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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.

Keywords

Charcot-Marie-Tooth type 2E; neuropathy exacerbation; nerve injury; functional recovery; gait alterations; neurofilament

1. Introduction

Charcot-Marie-Tooth (CMT) disease is the most commonly inherited peripheral neuropathy [1, 2], and it is grouped into four main types, CMT1-4, depending on the specific genetic defect. Despite these different classifications, most CMT patients present wasting of distal limb muscles, reduction in axonal diameters and nerve conduction velocities, sensory

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defects, deafness, and abnormal gaiting [2–5]. CMT age of onset and severity can vary between sub-types and can vary within a single family [5].

CMT patients can also experience exacerbation of their existing neuropathy. Evidence suggests administration non-toxic doses of prescribed drugs can exacerbate CMT disease neuropathy [6–9]. 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 [7–9]. The typical intervention in such cases is discontinued administration of agent, which typically leads to incomplete to no recovery [6, 8]. 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 [10, 11]. Neurofilament light (NF-L) protein associates with either neurofilament medium (NF-M) or heavy (NF-H) to form a 10nm filament (NF) [12]. Once formed, NFs are intrinsic determinants of axonal diameter [13–16], a major axonal property influencing neuronal conduction velocity [17, 18]. Furthermore, NFs provide the axons with structural stability and tensile strength in response to physical stress [10, 19–21]. Currently, there are two well established mouse models of CMT 2E, hNF-L^{P22S} [22] and hNF-L^{E397K} [23]. hNF-L^{P22S} mice develop hypertrophy of muscle fibers and muscle denervation without neuronal loss [22]. hNF-L^{E397K} mice develop muscle atrophy without muscle denervation or significant neuronal loss [23]. Interestingly, both models develop aberrant hind limb posture, altered gait, and sensorimotor defects, which recapitulate the disease pathology seen in human patients [22–25]. 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 [26].

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 [27]. 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 [7]. Moreover, many prescribed drugs can exacerbate CMT disease neuropathy in humans [6, 8]. Stopping administration of exacerbating compounds leads to reduced or no recovery in CMT patients [7, 8]. 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 the 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 recover to only about 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 [27]. Moreover, evidence shows that in human patients exacerbation of CMT disease results in sensorimotor defects that cause severe gait alterations [7–9]. To investigate if nerve injury leads to disease exacerbation and alterations to gait in our hNF-L^{E397K} model, we crushed the left sciatic nerve and analyzed gait. Gait was analyzed pre-injury, 5, 10, 15, and 25 days post-injury (Supplemental Figures). Functional recovery analysis showed maximal recovery 25 days after injury (Fig. 1C). Therefore, we have focused the present studies on pre-injury and 25 days post-injury time points. Since there were no differences in functional recovery between wild type and hNF-L control groups, only hNF-L animals were used as controls for gait analysis. The automated CatWalk system has been shown to be effective for objective, rapid and reproducible assessment of individual paw parameters as well as interlimb coordination on freely ambulating animals [28, 29].

2.2.1. Decreased ability to support weight in injured limb affects gait in diagonal limb

Print Width: Evidence from rat and mouse neuropathic pain models suggest that decreased ability to bear weight on a limb is strongly correlated with alterations to Print Width, Print Length and Max Contact Area metrics during voluntary locomotion [30–34]. Print Width is defined as the width of the paw from the first to the fifth digit. Nerve crush recovery analyses showed that hNF-L^{E397K} mice recovered toe spread ability to ~90% of their pre-injury value. Therefore, to investigate if these deficits were also reflected on the Catwalk system, we analyzed the print width of the mice during ambulation. Catwalk analysis

revealed that Print Width in both hNF-L and hNF-L^{E397K} mice did not recover to pre-injured values (Fig. 2A). Similar to toe spread analysis, hNF-L mice recovered Print Width better than hNF-L^{E397K} mice (Fig. 2A). Print Width was also reduced after recovery in hNF-L and hNF-L^{E397K} right, hind limbs (Fig. 2B). However, none of the changes reached statistical significance. Interestingly, both front paws had reduced Print Width in hNF-L^{E397K} mice following recovery (Fig. 2C and D).

