

A close-up, shallow depth-of-field photograph of a microscope's objective lens and eyepiece. A hand wearing a blue nitrile glove is visible in the background, holding the microscope. The scene is lit with a warm, golden light from below, creating a glow on the microscope's base and the glove.

THE CENTER FOR RESEARCH IN FOP & RELATED DISORDERS

27TH Annual Report of the
Fibrodysplasia Ossificans Progressiva (FOP)
Collaborative Research Project

FALL 2018



Penn Medicine

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IFOPA'S FOP FAMILY GATHERING (SAN FRANCISCO, CALIF., USA)



1 Emma Albee (Seal Cove, Maine) & Dr. Kaplan; 2 Drs. Kaplan & Grunwald confer with Claudia Cabrera (Everett, Mass.) and her mother Maria Fuentes; 3 Dr. Jeffrey Tabas (UCSF School of Medicine), the first FOP Research Fellow at the FOP Laboratory at the University of Pennsylvania (1989-1991), and his wife Dr. Michiko Shibata have a reunion with Dr. Kaplan; 4 Cassie Eckart (Lompoc, Calif.) and her parents Karen and Glenn Eckart meet with Drs. Grunwald, Kaplan, Hsiao and Pignolo; 5 Miriam Rocke (Davis, Calif.) and her father Professor David Rocke (IFOPA Board Member, far right) meet with Drs. Donna Grogan (Clementia Pharmaceuticals), Bob Pignolo (Mayo Clinic), Fred Kaplan (University of Pennsylvania), Zvi Grunwald (Thomas Jefferson University), Ed Hsiao (University of California, San Francisco) and Eileen Shore (University of Pennsylvania); 6 Kim Shields (Jonesborough, Tenn.) meets with Anitha Devadason (2017 IFOPA Program Manager) and Drs. Kaplan, Pignolo, Grunwald and Hsiao; 7 Adrienne & Larry Bailin (Sarasota, Fla.) meet with Drs. Kaplan, Grunwald, Pignolo, Shore and Hsiao; 8 Karina Chaikhoutdinov (Brooklyn, N.Y.) with her sister, Dr. Irina Chaikov, her father, Dr. Marat Chaikhoutdinov, and her brother-in-law, Dr. Sohrab Sohrabi



IFOPA'S FOP FAMILY GATHERING (SAN FRANCISCO, CALIF., USA)



9 Dr. Vincent Whelan (Fresno, Calif.) & Dr. Fred Kaplan; **10** Liam Rump (Littleton, Colo.) & Dr. Kaplan exchange ties; **11** Philadelphia Eagle's fan Adrian Bailon (El Paso, Texas) and his father Gerardo meet with Drs. Sheila Adami (Stanford), Bob Pignolo (Mayo Clinic), Zvi Grunwald (Thomas Jefferson University), Ed Hsiao (University of California, San Francisco), and Eileen Shore and Fred Kaplan (University of Pennsylvania); **12** Hayden Pheif (Mill Valley, Calif.) and his parents Megan Olsen (IFOPA Board Member) and John Pheif meet with Drs. Joyce Tang (Stanford), Eileen Shore (University of Pennsylvania), Ed Hsiao (University of California, San Francisco), Fred Kaplan (University of Pennsylvania) and Bob Pignolo (Mayo Clinic); **13** The Williams Family (Houston, Texas; from left to right: Evan, Daniel, Jeff & Tiffanie) meet with Amy Ton (UCSF Medical Student) and Drs. Shore, Kitterman, Grunwald, Pignolo, Kaplan and Hsiao; **14** Dilyn Martin (Kotzebue, Alaska), sisters Ana & Alex and mother Jade Hill meet with Drs. Pignolo, Kitterman, Grunwald, Kaplan, Hsiao and Shore; **15** The FOP Clinic staff (left to right): Dr. Eileen Shore (University of Pennsylvania), Mrs. Amanda Cali, Mrs. Julie Schmidt, and Dr. Ed Hsiao (University of California, San Francisco), Fred Kaplan (University of Pennsylvania), Zvi Grunwald (Thomas Jefferson University) and Bob Pignolo (Mayo Clinic)





ANNUAL MEETING OF FOP GERMANY (VALBERT, GERMANY)

1 Vanya & Dulce Alfonso (Angola) meet with the staff of the FOP Clinic; 2 Tuana Celik (Schifferstadt, Germany) and family meet with doctors at the FOP Clinic; 3 Tim and Virginia Wagner (Muhlthal, Germany) meet with Drs. Morhart and Kaplan; 4 Dr. Kaplan meets with Saskia Muehlhoff and her sister Franziska Muehlhoff (Plettenberg, Germany)



CANADIAN FOP FAMILY MEETING (LONDON, ONTARIO)



1 Jaxon Hamilton (Ottawa, Canada) meets with Drs. Kaplan & Pignolo and medical student Adam Jacob; 2 Kathleen & Karen Degenhardt (Goodsoil, Saskatchewan) with Drs. Pignolo & Kaplan; 3 James Dizon (Richmond Hill, Ontario) with his parents Joe & Debbie Dizon and Drs. Kaplan & Pignolo; 4 IFOPA Founding Member and Board Member Nancy Sando (Petoskey, MI, USA) & Dr. Kaplan; 5 Ian Brodie (Sault Ste. Marie, Ontario) with Drs. Pignolo & Kaplan; 6 Clara Bouchard (Montreal, Quebec) & Dr. Kaplan



THIRD INTERNATIONAL FOP RUSSIA FAMILY MEETING (MOSCOW, RUSSIA)



1 Irina Verugina (Almaty, Kazakhstan) & her mother Eugeniya meet with Drs. Rolf Morhart and Fred Kaplan; 2 Alexy Lensky (St. Petersburg, Russia) and his mother Olga meet with Drs. Margarita Dubko (St. Petersburg, Russia), Rolf Morhart (Garmisch-Partenkirchen, Germany), Fred Kaplan (Philadelphia, Pa., USA) and Eileen Shore (Philadelphia, Pa., USA); 3 Dimitry Baranov and his parents (Moscow, Russia) meet with Drs. Shore, Morhart, Nikishina and Kaplan; 4 Olesya Radushko (Kemerovo, Siberia, Russia) and Dr. Kaplan; 5 Igor Zahvatov (Rakvere, Estonia) and his mother Nadezhda meet with Drs. Kaplan & Morhart; 6 Zharylgap Sultangazi (Kostanay, Kazakhstan) and his parents meet with Drs. Shore, Morhart & Kaplan; 7 Daniel Eremin (Moscow, Russia) and Dr. Kaplan; 8 Alexy Onishchenko (Moscow, Russia) and his parents Vladislav Grachev & Svetlana Onishchenko meet with Drs. Kaplan, Shore & Morhart; 9 High school student Lili Voloncs-Mindszenthy (Budapest, Hungary) meets with Dr. Eileen Shore to discuss FOP basic science research; 10 The medical, administrative and interpretive staff of the FOP Clinic

FOP ITALIA AND IFOPA'S FOP DRUG DEVELOPMENT FORUM (SARDINIA, ITALY)



1 Federica Moresco and her father Luca meet with Dr. Kaplan; **2** Hugo Fahlberg (Eskilstuna, Sweden) and his parents Marie and Per meet with Drs. Ed Hsiao, Bob Pignolo, Zvi Grunwald and Fred Kaplan; **3** Elisa Cristoforetti (Trento, Italy) and Dr. Kaplan; **4** Isabelle Alfieri (Parma, Italy) and her parents Eleonora and Massimo Alfieri (Massimo is Co-president, FOP Italia) with Drs. Kaplan and Pignolo

THIRD LATIN AMERICAN FOP MEETING (BUENOS AIRES, ARGENTINA)



1 Jasmin Strack (Santa Cruz, Bolivia) and her parents meet with the clinical staff; **2** Manuel Robert meets with Drs. Clive Friedman, Fred Kaplan and Bob Pignolo; **3** Manuel Robert and his parents Moira Liljesthröm and Fredy Robert (organizers of the Third Latin American FOP Meeting) gather with doctors and staff for a photo at the conclusion of the FOP Clinics in Buenos Aires, Argentina



