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Chapter 19

Intermediate Filaments in Neurodegenerative Diseases

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Additional information is available at the end of the chapter

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1. Introduction

In adult central (CNS) and peripheral nervous system (PNS), intermediate filaments (IFs) are the most abundant cytoskeletal components [1]. Neurons express differentially several IF proteins depending on their developing stage or their localization in the nervous system. In CNS, IFs are principally composed of the neurofilament (NF) triplet proteins (called NFL (light, 68 kDa), NFM (medium, 160 kDa) and NFH (heavy, 205 kDa); type IV) and α-internexin (66 kDa; type IV), while in the PNS, NFs are made up of NFL, NFM, NFH and peripherin (57 kDa; type III) [2, 3]. Neurons may also express other IF proteins, including nestin (200 kDa; type IV), vimentin (57 kDa, type III) syncoilin isoforms (Sync1 (64 kDa), Sync2 (64 kDa); type III) and synemin isoforms (Low synemin (41 kDa), Middle or beta synemin (150 kDa), High or alpha synemin (180 kDa); type IV). While present in perikarya and dendrites, IFs are particularly abundant in large myelinated axons, where they are essential for axon radial growth during development and axon caliber maintenance [4]. Consequently they are crucial to optimize the conduction velocity of the nerve impulse. They also contribute to the dynamic properties of the axonal cytoskeleton during neuronal differentiation, axon outgrowth, regeneration and guidance [5]. The IF proteins share a common tripartite structure with a globular head, a central α-helical rod domain and variable tail domains that differ in length and amino acids composition. The central rod domain of approximately 310 amino acids contains four α-helical regions and is involved in the assembly of 10 nm filaments [6]. NFM and NFH subunits differ from other neuronal IF proteins by their long tail domains containing numerous repeats of Lys-Ser-Pro (KSP) phosphorylation sites [4].

An increasing body of evidence supports the view that the most common mechanism of chronic neurodegenerative disorders involves abnormal protein production, processing or misfolding and subsequent accumulation in nervous system. Alterations in the metabolism and/or organization of neuronal IFs are frequently associated, directly or indirectly, with various neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Charcot-Mar-

ie-Tooth (CMT) disease, giant axonal neuropathy (GAN), neuronal intermediate filament inclusion disease (NIFID), Parkinson disease (PD), diabetic neuropathy, dementia with Lewy bodies and spinal muscular atrophy [7]. While IF abnormalities in neurodegenerative disorders could simply reflect a pathological consequence of neuronal dysfunction, recent studies using transgenic mouse models suggested that IF disorganization itself can also produce deleterious effects and therefore could contribute to the neurodegeneration process. Glial IF, and more particularly GFAP in astrocytes, is also the target of mutations leading to neurodegenerative diseases. Astrocytes express various IF proteins, including nestin, vimentin and synemin, but GFAP is the most abundant. GFAP is a type III IF protein existing under different spliced forms. The relative abundance of these GFAP transcripts is variable and can be dependent upon astrocyte location or pathological states [8]. *GFAP* mutations lead to accumulations of GFAP protein and cause Alexander disease (AXD), a rare leukodystrophy. Here, we tempted to cover the current knowledge related to neuronal and glial IF involvement in human neurodegenerative diseases (Table 1).

Disease	Mutations in IF genes	Accumulation of IFs	Possible causes of IF accumulation	Possible roles of IF in disease pathogenesis
ALS	- Only 3 variants identified in *NEFH* gene. - Peripherin mutations identified in 3 sporadic ALS patients.	Accumulation of peripherin and extensively phosphorylated NFs in the perikaryon of motor neurons and in axonal spheroids.	- Defect of axonal transport caused by abnormal phosphorylation of NFs and/or alteration of the molecular motors. - Modification of NF stoichiometry. - Alteration of post-translational IF proteins modifications.	Paradoxically, perikaryal NF aggregates appeared protective in mouse models of ALS, slowing disease progression in these animals. - NFs may act as calcium chelators or phosphorylation sink. - Removal of NFs from the axonal compartment could enhance axonal transport.
CMT	20 mutations in the *NEFL* gene have been linked to CMT2E and CMT1F (represent ≈ 2% of CMT cases).	- Axonal swellings containing disorganized NFs. - *In vitro*, co-expression of most NFL mutants with Wt NFM or NFH results in the formation of aggregates within the cell body.	- Most mutated NFL proteins fail to self-assemble or co-assemble with Wt subunits, and affect axonal transport of Wt and mutant NFs. - Mutations of heat-shock protein can also cause NFL aggregate.	- Perturbation of the axonal transport by trapping molecular motors and organelles in the cell body. - Alteration of mitochondrial morphology and dynamic.
GAN	No	- Enlarged axons filled with abnormally packed NFs.	- An acceleration of NF transport concomitant with a normal rate of NF protein synthesis and insertion into transport system would lead	- Perturbation of the axonal transport.

Disease	Mutations in IF genes	Accumulation of IFs	Possible causes of IF accumulation	Possible roles of IF in disease pathogenesis
		- Generalized aggregation of other neuronal and non-neuronal IFs.	to the formation of distal axonal swelling with packed NFs. - Disturbed cytoskeleton regulation and modulation.	
NIFID	No	Neuronal cytoplasmic inclusions containing all type IV neuronal IFs, and especially α-internexin.	Not determined.	Not determined. Abnormal accumulation of IFs may only be a secondary phenomenon.
PD	A point mutation in the *NEFM* gene was reported in only one case of PD with early onset.	Cytoplasmic inclusion bodies (Lewy bodies) composed of α-synuclein, NF proteins, ubiquitin and proteasome subunits. Inappropriate phosphorylation and proteolysis of NFs occur in Lewy bodies.	Not determined.	Data suggest no direct implication of IFs in pathogenesis of PD.
AXD	≈ 100 mutations in the *GFAP* gene were identified in AXD patients. ≈ 95% of AXD cases are due to GFAP mutation.	Presence of protein aggregates (Rosenthal fibers) composed of GFAP, αB-crystallin, HSP27 and ubiquitin within the cytoplasm of astrocytes throughout the CNS.	-Decreased degradation of GFAP. - Up-regulation of αB-crystallin and HSP27 are associated to the aggregation of GFAP. - Some mutations could impair interaction of GFAP with partners which normally prevent its assembly, resulting in the accumulation of GFAP polymers. - Insufficient amount of plectin seems promote GFAP aggregates.	The specific aspects of astrocytes functions that are compromised by the mutations of GFAP have not yet been discovered, but inhibited proteasome activity and activated stress pathways seemed to be important consequences of GFAP accumulation.

Table 1. IFs in neurodegenerative diseases.

