ACVR1-Induced Sensory Neuron Dysfunction in Fibrodysplasia Ossificans Progressiva

Amy N. Ton*[1], Xiaobing Yu*[2], Blanca M. Morales*[1], Jiadong Chen[3], Emilie Barruet[1], Hongju Liu[2,5], Kin Cheung[4], Syed Ali[2], Tea Chan[1], Katie Bigay[1], Jennifer Ho[1], Ina Nikolli[1], Arnold Kriegstein[3], and Edward C. Hsiao[1]

[1]Division of Endocrinology and Metabolism, Department of Medicine; the Institute for Human Genetics; and the Program in Craniofacial Biology, [2]Department of Anesthesia and Perioperative Care, [3]Department of Neurology, University of California, San Francisco, California, USA; [4]International FOP Association FOP Connection Registry; [5]Department of Anesthesiology, Peking Union Medical College Hospital, Beijing, China

Introduction

Chronic pain is a significant complication of musculoskeletal diseases; however, few genetic associations are established. Animal studies implicate the BMP pathway in nervous system development and pain modulation. Whether humans show abnormal pain modulation in bone morphogenetic protein (BMP)-related disorders is unclear.

Fibrodysplasia ossificans progressiva (FOP) is a congenital disease caused by activating point mutations in the intracellular domain of the Activin Type 1 receptor ACVR1 (1). In addition to massive and progressive heterotopic ossification (HO), **some patients also show neurological symptoms**, including myoclonus, headache, and chronic pain with neuropathic characteristics.

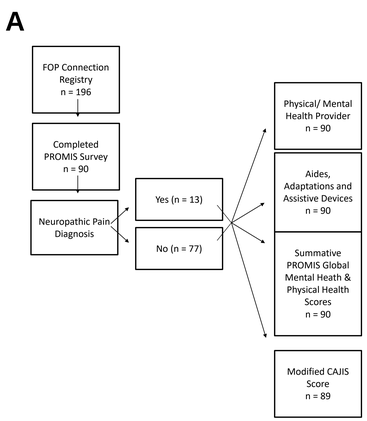
Because access to human neuronal tissue is limiting, human induced pluripotent stem cell (iPSC)-derived sensory neurons provide opportunities to identify molecular causes and new therapeutics for pain conditions (3, 4).

GOALS OF THIS STUDY: The main goals of this study were to identify and define the type of sensory dysfunction present in patients with FOP and to identify potential mechanisms.

1. We examined the incidence of neuropathic pain in a cohort of patients with FOP, identifying the type of sensory dysfunction in patients with FOP,
2. We used human iPSCs to determine potential cellular mechanisms.

Results

Figure 1: Self-Reported Neuropathic Pain is Associated With Increased Functional Disability in a Registry of Patients With FOP.



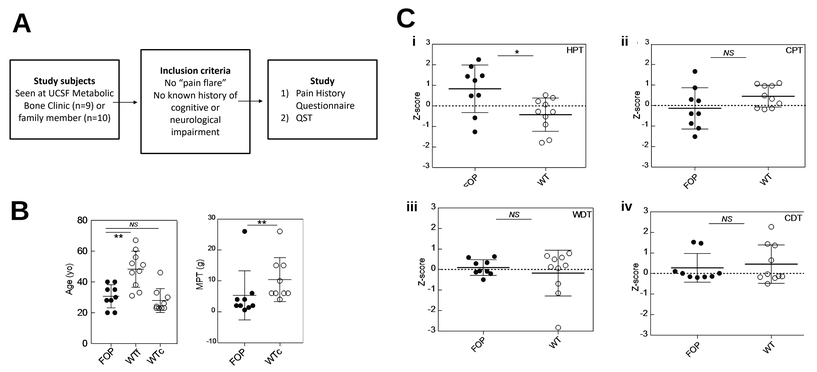
B

- 14.4% of patients with FOP suffer from neuropathic pain, compared to 6.9-10% of the general population (5, 6, 7).

Total	Statistics N	Neuropathic Pain (+)	Neuropathic Pain (-)	P value
Total Modified CAJIS Score[6]	n	12	67	
	Mean±SD	18.8±9.77	15.3±7.80	p = 0.2620
	Median	17.5	15.0	
	Min, Max	5.0, 33.0	0.0, 34.0	
Aides, Assistive Devices, and Adaptations	n	13	77	
	Mean±SD	22.5±14.52	14.7±10.04	p = 0.0426*
	Median	25.0	13.0	
	Min, Max	1.0, 51.0	0.0, 49.0	
# Times Visited a Doctor for Physical Health	n	13	77	
	Mean±SD	6.6±5.01	4.2±5.18	p = 0.0255*
	Median	6.0	2.0	
	Min, Max	1.0, 16.0	0.0, 25.0	
# Times Visited a Doctor for Mental Health	n	13	77	
	Mean±SD	4.0±8.15	2.0±7.72	p = 0.022*
	Median	1.0	0.0	
	Min, Max	0, 30.0	0, 60.0	
PROMIS Global Mental Health Score without Quality of Life	n	13	77	
	Mean±SD	2.8±0.92	3.3±0.90	p = 0.0997
	Median	3.0	3.3	
	Min, Max	1.3, 4.3	1.0, 4.7	

