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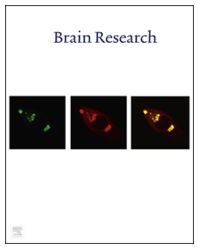

Research Report

Exacerbation of Charcot–Marie–Tooth type 2E neuropathy following traumatic nerve injury

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ABSTRACT

Charcot–Marie–Tooth disease (CMT) is the most commonly inherited peripheral neuropathy. CMT disease signs include distal limb neuropathy, abnormal gait, sensory defects, and deafness. We generated a novel line of CMT2E mice expressing hNF-L^{E397K}, which displayed muscle atrophy of the lower limbs without denervation, proximal reduction in large caliber axons, and decreased nerve conduction velocity. In this study, we challenged wild type, hNF-L and hNF-L^{E397K} mice with crush injury to the sciatic nerve. We analyzed functional recovery by measuring toe spread and analyzed gait using the Catwalk system. hNF-L^{E397K} mice demonstrated reduced recovery from nerve injury consistent with increased susceptibility to neuropathy observed in CMT patients. In addition, hNF-L^{E397K} developed a permanent reduction in their ability to weight bear, increased mechanical allodynia, and premature gait shift in the injured limb, which led to increasingly disrupted interlimb coordination in hNF-L^{E397K}. Exacerbation of neuropathy after injury and identification of gait alterations in combination with previously described pathology suggests that hNF-L^{E397K} mice recapitulate many of clinical signs associated with CMT2. Therefore, hNF-L^{E397K} mice provide a model for determining the efficacy of novel therapies.

Abbreviations: CMT, Charcot–Marie–Tooth; CMT2E, Charcot–Marie–Tooth type 2E; NF, Neurofilaments; NF-L, Neurofilament light; *nefl*, Neurofilament light gene; hNF-L, Human neurofilament light; IC, Initial contact; LH, Left hind; RH, Right hind; LF, Left front; RF, Right front

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1. Introduction

Charcot–Marie–Tooth (CMT) disease is the most commonly inherited peripheral neuropathy (Skre, 1974; Emery, 1991), and it is grouped into four main types, CMT1–4, depending on the specific genetic defect. Despite these different classifications, most CMT patients present with wasting of distal limb muscles, reduction in axonal diameters and nerve conduction velocities, sensory defects, deafness, and abnormal gaiting (Emery, 1991; Shy and Patzko, 2011; Georgiou et al., 2002; Züchner et al., 2004). CMT age of onset and severity can vary between sub-types and can vary within a single family (Züchner et al., 2004).

CMT patients can also experience exacerbation of their existing neuropathy. Evidence suggests administration non-toxic doses of prescribed medications can exacerbate CMT disease neuropathy (Weimer and Podwall, 2006; Chaudhry et al., 2003; Hildebrand et al., 2000; Martino et al., 2005). Patients that were administered such agents developed deterioration of nerve conduction velocity, severe pain, sensory impairments of upper and lower extremities, ataxia of gait, and in some cases inability to walk (Chaudhry et al., 2003; Hildebrand et al., 2000; Martino et al., 2005). The typical intervention in such cases is discontinued administration of the agent, which typically leads to incomplete to no recovery (Weimer and Podwall, 2006; Hildebrand et al., 2000). Moreover, upon exacerbation of the neuropathy, the mechanisms leading to poor or incomplete recovery are not well understood and poorly studied due to the lack of a reliable model of neuropathy exacerbation.