2. Neuronal intermediate filaments and neurodegenerative diseases

2.1. Amyotrophic lateral sclerosis

ALS, also referred to as Lou Gehrig's disease, is a neurodegenerative disease which, by affecting the motor neurons in the motor cortex, brain stem and spinal cord, causes progressive physical impairment, together with worsening limitations in the functions of breathing, swallowing and communication. The disease has an incidence rate of 1-2 per 100,000, with a higher occurrence in men than in women. There is no cure and death usually occurs within 3 to 5

years from symptom onset. Only 10% of cases are inherited in an autosomal dominant pattern with the remaining 90% sporadic. 20% of all the familial cases are due to mutations in Cu/Zn superoxide dismutase 1 (SOD1), the most abundant cytosolic enzyme.

One common pathological finding of both sporadic and familial ALS is the accumulation of NFs and/or peripherin in the perikaryon of motor neurons and in axonal spheroids [9]. Because of the presence of IF proteins in these aggregates, several studies have searched for mutations in genes coding for NF proteins and peripherin. The discovery of a small number of NF gene variants in ALS patients suggested the involvement of NFs in the pathogenesis of the disease. Indeed, codon deletions or insertions in the KSP repeat motifs of NFH have been identified in patients with sporadic ALS [10-12]. However, two others studies failed to identify such variants in the NF genes linked to sporadic and familial ALS [13, 14], suggesting that mutations in the NF genes are not a systematic common cause of ALS but could be a risk factor for sporadic ALS. Peripherin mutations have also been identified in three sporadic ALS patients [15-17], including a frameshift mutation in the *PRPH* gene able to disrupt the NF network assembly *in vitro*, reinforcing the view that NF disorganization may contribute to pathogenesis. These results suggest that peripherin mutations may be responsible for a small percentage of ALS cases. Two peripherin isoforms have been linked to ALS: aggregate-inducing Per28 is upregulated in patients with ALS, at both the mRNA and protein levels, and an antibody specific for Per28 stained the filamentous inclusions [18]. The Per61 splice variant is neurotoxic and has been observed in ALS mouse models and human patients [19]. These observations raise the possibility that missplicing of peripherin could lead to disease. It is also of interest to note the presence of high NFL and NFH levels and auto-antibodies against NFL in cerebrospinal fluid of ALS patients [20-22]. Furthermore, plasma NFH levels closely reflect later stages of disease progression and therapeutic response in a mouse model of ALS [23]. In the same way, a significant relation exists between cerebrospinal fluid NFL levels and disease progression in ALS patients [24]. Accordingly, it seems that NFs levels may be valuable biomarkers of later disease progression in ALS.

NFs found in perikaryal aggregates are extensively phosphorylated, a process that occurs normally only within the axon [25]. The mechanisms governing the formation of IF aggregates are still not clearly established but defects of axonal transport or abnormal stoichiometry of IF proteins could be involved. Perturbations of the axonal transport of NFs and organelles are one of the earliest pathological changes seen in several transgenic mouse models of ALS [26-29]. The premature phosphorylation of NF tail-domains in motor neurons cell bodies could directly mediate their accumulation in this region. Glutamate excitotoxicity, another pathogenic process in ALS, may induce this abnormal phosphorylation of NFs. Treatment of primary neurons with glutamate activates members of the mitogen-activated protein kinase family which phosphorylate NFs with ensuing slowing of their axonal transport [30]. In addition, glutamate leads to caspase cleavage and activation of protein kinase N1 (PKN1), a NF head-rod domain kinase [31]. This cleaved form of PKN1 disrupts NF organization and axonal transport. Excitotoxicity mediated by non-N-methyl-D-aspartic acid (NMDA) receptor is also associated with the aberrant colocalization of phosphorylated and dephosphorylated NF proteins [32]. Inhibition of Pin1, a prolyl isomerase, was suggested as a possible therapeutic target

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to reduce pathological accumulation of phosphorylated NFs. Pin1 associates with phosphorylated NFH in neurons and is found in aggregates in spinal cord from ALS patients [33]. Its inhibition by inhibitor or down-regulating Pin1 levels reduces glutamate-induced perikaryal accumulation of phosphorylated NFH. Finally, riluzole protects against glutamate-induced slowing of NF axonal transport by decreasing perikaryal NF side-arm phosphorylation [34], probably via the inhibition of ERK and p38 activities, two NF kinases activated in ALS.

Alterations of the anterograde or retrograde molecular motors may also be responsible for aggregation of IFs. Mutation of dynein or $p150^{glued}$ [35], overexpression of dynamitin [36] and absence of kinesin heavy chain isoform 5A (KIF5A) [37] induce NF accumulations in mice. Recent studies suggest that inhibition of retrograde transport is more susceptible to cause accumulation of NFs than inhibition of anterograde transport. The inhibition of dynein by increasing the level of dynamitin induces aberrant focal accumulation of NFs within axonal neurites whereas inhibition of kinesin inhibits anterograde transport but does not induce similar focal aggregations [38]. Similarly, the neuron-specific expression of Bicaudal D2 N-terminus (BICD2-N), a motor-adaptor protein, impairs dynein-dynactin function, causing the appearance of giant NF swellings in the proximal axons [39]. However these mice did not develop signs of motor neuron degeneration and motor abnormalities.

Modification in NF stoichiometry was also proposed to induce accumulation of NFs. Singly overexpressing any of the NF subunit in transgenic mice led to prominent motor neuropathy characterized by the presence of abnormal NF accumulations resembling those found in ALS [40-42]. Remarkably, the motor neuron disease caused by excess human NFH (hNFH) can be rescued by restoring a correct stoichiometry of NF subunits via the overexpression of hNFL in a dosage-dependent fashion [43]. Overexpression of peripherin in mice also provokes the formation of cytoplasmic protein aggregates and the subsequent selective loss of motor neurons during ageing [44, 45]. This loss is preceded by axonal transport defects and formation of axonal spheroids [46]. Because NFL mRNA levels are reduced in cases of ALS, Beaulieu et al. [45] generated double transgenic mice overexpressing peripherin and deficient for NFL (Per;NFL-/- mice). Here, the onset of peripherin-mediated disease is accelerated by the deficiency of NFL. Without NFL, peripherin interacts with NFM and NFH to form disorganized IF structures. This could explain why the number of IF inclusion bodies is increased in Per;NFL-/- mice, leading to an earlier neuronal death and to defects of fast axonal transport in cultured Per;NFL-/- neurons [47]. In contrast, peripherin toxicity can be attenuated by coexpression of NFL or NFH [48, 49], illustrating once again the importance of IF protein stoichiometry. NFH overexpression shifted the intracellular localization of inclusion bodies from the axonal to the perikaryal compartment of motor neurons, suggesting that the toxicity of peripherin inclusions may be related to their axonal localization, possibly by altering the axonal transport. However, it should be noted that peripherin is not a key contributing factor to the neuronal death in disease caused by SOD1 mutations because absence or overexpression of peripherin in $SOD1^{G37R}$ mice do not affect the onset and progression of motor neuron disease [50].