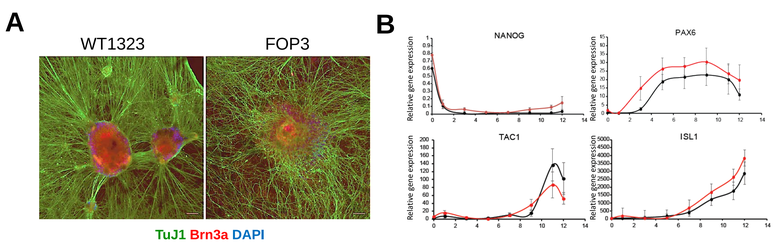
(A) Flowchart showing study design of included patients in study. **(B)** Total modified Cumulative Analogue Joint Involvement Scale (CAJIS) (6); Aids, Assistive Devices & Adaptations; health care provider utilization; and PROMIS Global Mental Health Scores based on patient-reported diagnosis of Neuropathic Pain at time of Enrollment into International FOP Patient Registry.

Figure 2: Quantitative Sensory Testing Shows Abnormal Mechanical Pain and Heat Hypersensitivity in Patients with FOP.



(A) Flow chart depicting patient enrollment in Quantitative Sensory Testing (QST), the clinical "gold standard" for assessing clinical function of small nerve fibers such as A-σ and C-fibers conducting thermal and mechanical sensation in humans. (**B**) Assessment of the average mechanical pain threshold (MPT) using QST assessing amount of force tolerated using von Frey filaments, showing significantly lower MPT in the FOP group than sex- and age-matched nonrelated healthy volunteer group (WTc). **(C)** Examination of thermal threshold of adult patients with FOP and healthy control family members using QST with a thermode with temperature range 0-50°C. To align QST data for age, gender and test site, raw thermal data of each subject were transformed into Z-score using the equation: Z-score = $(Value_{patient}-Mean_{controls})/SD_{controls}$ and published data obtained from healthy volunteers. Adult patients with FOP had lower heat pain threshold (HPT) compared to their healthy family members **(i)**. There were no differences in Z-scores between patients with FOP and family members regarding the **(ii)** cold pain threshold (CPT), **(iii)** warm detection threshold (WDT), and **(iv)** cold detection threshold (CDT).

Figure 3: Generation of Sensory Neurons from Patient-Derived Induced Pluripotent Stem Cells (iPSCs).



(A) Representative images of differentiated iSNs coexpressing TuJ1 (green, neuronal marker) and Brn3a (red, sensory neuron marker) at day 15 of differentiation. Nuclei were labelled with DAPI (blue). Scale bar: 15 mm. **(B)** Time course of qRT-PCR analysis of gene expression during iSN differentiation. show increased expression of Pax6 (neuroectoderm marker), Isl1 (sensory neuron marker), and Tac1 (nociceptor marker), and decreased expression of Nanog, a pluripotency marker.

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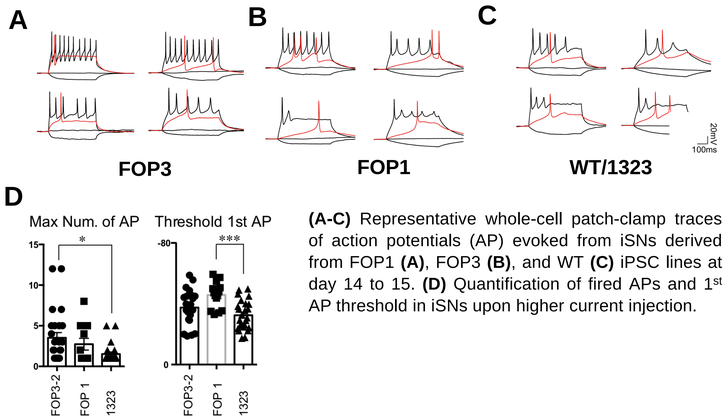
Figure 4: Increased Action Potential Firing Activity in FOP iPSC-derived Sensory Neurons.



(A-C) Representative whole-cell patch-clamp traces of action potentials (AP) evoked from iSNs derived from FOP1 **(A)**, FOP3 **(B)**, and WT **(C)** iPSC lines at day 14 to 15. **(D)** Quantification of fired APs and 1st AP threshold in iSNs upon higher current injection.

Conclusions

- **Neuropathic pain is common in patients with FOP**
  - Data from the International FOP Association Registry showed that 14.4% of patients with FOP suffer from neuropathic pain, compared to 6.9-10% of the general population.
  - QST on a cohort of patients with FOP demonstrated the heat pain and mechanical pain hypersensitivity.
- **Sensory neurons can be created from FOP iPSCs** (iSNs).
- **FOP iSNs show increased neuronal excitability** on electrophysiology compared to control iSNs.
- The results have implications for other conditions of chronic pain

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