4 Mariana Gomes Santos (Praia Grande, Brazil) meets with Drs. Yu, Kaplan, Delai, Shore and DeCunto; **5** Gisela Romano (Castelar, Argentina) greets a cheerful clinic staff; **6** Anastasia Moroz (Tel Aviv, Israel) and her father meet with Drs. Kaplan, Delai and Shore; **7** Dr. Kaplan enjoys a special moment with Cecelia Musso Weber (Rosario, Argentina), Valentino Fabrizio Fello (Tarpoto, Peru), Juan Pablo Musso Weber (Rosario, Argentina) and Julietta Sanchez (Tibasosa, Colombia)





1 University of Pennsylvania and Children's Hospital of Philadelphia (CHOP) Clinical Research Trials Coordinators: Rob Burgese, Renee Jurek, Katherine Toder, Jennifer Pizza, Vashisht Arshanapally;
2 The Center for Research in FOP & Related Disorders at the University of Pennsylvania (Seated): Amber Hamilton, Robyn Allen, Dr. Eileen Shore, Dr. Fred Kaplan, Meiqi Xu, Ruth McCarrick-Walmsley. (Standing): Bob Caron, Dr. Salin Chakkalakal, Dr. Deyu Zhang, Katherine Toder, Dr. Sun Peck, Kamlesh (Kay) Rai, Alexandra Stanley, Will Towler, Niambi Brewer, Dr. Vitali Lounev, Renee Jurek, Patsy Hooker

PART 1 Introduction — The Twilight Zone



“The longer you can look back,
the farther you can look forward.”

— Winston Churchill



Dr. Fred Kaplan and his father Harold Kaplan (far left) with Neil Armstrong in New York City; May 1969. Neil Armstrong was the spacecraft commander for Apollo 11, the first to land a spacecraft on the moon and the first human being to step on its surface.

On Wednesday morning, July 16, 1969, Apollo 11 astronauts Neil Armstrong, Buzz Aldrin and Michael Collins blasted off atop a massive Saturn V rocket from the Cape Canaveral launch site at Kennedy Space Center, Florida, on their four-day journey to the moon.

Two days later, on Friday July 18, 1969, an announcer on the morning news reported calmly that the Apollo 11 astronauts had entered the twilight zone on their journey to the moon.

The twilight zone! Breakfast dishes and coffee cups dropped on the floor. Certainly disaster had struck!

The popular TV science-fiction series was immediately thrust into the forefront of

consciousness. “It is a dimension as vast as space – and as timeless as infinity. It is the middle ground between light and shadow, between science and superstition, and it lies between the pit of man’s fears and the summit of his knowledge. This is the dimension of imagination. It is an area which we call ‘The Twilight Zone.’”

The announcer quickly and calmly clarified that all was well with the astronauts. The “twilight zone” that he was referring to was that zone between the Earth and the moon where the gravitational pull of the two celestial bodies on the Apollo 11 spaceship was equal.

Thus, while speeding towards the moon, the Apollo 11 astronauts were in a sort of limbo. Their speed had been slowed by the gravitational pull of the Earth, but had not yet been accelerated by the



gravitational pull of the moon. They had reached that point in the journey where the gravitational tug-of-war between the Earth and the moon were equal. As their spaceship moved at ever slowing speeds, the moon's gravitational pull would begin to exert its force and accelerate Apollo 11 into lunar orbit. The astronauts were on a historic rendezvous with destiny, but they were not there yet. They were half-way between nowhere. They were, in fact, in the twilight zone.

In many ways, our little FOP spaceship is like Apollo 11 on that Friday morning, two days after the historic launch, speeding through the cosmos between the Earth and the moon – in a twilight zone at the nadir of our journey – neither here nor there – on a hopeful, promising, but dangerous journey to a new frontier.

We have entered the twilight zone. That place in our journey between two worlds – the world of anecdotal and symptomatic management of FOP at one end and the newly discovered, but not yet accessible, world of new age treatments and targeted therapies for FOP at the other end. The gravitational pull of both worlds seems equal at this point in our journey – the world we are leaving and the world we are going to.

Clinical trials are our new world – the moon and beyond – and they are beginning to exert their gravitational pull on our imagination and on our lives. They are real and they are here, and they will continue to exert a stronger and stronger pull on our FOP spaceship as we journey to this new frontier. Unlike the Apollo 11 astronauts, we will not be going home once we arrive at our new frontier. At this point in our journey, there is no going back. We will stay and make a new reality. Some trials may fail and some may succeed, and those that succeed will undoubtedly succeed in different ways and to different degrees.

We have identified the central cause of FOP – the FOP gene. All of the clinical trials and every rationale for our journey to the new frontier are directly or indirectly based on that reality. But there is unlikely to be one treatment and one cure for FOP; FOP is too complex for that. What works for one person may not work for, or be tolerated by, another. What works at one stage of FOP may not work at another.

Like the Apollo 11 astronauts on the second day of their journey to the moon, we too have entered the twilight zone. We have a presence in both worlds, but we are speeding to a brave new one. Our science has made that possible. There will be even more and unimaginable discoveries ahead. The puzzle is not yet complete, and our destination is not yet determined. What is certain is that we are heading to a new frontier. We will not be coming home.

PART 2 The Center



Since its establishment in 1989, the FOP Collaborative Research Project has had a singular mission – to determine the cause of FOP and to use that knowledge to advance the treatment and a cure for FOP. During the past 29 years, we have moved from the wastelands of a little understood rare disease to the watershed of clinical trials. We identified the genetic cause of FOP and used that knowledge to spearhead worldwide research efforts to develop therapies that will transform the care, and the lives of individuals with FOP.

In partnership with our benefactors, we have expanded the frontiers of drug discovery and development in this rare and disabling condition, dismantled physical and perceptual barriers that have impeded progress, and inspired global research into small molecules, antibodies and gene therapy for FOP. We not only support the FOP dream, we helped create it.

Here, at The Center for Research in FOP & Related Disorders, our work is broad and comprehensive while focused on seven spheres of FOP activity:

- Clinical care and consultation worldwide
- Clinical research and infrastructure development
- Basic research (identification of therapeutic targets)
- Translational research (preclinical drug testing and biomarker discovery)
- Cali Developmental Research Grants Program
- Clinical trial development and proof-of-principle investigation in patients
- Education

The Center for Research in FOP & Related Disorders is unique. It is the world's first and only comprehensive center for FOP. Here at The Center, we have had a very busy year. During the past year, we achieved tremendous milestones in our FOP program.

OUR IMPACT:

Clinical Care and Consultation Worldwide

- » Guided patients, families and doctors worldwide in their daily battles with FOP
- » Directed the world's largest FOP clinic and referral center
- » Coordinated medical management of FOP patients worldwide
- » Conducted international FOP clinics for patients and families in London, Ontario, Canada; Shippensburg, Pennsylvania, USA; Valbert, Germany; Moscow, Russia; Sioux City, Iowa, USA; Buenos Aires, Argentina; Sardinia, Italy; and San Francisco, California, USA



1 A.J. Gonzales (Bellmawr, N.J.) visits Dr. Kaplan at Penn; 2 Kyle McWilliams (Victor, Iowa) visits with Drs. Al Mukaddam and Kaplan at Penn; 3 Justin Henke (Middletown, De.) visits with Dr. Kaplan at Penn; 4 Joey Hollywood (Bridgewater, N.J.) and Dr. Kaplan; 5 Adnan Hai (center; Queens, N.Y.) with his brother Aban and his mother Akter at Penn; 6 Ethan Szumetz (Greencastle, Pa.) at Dr. Kaplan's desk; 7 Cade Russell (Allen Park, Mich.) at Dr. Kaplan's desk; 8 Amber Hamilton from the FOP Laboratory (standing, left) visits with Mya and Tamara Watts and niece Kennedy at Penn; 9 Jordyn Bugarin (Baltimore, Md.) visits with Dr. Kaplan at Penn; 10 Eli Wallace (Madison, Wis.) celebrates the opening of The BIG Eli Ferris Wheel