Changes in stoichiometry were reported in ALS motor neurons as the levels of NFL, α-internexin and peripherin mRNA are decreased, while in familial ALS the levels of peripherin mRNA appear to be abnormally elevated [51-53]. This suggests a change in the stoichiometry of cytoskeletal protein expression which could be conducive to the formation of neurofilamentous aggregates in ALS. This decrease of IF mRNA could be due in part to modification in their stability. Several NFL mRNA binding proteins have been identified in human, including 14-3-3 proteins [54], TAR (trans-active regulatory) DNA-binding protein (TDP43) [55], both mutant and wild-type SOD1 [56] and Rho guanine nucleotide exchange factor (RGNEF) [57]. These proteins are incorporated in ALS intraneuronal aggregates and affect the stability of NFL mRNA. Mice expressing human TDP-43 displayed reduced NF mRNAs and proteins contents, inducing a decrease of caliber of their motor axons [58]. The involvement of TDP-43 in ALS pathogenesis was reinforced by the recent discovery of several mutant forms of this protein in familial and sporadic ALS [59]. Motor neurons from mice expressing such mutated TDP-43 displayed peripherin and NFs (NFM and NFH) aggregates, concomitant with a down-regulation of NFL and an overexpression of peripherin [60]. In addition, they detected in these mice the presence of abnormal splicing variants of peripherin, such as Per61, that can contribute to formation of IF aggregates. RGNEF is another RNA binding protein that acts as an NFL mRNA stability factor via 3′ untranslated region destabilization and reduces NFL protein levels when overexpressed in a stable cell line. Furthermore, RGNEF cytoplasmic inclusions were detected in ALS spinal motor neurons that colocalized with ubiquitin, p62/sequestosome-1, and TDP-43 [61]. These observations provide a possible mechanism for NF aggregate formation together with a link between ALS and Rho signaling pathways.

Neuronal IF abnormalities in ALS may also occur as a result of post-translational protein modifications. Indeed, advanced glycation endproducts were detected in NF aggregates of motor neurons in familial and sporadic ALS [62]. *O*-glycosylation of NFM is strongly decreased in spinal cord of different models for ALS, whereas phosphorylation is increased relative to total NFM [63, 64], suggesting competition of the binding sites of these two modifications and a potential mechanism for the formation of NF protein accumulations in ALS. Interestingly, inhibition of *O*-GlcNAcase (OGA), the enzyme catalyzing removal of *O*-GlcNAc, increased levels of *O*-GlcNAc modified NFM in spinal cords of control mice, but not in mutant SOD1 mice. Moreover, phosphorylation state of NFM appeared unchanged in these mutant mice [64]. The authors speculate that this lack of difference in NFM phosphorylation in mutant SOD1 mice may arise from the aggregation of hyperphosphorylated NFs, which may prevent dephosphorylation and subsequent *O*-GlcNAc modification. It was also showed that SOD1 can catalyze nitration of tyrosines by peroxynitrite in the rod and head domains of NFL [65]. However, no significant changes were detected in the nitration of NFL isolated from cervical spinal cord tissue of sporadic ALS cases [66].

Finally, it seems that non-neuronal cells could be directly involved in the formation of cytoskeletal aggregates within proximal axon from motor neurons. Indeed, cultured mouse spinal motor neurons in contact with non-neuronal cells displayed swellings that were morphologically and neurochemically comparable to axonal spheroids that develop *in vivo* in ALS transgenic mouse models [67]. These swellings contained NFL, NFM,

NFH, α-internexin and peripherin, and induced the accumulation of mitochondria and vesicle-like structures, suggesting a disruption of the axonal transport. Moreover, the severity of this axonopathy correlated with the phenotype of the glial cells, with a significant increase being induced by a glial feeder layer expressing mutant SOD1 or that was pre-aged prior to plating the motor neurons [67].

To further determine whether NFs are directly involved in SOD1-mediated disease, mice expressing mutant SOD1 were mated with transgenic mice deficient for axonal NFs. The withdrawal of NFs from the axonal compartment and their perikaryal accumulation induced by the expression of NFH-β-galactosidase fusion protein conferred no beneficial effect to $SOD1^{G37R}$ mice [68], indicating that axonal NFs are not necessary for SOD1-mediated disease. This was also observed in $SOD1^{G85R}$ mice deprived of NFL, but the absence of axonal NFs in these animals prolongs their life span by approximately 15% [69]. Surprisingly, overexpression of mouse NFL or mouse NFH in $SOD1^{G93A}$ mice [70], and overexpression of hNFH in $SOD1^{G37R}$ mice [71], also increase their life span by respectively 15% and 65%. This suggests a protective effect of NF perikaryal accumulation in motor neuron disease caused by mutant SOD1. While the mechanism of protection is unclear, it seems that perikaryal accumulation of NFs rather than their axonal deficiency is responsible for slowing disease in these models. Indeed, the formation of large perikaryal aggregates and a massive depletion of axonal NFs due to the expression of the human NFH43 allele cause more positive effects than human NFH44 allele which induces smaller aggregates and more axonal NFs [71]. Moreover, the disruption of one allele for each NF gene induces a 40% decrease of axonal NF proteins content and an important axonal atrophy without perikaryal accumulation of NFs in $SOD1^{G37R}$ mice, but it does not extend their life span nor does it alleviate the loss of motor axons [72]. Several hypotheses were proposed to explain this protective effect of perikaryal aggregates in SOD1-mediated disease. Through their multiple calcium-binding sites NFs may act as calcium chelators. Supporting this hypothesis, a significant neuroprotection was obtained by overexpressing the calcium-binding protein calbindin-D28k in cultured motor neurons [73]. It was also proposed that perikaryal accumulations of NFs in motor neurons may alleviate ALS pathogenesis by acting as a phosphorylation sink for cyclin-dependent kinase 5 dysregulation induced by mutant SOD1, thereby reducing the excessive phosphorylation of tau and other neuronal substrates [72]. This was supported by the fact that NF accumulations contain hyperphosphorylated NFM and NFH subunits in ALS patients [25] and in SOD1 mutant mice [74]. However, removal of NFM and NFH sidearms led to a delay of disease in SOD1 mutant mice rather than the acceleration predicted by a kinase dysregulation model [75], indicating that perikaryal phosphorylation of NFs is not an essential contributor to reduced toxicity of SOD1 mutants and that abnormal phosphorylation of NF proteins may be a detrimental factor. Alternatively, axonal removal of NFs could enhance axonal transport, which is impaired in SOD1 mice, by providing a more flexible axoplasm.

