The Link Between Lysosomal Storage Disorders and More Common Diseases

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Abstract

In the last decades, it has become more and more evident that lysosomal storage disorders and common neurodegenerative diseases such as Alzheimer and Parkinson diseases have clinical, neuropathological, and genetic features in common, including lysosomal dysfunction and impaired autophagy. Patients with Gaucher and even carriers of Gaucher disease have an increased risk to develop Parkinson disease. Likewise, individuals who are heterozygous for a mutation of a gene that causes an adult form of neuronal ceroid lipofuscinosis are more likely to be affected by a form of frontotemporal dementia in their later life. A further example is the gene *NAGLU* encoding the enzyme α-*N*-acetylglucosaminidase, which is deficient in patients with mucopolysaccharidosis type IIIB. Mutations of the *NAGLU* gene have been observed in patients affected by an axonal neuropathy. An interesting unexpected finding was the link between stuttering and genes that are essential for the function of all lysosomal enzymes. This review will present some example of the association of lysosomal storage disorders and neurodegenerative disease and discuss possible pathogenic mechanisms that are common to both conditions. The understanding of the pathophysiology of the endosomal–lysosomal–autophagic system may help to develop drugs, which might provide benefit not only for patients with rare lysosomal storage disorders but also for individuals affected by more common diseases.

Introduction

Lysosomes cannot be regarded anymore as simple waste bags that have the singular task of degrading high-molecular-weight compounds but must now be recognized as the central regulator of cell homeostasis, with a functional role in many cellular processes. Lysosomes continuously fuse or interact transiently with other compartments in the autophagic and endocytic pathways and even with the plasma membrane, thereby forming a complex cellular system, the endosomal–lysosomal–autophagic pathway, which plays an essential role in signaling, growth, cell dynamics, defense, and regulation of metabolic homeostasis.[1-3]

It has been shown that in lysosomal storage disorders, the primary storage material can impair also the clearance of other cellular cargo, for example, amyloid β and α synuclein, processed through the endocytic and autophagic routes.

From the other side, a dysfunction of the endosomal–lysosomal (E/L) network plays an essential role in the pathogenesis of secondary storage disorders such as Alzheimer and Huntington diseases, as these conditions are caused by mutations of genes whose function directly or indirectly regulates part of the autophagic pathway, even though they do not encode lysosomal enzymes.

All storage disorders are characterized by the accumulation of material such as gangliosides or undegraded proteins within the endosomal–lysosomal–autophagic system. At the end, the accumulations of these compounds trigger degenerative processes. Postmitotic neurons are particularly vulnerable to the effect of toxic substances because they are unable to dilute the

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storage material, unlike mitotic cells, which can remove their accumulated cargo through cell division.

In this review, clinical, pathophysiological, genetic, and therapeutic aspects of the association between lysosomal storage disorders and common neurodegenerative diseases will be discussed.

Common Neurodegenerative Diseases in the Context of Lysosomal Storage Disorders

Alzheimer Disease

Alzheimer, Parkinson, and Huntington diseases are neurodegenerative disorders, called proteinopathies, that are characterized by the presence of insoluble protein aggregates in the brain. A typical neuropathological feature of these conditions is the accumulation of cellular substances in neuronal cells that become toxic aggregates. In Parkinson disease, the aggregates consist of α-synuclein; in Huntington disease, Huntingtin is the accumulating compound; and Alzheimer disease is characterized by the deposition of neurofibrillary tangles (NFTs) and amyloid β (Aβ). The major component of the NFTs is hyperphosphorylated tau, a microtubule-stabilizing protein.