Clinical Research and Infrastructure Development

- » Founded and established the International Clinical Council on FOP
- » Published the first patient-reported longitudinal Natural History Study in FOP – a simple and validated tool that is used in the design and evaluation of clinical trials in FOP
- » Developed, validated and published the FOP Cumulative Analogue Joint Involvement Scale (CAJIS) – a novel, universally accessible and rapidly administered evaluation tool for FOP. This staging tool can be used to develop or revise clinical plans of care, define operational research criteria, and identify the effectiveness of interventions. CAJIS is now used in the clinical evaluation of FOP patients worldwide and has been incorporated into four ongoing clinical studies and trials on six continents.
- » Developed, validated and published an analog method for radiographic assessment of heterotopic ossification (HO) in FOP. This method enables practical, quantitative assessment of HO in clinical trials and has been incorporated in the ongoing interventional trials and the Natural History Study.
- » Published a paper on the value of imaging studies in early and late FOP lesions
- » Published comprehensive joint survival curves from most of the world's known population of FOP patients. These joint-specific survival curves are being used to facilitate clinical trial design and to determine if potential treatments can modify the predicted trajectory of progressive joint dysfunction and immobility.
- » Co-authored a major collaborative paper on clinical-pathological correlations in three patients with FOP highlighting the importance of post-mortem examinations and their contribution to our current knowledge of FOP
- » Published a major paper on acute unilateral hip pain in FOP describing bone and joint pathology that may confound and inform the evaluation of acute flare-ups
- » Published a major paper outlining the prevalence, risk factors, prevention and treatment of kidney stones in patients with FOP
- » Were instrumental in developing and implementing a patient-reported physical function outcome measure for children and adults with FOP
- » Were instrumental in implementing the Clementia longitudinal Natural History Study
- » Championed and brokered the prospective deposit of data from the Clementia longitudinal Natural History Study into the IFOPA's FOP Registry
- » Advocated for the direct deposit and open access of annotated whole genome sequence data from a sponsored clinical trial into the National Institutes of Health (NIH) database of Genotypes and Phenotypes (dbGaP)
- » Championed a single, unified international registry for FOP by the IFOPA, and owned by the FOP community
- » Co-authored a paper on the formulation and operation of the global FOP Registry



Basic Research (Identification of Therapeutic Targets)

- » Developed a new and improved conditional knock-in mouse with the classic FOP mutation
- » Demonstrated and published that Activin A amplifies dysregulated BMP signaling and induces chondro-osseous differentiation of primary connective tissue progenitor cells (CTPCs) in patients with FOP
- » Showed that both canonical ligands like BMP4 and non-canonical ligands like Activin A synergistically amplify BMP pathway signaling in FOP connective tissue progenitor cells and may cooperate to alter thresholds for HO in FOP
- » Showed and published for the first time that anti-Activin A monoclonal antibodies block the BMP signaling pathway in primary human connective tissue progenitor cells from people with FOP – opening the way for the use of blocking antibodies to Activin A in clinical trials for the prevention and treatment of FOP
- » Published case reports of two FOP patients in whom acute and chronic treatment with rapamycin was ineffective in stopping progression of FOP
- » Investigated cellular inflammatory triggers of early FOP lesions using novel triple knock-in FOP mouse models and identified key cellular targets in the innate immune system in the progression of FOP
- » Defined stages of HO in tissues in a knock-in mouse model of FOP and identified FOP mutation-induced effects prior to formation of ectopic cartilage and bone
- » Discovered and published that depletion of mast cells and macrophages impairs HO in FOP mice
- » Established that FOP is a type of localized mastocytosis and that pharmacologic inhibition of mast cells is a viable therapeutic approach in FOP
- » Published a major paper describing wide variability in the enhancement of BMP pathway signaling in a spectrum of ACVR1 mutations seen in patients with variant FOP
- » Investigated molecular mechanisms by which ultra-rare FOP variants trigger promiscuous BMP signaling and subsequent HO
- » Investigated the molecular mechanisms by which the innate immune system amplifies inductive BMP signaling in FOP connective tissue progenitor cells
- » Investigated molecular mechanisms and immunologic triggers of FOP flare-ups in state-of-the-art knock-in mouse models of classic FOP
- » Discovered and published that inflammatory stimuli broadly activate the innate immune system in FOP connective tissue progenitor cells
- » Discovered and published that toll-like receptors (TLRs) of the innate immune system amplify BMP pathway signaling
- » Discovered that Evolutionarily Conserved Signaling Intermediate in the Toll Pathway (ECSIT) integrates injury and tissue damage signals with dysregulated BMP pathway signals in FOP connective tissue progenitor cells – providing novel insight into the cell autonomous integration of injury signals from the innate immune system with dysregulated response signals from the BMP signaling pathway
- » Published a major paper describing how the innate immune system triggers and amplifies dysregulated BMP pathway signaling in FOP, providing new targets for therapeutic approaches to blocking the induction and amplification of FOP lesions

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- » Expanded collaborative investigations with developmental neurobiologists and pediatric oncologists on mechanisms of disease activity in malignant brainstem gliomas and in FOP, two catastrophic childhood diseases associated with common mutations in ACVR1
 - » Investigated the molecular mechanisms by which the mutant FOP gene alters the response of cells to their physical environment. These studies suggest that FOP cells are predisposed to misinterpret signals from their tissue microenvironment as instructions to form cartilage and bone.
 - » Continued to expand and develop the FOP SHED Cell Tooth Fairy Program – this limited and precious library of primary connective tissue progenitor cells from FOP patients is essential for ongoing and future studies in therapeutic target identification and drug discovery in FOP
 - » Continued collaborative studies to identify modifier genes in several patients with the FOP mutation, but who have relatively little HO; intense investigation will continue on this project
 - » Investigated the role of mutant ACVR1 on the regulation of joint formation, development and degeneration

Translational Research

(Preclinical Drug Testing & Biomarker Discovery Program)

- » Screened new categories of compounds for efficacy in preventing HO in FOP mouse models
- » Annotated an extensive library of plasma biomarker samples in a large cohort of classically-affected FOP patients and non-FOP age and sex-matched controls
- » Analyzed and are preparing to publish detailed biomarker analysis on these plasma samples
- » Published a paper showing that cartilage-derived retinoic acid-sensitive protein (CD-RAP) is a stage-specific biomarker of heterotopic endochondral ossification in a mouse model of FOP and in humans

Developmental Research Grants Program

- » Continued to support three highly innovative developmental research projects in the Cali Developmental Research Grants Program
- » "Molecular Basis of Pathogenic Signaling and High Throughput Testing of FOP Therapies in a Zebrafish Model System" (Mary Mullins, PhD, University of Pennsylvania)
- » "Identifying Alternative Therapeutic Targets and Genetic Interactors in FOP" (Ed Hsiao, MD, PhD, University of California, San Francisco)
- » "Novel Allosteric Destabilizers as Therapeutics for FOP" (Jay Groppe, PhD, Texas A&M University)

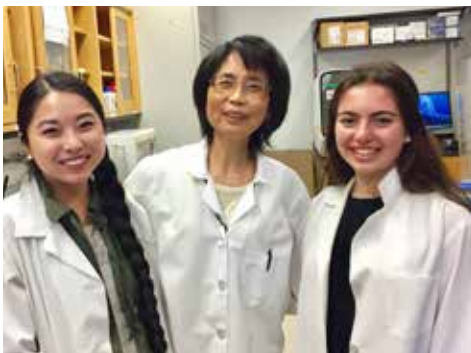


Dr. Jay Groppe with Diane Weiss and Amanda Cali at the IFOPA's FOP Drug Development Forum in Sardinia, Italy

Clinical Trial Development and Proof-of-Principle Investigation in Patients

- » Introduced a medication that targets the cellular response to tissue hypoxia and inflammation (specifically mast cells) into the clinic on a compassionate off-label basis for the management of FOP in children
- » Consulted on the study design of five clinical trials in development by three pharmaceutical and biotech companies
- » Advised 30 pharmaceutical and biotech companies on the development of novel drugs for clinical trials in children and adults with FOP, based on identified targets
- » Advanced understanding of small molecule inhibitors in physiologic and pathologic chondrogenesis in children – knowledge and approaches vital to future clinical trials for FOP
- » Enrolled and followed patients in two sponsored interventional clinical trials
- » Enrolled and followed patients in a sponsored longitudinal Natural History Study
- » Expanded The FOP Center to include a new pediatric clinical trials site at The Children's Hospital of Pennsylvania (CHOP)

Education



Summer students and junior scientists Kelly Li (undergraduate, Yale University) & Megan Martucci (high school student, Slingerlands, N.Y.) with mentor Meiqi Xu

- » Co-edited a special edition of *BONE* on heterotopic ossification (published in April 2018)
- » Mentored the next generation of physicians and scientists working on FOP in the classroom, clinic and laboratory
- » Mentored high school, college, medical and graduate students on research projects to expand vital knowledge and scientific and public awareness of FOP

- » Educated physicians, scientists, researchers and regulators at medical and scientific forums, meetings and conferences worldwide
- » Educated FOP experts worldwide on the use of the CAJIS evaluation for clinical management and clinical trials of FOP patients

Our work at The Center is continually evolving as we cross the bridge daily between the clinic and the laboratory and back again in a process that builds knowledge and deep understanding of FOP to help us accomplish our ultimate mission.