Finally, it was shown that NFs are involved in the localization of NMDA receptors in the neuronal plasma membrane by interacting with the NMDA NR1 subunit [76]. Thus, accumulation of NFs could interfere with glutamate receptor function and prevent glutamate excitotoxicity. However, NF aggregate-bearing neurons demonstrate increased intracellular calcium

levels and enhanced cell death in response to NMDA receptor activation without increased NMDA receptor expression. These results suggest that the presence of NF aggregates renders motor neurons more susceptible to NMDA-mediated excitotoxicity [77].

2.2. Charcot-Marie-Tooth disease

CMT represents a heterogeneous group of inherited peripheral neuropathies affecting both motor and sensory neurons to the muscles. CMT is the most common inherited disorder of the PNS, with approximately 1 per 2,500 people affected. Patients slowly lose function of their feet/legs and hand/arms as nerves to the extremities degenerate. First signs typically appear in the first or second decade of life, although it may be detected in infancy. This disease shows a high degree of heterogeneity, both in the clinical presentation and at the genetic level. CMT was originally subclassified into CMT1 and CMT2 on the basis of electrophysiological properties and histopathology. CMT1 is a demyelinating disease with reduced nerve conduction velocity whereas CMT2 is an axonal neuropathy with relatively normal nerve conduction velocity. CMT patients show a high degree of heterogeneity, due to mutations in multiple genes. This led to the distinction of other subtypes of CMT, including CMT3 (or Dejerine-Sottas disease, a particularly severe demyelinating form of CMT), CMT4 (autosomal recessive form of demyelinating CMT) and CMTX (X-linked form of CMT with both demyelinating and axonal features). Moreover, each type of CMT has several subtypes.

Vogel et al. [78] reported the presence of NF accumulations in CMT. Evidence for the involvement of IFs in the pathogenesis of CMT was provided by the identification of 20 mutations of the *NEFL* gene on chromosome 8 in patients with CMT1F and CMT2E. Mutations in *NEFL* gene are responsible for approximately 2% of CMT cases and a high percentage of CMT2 cases. These mutations are located throughout the three functional domains of this protein (head, rod and tail) and consist of substitutions, deletions and frame-shift mutations. Co-expression of most NFL mutants with wild-type NFM or NFH subunits disrupted the NF cytoskeleton *in vitro*, resulting in the formation of aggregates within the cell body [79, 80]. The first two CMT-associated *NEFL* mutations, NFL^{P8R} and NFL^{Q333P}, were identified in respectively a Belgian and a Russian family. In addition to disturb the assembly of NFs, these mutations affect the axonal transport of wild-type and mutant NFs, but also the transport of mitochondria and human amyloid β protein precursor, resulting in alterations of retrograde axonal transport, fragmentation of the Golgi apparatus and increased neuritic degeneration [79, 80]. The effect of these mutant proteins on filament assembly was dominant, since wild-type NFL could not rescue the assembly defect. Filament formation was also abolished in SW13 cells by the rod domain A148V mutation [81]. These data provide possible mechanisms by which these mutants could be involved in axonal degeneration and CMT pathogenesis.

The Pro-22 residue of NFL is also the target of several mutations: P22R, P22S and P22T. The P22R mutation, identified in a Korean family, is associated with demyelinating neuropathy features of CMT1F [82]. The P22S substitution was first described in a Slovenian CMT2 family [83], then in an Italian family developing a primary axonopathy characterized by giant axons with swellings composed essentially of aggregated NFs [84]. Interestingly, clinical and electrophysiological studies from patients with P22S mu-

tation revealed a mixed axonal and demyelinating neuropathy [85], emphasizing the complexity of genotype-phenotype correlations in CMT. Finally, the P22T mutation was detected in unrelated Japanese patients with CMT disease [86]. The formation of NF aggregates in patients expressing NFL^{P22S} and NFL^{P22T} mutant proteins could be explain by the ability of these mutations to abolish the phosphorylation of the adjacent Thr21 by cyclin-dependent kinase 5, which normally inhibits filament assembly [87]. The phosphorylation of NFL head domain by PKA alleviated aggregates in cortical neurons, providing a potential therapeutic approach to dissociate NF aggregates in CMT disease [87].

The screening of 323 patients with CMT or related peripheral neuropathies allowed the identification of six disease-associated missense mutations and one 3-bp in-frame deletion in the *NEFL* gene [88]. Other mutations were also detected in Korean CMT patients [89], in a German family [90], and four mutations in the head and rod domains of NFL, including a L268P substitution and a del322Cys_326 Asn deletion, were identified by the screening of 177 patients [91]. Most of these mutated proteins (except E7K and D469N) form aggregates, and thus could alter the axonal transport following their abnormal aggregation in cell bodies and axons. A duplication-insertion mutation of NFL in a patient with CMT was also reported [92], which probably provoked neuronal degeneration through both aggregation and destabilization of the IF network. Finally, new mutations in the *NEFL* gene were identified following the screening of 223 Japanese CMT patients [93]. Four heterozygous missense mutations (P8L, E90K, N98S and E396K) were detected in five unrelated patients as well as a homozygous nonsense mutation (E140Stop) in one patient. All these patients displayed moderate delayed nerve conduction velocities, possibly caused by a loss of large diameter fibers. This study suggested that nonsense *NEFL* mutations probably cause a recessive phenotype, while missense mutations cause a dominant phenotype [93]. The majority of NFL mutations are linked to axonal forms of CMT but their implication in demyelinating CMT cannot be excluded since nerves from patients expressing NFL^{L268P} or NFL^{E90K} showed evidence of Schwann cell abnormalities [88, 91].

The first mouse model of the CMT2E disease expressed the $hNFL^{P22S}$ mutant protein specifically in the nervous system and recapitulate many of the overt phenotypes observed in CMT2E patients, including aberrant hind limb posture, motor deficits, hypertrophy of muscle fibres and loss of muscle innervation without neuronal loss [94]. To address whether CMT2E disease is potentially reversible, this mouse model was based on the tetracycline-responsive gene system that allows the suppression of mutant $hNFL^{P22S}$ expression in mature neurons through administration of doxycycline. Remarkably, a 3-month treatment of these mice with doxycycline after disease onset efficiently down-regulated expression of $hNFL^{P22S}$ and reversed the neurological phenotype [94], providing hope that future therapeutic strategies might not only stop progress of CMT2E disease but also reverse it. A novel line of CMT2E mice that constitutively express $hNFL^{E397K}$ was recently generated [95]. As with the $hNFL^{P22S}$ mice, these mice developed as early as 4 months signs consistent with CMT2E patients, such as aberrant hind limb posture, digit deformities, reduced locomotor activity and reduced motor nerve conduction velocities. However, some aspects differed between the two lines of CMT2E mice. Indeed, $hNFL^{E397K}$ mice showed no significant denervation and their muscles were atrophied. More-

over, they showed only relatively mild signs of nerve pathology, including ectopic accumulations of phosphorylated NFs in motor neuron cell bodies, NF disorganization in motor and sensory roots, and reduced axonal caliber [95]. The divergence in cellular pathology between the two animal models may suggests that overt CMT2E phenotypes may arise through different cellular mechanisms.