From pathological and genetic studies, it has become more and more clear that dysfunction of the E/L network plays an essential role in the pathogenesis of Alzheimer disease. It was first recognized 20 years ago that the E/L system is upregulated in this neurodegenerative disorder.[4] Alterations of the E/L system are evident in familiar forms of Alzheimer disease: The most common cause of early-onset familial Alzheimer disease is mutations of presenilin 1 (*PS1*), a transmembrane protein (TMEM) that is involved in the autophagic turnover of proteins. The PS1 has been shown to be essential for lysosomal acidification and proteolysis during autophagy, and defective lysosomal proteolysis is apparently responsible for pathogenic protein accumulations and neuronal cell death in Alzheimer disease.[5]

In some families, the duplication of the amyloid precursor protein (*APP*) gene is the cause of early-onset Alzheimer disease, and in an animal model of Down syndrome, it was shown that the triplication of the *APP* gene leads to the dysfunction of the endocytic pathway, resulting in defective neurotrophic signaling and neurodegeneration.[6] The epsilon4 allele of ApoE polymorphism represents the strongest genetic risk factor for late-onset Alzheimer disease, and Cataldo et al were able to demonstrate that inheritance of this polymorphism accelerates the abnormal endocytic upregulation seen in early Alzheimer disease.[7]

Studies in patients and mouse models have demonstrated that the disruption of the E/L system is an essential component in the pathophysiology of Alzheimer disease; conversely, a dysfunction of the E/L network results in the formation of tau insoluble aggregates, which leads to neurodegeneration, as seen in human neuroblastoma cells.[8] And Ohmi et al have detected hyperphosphorylated tau in a mouse model of Sanfilippo syndrome type B (mucopolysaccharidosis type IIIB

[MPS IIIB]), and based on these findings, the author speculated that this lysosomal storage disorder should be regarded as a tauopathy.[9] Pathological abnormalities characteristic of Alzheimer disease, such as fragments of Aβ within enlarged neurons, have been demonstrated in the brain of patients affected by Niemann-Pick type C, thus reinforcing the close link between lysosomal storage disorders and more common neurodegenerative diseases.[10]

Parkinson Disease

In Parkinson disease, the main symptoms are rest tremor, rigidity, bradykinesia, postural instability, and nonmotor impairments such as autonomic dysfunction, depression, and cognitive deficits. Characteristic neuropathological changes are marked degeneration of dopamonergic neurons in the substantia nigra and the presence of Lewy body (LB) inclusions. Lewy bodies consist mainly of α-synuclein that is abnormally phosphorylated and tends to form insoluble fibrils.

For a long time, Parkinson disease was assumed to be a single, non-genetic disorder, but genetic analysis in a great number of affected individuals and their families has revealed a great number of genes associated with this condition.[11]

Clinical features similar to that of Parkinson disease are also observed in some lysosomal storage disorders, for example, bradykinesia and cognitive decline in Gaucher disease (defect of β-glucocerebrosidase). In 1996, Neudorfer et al reported that the incidence of Parkinson disease in patients with type I Gaucher was higher than expected.[12] Based on this and other observations, Tayebi et al studied the association of mutations of the β-glucocerebrosidase gene and Parkinson disease in 17 patients who, in addition to type I Gaucher disease, had symptoms of this neurodegenerative disorder such as rigidity, bradykinesia, and tremor. Genotyping in 14 patients revealed the common "non-neuronopathic" N370S mutation.[13] Using data from the International Gaucher Registry, it was calculated that patients with type I Gaucher have a 9% to 12% probability of developing Parkinson disease before the eighth decade of life,[14] as compared to a probability of 2.6% in the general population.[15] A study performed by Goker-Alpan et al in relatives of patients with Gaucher revealed that also heterozygotes of this lysosomal storage disorder have an increased risk for developing Parkinson disease.[16]