The scope of research in the FOP laboratory covers a range of investigations that are focused on identifying and characterizing transformative targets for therapy.

THE COLLABORATIVE ACTIVITIES OF THE FOP LABORATORY FOCUS ON SIX MAJOR RESEARCH AREAS:

1 Identifying and characterizing central signaling targets in the induction and amplification of FOP lesions

These studies are conducted by Meiqi Xu, Dr. Salin Chakkalakal, Michael Convente, Dr. Haitao Wang, Alexandra Stanley, Robyn Allen, Will Towler and Niambi Brewer. This vital research enables the development of drugs that target these pathways.

2 Identifying and characterizing immunologic and microenvironmental targets that amplify FOP flare-ups

These studies are conducted by graduate students Michael Convente, Niambi Brewer and Alexandra Stanley, and research scientists Amber Hamilton, and Drs. Haitao Wang and Vitali Lounev. Their projects investigate the cellular response to the immunologic, biochemical and biomechanical microenvironments of early (pre-cartilage/bone) FOP lesions. Stunning new therapeutic targets are emerging from their work, and it is possible that one or more such targets will become the basis for clinical trials with re-purposed drugs.

3 Identifying cell and tissue targets in FOP lesions

These studies are conducted by Dr. Vitali Lounev, Michael Convente, Dr. Salin Chakkalakal and Dr. Haitao Wang, along with Will Towler, Alexandra Stanley, Ruth McCarrick-Walmsley and Bob Caron. These studies identify the specific cells and mechanisms that can be targeted to block heterotopic ossification.



Bob Caron, Meiqi Xu, Dr. Vitali Lounev and Ruth McCarrick-Walmsley move a refrigerator into the temporary lab space at The Center for Research in FOP & Related Disorders at the University of Pennsylvania

4 Identifying and characterizing developmental targets in FOP that impact joint development and degenerative joint disease

These studies are conducted by Will Towler and Dr. Salin Chakkalakal.

5 Developing *in vitro* and *in vivo* FOP models for drug “target testing”

These studies are conducted by post-doctoral fellow Dr. Salin Chakkalakal, by graduate students Robyn Allen and Alexandra Stanley, and by research scientists Drs. Vitali Lounev and Deyu Zhang and Meiqi Xu and Ruth McCarrick-Walmsley. Their projects are centered on developing new resources for FOP research that will be used in multiple other projects as well as used for *in vivo* screening of drug candidates. This work is a vital part of the infrastructure for drug discovery and development – the infrastructure for a cure.

6 Pre-clinical drug testing

Pre-clinical drug testing in FOP mouse models is conducted by Drs. Vitali Lounev, Deyu Zhang and Salin Chakkalakal.

DESPITE REMARKABLE ADVANCES IN FOP RESEARCH OVER THE PAST SEVERAL YEARS, WE REMAIN FAR FROM UNDERSTANDING SOME OF THE MOST BASIC AND FUNDAMENTAL MYSTERIES OF FOP:

- › What are the cellular and molecular triggers of FOP flare-ups?
- › How does FOP progress in the absence of flare-ups?
- › How do the immune system and the lesional tissue microenvironment influence the progression of FOP?
- › What is the relationship between the innate immune system and the skeletal progenitor cells that initiate FOP flare-ups?
- › What insights do the ultra-rare genetic variants of FOP (which affect only two to three percent of FOP patients worldwide) teach us about the function of the genetic switch that drives heterotopic ossification in FOP, and how do these ultra-rare insights inform the identification of new targets for drug development?

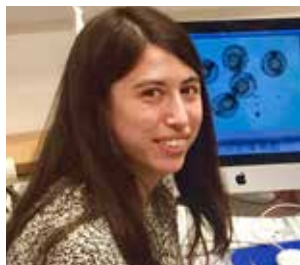
These questions and more continue under intense investigation at The Center, and their answers will help identify and confirm novel targets for drug discovery and development.

"Our heart is FOP" at the FOP Lab (from left to right): Alexandra Stanley, Niambi Brewer, Dr. Sun Peck, Amber Hamilton & Will Towler



WE THE PEOPLE

The Center for Research in FOP & Related Disorders is only as strong as its people. We are very proud of our team.



FOP Laboratory Team

Robyn Allen

Robyn Allen hails from Maryland where she trained horses and aspired to become a veterinarian. She became interested in research during college while working at the NIH, where she experienced translational medical research from animals to humans in action. As a dual VMD-PhD student at the University of Pennsylvania, studying FOP with Drs. Mullins and Shore, she has had a wonderful opportunity to apply both her clinical and research training. Her goal is to become an academic researcher in developmental biology and genetics.



Niambi Brewer

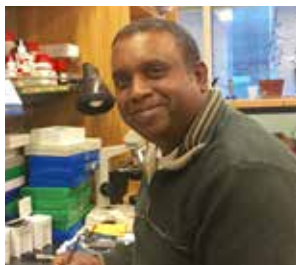
Niambi Brewer is from Bowie, Maryland and received her undergraduate degree in Biology from the University of Maryland. She is a 4th year PhD student in Biomedical Graduate Studies at University of Pennsylvania with her focus on Genetics and Epigenetics. She greatly enjoys working in the FOP laboratory to discover pathways for treatment of genetic and non-genetic forms of HO. She greatly enjoys her interactions with the FOP patient community. "In my free time, I like to explore Philadelphia and all of the great food!"



Bob Caron

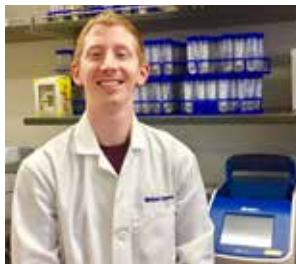
Bob Caron grew up in Havertown, Pennsylvania. He attended Widener University and graduated with a degree in science administration with minors in biology, finance and accounting. Bob has worked in the FOP laboratory for almost 20 years on histopathology, informatics and communications, and recently, cell culture. On this journey, he hopes to continue to make discoveries that will help improve patient's lives. When he is not in the FOP laboratory, he enjoys exercising and bodybuilding.

From top to bottom:
Graduate student Robyn Allen, graduate student Niambi Brewer, Bob Caron



Salin Chakkalakal, PhD

Salin Chakkalakal is a senior research investigator at the University of Pennsylvania. His academic background began with a Bachelor of Pharmaceutical Sciences degree and a Masters in Biotechnology during his studies at universities in India. He was one of ten research scholars selected internationally to pursue a fully-funded PhD program in genetics at the University of Cologne, Germany where he completed doctoral work in medical biochemistry. While working on his doctoral thesis, he began to learn about genetic diseases in cartilage and bone that have no cure and are poorly understood – motivating him to work on FOP which provides meaningful opportunities to develop cures. After postdoctoral training in the FOP laboratory, Salin has helped develop animal models for FOP and conducted preclinical drug testing to stop heterotopic ossification. When not working in the lab, Salin spends time with his family – a little daughter and his wife – who also works in the medical field.