Mutations of myotubularin-related protein 2 (MTMR2) (CMT4B), heat-shock protein B1 (HSPB1) (CMT2F) or HSPB8 (CMT2L) can also cause NFL aggregation [96-99], indicating that mutation of NFs is not the only mechanism inducing their accumulation in CMT. Co-expression of Wt HSPB1 with P8R or Q333P CMT mutant NFL reduced their aggregation, induced reversal of mutant NFL aggregates and decreased mutant NFL-induced loss of motor neuron viability [100]. On the opposite, mutant HSPB1 was found to have an inhibitory effect on the assembly of NFL in transfected cells. Zhai et al. [100] showed that deletion of NFL markedly reduces degeneration and loss of motor neurons induced by mutant HSPB1. Finally, mice expressing mutant HSPB1 throughout the nervous system showed axonal pathology in spinal cord and peripheral nerve that was age-dependent, with evidence of impaired NF cytoskeleton, associated with organelle accumulation. These data suggest that overexpression of mutant HSPB1 in neurons is sufficient to cause pathological changes in mice that are seen in patients with CMT. Mutant MTMR2 also induces abnormal NFL assembly in transfected cells [98] and mice lacking MTMR2 develop a CMT-like neuropathy, including several characteristics of dysmyelination [101]. A similar phenotype was observed following Schwann cell-specific *MTMR2* inactivation, whereas neuron-specific inactivation did not provoke myelin outfoldings nor axonal defects, suggesting that loss of MTMR2 in Schwann cells, but not in motor neurons, is both sufficient and necessary to cause CMT4B neuropathy [102]. In addition to disrupt the NF network, recent studies showed that expression of NFL^{P8R} or NFL^{Q333P} in cultured motor neurons caused the rounding of mitochondria and decreased their rate of fusion concomitant with increased motility [103, 104], indicating an important function of NFs in mitochondrial dynamics. Cotransfection of HSPB1 helped to maintain normal NF network, axonal caliber and mitochondrial morphology. On the other hand, the cotransfection of HSPA1 was effective in neurons expressing NFL^{Q333P}, but not NFL^{P8R}, suggesting that chaperone-based therapies have potential for the treatment of CMT2E but their efficacy would depend on the profile of HSPs induced and the type of *NEFL* mutation.

2.3. Giant axonal neuropathy

GAN is a rare progressive neurodegenerative disorder with early onset affecting both PNS and CNS. Phenotypic variability has been reported but typical clinical features include distal limb weakness, areflexia and a marked gait disturbance. The motor deficits encompass amyotrophy, muscle weakness and evolve with skeletal deformations and loss of ambulation by the adolescence. As the disorder progresses, CNS involvement includes electroencephalographic abnormalities, mental retardation, speech defect, seizures and defective upper motor neuron function. GAN is caused by mutations in the *GAN* gene encoding the ubiquitously expressed protein gigaxonin. Gigaxonin belongs to a protein family that is characterized by an N-terminal BTB (broad-complex, Tramtrack, and Bric a brac) domain and six kelch repeats [105]. BTB/

kelch proteins are organizers of the cytoskeletal network and closely linked to the ubiquitin degradation pathway. More than 45 distinct mutations of the gigaxonin have been identified to date along the entire *GAN* gene in patients. By revealing a high instability of gigaxonin in multiple lymphoblasts cell lines from unrelated patients, Cleveland et al. [106] showed that GAN is caused by a loss of function of gigaxonin.

The major cytopathological hallmark of GAN is the presence of distal enlarged axons, also called giant axons, filled with abnormally packed IFs associated with a reduced number of MTs [107]. In contrast, axonal segments proximal to the swellings exhibit a reduced number of NFs [108]. Disorganization and accumulation of other types of IFs are also found in skin fibroblasts, Schwann cells and muscle fibers [109-111], suggesting a critical role of gigaxonin in maintaining cytoskeletal architecture. A decreased inter-NF distance was observed in sural nerve axons of a GAN patient and, more surprisingly, the mean diameter of NFs was increased (12.4 nm in GAN compared with 10.1 nm in controls) [112]. Although the mechanism leading to the distal axonal accumulation of NFs is still unclear, an acceleration of their axonal transport was observed in optic nerve from experimentally induced GAN rat model, concomitant with a proximal decreased content of NFs and their distal accumulation [113]. The authors proposed that acceleration of NF transport in the presence of a normal rate of NF protein synthesis and insertion into transport system would lead to the formation of distal axonal swellings with packed NFs.

In order to determine how loss of gigaxonin's function leads to GAN, mice deleted in exons 3-5 of the *GAN* gene ($GAN^{\Delta 3\text{-}5}$ mice) were produced [114]. These mice develop strong motor deficits as early as 6 months of age, including reduction of spontaneous movement, bizarre limb posture and overall weakness. However, they displayed normal life span and fertility, and giant axons were never seen. Nevertheless these mice exhibited enlarged axons with densely packed NF, leading to the segregation of axonal organelles, a feature characteristic of human GAN pathology. This was accompanied by an axonal loss at the age of 9-12 months. However, it should be noted that some null mice showed no overt neurological phenotypes, suggesting that some genetic modifiers may exist [115]. Another mouse model with deletion of exon 1 of the *GAN* gene was generated [116] which exhibited no overt phenotype over 15 months in contrast to $GAN^{\Delta 3\text{-}5}$ mice. Nevertheless, they developed aggregates composed of non-phosphorylated NFH and α-internexin in cerebral cortex and thalamus. Small aggregates of NFL and peripherin also formed in cell bodies of dorsal root ganglion neurons. Moreover, increased levels of neuronal IF proteins were detected in various regions of the nervous system, confirming the importance of gigaxonin in modulating the levels and organization of IF proteins. Given the very different phenotypes between these two GAN models, Ganay et al. [117] conducted a behavioral analysis over a 72-week period in their own $GAN^{\Delta 3\text{-}5}$ mice as well as in $GAN^{\Delta 3\text{-}5}$ mice developed by Ding et al [114]. Analysis performed on their own model revealed difference depending on the genetic background. Indeed, a mild but persistent motor impairment was reported in the 129/SvJ genetic background, while C57BL/6 animals displayed rather a deterioration of sensory functions. Despite the modest phenotypic manifestation and no pronounced signs of neurodegeneration, these mice exhibited severe cytoskeletal alterations, including an increase in the diameter of NFs, an overt impairment in their orientation

and a strikingly increased abundance of the three NF subunits. Finally, they tested motor deficits in GAN$^{\Delta 3\text{-}5}$ mice produced by Ding et al [114] and detected no clinical signs within the first year. This is consistent with a mild progression of the disease in mice and suggests that the three existing models probably display a phenotype of similar intensity. Altogether, these data shown that the absence of gigaxonin results in a milder version of the GAN disease in mice at the behavioral level, associated with a severe disorganization of the NF network that recapitulates what is observed in patients [117].