Aharon-Peretz investigated 99 Ashkenazi patients with idiopathic Parkinson disease and 1543 healthy Ashkenazi Jews for the occurrence of the 6 most common mutations of the *GBA* gene. Her results showed that 31 (31.3%) patients with Parkinson disease had 1 or 2 mutant *GBA* alleles, but only 6.2% (95 of 1543) of control participants carried a *GBA* mutation (92 were heterozygous for the mutation N370S and 3 were heterozygous for the mutation 84GG).[17] The suggestion that heterozygosity for a *GBA* mutation predisposes to Parkinson disease was confirmed in an international study in which 5691 patients with Parkinson disease (780 Ashkenazi Jews) and 4898 controls (387 Ashkenazi Jews) from 16 centers were enrolled. In both Jewish and non-Jewish patients with Parkinson disease, the

frequency of a *GBA* mutation was significantly higher than in the control group. Furthermore, individuals who carried a *GBA* mutation had an earlier onset of symptoms and more often demonstrated atypical clinical manifestations compared to patients who were not carriers.[18]

The concept of an association of *GBA* mutations and Parkinson disease is based not only on clinical observations but also on neuropathology findings. In the brain of 4 patients who had symptoms of Parkinson disease in addition to type I Gaucher disease, LBs were detected. The level of the storage compound glucosylsphingosine, however, was not elevated.[13] When neuropathological, genetic, and immunofluorescence studies were performed in brain samples from 7 patients with Parkinson disease who had Gaucher disease or were heterozygous for a *GBA* mutation, immunofluorescence showed the presence of glucocerebrosidase in 75% of LBs. In samples from 7 participants without a GBA mutation, however, less than 10% of LBs were glucocerebrosidase positive.[19] These results demonstrate again that β-glucocerebrosidase represents an important component of pathological α-synuclein-positive inclusions.

Although the role of *GBA* mutations in the pathogenesis of Parkinson disease is not yet fully understood, several possible mechanisms have been proposed. It has been shown, for example, that α-synuclein is predominantly degraded by lysosomes, in part by chaperone-mediated autophagy.[20] In mouse models of lysosomal storage disorders that are associated with severe neurodegeneration (MPS type IIIA and multiple sulfatase deficiency), an impairment of the autophagic pathway was observed, leading to insufficient degradation of proteins such as α-synuclein or huntingtin.[21] Conversely, abnormalities of α-synuclein have an influence on the lysosomal function, as demonstrated by the studies of Cuervo et al who have shown that the pathogenic A53T and A30P α-synuclein mutants, bound to the lysosomal membrane via the receptor LAMP2A, inhibit both their own degradation and that of other proteins.[20] This observation, however, cannot explain why in the cerebrospinal fluid of patients with Parkinson disease a significant decrease in the activity of several lysosomal enzymes (including β-glucocerebrosidase) was observed.[22]

A direct physical interaction between β-glucocerebrosidase and α-synuclein at acidic pH has been detected by the use of fluorescence and nuclear magnetic resonance spectroscopy. This interaction apparently enables lysosomal degradation or prevents aggregation of α-synuclein, whereas the N370S mutant form of β-glucocerebrosidase has shown a reduced affinity for α-synuclein, possibly impairing its degradation.[23] In mice, elevated α-synuclein levels were observed within nigral cell bodies as well as in astroglia after the inhibition of β-glucocerebrosidase with conduritol B epoxide.[24]

Based on a comprehensive literature search, Shachar et al found that not only patients with Gaucher disease but also individuals affected by another neurodegenerative lysosomal storage disorder might develop symptoms of Parkinson disease and show pathological abnormalities such as α-synuclein aggregation in the brain and substantia nigra pathology.[25] An association with Parkinson disease has been described in GM1- and GM2-gangliosidosis, Niemann-Pick disease type C, neuronal ceroid lipofuscinoses (NCLs), and mucopolysaccharidoses, for example.

It has already been reported that mechanisms that might be implicated in the link between a rare lysosomal storage disorder and a more common neurodegenerative disease include impairment of autophagy, disturbance of calcium homeostasis, oxidative stress, and dysfunction of the ubiquitin–proteasome system.[26] All in all, one has to state that the present theories that try to explain the link between a group of lysosomal storage disorders and a synucleinopathy still have their limitations.