Print Length: Print Length is measured from the beginning of the heel to the tip of the longest digit. CMT patients and mouse models develop alterations in foot structure [1, 5, 22, 23, 25, 26], such as hammertoes and high arches, which directly influence paw print length. Therefore, we analyzed Print Length to investigate if our mice develop alterations to this metric following recovery. Moreover, simultaneous reductions in both Print Width and Print Length could indicate the development of neuropathic pain [30–34]. Similar to changes in Print Width, hNF-L and hNF-L^{E397K} mice showed a significant reduction in Print Length following recovery. Moreover, hNF-L mice recovered better than hNF-L^{E397K} mice (Fig. 3A). On the right, hind paw, hNF-L mice showed a slight (not significant) decrease in Print Length, while hNF-L^{E397K} showed no alterations post-injury (Fig. 3B). Print Length on the left front paw showed slight (not significant) reductions post-injury in both hNF-L and hNF-L^{E397K} (Fig. 3C). On the right front paw, hNF-L mice showed no Print Length alterations post-injury. However, Print Length of hNF-L^{E397K} mice was significantly reduced compared to hNF-L Print Length following recovery (Fig. 3D).

Max Contact Area: Max Contact Area is a measure of the area contacted by a paw when the most pressure is being applied. On the injured paw, hNF-L and hNF-L^{E397K} mice showed a significant decrease in Max Contact Area following recovery. However, Max Contact Area recovered better in hNF-L mice relative to hNF-L^{E397K} mice (Fig. 4A). Reductions in Max Contact Area were observed in the right hind paw of both hNF-L and hNF-L^{E397K} mice, but these changes were not statistically significant (Fig. 4B). On the left, front paw, hNF-L mice showed no alterations to Max Contact Area. However, hNF-L^{E397K} Max Contact Area was significantly reduced following recovery compared to hNF-L controls (Fig. 4C). Interestingly, recovery of Max Contact Area of the diagonal, right front limb was significantly reduced in hNF-L^{E397K} mice relative to hNF-L mice (Fig. 4D).

Statistical analyses of pre-injury compared to post-injury values are reported in the main text and figures. For a time course analysis including pre-injury, 5, 10, 15, and 25 days post-injury of Print Width, Print Length, and Max Contact area, refer to Supplemental Figures 1, 2, and 3. For statistical analyses of changes within a genotype refer to Supplemental Tables 1, 2, and 3.

2.2.2. Increased mechanical allodynia to injured paw alters weight bearing on diagonal paw after recovery from injury—Max Intensity is a measure of the maximum light intensity of a paw print, and it correlates with the amount of pressure placed on a paw while the paw is in contact with the glass regardless of the area of contact. This is a parameter that is commonly used to evaluate effects of neuropathic pain, specifically mechanical allodynia [30, 32, 35, 36]. Max Intensity on the injured paw (left hind) of hNF-L mice recovered to values that were similar to pre-injury values (Fig 5A). Unlike hNF-L

mice, Max Intensity failed to recover in hNF-L^{E397K} mice to pre-injured values (Fig. 5A), indicating decreased ability to bear weight. Max Intensity was unaffected in the right hind (Fig. 5B) and left front (Fig. 5C) paws in either hNF-L or hNF-L^{E397K} mice. Interestingly, the Max Intensity on the right front paw of hNF-L^{E397K} mice was significantly reduced following recovery relative to pre-injury and hNF-L control values (Fig. 5D).

These results suggest that hNF-L^{E397K} develop alterations to Max Intensity, which translates into decreased ability to bear weight, on the injured paw that do not return to baseline levels after recovery from nerve injury. Moreover, our results also suggest that hNF-L^{E397K} mice develop similar weight bearing deficits on the uninjured diagonal paw (right front) after recovery from injury.

For a time course analysis and statistical analyses of changes within a genotype of Max Intensity refer to Supplemental Figure 4 and Supplemental Table 4, respectively.

2.2.3. Premature phase shift during ambulation after recovery from nerve injury—A model of a typical step cycle is provided (Fig. 6A). In the step cycle (Fig. 6A), the Stand phase of ambulation can be separated into braking and propulsion sub-phases (Fig. 6B). The point at which the braking sub-phase transitions into the propulsion sub-phase is defined by the maximum contact of a paw. The time it takes for a *contacting* paw to reach its maximum contact is referred to as Max Contact At (s) (Fig. 6B). Max Contact At (%) can be regarded as the point where the braking phase transitions into the propulsion phase during the Stand phase.