Michael Convente, PhD

Michael Convente is from Midland Park, NJ. He attended Rutgers University, completing a BA degree in molecular biology and biochemistry, with a minor in psychology. Michael joined the FOP lab in March 2010 and conducted original research that led to the successful defense of his acclaimed dissertation, “The Immunological Contributions to Heterotopic Ossification Disorders - Insights from Fibrodysplasia Ossificans Progressiva” and a PhD degree in 2017. During his time in the lab, Michael most enjoyed meeting with FOP patients and families and learning about their experiences, which guided his research throughout his project. Michael is an Ashley Martucci Fellow in FOP Research. In his free time, Michael enjoys following Rutgers athletics, riding his bike and traveling.



*From top to bottom:
Dr. Salin Chakkalakal,
Dr. Michael Convente,
Amber Hamilton,
Dr. Vitali Lounev*

Amber Hamilton

Amber Hamilton graduated from the University of Pennsylvania in 2016 and began working in the FOP lab right after graduation. She will begin medical school in Fall 2018 and has dreams of becoming an orthopedic endocrinologist. She enjoys studying FOP “because every discovery made in our lab motivates me to one day treat people with rare metabolic bone disorders.”

Vitali Lounev, PhD

Vitali Lounev is from Belarus (previously part of the former Soviet Union) and received his M.S. in Minsk, Belarus and his PhD in Moscow, Russia. He joined the FOP laboratory in 2005 as a post-doctoral fellow. He is interested in and motivated by developing new knowledge in the lab’s ongoing work to improve the lives of people with FOP and to find a cure for FOP. He works on projects to understand mechanisms of FOP and to screen new drugs to prevent HO. Additionally, Vitali provides support to FOP patients from Russia with translation and interpretation of information about FOP.

Ruth McCarrick-Walmsley

Ruth McCarrick-Walmsley grew up in the suburbs of Washington, D.C. and attended the University of Virginia where she obtained a bachelor's degree in biology. She had considered a career in medical illustration and is currently studying art with local artists. Outside of the lab, she enjoys painting, running, skiing and spending time outdoors with her family. In the lab, many will know Ruth from her work with our "Tooth Ferry" Program – one of her many valuable contributions. "What I most enjoy about working in the FOP laboratory is the great relationships that I have formed with colleagues and with FOP families and friends."



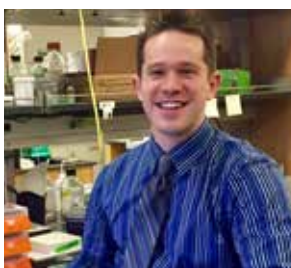
Alexandra Stanley

Alexandra Stanley grew up in San Diego, California. She attended the University of California, San Diego and earned a B.S. degree in Human Biology. As a student in the Developmental, Stem Cell and Regenerative Biology graduate program at the University of Pennsylvania, she joined the FOP laboratory motivated by research that focused on therapies to treat genetic disease. "My favorite part about the lab is meeting patients and being reminded why we do the work we do." When she's not in lab, she's playing with her dog, kickboxing or wine tasting.



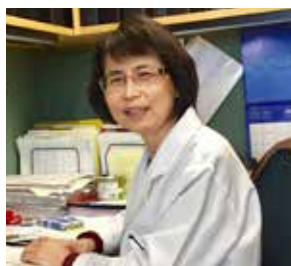
Will Towler

Will Towler is from Greenville, South Carolina. He is a PhD candidate in Developmental, Stem Cell and Regenerative Biology at the University of Pennsylvania. His thesis project focuses on the big toe mystery of FOP – what it is, how it happens and what it can tell us about FOP. "I joined the FOP laboratory because I love figuring out the puzzle of how the body takes the shape it does and how we can change or mimic that process – from big concepts like why we have arms, to focused questions like what makes the big toe so unique in FOP." When not in the lab, Will likes video and board games and "as many fun things as I can fit into a day."



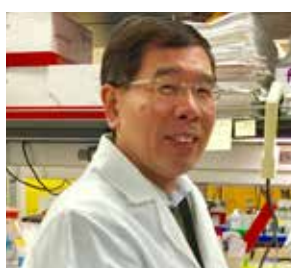
Meiqi Xu

Meiqi Xu was born and raised in Shanghai, China where she received her BS degree. "When the FOP lab was starting, I was the first person hired to work in the lab with Drs. Kaplan and Shore; we have worked together for more than 20 years. I love being an FOP researcher and am motivated by the good feelings of accomplishment." In her spare time, Meiqi enjoys traveling so that she can see and understand different countries.



Deyu Zhang, PhD

Deyu Zhang grew up in Beijing and graduated from Nanjing University, China. He has worked in the FOP lab for more than 17 years. He provides valuable expertise and support for most projects in the laboratory that use mouse models of FOP, including preclinical drug testing.



*From top to bottom:
Ruth McCarrick-Walmsley,
graduate student
Alexandra Stanley,
graduate student
Will Towler, Meiqi Xu,
Dr. Deyu Zhang*



Holly LaPrade (North Haven, Conn.)
and the clinical research team
at University of Pennsylvania.
Below (top to bottom):
Dr. Mona Al Mukaddam,
Mrs. Kay Rai

FOP Clinical Trials Team

To accomplish our goal we need a dedicated team of physicians and research coordinators to accommodate the expanding clinical and research needs. We have also expanded our team to include specialists at the Children's Hospital of Philadelphia (CHOP).

Meet our clinical team:

Mona Al Mukaddam, MD

In April 2016, Mona Al Mukaddam, MD was approached by Dr. Kaplan asking if she would be interested in clinical trials in FOP. She immediately responded "yes" as she knew this was going to be an opportunity of a lifetime. However, little did she know the impact it would have on her professional, academic and personal life.

In October 2016, she took over the role of principal investigator of ongoing clinical trials in FOP at The Center for Research in FOP & Related Disorders. Since then, she has attended the IFOPA's FOP Drug Development Forum in Boston, Massachusetts in October 2016 and Sardinia, Italy in October 2017, and the IFOPA-sponsored Midwest Family Gathering and Clinics in St. Louis in November 2016. She had the opportunity to meet with FOP patients and their families from around the world. Being a member of the International Clinical Council on FOP (ICC) and serving as chairperson of the Communications & Relations Committee and a member of the Governance & Membership Committee provided an even deeper understanding of what it truly means to be a part of the FOP community.



Dr. Al Mukaddam states, "The dedication, hard work and creative intelligence of everyone in the FOP community is tremendous and inspirational and it is all devoted towards the goal of better care of our FOP patients and a treatment and cure for FOP. I have witnessed my personal and my work families grow, and I could not be prouder."

Kamlesh (Kay) Rai

Kay Rai is a clinical research assistant in the University of Pennsylvania's Perelman School of Medicine's Department of Orthopaedic Surgery. Kay has worked with Dr. Kaplan for 37 years and is the key to starting this clinical team. Kay is Indian-born and was raised in Scotland before moving to the United States in her early twenties.

Kay started working with Dr. Kaplan in 1981, the day he became an attending at the University of Pennsylvania. She has met most of the FOP patients and families who have come to the University of Pennsylvania since the FOP program was started in 1989. She coordinates new patient visits, obtains clinical information, schedules appointments and assists in any of the needs of the FOP community that may come her way. Kay always does this with a smile and kind demeanor. In her spare time, Kay enjoys music, art, books, gardening and meeting people, and most of all, spending time with her grandchildren. Kay notes, "I have found this to be an extremely rewarding experience. I am very humbled and honored and feel privileged to have worked with the wonderful FOP community over the years."

Staci Kallish, DO

Stacy Kallish is a medical geneticist at the Perelman School of Medicine at the University of Pennsylvania. She is the vice president of the National Tay-Sachs and Allied Diseases Association and a member of the Society of Inherited Metabolic Disorders and American College of Medical Genetics and Genomics. Her clinical expertise in rare genetic diseases will be an extremely valuable addition to our team. Dr. Kallish received her Bachelor of Science at Emory University and Doctor of Osteopathic Medicine at the University of Medicine and Dentistry of New Jersey. She completed her Pediatric residency at Cooper University Hospital and a fellowship in medical genetics at Children's Hospital of Philadelphia. Dr. Kallish is board certified in medical genetics - both clinical and biochemical genetics. We are thrilled to have Dr. Staci Kallish join our team.