Gigaxonin was shown to be a direct key player in the Ubiquitin Proteasome System (UPS). Indeed, BTB-containing proteins, including gigaxonin have been found to be the substrate adaptors of Cul3-dependant E3 ubiquitin ligases, interacting with Cul3 and the substrates through the BTB and the C-terminal domains, respectively [118-120]. Gigaxonin was shown to promote the ubiquitin-mediated degradation of its three known substrates, the microtubule-associated protein 1B (MAP1B) [121], tubulin folding cofactor B (TBCB) [122] and MAP1S (also called MAP8) [114]. Disease associated gigaxonin mutations perturb its association with these partners while gigaxonin ablation results in their accumulation [114, 122, 123]. This raised the possibility that IF accumulation in GAN results from a MT reorganization/destabilization. However, it is intriguing to note that these proteins have opposite effects on MT network: MAP1B is a MT-stabilizing phosphoprotein, whereas overexpression of TBCB depolymerizes MTs. Using primary fibroblasts derived from skin biopsies of multiple GAN patients with aberrant aggregates of vimentin, Cleveland et al. [106] demonstrated that vimentin aggregation is greatly enhanced in conditions driving quiescence and is not caused by an abnormal accumulation of the tubulin chaperone TBCB and its effect on MT stability. Moreover, the prolonged depletion of the MT network did not induce GAN-like aggregates of vimentin in normal fibroblasts. These results indicated that the generalized disorganization of IFs in GAN patients may not involve TBCB-mediated MT disassembly and must be regulated by a yet unidentified mechanism [106]. Recently, proteomic analysis performed in fibroblasts from four GAN patients provided new insights into disease mechanisms [124]. Although the major role of gigaxonin is reported to be degradation of cytoskeleton-associated proteins, the amount of 76 structural cytoskeletal proteins was unaltered. However, several proteins linked to regulation of the cytoskeleton network were found to be upregulated or downregulated. The authors speculated that in GAN, dysregulation of the cytoskeletal network is responsible for formation of aggregates of IFs. In the case of fibroblasts, disturbed cytoskeletal regulation could lead to a hyperphosphorylation state of vimentin that results in massive depolymerization of vimentin filaments and finally in collapse of the vimentin network. The unpolymerized filaments are collected in the aggresome near the nucleus where they form the typical aggregates [124].

2.4. Neuronal intermediate filament inclusion disease

NIFID is a recently described uncommon neurological disorder of early onset with a heterogeneous clinical phenotype, including sporadic fronto-temporal dementia associated with a pyramidal and/or extrapyramidal movement disorder. The symptoms comprise behavioural

and personality changes, which can be associated to memory loss, cognitive impairment, language impairment, hyperreflexia and motor weakness. Neuropathologically, NIFID is characterized by widespread degeneration of the frontal and temporal lobes. The cytopathological characteristics consist of neuronal loss, gliosis, swollen neurons and presence of large inclusions in the cell body of many neurons that are immunoreactive for all of the class IV neuronal IFs and especially in α-internexin [125, 126]. These inclusions of α-internexin but negative for tau or synuclein distinguish NIFID from other disease that involve IF inclusions, such as synucleinopathies (e.g., PD), tauopathies (e.g., AD and frontotemporal dementia), and motor neuron disease. Although α-internexin has been observed in neuronal inclusions in other neurodegenerative disorders, it is generally a relatively minor component. This raises the question whether α-internexin-positive neuronal inclusions in NIFID reflect any selective neuronal dysfunction, and as such if they are associated with some specific clinical symptoms. Genetic screening revealed no pathogenic variants for all type IV neuronal IFs, SOD1, NUDEL and gigaxonin [127, 128]. To date, no genetic mutations leading to NIFID have been described.

Interestingly, the number of IFs aggregates is high in areas with reduced neuronal loss, and low in sites of intense neuronal degeneration. Cairns et al. [125] proposed that the formation of these inclusions is an early event in the pathogenesis of NIFID, and these aggregates are then released and degraded into the extracellular space following degeneration of the neuron. The mechanism of IF aggregation and the role they play in neuronal dysfunction and cell death are still unclear. Although immunoreactivity for IFs was initially described as the defining pathological feature of NIFID, not all the inclusions in NIFID are IF-positive. It now appears that aggregates of FUS (fused in sarcoma) protein, is a more consistent feature of NIFID. Indeed, intracellular accumulations of FUS are more often encountered than IF inclusions and all neurons that contained abnormal IF aggregates also contained FUS inclusions [129]. It should also be noted that clusters of FUS-immunoreactive inclusions are larger than those revealed by NFH or α-internexin [130]. The authors interpreted this finding as suggesting that FUS plays a more central role in the pathogenesis of NIFID and that the abnormal accumulation of IFs is likely a secondary phenomenon. It now remains to determine the exact implication of FUS in the pathogenesis of NIFID.

2.5. Diabetic neuropathy

Diabetes is the leading cause of peripheral neuropathy worldwide. About 60 to 70 percent of people with diabetes have some form of neuropathy. People with diabetes can develop nerve problems at any time, but risk rises with age and longer duration of diabetes. Diabetic neuropathies are complex, heterogeneous disorders that affect dorsal root ganglia and sensory axons more so than motor fibers. Nerve damage is likely due to a combination of factors, including metabolic factors (e.g., high blood glucose, abnormal blood fat levels), neurovascular factors leading to damage to the blood vessels, autoimmune factors, lifestyle factors and inherited traits that increase susceptibility to nerve disease. Although its pathogenesis has not been fully elucidated, diabetic neuropathy is characterized by slower conduction velocity, impairment of axonal transport, axonal atrophy and reduced capacity for nerve regeneration. All these