Following recovery, hNF-L Max Contact At (%) returned to pre-injury values (Fig. 7A). Interestingly, hNF-L^{E397K} mice Max Contact At (%) values failed to recover to pre-injury values, and recovered less than hNF-L mice (Fig. 7A). Max Contact At (%) was not affected on the right hind (Fig. 7B) and left front paws (Fig. 7C). On the right, front paw, Max Contact At (%) recovered to pre-injury values in hNF-L animals, but not in hNF-L^{E397K} animals (Fig. 7D).

Altered Max Contact At (%) in the left hind and right front paws of hNF-L^{E397K} mice can result from either a decreased Stand (s) time or increased Max Contact At (s) time (Fig. 6). Therefore, the relative stand (s) and Max Contact At (s) for each paw and time point were examined and revealed no alterations to these metrics (data not shown) suggesting that the decreased Max Contact At (%) values are due to a premature phase shift during the Stand phase.

For time course analysis and statistical analyses of changes within a genotype refer to Supplemental Figure 5 and Supplemental Table 5, respectively.

2.2.4. Altered interlimb coordination after recovery from nerve injury—Limb coupling is a measurement of interlimb coordination. Coordinated diagonal limbs (LH→RF, RH→LF) should produce coupling values of ~ 0% as they move synchronously and their paws should be in contact with the glass at the same time. Girdle (LH→RH and LF→RF) and ipsilateral (LH→LF and RF→RH), which move alternatively, should produce coupling

values of ~50%. Animals which have uncoordinated limb couplings present values that deviate from these expected percentages [24, 37].

hNF-L^{E397K} mice displayed significantly altered coordination in the girdle (LH→RH, Fig. 8A), ipsilateral (LH→LF, Fig. 8B), and diagonal (LH→RF, Fig. 8F) limbs following recovery. Interestingly, in hNF-L^{E397K} ipsilateral (RH→RF, Fig. 8C), girdle (LF→RF, Fig. 8D) and diagonal (RH→LF, Fig. 8E) limbs, which do not involve the LH limb, showed no alterations to limb coordination (Fig. 8 A–F). hNF-L control mice showed no alterations to interlimb coordination. Analysis of alternative coupling patterns showed similar alterations (data not shown). These results indicate that interlimb coordination was significantly altered, and did not return to pre-injury coordination for limb combinations involving the injured limb in the hNF-L^{E397K} mice.

Time course analyses and statistical analyses of changes within a genotype are reported in Supplemental Figure 6 and Supplemental Table 6, respectively.

3. Discussion

Evidence suggests that pre-existing neuropathies can be exacerbated by non-toxic dosages of neurotoxic agents and by administration of prescribed drugs [7–9]. In human patients, exacerbation of neuropathy has been reported to result in muscle weakness in both upper and lower limbs, inability to support weight [8], development of pain, and decreased coordination while walking [7, 9]. Currently, it is not known whether exacerbation is due to functional or structural alterations to the nerves, thus no current treatments, other than physical therapy, are available [6, 8]. We investigated if CMT2E neuropathy is exacerbated following a nerve challenge. We challenged these mice with a crush to the left sciatic nerve, and analyzed function and gait during (supplemental figures) and after recovery.

Previous analyses of hNF-L^{E397K} animals, by Catwalk, revealed alterations in interlimb coordination in symptomatic animals without alterations to Print Width, Print Length, or Max Contact Area [24]. During recovery from nerve injury, hNF-L^{E397K} mice developed alterations to Print Width, Print Length, Max Contact Area, and Max Intensity, all of which are indicative of decreased ability to bear weight and mechanical allodynia on the injured paw. Decreased ability to bear weight or increased mechanical allodynia, or a combination of both, could have resulted in the development of a premature phase shift observed during the Stand phase, as indicated by altered Max Contact At (%) values, in hNF-L^{E397K} mice. Development of such alterations ultimately caused disruption to interlimb coordination that were more pronounced than those previously seen in normal (uninjured) hNF-L^{E397K} mice [24].