From top to bottom:
Dr. Staci Kallish,
Dr. Edna Mancilla,

Edna Mancilla, MD

Edna Mancilla is a renowned pediatric endocrinologist at the Perelman School of Medicine and the Children's Hospital of Philadelphia (CHOP) and she will be leading the clinical trials in FOP at CHOP. Dr. Mancilla has the research and clinical expertise in metabolic bone health in children and has performed research on the effects of retinoic acid on the growth plate. Dr. Mancilla received her medical degree at the University of Chile, and worked as visiting fellow at National Institutes of Health in the laboratory of Cell Biology and Genetics. Dr. Mancilla completed her residency in pediatrics at NYU Bellevue Hospital Center and Georgetown University Hospital. She completed a fellowship in pediatric endocrinology at Children's Hospital of Pittsburgh and the National Institutes of Health. Dr. Mancilla practiced in Chile from 1998 till 2009 when she moved to the United States and was appointed to the faculty at CHOP. She has lectured both nationally and internationally and has published articles in *The Journal of Clinical Endocrinology & Metabolism*; *Endocrinology*; *Human Mutation*; and *Lancet*. Dr. Mancilla attended the IFOPA Drug Development Forum in Sardinia, Italy in October 2017. We are thrilled to have Dr. Edna Mancilla join our team.



The Wray Family (Pittsford, N.Y.) visits with Dr. Edna Mancilla, Children's Hospital of Philadelphia (CHOP) at University of Pennsylvania (from left to right): Taylor, Jr.; Philip; Taylor, III; Felicia; Maria and Dr. Mancilla



Above, top left: Katherine Toder; Bottom left: Renee Jurek; Right: Renee Jurek, Katherine Toder, Kay Rai and Drs. Fred Kaplan and Mona Al Mukaddam at "The Crooked Little Tree," the symbol of Orthopaedics

Katherine Toder

Katherine Toder is a Research Project Manager in the University of Pennsylvania's Perelman School of Medicine's Department of Orthopaedic Surgery. Katherine moved to Philadelphia from Zimbabwe in 2004 and has enjoyed exploring the city's diverse art and restaurant scene ever since. She studied psychology and sociology at the University of Pennsylvania, and started exploring different types of research after graduating with a BA in psychology in 2008. Her research background includes suicide risk assessment and prevention, the dissemination of cognitive behavioral therapy and the epidemiology of various reproductive cancers. She has been a member of the FOP clinical research team since 2015 and has been the project manager of the clinical research team since 2017. Katherine's knowledge, dedication and meticulous work ethic are instrumental for the success of our clinical trials. Katherine goes above and beyond to ensure that our patients are well taken care of in every detail. She is frequently asked for advice on places and restaurants in Philadelphia. Katherine notes, "I feel privileged to meet so many inspiring FOP patients and their caregivers, families and advocates through my involvement in these groundbreaking projects."

Renee Jurek

Renee Jurek is a Clinical Research Coordinator at the Perelman School of Medicine at the University of Pennsylvania. Renee grew up in Michigan and received her bachelor's degree in athletic training from the University of Michigan in 2015. She moved to Philadelphia soon after graduation and began working at the University of Pennsylvania. In her free time, Renee plays Ultimate Frisbee in recreational leagues and on a club team in Philadelphia. She has been playing Ultimate Frisbee for seven years and her favorite part is the community of people she has met. Renee is a vital member of our team. She quietly and meticulously coordinates and orchestrates many aspects of the clinical trials with amazing attention to detail and with superb memory. Renee notes, "I have learned so much during my two years working on the FOP studies and feel very fortunate to collaborate with such an amazing staff. I have also thoroughly enjoyed meeting so many amazing patients, hearing their stories and working closely with them during the studies."

Jennifer Pizza

Jennifer Pizza is a Research Nurse Coordinator at the Children's Hospital of Philadelphia (CHOP). Jennifer obtained a nursing degree at Widener University, Chester, Pennsylvania and a Certificate in Clinical Trials Management at the University of Delaware, Wilmington, Delaware. She has enjoyed working as a registered nurse in different pediatric settings such as The Chester County Hospital, West Chester, Pennsylvania; A.I. Dupont Hospital for Children, Wilmington, Delaware; and Bayada Nursing Home Care, Malvern, Pennsylvania.

Jennifer has been a Research Nurse Coordinator at CHOP since 2006, initially in the Division of Cardiothoracic Surgery, joining the Division of Endocrinology in 2014. During her time in Endocrinology she has worked on various projects relating to thyroid, bone and calcium disorders. She is extremely devoted to research and pediatric care and is very excited about this opportunity to work on the FOP clinical trials team.



Jennifer Pizza

In the next two sections of the annual report, we will review the historical background and present status of novel clinical strategies for the treatment of FOP.



PART 3 Are We Ready?

It was a year before the discovery of the FOP gene. In the history of FOP, it was a different era and had a vastly different feel than now. Hope even had a different name – and loomed far below the horizon.

The only glimmer of light on a landscape was the FOP lab. The BMP pathway was implicated in FOP, but no direct cause could be found. There were experimental leads and new clues from patients and families, but there were also blind alleys and dead ends.

Outside of the FOP Lab, there wasn't much interest in FOP – not in the medical community, not in the scientific community – and certainly not in the pharmaceutical community – except for one.

It was in that context that a small European-based pharmaceutical company contacted us. They had read some of our early papers and heard about our efforts on FOP in the popular press. They were scheduled to be in Philadelphia for another meeting and wondered if they could stop by and “chat.” We were surprised by their overture, but welcomed the visit and were interested to see if they might be able to help in the effort.

It was a cordial meeting that lasted about an hour – a rest stop, no doubt, on their way to a more important meeting. We learned what they were interested in, and we were eager to share our findings and advances – however fundamental.

At the conclusion, a senior member of the group smiled and thanked us for our time. Then he sighed deeply and exclaimed, “Your work is very promising, but you’re not ready for us yet.”



We nodded, shook hands, thanked them for their interest and bid them farewell.

The words resounded with the force of an earthquake:

“You’re not ready for us yet.”

A little over a year later, things were a bit different. We had discovered the FOP gene. The bold and glorious headline in *The New York Times* proclaimed:

“Finally, With Genetic Discovery, Hope for Escape from a Prison of Bone.”

In little more than a year we went from “You’re not ready for us yet” to “Finally, With Genetic Discovery, Hope for Escape from a Prison of Bone.”



Our small world had changed forever. Things were suddenly and remarkably different.

Worldwide interest in FOP skyrocketed in the wake of the historic discovery of the FOP gene in 2006 – the first hard target for FOP therapy. The FOP gene discovery launched an industry that catapulted the field to clinical trials.

FOP research is now a worldwide enterprise. As of January 1, 2018, there were 37 research universities and/or clinical centers actively engaged in FOP research; 18 in the United States & Canada, 12 in Europe, five in Asia, one in Africa and one in Australia. As of January 1, 2018, there were 12 pharmaceutical and biotechnology companies actively developing drugs for FOP based on a multitude of hard targets, and over 30 companies that have expressed interest.

Presently, there are over 25 FOP medical experts on six continents, 14 active FOP support groups worldwide representing nearly 900 patients, and 25 sites actively involved in four pharmaceutical company-sponsored or investigator-sponsored clinical studies and trials for FOP. There has been a remarkable sea change in the past few years that has occurred in a rapidly-evolving area of target discovery and drug development – all directly or indirectly based on the FOP gene discovery – a druggable target of remarkable fidelity. In fact, every single one of the current clinical trials emanate directly or indirectly from this seminal discovery.

The concept of orphan drugs for orphan diseases has been widely embraced in just the past ten years. This new vision is leading to massive research investment in FOP on the part of academia and industry worldwide – all propelled by the discovery of the FOP gene and by the identification of its robust therapeutic target – the ACVR1 receptor and its interacting molecules and pathways.

“You’re not ready for us yet.”

...to...

“Finally, With Genetic Discovery, Hope for Escape from a Prison of Bone.”