features of nerve function depend on the integrity of the axonal cytoskeleton and particularly on NFs. In agreement with this, multiple abnormalities of NF biology were identified in models of diabetes. An impairment of the axonal transport of NFs, actin and tubulin concomitant with a proximal increase and a distal decrease of axonal calibers were observed in rats with streptozotocin-induced diabetes and in BioBreeding rats (a model of spontaneous type I diabetes) [131, 132]. The distal axonal atrophy is accompanied by a concomitant NF loss in this region [133], and accumulations of highly phosphorylated NF epitopes are present in proximal axonal segments of dorsal root ganglia sensory neurons from diabetic patients [134]. An increase of NF phosphorylation, correlated with activation of JNK, was also detected in lumbar dorsal root ganglia from rat models [135]. Finally, there were a substantial decline in the mRNA levels of all three NF subunits as well as reduced NF numbers and densities within large myelinated sensory of long-term diabetic models [136]. All these results suggest that NF abnormalities may contribute to the development of diabetic neuropathy, or may be affected by this disease. However, slowing of conduction velocity in diabetic models occurs much earlier than loss of NF investment or axonal atrophy [136]. To further elucidate the contribution of NFs to diabetic neuropathy pathogenesis, the effect of streptozotocin-induced diabetes was analyzed in NFH-LacZ transgenic mice characterized by axons completely lacking NFs [137]. Interestingly, diabetic mice lacking NFs developed progressive slowing of conduction velocity in their motor and sensory fibres and displayed decreased nerve action potential amplitudes earlier than diabetic mice with normal IF cytoskeleton. Moreover, superimposing diabetes on axons without NFs also accentuated axonal atrophy. Administration of insulin that restored normoglycemia reversed conduction slowing and restored sensory axon caliber. These findings indicate that changes in NF expression, transport or post-translational modifications cannot account alone for neurological features of diabetic neuropathy, but these IFs may help axons to better resist the negative effects of diabetes [137].

2.6. Parkinson disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD, with a prevalence of about 2% among people over the age of 65 years. This disease is marked by the depletion of dopaminergic melanin-containing neurons in the substantia nigra pars compacta and a consequent loss of dopamine in the striatum. Another important pathological feature is the presence, especially in substantia nigra pars compacta neurons, of eosinophilic cytoplasmic inclusion bodies named Lewy bodies, composed of α-synuclein, NF proteins, ubiquitin and proteasome subunits. Various features distinguish NFs in PD, including inappropriate phosphorylation and proteolysis in Lewy bodies [138, 139], decreased NFL and NFH mRNA levels [140], and reduced protein level of NFL and NFM [141]. A point mutation in the *NEFM* gene was reported in a case of PD with early onset [142]. This mutation consisted in a substitution of Ser for Gly at residue 336, a highly conserved region in the rod domain 2B of NFM, and was argued to disrupt NF assembly. Although three other unaffected family members also carried this mutation, the authors had then proposed that aberrations in neuronal IFs could lead to the development of the pathology seen in PD. However, the G336S mutation does not disrupt the assembly and the distribution of NFs *in vitro* [143] and the screenings of PD patients of similar or different ethnic background failed to identify this mutations [144,

145], arguing against the implication of this *NEFM* mutation in pathogenesis of PD. Interestingly, research has shown that changes in the levels of NFL in the cerebrospinal fluid may be used as a biomarker for the identification of PD [146] and that the serum levels of anti-NF protein antibodies increase significantly in patients with PD [147]. Finally, it seemed that the serum level of NFs in patients with PD was significantly correlated with duration of the disease and age [148]. These findings support the idea that axonal injury causes the release of cytoskeleton proteins, and changes in the concentrations of serum NFs are probably related to the severity of axonal injuries.

3. Glial intermediate filament GFAP and Alexander disease

Neuronal IFs are not the only class of IF to be responsible for the development of neurological disorders. Glial IF can also be the primary cause of a CNS disorder. Indeed, GFAP, the major constituent of astrocytic IFs, is directly involved in the development of the AXD. This disease is a fatal, progressive white matter disorder that has been classified into three types based on the age of onset: infantile, juvenile and adult. The infantile type, with onset between birth and about two years of age, is the most frequent form of the disease and is fatal either within that period or by around the age of 10 years. Clinical symptoms comprise progressive megalencephaly, seizures and impaired cognitive function, which may be associated with ataxia and hydrocephalus. Such phenotypes become progressively less common for the juvenile and adult forms (for recent reviews, see [149, 150]). Both the infantile and juvenile forms usually appear to be sporadic while the adult form is often familial.

AXD is a primary astrocytic disease and its manifestations are the result of astrocyte dysfunctions leading to both myelin damage and neuron dysfunction. Neuronal loss is often reported but axons are relatively well preserved in demyelinated areas. The pathological hallmark of AXD is the presence of protein aggregates known as Rosenthal fibers within the cytoplasm of astrocytes throughout the CNS, but especially those located in the subpial, periventricular and subependymal zones. Different constituents were identified in Rosenthal fibers: GFAP, αB-crystallin, HSP27 and ubiquitin [151-153]. Although GFAP is also expressed in glial cells of the PNS and in several other organs, Rosenthal fibers were not reported outside the CNS of AXD patients.

To examine the function of GFAP *in vivo*, GFAP knock-out mice were generated [154-157]. These studies showed that mice lacking GFAP displayed astrocytes devoid of the IF, but still developed and reproduced normally. Only subtle phenotypes emerged with age, arguing for a role of GFAP in the white matter architecture, blood-brain barrier integrity, astrocyte-neuronal interactions and in modulating synaptic efficacy in the CNS [156, 157]. This is consistent with the known roles of astrocytes that help to form blood brain barrier, promote synaptic plasticity and coordinate neuronal activity. To determine the influence of increased GFAP expression on astrocyte function, mice overexpressing the human *GFAP* gene were produced [158]. Mice in the highest expressing lines developed a phenotype close to that observed in AXD. Indeed, their brains contain many inclusion bodies indistinguishable from human

Rosenthal fibers, astrocytes are hypertrophic and these animals died from an encephalopathy at an age that is inversely correlated with the level of expression of the transgene. However, no myelin abnormalities were observed. Microarray analysis performed on olfactory bulbs of these animals recently highlighted the appearance of an initial stress response by astrocytes which results in the activation of microglia and compromised neuronal function [159]. All these results suggested that a primary alteration in GFAP may be responsible for AXD.