Following a nerve crush injury, nerves degenerate from the day of injury to about 7 days post injury, which is followed by a period of nerve regeneration and re-innervation from 7 to about 20 days post-injury [38–41]. After re-innervation, axons undergo radial growth to regain pre-injury axonal diameters and recover normal nerve conduction velocity [40–43]. Our functional recovery analyses suggest hNF-L^{E397K} mice recover similar to hNF-L mice up to day 20 post-injury. From day 20 to day 25, hNF-L mice further improve, whereas

hNF-L^{E397K} mice do not continue to recover. In some instances, hNF-L^{E397K} recovery declines. This suggests that, following nerve injury, axons of hNF-L^{E397K} animals are able to regenerate and re-innervate their targets similarly to control animals. However, the lack of, or decline in, recovery from day 20 onwards in hNF-L^{E397K} animals suggests possible defects in axonal radial growth. Initial characterization of symptomatic hNF-L^{E397K} mice revealed a reduction nerve conduction velocity and axonal diameters in the motor nerves [23]. This suggests that a possible mechanism of disease exacerbation following a nerve injury could be the inability of axons to expand radially leading to even further reduced nerve conduction velocities. Further analyses will be required to investigate the molecular mechanisms leading neuropathy exacerbation in our CMT2E mouse model.

Taken together, our results suggest that nerve injury exacerbates CMT2E neuropathy, which manifests as alterations in sensory and motor coordination pathways on the injured limb. This provides a possible new approach for investigating exacerbation of neuropathies in patients suffering from a peripheral neuropathy. Furthermore, our data suggests that hNF-L^{E397K} mice may be a useful model for developing and screening potential therapies for treating or preventing disease exacerbation. Given that neuronal recovery is altered, hNF-L^{E397K} mice may also provide valuable insight into potential neurological toxicity of new and existing compounds.

4. Conclusion

Similar to CMT patients, our hNF-L^{E397K} mouse model develops functional deficits that are exacerbated following nerve injury. In view of the fact that hNF-L^{E397K} mice develop neuropathy that is similar to CMT patients, hNF-L^{E397K} mice provide a good model for determining the efficacy of novel therapies for treating CMT or for preventing exacerbation of the preexisting neuropathy.

5. Methods and materials

5.1. Animals

All procedures were in compliance with the University of Missouri Animal Care and Use Committee and with all local and federal laws governing the humane treatment of animals. Mice were housed in microisolator cages on a 12-h light/dark cycle and were given food and water *ad libitum*.

5.2. Sciatic nerve crush

Mice were placed under isoflurane anesthesia and the sciatic nerve was exposed via an incision in the flank followed by separation of underlying musculature by blunt dissection. The nerve was crushed using fine jewelers forceps at the level of the obturator tendon. To assess functional recovery of the injured limb, the mouse was induced to spread its toes by briefly lifting the hind limbs off the bench and slowly placing the mouse hind paws back on the floor. During this process the mouse spreads its toes when lifted by the tail and maintains them spread while it is being lowered and placed down on the floor again. The distance between the first and fifth digits was then measured using a compass and a ruler. Measurements were expressed as a percentage of pre-crush spread distance. Measurements

were made in triplicate for up to 30 days. Means for each daily toe spread measurement were analyzed for overall statistical differences by two-way repeated measures ANOVA with Holm-Sidak post hoc analysis (SigmaPlot, Systat Software Inc., San Jose, CA, US).

5.3. Gait

Gait for hNF-L (n = 9) and symptomatic hNF-L^{E397K} (n = 11) mice was monitored using the Catwalk XT gait analysis system (Noldus Information Technology, Asheville, NC) during the animal's light phase. Gait was analyzed on both hNF-L and hNF-L^{E397K} mice before nerve crush, and at 5, 10, 20 and 25 days after crush. Animals were allowed to ambulate freely on a restricted area of an illuminated glass walkway. Footprints were recorded using a high-speed camera. Runs with average speeds of 2.0 ± 0.5 cm/s were recorded for each animal and mean values were calculated. All means were analyzed for statistical differences by a two way ANOVA followed by a Holm-Sidak *post hoc* analysis for pair wise comparisons. For time course analysis (Supplemental Figures) all means were analyzed for statistical differences by a two way repeated measures ANOVA followed by a Holm-Sidak *post hoc* analysis for pairwise comparisons (SigmaPlot, Systat Software Inc., San Jose, CA, US).

