG5 variants leading to SLE also alter LAP, a pathway that is clearly linked to the disease progression. Thus, while learning more about the etiology of several diseases and their genetic predispositions, we might acquire insights not only into autophagy and its roles, but also into non-canonical functions of ATG proteins. Conversely, increasing our knowledge about the mechanisms and physiological roles of autophagy, but also about the non-canonical functions of ATG proteins, could help in deciphering the pathophysiology of some diseases.

We are just at the beginning of a long journey, but the combination of genetic and functional information could pave the way to the development of optimal therapies to cure, or at least delay, the onset of specific illnesses. Most of the current autophagy-based therapies rely on drugs such as rapamycin or chloroquine, which are far from being specific as they alter numerous cellular pathways other than autophagy [168,169]. Although there is a global effort in developing more specific compounds that exclusively target ATG proteins, their involvement in other processes and the multiple roles of autophagy, makes this task very difficult. Understanding the exact molecular principles underlying both autophagy and the associated disease could be of great help to achieve this task, as well as exploring combinatorial approaches were autophagy is modulated by physiological means, for example with a protein-poor diet and/or exercise [170,171].

Table 4

| Neurodegenerative disorders | Myopathies | Inflammation and infection | Other diseases |
|----------------------------|-----------|---------------------------|---------------|
| ALS                        | DMRV      | CD                        | PDB           |
| FTD                        | SIBM      | Infectious diseases       | IPF           |
| FTD-ALS                    | AAS       | FA                        |               |
| PD                         | NTG       |                           |               |
| HSAN1IB                    |           |                           |               |

Table 3

| Neurodegenerative disorders | Inflammation and infection | Respiratory | Other diseases |
|-----------------------------|---------------------------|-------------|---------------|
| Rett-like syndrome          | VKHS                      | Asthma      | KB            |
| NMO                         | Systemic sclerosis        | COPD        | NAFLD         |
| BPAN                        | BD                        | PDB         |               |
| FD                          | CED                       | RA          | DEE           |
| Ataxia                      |                           | SLE         | EOE           |
| EOE                         |                           | CD          |               |

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