Frontotemporal Dementia and NCL

Frontotemporal dementia (FTD), the second most common form of young-onset dementia, is characterized by atrophy of the frontal and temporal lobes and is clinically, neuropathologically, and genetically heterogeneous.[27] The clinicopathological spectrum named frontotemporal lobar degeneration (FTLD) can be divided into 3 clinical syndromes: frontal variant (fvFTD) with predominant behavioral disorders, semantic dementia, and progressive nonfluent aphasia, in which language disturbances predominate. Two major pathological categories of FTLD can be distinguished. There are FTLD with tau-positive (FTLD-tau) inclusions and FTLD with ubiquitin and TAR DNA-binding protein of 43 kDa (TDP-43) positive, but tau-negative inclusions (FTLD-TDP). In only a small number of cases, immunoreactivity with the fused in sarcoma (FUS) antibody (FTLD-FUS) has been detected, and FUS-positive inclusions were additionally found in patients with neuronal filament inclusion disease. About a half of the familial FTD cases are caused by mutations in the microtubule associated protein tau (*MAPT*) and *GRN* (progranulin) genes, whereas mutations valosin-containing protein (*VCP*), charged multivesicular body protein 2B (*CHMP2B*), *TDP-43*, and *FUS* genes are found in less than 5% of cases.[27]

The assumption that dysfunction of the E/L pathway contributes to the pathogenesis of not only Alzheimer and Parkinson diseases but also of frontal lobar dementia was supported by many morphological and biochemical studies in the brains and cell culture models of affected patients. In fibroblasts of patients with FTD who carried a *CHMP2B* mutation, a malfunction of endosomal trafficking, namely the disruption of the fusion of endosomes with lysosomes, was detected.[28] Tresse et al have demonstrated an accumulation of large autophagic vesicles with cathepsin B activity in cells (myoblasts) from patients with FTD having a mutation of the *VCP* gene, indicating an autophagy defect. From this finding, the authors concluded that *VCP* is essential for maturation of ubiquitin-containing autophagosomes and that a defect in this function may contribute to FTD pathogenesis.[29]

The assumption that FTD and lysosomal storage disorders share common pathophysiological mechanisms was confirmed by the observation of a co-occurrence of NCL and FTD in 1 family[30]: In this family, the index cases were 2 adult siblings

who had typical features of NGL, but in whom no mutation in known NCL genes could be found. Using linkage analysis and exome sequencing in the siblings, the homozygous deletion c.813_816del (p.Thr272Serfs *10) in the progranulin (*GRN*) gene was detected, and a similar 4 base pair (bp) deletion in exon 7 (c.811–814delCTCA) was already known as a pathogenic mutation, causing FTD when present in heterozygosity.[31] Later on, the ceroid lipofuscinosis, caused by a progranulin mutation, has been designated as CLN11.[32]

Neither parent of the siblings described by Smith et al had symptoms of dementia at the time of their clinical evaluation, although they were heterozygous for the 4 bp deletion, but one has to take into account that they were still in their 50s. There was, however, a history of dementia in several ancestors of both families.[30]

Additionally, studies in several animal models of FTD and NCL showed an overlap of morphological and biochemical abnormalities. In Grn$^{(-/-)}$ mice, in which the progranulin gene is completely absent, not only increased levels of TMEM 106B (a known risk factor for FTLD-TDP) but also an accumulation of lipofuscin and other components characteristic of NCL were detected.[33] Vice versa, in the brain of mice deficient in cathepsin D, which are a model for the most severe form of ceroid lipofuscinosis (congenital NCL10), an accumulation not only of saposin D, the typical storage material of patients with NCL10, but also increased levels of the FTLD-associated proteins progranulin and TMEM106B have been found.[33] The observation that in the brain of several patients with NCL, phosphorylated TDP-43 was detected in addition to the characteristic accumulation of saposin D, again implicates that lysosomal storage disorders and FTD share common features. This applies not only for *GRN*-associated frontal lobar dementia but also for FTLD cases caused by *CHMP2B* and *VCP* mutations that apparently also impair lysosomal function.[28,29] However, the mechanisms that lead to a malfunction of the lysosomal–endosomal–autophagic pathway in mutations of several genes of frontal lobar dementia are still unclear.

