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# Intermediate filaments

## A common thread in neuromuscular disorders

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In this issue of *Neurology*, Kulhenbäumer et al.<sup>1</sup> describe novel mutations of the gene responsible for giant axonal neuropathy (GAN). GAN, a recessive multisystem disorder, presents with a variable phenotype that includes tightly curled hair, CNS involvement, and a progressive sensorimotor axonal neuropathy.<sup>2</sup> GAN is an especially instructive reminder of the roles played by one class of cytoskeletal element: the intermediate filament. Intermediate filaments (IF) are a family of 10-nm diameter structures that interact with actin microfilaments (7 nm) and microtubules (24 nm) to form the cytoskeletal scaffolding in eukaryotic cells.<sup>3,4</sup> The most basic function of IF is to impart structural resilience to cells. This is exemplified in keratinocytes, where mutations in the major IF, keratin, result in bullous disorders of skin, including epidermolysis bullosa.<sup>3</sup> In various instances it is now clear that abnormalities of IF or IF association proteins with disruption of the cytoskeletal network are pathogenic (table). Of special interest to neurologists is that mutations in desmin, the dominant IF in muscle, produce a recessive distal myopathy.<sup>3</sup> In other filamentopathies, the primary defect is one of organization of the cytoskeletal network. Mutations in the plectin gene, a large IF association protein, produces a muscular dystrophy and epidermolysis bullosa.<sup>3</sup>

Neurofilaments (NF) are the major IF in neurons, where they account for up to 85% of protein content. NF are composed of 3 subunits: NF light chain (NFL, 68 kDa), which forms the backbone and initiates assembly of NF, NF medium (NFM, 160 kDa), and NF heavy (NFH, 200 kDa) chains.<sup>3</sup> The carboxy terminals of NFH as well as NFM form side arm projections of NF that allow interactions among neurofilaments and underlie the "cross links" between adjacent filaments seen by electron microscopy.<sup>3-7</sup> Various IF association molecules, including plectin and BPAG1, mediate the cross-linking of NF and microtubules to produce the cytoskeletal lattice.<sup>3</sup> Proper stoichiometry of NF sub-

units as well as their phosphorylation state appears important for their assembly into 10-nm NF, transport from the perikaryon to the axon through slow transport mechanisms, and incorporation into a cytoskeletal network.<sup>5</sup>

In the nervous system, NF are not required for axonogenesis, but are critical influences on axonal diameter. In myelinated axons there is a nearly linear relationship between axonal cross-sectional area and neurofilament number.<sup>6</sup> Nerve conduction velocity is directly related to axonal caliber, so that neurofilament content has a major influence on nerve conduction velocity in individual fibers. Neurofilament content, axonal caliber, and conduction velocities are markedly reduced in animals lacking axonal NF.<sup>4,6</sup>

NFH is one of the most highly phosphorylated proteins known, with 40-70 Lys-Ser-Pro (KSP) repeats providing a potential phosphorylation state in the C-terminus domains. NFM also has phosphorylation sites. Phosphorylation is involved in the regulation of the orientation of NF side arm projections and consequently of NF spacing and axonal diameter.<sup>4</sup>

The neurology of NF can be classified into disorders of NF synthesis, phosphorylation, distribution by axonal transport, and, at least in theory, of their breakdown (see the table). NF are not required for neuronal survival.<sup>3</sup> Spontaneous mutations and genetically engineered mice that lack NF show only a modest loss of neurons and axons, and neurons can survive for long periods with massive accumulations of neurofilaments. Transgenic mice that overexpress human wild type neurofilament subunit genes and that have one of several NF murine mutants (e.g., a missense mutation of the N-terminus of NFL) develop massive accumulations of neurofilaments in their motor and large sensory neurons, and ultimately undergo motor neuron disease.<sup>7-9</sup> In Charcot-Marie-Tooth Disease (CMT) 2E, a mutation in the highly conserved rod domain of NFL is causative, likely through a dominant gain of function.<sup>10</sup> In rare

