Charcot-Marie-Tooth (CMT) is the most common inherited neuropathy of the PNS affecting approximately 2.8 million people. CMT is generally grouped into demyelinating (type 1), axonal (type2) or intermediate forms (type 3, 4 and X-linked). CMT type 2E, an axonal form of CMT, has been linked to mutations in the neurofilament light gene (nefl) and leads to distal neuropathy characterized by reduced nerve conduction velocity, muscle atrophy and sensory loss. However, the mechanisms of disease pathogenesis are not well understood. We generated a mouse model of CMT2E expressing human neurofilament light with the E396K mutation (hNF-LE396K), which develops decreased motor nerve conduction velocity, ataxia, and muscle atrophy by 4 months of age.

My work further characterizes this CMT2E mouse model and shows that symptomatic hNF-LE396K mice developed phenotypes that were consistent with proprioceptive sensory defects as well as reduced sensitivity to mechanical stimulation, while thermal sensitivity and auditory brainstem responses were unaltered. Progression from pre-symptomatic to symptomatic included a 50% loss of large diameter sensory axons within the fifth lumbar dorsal root of hNF-LE396K mice. Due to proprioceptive deficits and loss of large diameter sensory axons, I analyzed muscle spindle morphology in pre-symptomatic and symptomatic hNF-LE396K and hNF-L control mice. Muscle spindle cross sectional area and volume were reduced in all hNF-LE396K mice analyzed, suggesting that alterations in muscle spindle morphology occurred prior to the onset of typical CMT pathology. These data suggested that CMT2E pathology initiates in the muscle spindles altering the proprioceptive sensory system.

A concern of patients suffering with CMT neuropathy is their increased susceptibility to neuropathy exacerbation after a traumatic event such as undergoing surgery or receiving prescribed drug treatments. Although this problem has been observed with more frequency in recent years, it is not well understood and poorly studied. To investigate if a traumatic event could exacerbate neuropathy in our CMT2E mouse model and possibly establish this as a model to study CMT neuropathy exacerbation, I challenged wild type, hNF-L, and hNF-LE396K mice with crush injury to the sciatic nerve. Then, I analyzed functional recovery by measuring toe spread and analyzed gait using the Catwalk system. hNF-LE396K mice showed reduced recovery from nerve injury consistent with increased susceptibility to neuropathy observed in CMT patients. In addition, hNF-LE396K developed a permanent reduction in their ability to bear weight, increased mechanical allodynia, and premature gait shift in the injured limb, which led to disrupted interlimb coordination in hNF-LE396K. Exacerbation of neuropathy after injury and identification of gait alterations in combination with previously described pathology suggests that hNF-LE396K mice recapitulate many of clinical signs associated with CMT2. The results of my work demonstrate that hNF-LE396K mice provide a model for determining the efficacy of novel therapies.

A complication of peripheral neuropathies or nerve injuries is the loss of myelin. During development, a positive correlation between internodal length and axonal diameter is established so that both are optimized for maximal conduction velocity. However, after recovery from injury this correlation is lost. While axonal diameters recover back normal levels, remyelination results in uniformly short internodes and reduced conduction velocity. Although this phenomenon has been known for over 70 years, the mechanisms leading to abnormally reduced internodal length after remyelination are not well understood. I investigated the potential role of neurofilament phosphorylation in regulating internode length during remyelination and myelination in mice. Following ethidium bromide demyelination in sciatic nerve, the levels of neurofilament medium (NF-M) and heavy (NF-H) phosphorylation were unaffected. Preventing NF-M lysine-serine-proline...
(KSP) repeat phosphorylation increased internode length by 30% after remyelination. Mimicking constitutive KSP phosphorylation reduced internode length by 16% during myelination and motor nerve conduction velocity by ~27% without altering sensory nerve structure or function. These results suggest that NF-M KSP phosphorylation is a negative regulator of Schwann cell elongation, and suggest that motor and sensory axons utilize different mechanisms to establish internode length. My work on this project provided the first molecular basis for an empirical observation made over 70 years ago, and identified a potential target for therapeutic intervention aimed at enhancing recovery from demyelinating diseases or nerve injuries.