Axonal Neuropathy and MPS Type IIIB (Sanfilippo Disease IIIB)

Genetic sensory neuropathies, which are often accompanied by varying degrees of autonomic dysfunction, are usually painless and have an early onset.[34] Late-onset painful polyneuropathies, however, rarely have a genetic basis. Most are associated with other systemic disorders such as diabetes or alcohol abuse.[35] Little is known about genetically determined adult-onset forms of sensory neuropathy, but the identification of the underlying genetic defects may become possible by the application of new molecular techniques such as next generation sequencing. Whole exome sequencing was used by Tetreault et al to discover the mutated gene in a large family with many members affected by a dominant late-onset painful neuropathy.[36] Of 45 individuals of this French Canadian family, 21 had symptoms. Pedigree analysis suggested a dominant mode of inheritance, whereby a significant intrafamilial variability in age of onset

and severity could be observed. All patients complained of painful sensations, and the diagnosis of a peripheral neuropathy was based upon the finding of decreased vibration sense and/or a loss of some deep tendon reflexes. Whole exome sequencing analysis in 4 affected individuals did not reveal a mutation in the genes for the types 1 to 5 of hereditary sensory and autonomic neuropathy, for Fabry disease, or for GM2-gangliosidosis. The great number of variants that was found by exome sequencing analysis was filtered against several databases in order to eliminate common polymorphisms. After the last step of filtering, 4 candidate variants were left, and only the missense mutation c.1208T>C (p.Ile403Thr) in exon 6 of the α-*N*-acetylglucosaminidase (*NAGLU*) gene was predicted to be pathogenic by the bioinformatics programs. This result was validated by Sanger sequencing, and segregation analysis in the whole family demonstrated that only the *NAGLU* mutation p.Ile403Thr segregated with the disease. This variant was not found in any available databases, but its location on the protein structure suggests an impact on the activity of α-*N*-acetyl-glucosaminidase. The activity of α-*N*-acetyl-glucosaminidase was measured in 42 individuals of this family and showed a reduction of about 50% of normal control, confirming the biological effect of the p.Ile403Thr mutation. No second *NAGLU* variant could be detected in any of the affected patients. In order to find additional patients affected by a neuropathy who might carry a *NAGLU* mutation, the authors searched a database of the University of Miami, the Genomes Management Application. In this database, a patient with Charcot-Marie-Tooth (CMT) was detected, who carried the nonsense p.Glu123X variant of the α-*N*-acetyl-glucosaminidase gene, that undoubtedly has a biological effect, as it leads to a truncation of the protein in the *N*-terminal domain before the catalytic domain. Many family members of the patient with CMT had diminished reflexes, reduced sense of vibration, and complained of pain. Segregation analysis, performed in 3 affected members from whom DNA was available, confirmed the presence of the NAGLU p.Glu123X variant.[36]

The lysosomal enzyme α-*N*-acetyl-glucosaminidase is involved in the degradation of the glycosaminoglycan heparan sulfate. A deficiency of this enzyme, caused by *NAGLU* mutations, leads to the neurodegenerative disorder Sanfilippo disease IIIB (MPS type IIIB), which is inherited in an autosomal recessive manner and characterized by a broad clinical heterogeneity.[37] Very mildly affected patients, described by Selmer et al, presented with sensory ataxia but had hyperactive tendon reflexes.[38] From the observation that *NAGLU* knockout mice develop significantly reduced response to painful stimuli by the age of about 4 months,[39] Tetreault and coworkers speculated that involvement of the peripheral nervous system could also be present in patients with MPS IIIB but that they are masked by the severe neurodegenerative symptoms.[36] Furthermore, it could be possible that the late-onset phenotype in heterozygotes of a *NAGLU* mutation is caused by the neurotoxic effects of slowly accumulating glycosaminoglycans, as in the brain of *NAGLU* knockout mice, an increased level of not only

glycosaminoglycans but also of GM3 ganglioside and P-tau has been detected.[9] However, it is obvious that the association of peripheral neuropathy and *NAGLU* mutations cannot be fully explained at the present time.

