



# Abstract 135: A Somatic GNA11 Mutation is Associated with Extremity Capillary Malformation and Overgrowth

## Citation

Couto, J. A., U. M. Ayturk, D. J. Konczyk, J. A. Goss, A. Y. Huang, S. Hann, J. L. Reeve, et al. 2017. "Abstract 135: A Somatic GNA11 Mutation is Associated with Extremity Capillary Malformation and Overgrowth." *Plastic and Reconstructive Surgery Global Open* 5 (4 Suppl): 99-100. doi:10.1097/01.GOX.0000516654.69082.be. <http://dx.doi.org/10.1097/01.GOX.0000516654.69082.be>.

## Published Version

[doi:10.1097/01.GOX.0000516654.69082.be](https://doi.org/10.1097/01.GOX.0000516654.69082.be)

## Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:33029801>

## Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

## Share Your Story

The Harvard community has made this article openly available. Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

134

## FK506 Delivering Conduit For Peripheral Nerve Regeneration

**Pratima Labroo, MS, Jill E. Shea, PhD, Brett Davis, BS, David Hilgart, BS, Christopher Lambert, BS, Himanshu Sant, PhD, Bruce Gale, PhD, Jayant Agarwal, MD**

**University of Utah, Salt Lake City, UT**

**PURPOSE:** Following a peripheral nerve injury, nerve gaps require grafts or conduits to connect the two nerve ends. There is a clinical need to improve functional recovery following nerve injury and local release of neurotrophic factors is one way to improve outcomes. FK506, an FDA approved small molecule, has been shown to enhance axon growth *in vitro* and peripheral nerve regeneration *in vivo*. Systemic delivery of FK506 has numerous potentially serious side effects such as central nervous system toxicity, infection and nephrotoxicity. Local delivery of FK506 from a nerve guide could provide the neurotrophic benefits of FK506 but prevent the negative consequence of systemic delivery. Here we describe the *in vitro* and *in vivo* outcomes when using a novel drug delivery apparatus integrated with a biocompatible polytetrafluoroethylene (PTFE) based nerve conduit for controlled local delivery of FK506.

**METHODS:** The device consists of two concentric PTFE tubes (Zeus Inc.) and a reservoir in between the tubes that stores FK506. A 120 $\mu$ m diffusion hole was drilled into the inner tube by pulsing a laser. *In vitro* release tests were performed over a 32-day period and the concentration of released FK506 was determined with an enzyme linked immunosorbent assay (ELISA, Abnova). The bioactivity of the released FK506 was evaluated using a chick dorsal root ganglion (DRG) cell based bioassay. *In vivo* testing of the FK506-delivering conduit was performed in a critical sized 9mm mouse sciatic nerve gap model and compared with negative control (empty conduit). The animals were sacrificed at 18 weeks postoperatively and functional outcomes such as nerve histomorphometry, neuromuscular junction connectivity and compound muscle action potential were evaluated.

**RESULTS:** FK506 was released at a consistent concentration,  $5.2 \pm 3.4$  ng/mL/day, over a period of 32 days. The released FK506 was found to be bioactive and enhanced neurite elongation by ~40% compared with DRGs exposed

to control media (n=4) (p<0.05) but comparable to freshly prepared FK506 (p>0.05). In the *in vivo* study, we measured a 53% increase in neuromuscular junction connectivity and a 40% increase in the amplitude of muscle compound action potential when a 9mm sciatic nerve gap injury was repaired with our FK506 releasing device compared to the (no drug) device alone (n=3) (p<0.05).

**CONCLUSION:** We successfully manufactured PTFE nerve conduits that can deliver bioactive FK506 in a controlled manner for 32 days. The released FK506 enhanced neurite extension *in vitro* and nerve regeneration *in vivo*. We are currently examining how locally released FK506 impacted the nerve histomorphometry by evaluating nerve myelination and retrograde labelling compared to treatment with an empty conduit. Future work will evaluate the efficacy of this local delivery of FK506 *in vivo* using a biodegradable biomaterial.

135

## A Somatic GNA11 Mutation is Associated with Extremity Capillary Malformation and Overgrowth

**Javier A. Couto, BS<sup>1</sup>, Ugur M. Ayturk, PhD<sup>2</sup>, Dennis J. Konczyk, BS<sup>1</sup>, Jeremy A. Goss, MD<sup>1</sup>, August Y. Huang, PhD<sup>2</sup>, Steven Hann, PhD<sup>2</sup>, Jennifer L. Reeve, MD, PhD<sup>3</sup>, Marilyn G. Liang, MD<sup>4</sup>, Joyce Bischoff, PhD<sup>5</sup>, Matthew L. Warman, MD<sup>2,6</sup>, Arin K. Greene, MD, MMSc<sup>1</sup>**