5.4. Definition of reported measures

5.4.1. Print Width—A rectangle is outlined around each paw so that the paw fits perfectly in the rectangle. Print width is measured by measuring the width (perpendicular to the paw axis) of the rectangle. This gives a measure of the distance between the first and last digit of the paw, perpendicular to the paw axis.

5.4.2. Print length—This is measured by measuring the length of the rectangle fitted to the paw print. This gives a measure of the distance between the beginning of the heel and the tip of the longest digit parallel to the paw axis.

5.4.3. Max contact area—Defined as the maximum area contacted by the paw when paw is at maximum contact with the floor during a stance phase.

5.4.4. Max contact at %—Defined as the percentage time (seconds) since the start of a run that a paw reaches maximum contact with the glass plate relative to the stand phase for that paw. It is defined by the following formula:

$$\text{Max contact at} = \frac{\text{Max Contact at (s)} - \text{Initial Contact (s)}}{\text{Stand(s)}} \times 100\% \quad (1)$$

5.4.5. Max intensity—Is the maximum print intensity that each paw reaches while in contact with the walking surface during the stance phase of the step cycle.

5.4.6. Duty Cycle %—Defined as the percentage of the Stand phase (s) in a step cycle (stand + swing) and it is defined by:

$$\text{Duty cycle} = \frac{\text{Stand (s)}}{\text{Stand (s)} + \text{Swing (s)}} \times 100\% \quad (2)$$

5.4.7. Couplings—Defined as the difference between initial contact (IC) of a target paw and the reference paw relative to a reference paw's stride cycle (time interval between first contact of the reference paw and the subsequent contact).

$$\frac{\text{IC}_{\text{target}} - \text{IC}_{\text{reference}}}{\text{Stride cycle}} \times 100 \quad (1)$$

For diagonal (**LH**→**RF** and **RH**→**LF**) and ipsilateral (**LH**→**LF** and **RH**→**RF**) limb pairs, we assigned the hind paw as the reference paw. For girdle (**LH**→**RH** and **LF**→**RF**) limb pairs, we assigned the left paw as the reference paw. For diagonal limb pairs values approaching 0 % are more coordinated and for ipsilateral and girdle limb pairs values approaching 50 % are more coordinated [24].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CMT	Charcot-Marie-Tooth
CMT2E	Charcot-Marie-Tooth type 2E
NF	Neurofilaments
NF-L	Neurofilament light
<i>nefl</i>	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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Highlights

- We investigated neuropathy exacerbation in our CMT 2E mouse model.
- Traumatic nerve injury exacerbates CMT2E neuropathy.
- Exacerbation of CMT2E neuropathy leads to development of mechanical allodynia.
- Reduced functional recovery after nerve injury in CMT2E mice.

Figure 1. Functional recovery from sciatic nerve injury was reduced in hNF-L^{E397K} mice
 Sciatic nerves were crushed at the level of the obturator tendon on four-month-old wild type, hNF-L and symptomatic hNF-L^{E397K} mice. Nerve injury prevented toe spread on the injured limb (A). Toe spread was quantified by measuring the distance between the first and fifth digit of the paw (B) over a time course for up to 25 days (C). Wild type and hNF-L mice fully recovered 25 days post-injury (C). Functional recovery was significantly reduced in hNF-L^{E397K} mice beginning at day 15(C), and hNF-L^{E397K} mice failed to fully recover (C). Means for each daily toe spread measurement were analyzed for overall statistical differences by two way repeated measures ANOVA with Holm-Sidak post hoc analysis., *, $p < 0.05$ as compared to wild type toe spread; †, $p < 0.05$ as compared to hNF-L toe spread.

Figure 2. Reduced Print Width after nerve crush recovery

Print Width is defined as the width of the paw from the first to the fifth digit. Print width (cm) values are reported for the left hind (A), right hind (B), left front (C), and right front (D) paws from pre-crush and post-injury analyses. Print Width failed to recover to pre-injury values for hNF-L mice only in the left hind paw (A). For hNF-L^{E397K} mice, Print Width was significantly decreased following recovery compared to its pre-injury values for left hind (A), left front (C), and right front (D). On the left hind (A) and right front (D) paw, hNF-L^{E397K} mice recovered less than hNF-L mice. The differences in means were analyzed for overall statistical differences using a two-way ANOVA with Holm-Sidak post hoc analysis. *, $P < 0.05$.