Stuttering and Mucolipidosis

Stuttering is a speech disorder characterized by excessive repetitions of sounds and syllables as well as blockages of the vocal tract. The disorder typically arises in childhood but generally stops spontaneously. Only in a minority of cases, stuttering persist into adulthood. The cause of stuttering is unknown. From twin and family studies, however, it has become clear that genetic factors also play role in the etiology of this condition. Genetic studies in large families have uncovered linkage with several loci across the genome, for example, linkage with chromosome 3,13, and 15.[40] Riaz et al have performed a linkage analysis in a group of 44 consanguineous Pakistani families, each of them containing numerous individuals affected by stuttering. In these families, a very strong linkage with a locus on the chromosome 12q, giving a LOD score above 3, was detected.[41] Kang et al analyzed this region in more detail in Pakistani and North American families who had affected members, as well as in control participants.[42] They found a high incidence of mutations of genes that are required for the generation of the mannose-6-phosphate residue, a signal that directs lysosomal enzymes to the lysosome. These genes are the *N*-acetylglucosamine-1-phosphate transferase gene *GNPTAB*, which encodes the α and β subunits of GlcNAc-phosphotransferase (GNPT), the *GNPTG* gene, which encodes the γ subunit of GNPT, and the gene *NAGPA*, which encodes the so-called uncovering enzyme (UCE) that is responsible for removing 1 *N*-acetylglucosamine group, thereby exposing the targeting signal mannose-6-phosphate.

Mutations of the *GNTAB* gene, localized in chromosome 12q.23.3, and of the *GNPTG* gene, localized in chromosome 16p13.3, are known to cause the lysosomal storage disorders such as mucolipidosis type II and type III, which are multisystemic diseases in which also the central nervous system is often involved.[43] However, the mutations that have been observed in stuttering cases are totally different from those found in mucolipidosis. To date, there are no known disorders caused by mutations the *NAGPA* gene (localization: chromosome 16p13.3).

Kang et al reported a high incidence of mutations of the *GNPTAB*, *GNTPG*, and *NAGPA* genes in patients who had stuttering.[42] In order to confirm these preliminary results, the frequency of rare mutations in these genes was reexamined in a larger number of patients.[44] In this study, 1013 individuals with persistent stuttering from the United States, England, Pakistan, and Brazil were included. And 164 (16%) of the patients were found to carry a rare, non-synonymous coding variant in 1 of these 3 genes, this frequency being significantly higher in comparison to population-matched controls. In total, 81 different variants were detected in the stuttering participants. Almost all coding variants were missense amino acid substitutions. Only 1

mutation, a 3-bp in-frame deletion in the *GNPTAB* gene, has been previously described in a mildly affected patient with mucolipidosis type III.[45] In summary, Raza et al demonstrated that rare non-synonymous coding variants of the genes *GNPTAB*, *GNTPG*, or *NAGPA* account for as much 16% of persistent stuttering cases.[44]

The question arises, however, whether the variants of these genes in a heterozygous status even have an impact on the activity of the enzymes they are encoding. In order to answer this question, Lee et al analyzed the consequences of 3 mutations in the *NAGPA* gene, detected in individuals with persistent stuttering, on the function of the UCE.[46] These mutations (2 missense and 1 deletion/frameshift) resulted in impaired folding and rapid degradation, respectively, of the synthesized protein and finally in a reduction of cellular enzyme activity.