The discovery of the FOP gene in 2006 was a transformative event in the history of FOP, and immediately revealed at least four druggable targets and approaches to treatment and/or prevention, including:

1. Diminishing the activity of the mutant receptor (ACVR1/ALK2) that causes increased BMP pathway signaling through inhibitory monoclonal antibodies, ligand traps, signal transduction inhibitors (STIs) or inhibitory RNA
2. Inhibiting inflammatory triggers of FOP flare-ups
3. Directing FOP stem cells away from cartilage or bone to alternate cell fates
4. Blocking the body's response to signals within the tissue microenvironment, such as hypoxia, that amplify dysregulated BMP signaling and promote the formation of FOP lesions

A contemporary view of hard targets for FOP places the mutated ACVR1 receptor at the center of the bullseye. But, ACVR1 is not the only potentially effective target for treating FOP. FOP is a complex disease, and it is unlikely that one magic bullet will effectively and safely neutralize such an enemy.

“You’re not ready for us yet.”

Yes we are!

Our main challenge is not the drugs to test, but how to test them in patients – safely, expeditiously and with clear resolution of their effectiveness. Our main challenge is how to work together to manage that readiness so that clinical trials do not descend into a chaotic jumble where lack of coordination



leads to a state where we fail to adequately enroll clinical trials and test promising and emerging therapies. Our greatest challenge may soon become not a lack of druggable targets but a lack of focus on a way forward. Our greatest challenge may soon become not a lack of targets and weapons, but a lack of battle plans to wage war on FOP. No one drug, no one weapon will win the war on FOP.

“You’re not ready for us yet.”

Oh, yes we are!

We are MORE than ready!

And now, a look at the pipeline!

PART 4 Pipeline Drugs for FOP That Made Headlines in 2017



PALOVAROTENE

In FOP, heterotopic bone forms by a mechanism called endochondral ossification. In other words, through an obligate cartilage scaffold. This is how most bones of the skeleton grow and how fractures heal – by forming a cartilage template and then transforming into bone. Palovarotene, a drug now in Phase 3 clinical trials for FOP, selectively targets cartilage formation. Pacifici and Iwamoto showed in 2011 that palovarotene inhibited HO in mice genetically engineered to form heterotopic bone. Chakkalakal, Shore and colleagues showed in 2016 that palovarotene inhibited both the spontaneous and trauma-induced HO in FOP mice. If the cartilage scaffold can be inhibited, bone will not form.

Palovarotene activates the turn-off signal for cartilage formation. Palovarotene does not target ACVR1, the mutated BMP pathway receptor encoded by the FOP mutation, but targets other molecules interacting with BMP signaling pathways in pre-cartilage cells that are directly downstream of ACVR1.

In 2014, Clementia Pharmaceuticals, after several years of consultation with The Center for Research in FOP & Related Disorders, began a Phase 2 clinical trial as well as an open-label extension trial of palovarotene in adults with FOP.

In 2016, Clementia Pharmaceuticals announced top line results from its Phase 2 clinical trial investigating palovarotene for the treatment of FOP. Several positive trends were detected in this 40-subject placebo-controlled trial, including reductions in the proportion of subjects who developed new HO, reductions in volume of new HO, improvement in patient-reported pain associated with flare-ups, and a decrease in the duration of flare-ups – although none of these outcomes reached statistical significance. Subjects tolerated palovarotene well – all subjects completed the 12-week trial and enrolled in the Phase 2 open-label extension trial. The results of this landmark clinical trial are encouraging and closely mirror what was observed in previously reported animal studies with palovarotene.

At the IFOPA's FOP Drug Development Forum in Sardinia, Italy in October 2017, Dr. Donna Grogan (Clementia Pharmaceuticals) and Dr. Fred Kaplan (University of Pennsylvania) highlighted Clementia's Phase 2 results with palovarotene and the imminent plans to initiate a Phase 3 clinical trial (MOVE trial) with palovarotene. The two-year, Phase 3 clinical trial of palovarotene will enroll 80 individuals (age >4 years old) and will be conducted at 20 clinical sites in 14 countries. The trial began enrollment in December 2017. Information can be found at clinicaltrials.gov.

The clinicians and scientists at The Center for Research in FOP & Related Disorders have been key thought leaders and directors of the basic, translational and clinical research on this novel target and potential therapy for FOP.

ACTIVIN A ANTIBODIES

Two recent studies described the role of Activin A (Act A) in the pathogenesis of heterotopic ossification (HO) in FOP. The investigators showed that mutant ACVR1 unexpectedly sensitized FOP cells to Act A, a ligand (hormone-like protein) that had been found to inhibit BMP signaling. Importantly, they showed that blocking antibodies to Act A abolished HO in mouse models of FOP. The authors cautioned about the paucity of data implicating Activin A as a driver of HO in FOP patients, largely due to the inability to safely acquire relevant human tissues and cells for testing.

The use of primary connective tissue progenitor cells [CTPCs; also known as stem cells from human exfoliated deciduous teeth (SHED) cells] from FOP patients provides an extraordinary opportunity to examine the proposed molecular pathophysiology of the disease in an *in vitro* system directly relevant to the human condition. Importantly, these cells are obtained innocuously from FOP patients (these are the good “Tooth Ferry” cells that FOP families have kindly donated) and are not transfected with viral agents that could alter the immune response to BMP pathway signaling in FOP.

Studies from The Center for Research in FOP & Related Disorders show unequivocally that Act A stimulates BMP signaling and enhances cartilage and bone differentiation in CTPCs from FOP patients and controls (but much more in cells from FOP patients), a finding relevant to the primary pathology of FOP in humans. These results also reveal that stimulation by BMP4 plays a vital role in hyperactive signaling from mutant ACVR1 in human CTPCs. Taken together, these results suggest that therapeutic strategies for FOP in humans needs to account for mutant receptor activation by both Act A and BMP4.

Our study (“Activin A amplifies dysregulated BMP signaling and induces chondro-osseous differentiation of primary connective tissue progenitor cells in patients with FOP”), published online in 2017 in the journal *BONE*, establishes the ability of Act A to stimulate BMP pathway signaling in human FOP CTPCs and suggests that mutant ACVR1 confers the ability of Act A to enhance BMP signaling that leads to HO.

A complementary study (“The FOP mutation p.R206H in ACVR1 confers an altered ligand response”), published online in 2017 in *BONE* by Laura Hildebrand, Petra Seemann and colleagues from Berlin-Brandenburg Center for Regenerative Therapies in Berlin, Germany, compared the signaling responses of wild type and mutant ACVR1 to different ligands. The authors also discovered that the FOP mutation is more sensitive to a number of natural ligands including Activin A. The mutant receptor appears to have lost some essential inhibitory interactions with its ligands and co-receptors, thereby conferring an enhanced ligand-dependent signaling and stimulating ectopic bone formation as observed in the patients.

Our data in primary native CTPCs from people with FOP suggest that Act A is relevant to the pathogenesis of HO in human FOP, as has been shown in FOP knock-in mouse models. Act A expression is induced in skeletal muscle following injury and overexpression of Act A causes significant damage to skeletal muscle. Conversely, Act A neutralization improves repair and regeneration of skeletal muscle.

Our studies also suggest plausible *in vivo* mechanisms based on BMP4 and Act A dependence in FOP CTPCs. We propose that pre-lesional tissue reflects the basal increased BMP pathway signaling associated with mutant ACVR1 and is likely responsible for the developmental features of FOP – similar to the basal state in FOP CTPCs where enhanced BMP pathway signaling is BMP and Act A independent. Our data also suggest that hypoxia (and by inference, early inflammatory lesions) that promote BMP pathway signaling are also BMP4 and Act A independent. Conversely, the induction of early tissue changes *in vivo* that lead ultimately to heterotopic bone formation in mature lesions is almost certainly related to enhanced BMP pathway signaling due to, at least, both BMP4 and Act A stimulation.

Our study shows that endogenous and exogenous Act A has a stimulatory effect on BMP pathway signaling in human FOP CTPCs, consistent with recent reports. Additionally, our data raise the cautionary note that proteins like BMP4 also play key stimulatory roles that should be considered in emerging therapeutics. The data intriguingly suggest that attenuation of both BMP4 and Act A, for example, have a synergistic effect on BMP pathway signaling in FOP in humans and thus may cooperatively alter the threshold for HO in FOP. The most important message here is that although much is learned from animal models of FOP, none fully reproduce the clinical course or intensity of HO in human FOP. It is thus imperative to complement lessons learned from FOP animal models in the study of human FOP. Translational studies in animal models can instruct us and guide us about studies in humans, but they cannot replace clinical trials. As a famous FOP scientist once said, “The best study of humans is humans.”