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**Table** Neuromuscular disorders that may be filamentopathies

| Disorder  | Mutation  | Gene product  | Intermediate filament pathology   |
|---|---|---|---|
| Giant axonal neuropathy                                 | GAN (16q24)                                     | Gigaxonin (BTB/kelch protein)   | Disorganized IF network, giant axonal swellings   |
| CMT 2E  | NFL gene (8p21)                                 | NFL   | Pathology not described   |
| ALS   | NFH gene (22q12.1-q13.1)–tail deletions         | NFH   | No pathology described for ALS with NFH mutations; prominent accumulation of NF occur in motor neurons in sporadic ALS  |
| Familial ALS  | NFH gene (22q12.1-q13.1)–tail deletions         | NFH   | A single kindred, no pathology described  |
| Myofibrillar myopathies                                 | Desmin (2 q35)<br>αβ-crystallin (11q22.3-q23.1) | Desmin (IF protein)<br>αβ-crystallin (heat shock protein–desmin associated) | Focal lysis of myofibril network with accumulation of desmin and other proteins   |
| Emery–Dreifuss muscular dystrophy                       | Lamins A/C (1q11-q23)<br>Emerin (Xq28)          | Nuclear lamins A/C (IF proteins)<br>Emerin (IF associated protein)          | Dystrophic features, detachment of the nuclear envelop on ultrastructural studies   |
| Congenital muscular dystrophy and epidermolysis bullosa | Plectin (8 q24.3)                               | Plectin (IF binding protein)  | Myonecrosis and regeneration, absent plectin staining at Z-lines  |
| Iminodipropionitrile (IDPN) neuropathy                  |   |   | Giant axonal swellings in the proximal axon, cytoskeletal reorganization with subaxolemmal segregation of NF, selective defect of slow axonal transport of NF |
| 2,5 Hexanedione (glue-sniffing) neuropathy              |   |   | Giant axonal swellings in the distal axon, NF accumulations, primary mechanism uncertain  |

CMT 2E = Charcot–Marie Tooth disease 2E; NFL = neurofilament light subunit; NFH = neurofilament heavy subunit; IF = intermediate filament.

instances of familial ALS and sporadic ALS, polymorphisms in the tail domain of NFH may contribute to development of the disease.<sup>7,11,12</sup>

Axonal accumulations of IF are a pathologic hallmark of several neurologic disorders, including GAN and glue-sniffing neuropathy caused by neurotoxic hexacarbons (see the table). These disorders are characterized by massive multifocal accumulations of neurofilaments in axons, producing large axonal swellings. When an axonal swelling is near a paranode, the myelin is typically pushed back into the internode, creating paranodal demyelination and leading to conduction velocities more typical of primary demyelinating than axonal disorders. The swellings alternate with stretches of axon that are depleted in neurofilaments and consequently are atrophic. Ultrastructural investigations in GAN show reduced NF spacing, an increase in NF thickness, and improper formation of sidearm projections.<sup>5,13</sup> Immunohistochemical studies confirm the presence of normal staining for NFH, M, and L in GAN.<sup>5</sup> These pathologic findings predicted a primary abnormality of cross-linking mechanisms of IF, with impaired (possibly because of abnormal post-translational modification) NF sidearm formation.<sup>13</sup>

The gene responsible for GAN encodes a novel protein, gigaxonin, whose exact function is unknown. The current article increases the number of pathogenic mutations described in GAN to 17.<sup>14</sup>

Gigaxonin is a 597-amino acid BTB (Broad-Complex, Tramtrack and Bric a brac)/Kelch) pro-

tein.<sup>14</sup> The N-terminal BTB domain likely mediates dimerization of the 6 kelch repeat sequences within the carboxy terminal portion of the protein. More than 20 Kelch repeat proteins (first described in *Drosophila*) have been cloned.<sup>15</sup> Kelch repeat proteins subserve diverse intracellular and extracellular functions. Among these, their function as cytoskeletal modulators, both through the cross-linking of actin, as well as by nonactinic mechanisms, predicts a cytoskeletal role for gigaxonin.<sup>14,15</sup> Gigaxonin may serve as an IF association protein, vital to proper sidearm orientation of IF, and to the cross-linking of IF, microtubules, and microfilaments to form a competent cytoskeleton. Several kelch proteins are regulators of gene expression—an alternative hypothesis to be explored is that gigaxonin may function as a regulator of genes as yet unidentified that are crucial to the proper organization of cytoskeletal elements.<sup>15</sup>

Future investigations in GAN will need to focus on genotype–phenotype correlations. GAN demonstrates phenotypic heterogeneity<sup>16</sup> and the effects, perhaps disparate, of different mutations of GAN may enhance current understanding of the regulation of the cytoskeleton in different tissues.

Identification of the precise subcellular localization of gigaxonin and the development of gigaxonin transgenes should provide additional insight into the basic mechanisms of a variety of acquired and hereditary disorders in which accumulation of neurofilaments is a prominent and early histopathologic finding.

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