It was even demonstrated in an animal model, that mutations in genes essential for the function of lysosomal enzymes can significantly contribute to stuttering: Barnes reported on experiments he performed in a mouse that carried a missense mutation in the *GNTPAB* gene. This mouse was shown to produce fewer vocalizations per unit time and had longer pauses between vocalizations in comparison with controls, speech abnormalities that are comparable to those exhibited by stutterers.[47]

Taking together all findings from genetic investigations in large families, biochemical analyses, and the animal model, there is no doubt anymore that mutations of the *GNTPAB*, *GNPTGG*, and *NAGPA* genes, which function sequentially in the metabolic pathway of lysosomal enzymes, play an important role in the pathogenesis of stuttering. However, the functional mechanism behind the association of mutations in these genes and stuttering remains unknown, and to be able to find an explanation for this association, a better understanding of the pathophysiology of stuttering and the identification of neurons that are crucial for this process will be required.

Therapeutic Aspects

As many common neurodegenerative disorders are associated with a dysfunction of the E/L system, therapeutic interventions that aim to restore lysosomal function may also be useful for the treatment of conditions such as Alzheimer or Parkinson disease. A positive lysosomal modulation can be achieved in different ways. One possibility is to increase the activity of lysosomal enzymes, since it is known that in neuronal cells the accumulation of toxic agents leads to upregulation of catabolic enzymes, as this is apparent in the brain of patients with Alzheimer and in related transgenic mouse models. The compensatory mechanism, however, which should increase the degradation of the accumulating material, is not sufficient to prevent damage to the neuronal cells. Therefore, a further increase in the level of catabolic enzymes could be a therapeutic target. One way to increase the activity of lysosomal enzymes is to use small molecules that act as specific chaperones. In Alzheimer disease, Aβ forms complexes with the gangliosides GM2 and GM3. Assuming that a reduction in

Aβ could be achieved by lowering the GM2 level, Knight et al used the iminosugar OT1001, a chaperone for β-hexosaminidase, in an in vivo model of Alzheimer disease (Dutch APP^{E693Q} mouse). These transgenic mice, which overexpress APP and show intraneuronal accumulation of Aβ, also display significant learning deficits.[48] Treatment of the mice with OT1001 for 3 months led to a significant increase in brain β-hexosaminidase and a substantial reduction in Aβ in the perirhinal cortex. Additionally, an improvement in learning behavior could be demonstrated.[49]

The increased risk of Parkinson disease in patients and carriers of Gaucher disease is explained by the fact that deficient β-glucocerebrosidase activity leads to increased accumulation of α-synuclein in the central nervous system. It could be shown that, on the other hand, the formation of the membrane-bound α-synuclein complex with glucocerebrosidase inhibits enzyme function,[50] suggesting that β-glucocerebrosidase deficiency may also play a role in sporadic Parkinson disease. In order to investigate whether an increase in wild-type β-glucocerebrosidase activity by the use of a chaperone could be a therapeutic strategy for sporadic Parkinson disease in the absence of a *GBA* mutation, Richter et al performed an experiment in transgenic mice overexpressing human wild-type α-synuclein (Thy1-aSyn mice).[51] These animals were treated with AT2101 (afegostat-tartrate, isofagomine) that has been demonstrated to be a potential pharmacological chaperone of β-glucocerebrosidase.[52] Thy1-aSyn mice that received AT2101 for 4 months showed a reduction in α-synuclein immunoreactivity in dopaminergic neurons and an improvement in mobility and nonmotor function, namely of olfactory deficits.[51]

A positive effect of enzyme enhancement on the level of α-synuclein, which is believed to be the major neurotoxic agent in the neurodegenerative process of Parkinson disease and other synucleinopathies, could be demonstrated not only in mouse models but also in patients affected by Gaucher disease. In these individuals, increased plasma levels of α-synuclein oligomers have been observed. Patients with Gaucher, however, who received enzyme replacement therapy for more than 5 years, showed much lower α-synuclein levels than untreated individuals.[53] From these results, it can be concluded that mutations in the *GBA* gene apparently are associated with an increase in plasma α-synuclein, and enzyme replacement therapy used for treatment of Gaucher disease might decrease the formation of this toxic compound.