Mutations in neurofilament light gene (*nefl*) cause CMT type 2E (Barry et al., 2007; Dale and Garcia, 2012). Neurofilament light (NF-L) protein associates with either neurofilament medium (NF-M) or heavy (NF-H) to form a 10 nm filament (NF) (Lee et al., 1993). Once formed, NFs are intrinsic determinants of axonal diameter (Xu et al., 1996; Eyer and Peterson, 1994; Zhu et al., 1997; Garcia et al., 2003), a major axonal property influencing neuronal conduction velocity (Waxman, 1980; Kriz et al., 2000). Furthermore, NFs provide the axons with structural stability and tensile strength in response to physical stress (Barry et al., 2007; Rammensee et al., 2007; Lee and Shea, 2014; Gilbert, 1975). Currently, there are two well established mouse models of CMT 2E, hNF-L^{P22S} (Dequen et al., 2010) and hNF-L^{E397K} (Shen et al., 2011). hNF-L^{P22S} mice develop hypertrophy of muscle fibers and muscle denervation without neuronal loss (Dequen et al., 2010). hNF-L^{E397K} mice develop muscle atrophy without muscle denervation or significant neuronal loss (Shen et al., 2011). Interestingly, both models develop aberrant hind limb posture, altered gait, and sensorimotor defects, which recapitulate the disease pathology seen in human patients (Dequen et al., 2010; Shen et al., 2011; Dale et al., 2012; Filali et al., 2011). More recently, two additional models of CMT2E have been generated, NF-L^{P8R} and NF-L^{N98S}, of which only NF-L^{N98S} displays molecular pathology similar to human patients (Adebola et al., 2015).

In this study, we investigated the effect of traumatic nerve injury on CMT neuropathy in our hNF-L^{E397K} mouse model by analyzing functional recovery and gait alterations. Evidence suggests that wild type animals develop gait alterations following sciatic nerve crush injury that disappear after recovery (Bozkurt et al., 2008). We found that hNF-L^{E397K} animals, following a nerve injury, develop functional and gait alterations that do not fully recover suggesting increased vulnerability to injury, decreased recovery following injury, or both. Moreover, this data shows that our hNF-L^{E397K} mouse model could be a viable model to study the mechanisms of neuropathy exacerbation after nerve injury.

2. Results

2.1. *Reduced functional recovery after nerve injury in hNF-L^{E397K} mice*

Evidence suggests that pre-existing neuropathies can be exacerbated by non-toxic dosages of neurotoxic agents (Chaudhry et al., 2003). Moreover, many prescribed medications can exacerbate CMT disease neuropathy in humans (Weimer and Podwall, 2006; Hildebrand et al., 2000). Stopping administration of exacerbating compounds leads to reduced or no recovery in CMT patients (Chaudhry et al., 2003; Hildebrand et al., 2000). It is unclear if reduced or failed recovery is due to enhanced susceptibility to injury or reduced recovery following exposure to noxious stimuli. Therefore, we analyzed functional recovery from sciatic nerve injury in wild type ($n=10$), hNF-L ($n=20$), and hNF-L^{E397K} ($n=12$) mice. The left sciatic nerve was crushed at the level of the obturator tendon, and toe spread was monitored in the ipsilateral paw (Fig. 1A). Distance between first and fifth digits was measured pre-injury and post-injury (Fig. 1B) over a time course of 25 days (Fig. 1C). Recovery was analyzed in aged matched wild type, aged matched hNF-L and symptomatic (4 month old) hNF-L^{E397K} mice. Post-injury measurements were plotted as a percentage of the pre-injury values (Fig. 1C).

Toe spread recovery in wild type and hNF-L mice was similar over the entire time course. Although, ultimately hNF-L mice recovered to only about 98% of their pre-injury values, this difference was not significant when compared to wild type controls. Recovery in hNF-L^{E397K} mice was similar to hNF-L and wild type controls from day 1 to day 14 post-injury. From day 15 post-injury, functional recovery in hNF-L^{E397K} mice began to plateau while hNF-L and wild type controls continued to recover. Functional recovery in hNF-L^{E397K} was significantly reduced compared to wild type but not compared to hNF-L controls at day 15. From day 16 to day 25 post-injury, recovery in hNF-L^{E397K} mice was significantly lower compared to both controls, with the exception of day 20 where hNF-L^{E397K} recovery was significantly lower only compared to wild type mice (Fig. 1C; $p<0.05$). Unlike wild type and hNF-L controls, hNF-L^{E397K} mice recovered to approximately 89% of their pre-injury toe spread values (Fig. 1C).

2.2. *Gait alterations after recovery from nerve injury*

Wild type animals develop gait alterations following nerve injury. Interestingly, wild type animals are able to completely recover resulting in complete reversal of gait alterations observed with nerve injury (Bozkurt et al., 2008). Moreover,