Sequence analysis of DNA samples from AXD patients was thus performed and revealed that most cases are associated with mutations in the *GFAP* gene [160]. Since then, numerous mutations of this gene were identified; many of them being located in highly conserved domains of the encoded protein that play specific roles in the assembly of IF network [8, 150]. It was estimated that more than 95% of AXD cases are due to *GFAP* mutation. To date, all the identified mutations are heterozygous and nearly all of them involve amino acid substitutions, but several insertion or deletion/insertion alterations have also been reported (a continually updated list of all published mutations is maintained at the Waisman Center of the University of Wisconsin-Madison; www.waisman.wisc.edu/alexander). Numerous mutations cluster in the coils 1A and 2B of GFAP and two sites (R79 and R239) account for approximately half of all patients affected. The comparison of mutations occurring in the various IF proteins revealed that frequent mutations lying in the 2A segment seem to be unique to GFAP. It is possible that molecular partners specifically interact with this region of GFAP but not with the equivalent region of other IFs. The calcium-binding protein S100B binds to the N-terminal part of GFAP-coil 2A [161]. As S100B prevents GFAP assembly [162], mutations in this domain could impair GFAP-S100B interactions, resulting in the accumulation of GFAP polymers and possibly aggregates. It seems that a correlation exists between the different mutations and the severity of the disease. However, there also exists significant phenotypic variability and age of onset for the same mutation [163], suggesting that epigenetic and environmental factors influence the appearance and timing of disease symptoms. It should also be noted that in rare cases of AXD, no mutations in the *GFAP* gene has been found [164], indicating that there may be additional causes of the disease.

The discovery of GFAP mutations led to the generation of knock-in mice with missense mutations homologous to those found in humans (R76H and R236H, which correspond to the R79H and R239H mutations in human) [165, 166]. If the presence of mutant GFAP *per se* seemed insufficient for aggregate formation, a 30% increase in GFAP content over that in wild-type induced the formation of Rosenthal fibers in multiples sites throughout the CNS [166]. These animals were also more susceptible to kainate-induced seizures. Nevertheless, they had a normal lifespan, showed no overt behavioral defects and general white matter architecture and myelination appeared normal. These features resemble those found in the adult form of AXD rather than in the infantile form. This indicates that the presence of GFAP aggregates containing mutant GFAP is not sufficient to induce a major phenotype of AXD, even though it causes some abnormalities in the mouse. Interestingly, further elevation of GFAP via crosses to GFAP transgenic animals led to a shift in GFAP solubility, an increased stress response, and ultimately death [165]. This correlates GFAP protein levels to the severity of the disease.

While the genetic basis for AXD is now firmly recognized, there is little information concerning the mechanisms by which GFAP mutations lead to disease. Several study showed that mutations of GFAP alters the normal solubility and organization of GFAP networks [163, 167]. When expressed alone, these mutant proteins lost their ability to form filament *in vitro*. But in presence of assembly partners, such as wild-type GFAP or vimentin, they were still capable of incorporation into filament networks in transfected cells. If wild-type GFAP is prone to aggregate, mutations of GFAP exacerbates this accumulation [168]. Insufficient amounts of plectin, due to R239C GFAP expression, were also proposed to promote GFAP aggregation and Rosenthal fibers formation in AXD [169]. Both inhibited proteasome activity and activated stress pathways seemed to be important consequences of GFAP accumulation [168]. As a positive feedback response, both the proteasome hypofunction and JNK activation exacerbated GFAP accumulation, increasing susceptibility of the cell to stressful stimuli. It thus appeared that accumulations of GFAP protein would be more deleterious to the astrocytes than the mutant protein itself. However, as a positive consequence, up-regulation of αB-crystallin and HSP27 were also associated to the aggregation of GFAP in AXD patients [153, 170] as well as in cell and animal models [159, 165, 168]. Increased αB-crystallin levels would contribute to the disaggregation of GFAP aggregates and could protect cells from apoptotic events [171]. Moreover, a recent study demonstrated that AXD mutant GFAP accumulation stimulates autophagy which in turn contributes to decrease GFAP levels [172]. The balance between the positive and negative effects of GFAP accumulation might define the survival or death of the cell. Compounds known to reduce GFAP expression *in vitro*, such as quercetin, might be useful as therapeutics. For instance, treatment with the antibiotic ceftriaxone alleviates intracytoplasmic aggregates of mutant GFAP by inducing the up-regulation of HSP27 and αB-crystallin, poly-ubiquitination and autophagy, and by reducing the *GFAP* promoter transcriptional regulation [173]. Curcumin was also reported to have beneficial effects in an *in vitro* model of AXD. Indeed, curcumin is able to induce both HSP27 and αB-crystallin, to reduce expression of both RNA and protein of endogenous GFAP, to induce autophagy and, finally, to rescue the filamentous organization of the GFAP mutant protein, thus suggesting a role of this spice in counteracting the pathogenic effects of *GFAP* mutations [174].

The *GFAP* gene is known to generate different splice variants, including the most abundant isoform GFAP-α, and seven other differentially expressed transcripts including GFAP-δ (human homologous GFAP-ε). GFAP-δ is incapable of self-assembly into IF *per se*, but can incorporate a filament network composed of GFAP-α if the proportion of GFAP-δ to GFAP-α remains <10% [175]. However, elevating the proportion of GFAP-δ perturbs association of αB-crystallin with the IF fraction and induced IF bundling and aggregation in transiently transfected cells. Interestingly, GFAP-δ isoform is preferentially expressed in the same populations of astrocytes that contain the most Rosenthal fibers in AXD. This raises the possibility that GFAP-δ may play a key role in aggregate formation in combination with mutated GFAP. It remains to determine whether GFAP-α:GFAP-δ ratio is perturbed in AXD tissues.

4. Conclusion

IFs abnormalities are reminiscent in multiple human neurodegenerative disorders. Despite extensive efforts over the past 40 years, processes leading to these abnormalities as well as their precise contribution to disease pathogenesis often remain poorly understood. For instance, if it is clearly established that mutation in IF genes can be a primary cause of neurodegenerative disorders, the question as to how they induce neurodegeneration frequently remain unsolved. Although transgenic mouse models have been somewhat helpful in understanding some mechanisms, most of these animals displayed a much less severe phenotype than patients and results have not always been completely clear-cut. A growing body of evidence suggests that perturbation of IF axonal transport and/or stoichiometry are directly involved in the formation of intracellular IF aggregates. Destabilization of IF mRNA could be responsible for alteration in IF stoichiometry whereas aberrant post-translational modifications could affect their transport. More investigations are also necessary to identify IF partners. The importance of IF-associated proteins in the development of neurodegenerative disorders was also highlighted by the identification of mutations in genes encoding IF partners that mimic IF-related disease. This is particularly the case of gigaxonin in GAN. A particular attention should also be paid to elucidate the role that IF proteins may play in signaling. Finally, it will be important to elucidate why certain types of IF accumulations appear more toxic than others. While perikaryal accumulations are generally well tolerated, axonal inclusions are often noxious. The more deleterious effect of axonal aggregates on axonal transport could be a promising avenue to explore in the future and the identification of compounds able to remove these IF aggregates is crucial to the development of new therapeutic approaches.

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