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# Laser-Induced Axonal Injury Reveals Gene Dosage-Dependent Mitochondrial Vulnerability in CMT2B Sensory Neurons

**Murong Su[1], Ian Chen[2], Joy Young[3], Yingli Gu[4], Veronica Gomez-Godinez[5], Linda Shi[5], Chengbiao Wu[4]**

[1] Department of Health, Society, and Behavior, Program in Public Health, University of California, Irvine, Irvine, CA 92697
[2] Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia Research Institute, Philadelphia, PA 19104
[3] College of Chemistry, university of California, Berkeley, CA 94720
[4] Department of Neurosciences, University of California San Diego, La Jolla, CA 92093
[5] Institute of Engineering in Medicine, University of California San Diego, La Jolla, CA, 92093

## Extended Abstract

Charcot-Marie-Tooth type 2B (CMT2B) is an inherited peripheral neuropathy characterized by axonal degeneration, progressive muscle weakness, and loss of pain sensation[1]. The disease is caused by missense mutations in the Rab7a gene, which disrupt vesicular transport, mitochondrial dynamics, and axonal stability[2]. While previous research has established a role for Rab7a-mediated mitochondrial dysfunction in CMT2B pathogenesis, the mechanisms underlying axonal vulnerability and response to injury remain insufficiently understood[3].

In this study, we applied laser nano-surgery to dorsal root ganglion (DRG) neurons derived from wild-type and CMT2B mutant mice to model and analyze axonal injury responses. A 740 nm femtosecond laser beam was focused at 120 mW to generate submicron axonal lesions, and neurons were monitored for 60 minutes post-ablation using phase-contrast microscopy. Damage was scored by morphology-based classification into three levels: level 0 (neuropraxia), level 1 (axonotmesis), and level 2 (neurotmesis). To examine the contribution of mitochondrial fission, select cultures were pretreated with Mdivi-1, a Drp1 inhibitor, at 100 μM for 60 minutes before laser exposure.

Results demonstrated that Mdivi-1 significantly reduced the frequency of severe axonal damage in heterozygous CMT2B neurons (fln/+), with level 2 injury rates decreasing from 26% to 3%. In homozygous neurons (fln/fln), level 2 injury decreased from 32% to 26%, suggesting a gene dosage effect in treatment efficacy. These data indicate that mitochondrial fission plays a critical role in the damage response of CMT2B neurons, and that Drp1 inhibition may have therapeutic potential.

This study establishes laser nano-surgery as a powerful and reproducible tool for inducing and analyzing axonal injury in vitro, and highlights mitochondrial dysfunction as a key contributor to axonal pathology in CMT2B.

**Keywords:** Charcot-Marie-Tooth 2B (CMT2B), Rab7a, Laser Nano-Surgery, Mdivi-1, Mitochondrial Fission, Drp1, Axonal Damage, Peripherin, Gene Dosage, Neurodegeneration

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