**<sup>1</sup>Department of Plastic & Oral Surgery, Boston Children's Hospital, Harvard Medical School, Boston, MA, <sup>2</sup>Department of Orthopedic, Boston Children's Hospital, Harvard Medical School, Boston, MA, <sup>3</sup>Department of Dermatology, University of Michigan, Ann Arbor, MI, <sup>4</sup>Department of Dermatology, Boston Children's Hospital, Harvard Medical School, Boston, MA, <sup>5</sup>Department of Surgery, Vascular Biology Program, Boston, MA, <sup>6</sup>Howard Hughes Medical Institute, Boston Children's Hospital, Boston, MA**

**PURPOSE:** Capillary malformation is a cutaneous vascular anomaly that is present at birth, darkens over time, and can cause overgrowth of tissues beneath the stain. The lesion is caused by a somatic activating mutation in *GNAQ*. In a previous study we were unable to identify a *GNAQ* mutation in patients with a capillary malformation involving an overgrown lower extremity. We hypothesized that mutations in *GNA11* or *GNA14*, genes closely related to *GNAQ*, also may cause capillary malformations.

**METHODS:** Human capillary malformation tissue obtained from 8 patients that had tested negative for *GNAQ* mutations were studied. Lesions involved an extremity (n=7) or trunk (n=1). Droplet digital PCR (ddPCR) was used to detect *GNA11* or *GNA14* mutant cells (p.Arg183) in the specimens. Single molecule molecular inversion probe sequencing (smMIP-seq) was performed to search for other mutations in *GNA11*. Mutations were validated by subcloning and sequencing amplimers.

**RESULTS:** We found a somatic *GNA11* missense mutation (c.547C>T; p.Arg183Cys) in 3 patients with a diffuse capillary malformation of an extremity. Mutant allelic frequencies ranged from 0.3%-5.0%. *GNA11* or *GNA14* mutations were not found in 5 affected tissues or in unaffected tissues (white blood cell DNA).

**CONCLUSION:** *GNA11* mutations are associated with extremity capillary malformations causing overgrowth. Pharmacotherapy that affects *GNA11* signaling may prevent the progression of capillary malformations.

136

### Short Duration 9-cis Retinoid Acid Therapy is Sufficient to Improve Functional Lymphatic Drainage in Post-Surgical Lymphedema

Gene K. Lee, MD, MPH, David P. Perrault, BS, Antoun Bouz, BS, Austin J. Pourmousa, MS, Daniel Gardner, BS, Maxwell Johnson, MS, Sun Young Park, MS, Athanasios Bramos, MD, Eun Kyung Park, MS, Young Jin N. Seong, BS, Sunju Lee, PhD, Lia E. Jung, MS, Dongwon Choi, PhD, Young-Kwon Hong, PhD, Alex K. Wong, MD, FACS

### University of Southern California, Los Angeles, CA

**PURPOSE:** 9-cis Retinoic Acid (9-cis RA), prevents post-surgical lymphedema and is associated with increased lymphangiogenesis and lymphatic clearance. In this study, we sought to determine if shorter durations of 9-cis RA therapy were sufficient to maintain the functional effect on post-surgical lymphatic clearance.

**METHODS:** Fifty C57BL/6 mice underwent surgical induction of tail lymphedema. Treatment groups (n=10) included injection of 9-cis RA starting on post-operative day (POD) zero for either 7, 7 days at POD 7, 14, and 45 days. The control group was injected with a vehicle control for 45 days. On POD 42, indocyanine green (ICG) lymphangiography was performed. Lymphatic fluid clearance was quantified over time with Image J and student-t tests were calculated between each group at various time points using GraphPad Prism 6.

**RESULTS:** \*Significant ( $p$ -value < 0.05) difference between the control group and all other treatment groups

**CONCLUSIONS:** The improvement in functional lymphatic flow in post-surgical lymphedema by 9-cis RA is present with 7 days, 7 days of delayed, 14 days and 45 days of therapy. The percent change in tail fluorescence on POD 42, upon injection of ICG as measured by SPY lymphangiography was significantly lower in all experimental groups overtime, compared to the vehicle control. 9-cis RA is a promising therapy for post-surgical lymphedema, even at shorter durations of therapy. A minimal effect dosing regimen has the potential to limit side effects of 9-cis RA systemic therapy.

137

### Contribution of the Non-Canonical BMP Receptor Signaling Pathway Osteogenesis on Bioinspired Nanoparticulate Mineralized Collagen Scaffolds

Qi Zhou, MD<sup>1</sup>, Xiaoyan Ren, MD, PhD<sup>1</sup>, David Bischoff, PhD<sup>2</sup>, Daniel Weisgerber, PhD<sup>3</sup>, Dean Yamaguchi, MD, PhD<sup>2</sup>, Timothy Miller, MD<sup>1</sup>, Brendan Harley, ScD<sup>3</sup>, Justine C. Lee, MD, PhD<sup>1</sup>