Figure 3. Reduced Print Length on the injured limb after recovery

Print Length is measured from the beginning of the heel to the tip of the longest digit. Print Length was significantly lower in hNF-L and hNF-L^{E397K} mice following recovery compared to pre-injury values (A). Additionally, hNF-L^{E397K} mice did not recover Print Length as well as hNF-L mice (A). Print Length values for all right hind and left front paws were unchanged after recovery (B, and C). On the right, front paw, hNF-L^{E397K} lengths were significantly lower compared to hNF-L lengths (D). The differences in means were analyzed for overall statistical differences using a two-way ANOVA with Holm-Sidak post hoc analysis. *, $P < 0.05$.

Figure 4. Max Contact Area is decreased in hind left and diagonal paw after recovery
 Max Contact Area is a measure of the area contacted by a paw when the most pressure is being applied. Max Contact Area was reduced following recovery in the left hind paw of hNF-L and hNF-L^{E397K} mice (A). Additionally, hNF-L^{E397K} mice failed to recover to the same extent as hNF-L (A). Max Contact Area was unaltered in the right hind paw of hNF-L and hNF-L^{E397K} mice (B). Interestingly, Max Contact Area was reduced in left (C) and right (D) front paws in hNF-L^{E397K} mice. The differences in means were analyzed for overall statistical differences using a two-way ANOVA with Holm-Sidak post hoc analysis. *, $P < 0.05$.

Figure 5. Reduced Max Intensity in right front and left hind limbs post-injury

Max Intensity is a measure of the maximum light intensity of a paw print. Max Intensity correlates with the amount of pressure placed on a paw while the paw is in contact with the glass regardless of the area of contact. Max intensity failed to recover to pre-injury levels in the left hind (A) and right front (D) paws of hNF-L^{E397K} mice. Moreover, hNF-L^{E397K} animals had significantly lower Max Intensity values compared to hNF-L following recovery (A and D). Max Intensity values were unchanged in the right hind (B) and left front (C) paws. The differences in means were analyzed for overall statistical differences using a two-way ANOVA with Holm-Sidak post hoc analysis. *, $P < 0.05$.

Figure 6. Schematic outlining parameters measured by CatWalk

Step cycle of all paws is composed of alternating contacting (black) and non-contacting (white) phases (A). Within a step cycle, the contacting phase is referred to as the stand phase [reported as Stand (s)] and the non-contacting phase is referred to as the swing phase [reported as Swing (s)] (A). Each stand phase contains both a braking phase and propulsion phase. Maximum contact or max value of a paw roughly defines the point at which the braking phase transitions into the propulsion phase. The time it takes a paw to reach the Max Value is reported as Max Intensity At (s). Max Contact At (%) can be regarded as the point where the braking phase transitions into the propulsion phase during the Stand phase (B).

Figure 7. Reduced Max Contact At (%) in right front and left hind paws following nerve injury recovery

As Max Contact At (%) is regarded as the point where gait transitions from braking to propulsion, Max Contact At (%) is also a measure of the point within a stand phase where the maximal contact area with the walking surface is reached. hNF-L^{E397K} Max Contact At (%) failed to recover to pre-injury values, and was recovered less than hNF-L controls (A). Max Contact At (%) was not altered on the right hind (B) or left front (C) paws. On the right front paw, hNF-L^{E397K} Max Contact At (%) was significantly reduced compared to hNF-L Max Contact At (%) following recovery (D). The differences in means were analyzed for overall statistical differences using a two-way ANOVA with Holm-Sidak post hoc analysis. *, $P < 0.05$.

Figure 8. Limb coordination in hNF-L^{E397K} mice is altered after recovery from nerve injury Perfectly coordinated diagonal (LH→RF and RH→LF) limbs have coupling values of ~0, whereas perfectly coordinated girdle (LH→RH and LF→RF) and ipsilateral (LH→LF and RH→RF) limbs have coupling values of ~50. Coordination failed to recover for all coupling values that included the left hind (LH) limb in hNF-L^{E397K} mice relative to pre-injury and hNF-L measurements (A, B and F). Coordination between limbs that did not include the left hind limb was unaffected following recovery (B, C and D). The differences in means were analyzed for overall statistical differences using a two-way ANOVA with Holm-Sidak post hoc analysis. *, $P < 0.05$.