Another way to improve lysosomal function, and thereby reverse abnormal aggregation of proteins, is the use of substances that have a direct effect on autophagic clearance. And in the last years, several non-toxic small molecules have been identified that are able to restore autophagy in neurons. Rapamycin, for example, is known to block mTOR, the master regulator of growth that is also an inhibitor of autophagy. Rapamycin has also been demonstrated to be able to ameliorate Aβ and tau pathology and rescue cognitive deficits in an animal model of Alzheimer disease.[54]

Latrepirdine, a Russian antihistaminic compound also known as Dimebon, has been assessed for its ability to regulate mTOR-dependent autophagy. In cultured cells, latrepirdine led to enhanced autophagy, and in a mouse model of Alzheimer disease, latrepirdine treatment was associated with a reduction in accumulation of Aβ- and α-synuclein.[55] In Russia, a randomized, placebo-controlled phase 2 clinical trial was performed in 134 patients with Alzheimer disease. Patients who were treated with Dimebon for 26 weeks showed a significant improvement in the Alzheimer disease assessment scale (ADAS-cog) in comparison to the placebo group.[56] The molecular mechanisms underlying the putative positive effects of latrepirdine in patients with Alzheimer are hardly understood. In a phase 3 clinical trial, however, Dimebon failed to show any significant effect in 2 coprimary and several secondary outcome measures.[57]

Many other autophagy-enhancing molecules have been studied in cell culture systems and mouse models that in the future may serve as therapeutics in neurodegenerative disorders.

Conclusion

Clinical, histological, biochemical, and genetic investigations in patients and animal models have clearly demonstrated that rare lysosomal storage disorders and more frequent neurodegenerative diseases share a common underlying feature, namely an impaired clearance of cellular cargo through the E/L network. However, the exact pathophysiological mechanisms tying together the various factors involved are not yet fully understood. In Alzheimer and Parkinson diseases, aggregates of undegraded proteins (Aβ, tau protein, α-synuclein) may impede the endosomal transport of not only these compounds but also of gangliosides and other possibly toxic macromolecules. On the other hand, a reduced activity of a lysosomal enzyme, as seen in heterozygous carriers of a lysosomal storage disorder causing mutation, might be insufficient to remove protein aggregates that should be degraded by the E/L. Examples of this possible mechanism are the link between frontotemporal lobar dementia and heterozygosity for *CLN11* mutations and the link between axonal neuropathy and heterozygosity for MPS IIIB mutations.

Up to now, there is no explanation for the association between stuttering and mutations of the genes sharing a role in the pathway of mannose-6-phosphate formation (an essential signaling marker for lysosomal enzymes). Patients who have stuttering and carry a mutation in one of these genes do not show any symptoms associated with mucolipidosis, and patients affected by mucolipidosis II or III do not stutter. Furthermore, the mutations in *GNPTAB* and *GNPTG* causing stuttering are completely different from those observed in ML II or ML III. One may hypothesize that the products of these gene have several functions and that different mutations have an influence on this function in different ways, leading to mucolipidosis II or III on one side and to stuttering on the other side.

The finding that there is a close link between neurodegenerative disorders and lysosomal storage disorders offers the

opportunity for new therapeutic strategies. Compounds that induce autophagy, such as rapamycin, may have a beneficial effect in Alzheimer disease. In animal models and even in humans, it has been shown that drugs used for the treatment of Gaucher disease, such as enzyme replacement therapy and the chaperone isofagomine, are able to reduce levels of α-synu-clein and therefore may be helpful in treating patients with Parkinson disease. It can be expected that in the future, drugs will be developed that are able to efficiently enhance protein clearance and slow down the progression of proteinopathies. Perhaps those drugs may provide a benefit for patients with a lysosomal storage disorder as well.

Declaration of Conflicting Interests

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