In summary, the unexpected discovery of Act A in the pathogenesis of FOP identifies a therapeutic target for FOP and excavates a foundation for clinical development. The stage is set for the next clinical trial. At the IFOPA’s FOP Drug Development Forum in Sardinia, Italy in October 2017, Dr. Xiaobing Qian from Regeneron Pharmaceuticals announced the details of a Regeneron-sponsored Phase 2 clinical trial (LUMINA-1) to investigate the safety, tolerability and efficacy of REGN2477, an anti-Activin A antibody, in 40 adults with FOP. Details of the trial can be found on clinicaltrials.gov.

RAPAMYCIN

Although Activin A (Act A) evokes enhanced chondrogenesis (pre-bone cartilage scaffold formation) *in vitro* and heterotopic ossification (HO) *in vivo*, the underlying mechanisms are unknown. Writing in *The Journal of Clinical Investigation* (“Activin-A enhances mTOR signaling to promote aberrant chondrogenesis in FOP”), Hino, Ikeya and colleagues from Kyoto University in Japan reported the development of a high-throughput screening system using FOP patient-derived induced pluripotent stem cells to identify pivotal pathways in enhanced chondrogenesis that are initiated by Act A. In a screen of 6,809 small molecules, the authors identified mTOR signaling (a molecular pathway involved in tissue injury and repair) as a critical pathway for the increased chondrogenesis of mesenchymal stromal cells derived from FOP induced pluripotent stem cells. Two different HO mouse models including an FOP mouse model revealed critical roles for mTOR signaling *in vivo*. By using the mTOR inhibitor rapamycin, a drug used to prevent the rejection of transplanted organs, the investigators were able to inhibit heterotopic ossification in FOP mice. These results uncovered an Act A - ACVR1 / mTOR axis in FOP pathogenesis and identified the widely used and approved drug rapamycin as an inhibitor of this pathway.

As a result of this work, Dr. Junya Toguchida, one of the authors on the study, reported at the IFOPA’s Drug Development Forum in Sardinia, Italy in October 2017, that he had begun a clinical trial with rapamycin in Japan. The one-year trial will enroll 20 individuals (ages 6-59) with FOP across four sites in Japan – Kyoto, Kyushu, Nagoya and Tokyo.

Reporting in *BONE* (“Acute and chronic rapamycin use in patients with FOP: A report of two cases”), Fred Kaplan and colleagues noted that recent studies in genetic mouse models of FOP support involvement of the mechanistic target of rapamycin (mTOR) pathway in the pathophysiology of FOP and proposed the repurposed use of rapamycin, an inhibitor of mTOR signaling in clinical trials for the management of FOP. The authors reported two patients with the classic FOP mutation who received rapamycin – one for four months on a compassionate basis for treatment of acute flare-ups of the neck and back that were refractory to corticosteroid therapy, and the other for 18 years for chronic immunosuppression following liver transplantation for intercurrent cytomegalovirus infection. In both patients, FOP progressed despite the use of rapamycin. This report highlights the

real-world use of rapamycin in two FOP patients and provides insight and caution into the use of rapamycin in clinical trials for the management of FOP.

IMATINIB

In 2017, Fred Kaplan, Bob Pignolo and colleagues reported in *BONE* (“Early clinical observations on the use of imatinib in FOP: A report of seven cases”) a combined case experience with off-label use of imatinib in seven children with relentless flare-ups and rapid symptomatic progression of FOP. Prior to publication, a reviewer wrote: “When treating patients with a very rare disease, reporting cases of off-label use of medications is extremely important. This paper presents very helpful information regarding the age of the patients taking the medication, presentation at time of starting imatinib, duration of treatment, side effects and rationale for stopping medications. Additionally, it provides compelling preclinical data on the possible utility of imatinib and very useful information regarding the safety and tolerability of the drug.” The full paper is available by contacting Dr. Kaplan at frederick.kaplan@uphs.upenn.edu. Here, we present a brief summary.

Research studies have identified multiple potential targets for therapy in FOP, and novel drug candidates are being developed for testing in clinical trials. A complementary approach seeks to identify approved drugs that could be repurposed for off-label use against defined targets in FOP. Imatinib is one such drug.

Imatinib is a tyrosine kinase inhibitor originally developed for use in patients with chronic myeloid leukemia (CML). Imatinib is a safe, well-tolerated medication that has been used in thousands of adults and children with CML. Importantly, imatinib has the desirable effect of down-regulating multiple targets relevant to the pathophysiology of FOP. Recent preclinical studies published last year from The Center for Research in FOP & Related Disorders and featured in the 25th and 26th Annual Report of the FOP Collaborative Research Project demonstrated that imatinib decreases HIF1- α /inflammation activity, mutant ACVR1 activity in hypoxic FOP stem cells, and is effective in reducing HO in a mouse model of FOP. In addition to the robust HIF1- α target, imatinib may have the added benefit of impacting other targets including c-Kit (an essential gene for mast cell generation and mast cell activity) which plays a major role in FOP lesion formation, PDGFR α (expressed by the FOP lesional fibroproliferative cells), and multiple MAP kinases involved in inflammation. Imatinib also has potential beneficial effects in lymphocytes, macrophages and mast cells by dampening multiple signaling pathways implicated in the pathophysiology of FOP lesions. Notably, imatinib is effective in the treatment of systemic mast cell disease and inhibits multiple inflammatory proteins implicated in the formation of heterotopic ossification. Thus, imatinib has potential impact on major therapeutic targets of FOP.

Based on compelling biologic rationale, strong preclinical data and a favorable safety profile, imatinib has been prescribed on an off-label basis in seven children with continuous FOP flare-ups, predominantly in the axial regions, and which were not responsive to standard-of-care regimens. All seven children failed to demonstrate any durable symptomatic response to the standard medications used to manage symptoms of FOP such as corticosteroids, non-steroidal anti-inflammatory agents, cromolyn or intravenous bisphosphonates. All seven children were referred to a pediatric hematologist-oncologist or a pediatric rheumatologist for consideration of imatinib therapy after detailed consultation with the parents on the relative risks and benefits of off-label use of imatinib for FOP. Parents were informed that imatinib use was on an off-label basis and was not part of a clinical research study. The parents were also informed that progress would be monitored clinically and that radiographs would not be performed routinely.



Anecdotal reports in these cases document that the medication was well-tolerated with an overall reported decrease in the intensity of flare-ups in the seven children who took the medication. Moreover, the parents of all seven children who were able to take imatinib on a daily basis noted subjective decreases in flare-up intensity after several weeks of use.

Clinical trials for rare diseases commonly focus on one target and one potential therapeutic at a time. However, the exigencies of clinical care in a real-world setting require flexibility in managing symptomatic disease, especially when no other alternatives are available. Approved medicines for one condition may have potential off-target effects for another and thereby be suitable for off-label use on a compassionate basis. Early anecdotal experience with such medications may suggest useful parameters for monitoring meaningful endpoints in future clinical trials.

As a result of our experience in these seven children, we strongly feel that imatinib should be evaluated in a controlled clinical trial in the pediatric FOP population who are experiencing relentless axial flare-ups and for whom few, if any tangible treatment opportunities presently exist. These early clinical observations support the implementation of clinical trials in children with uncontrolled FOP flare-ups to determine if imatinib may ameliorate symptoms or alter the natural history of this debilitating and life-threatening disease. Alternatively, imatinib may be used by physicians on an off-label basis to manage symptoms in children with FOP.

ON THE HORIZON

“We ran, as if to meet the moon.” – Robert Frost

“Molecularly targeted therapy will have truly arrived when patients are matched with a treatment regimen predicated by their genetic attributes.” – Sebolt-Leopold & English (“Mechanisms of drug inhibition of signaling molecules;” *Nature*)

Blocking Antibodies against ACVR1

Mutant ACVR1 demonstrates leaky BMP pathway signaling and ligand hyper-responsiveness, providing a rationale for using blocking antibodies to ACVR1 in the prevention and treatment of FOP. Therapeutic monoclonal antibodies specific for ACVR1 are under development by at least two pharmaceutical companies. This approach was highlighted at the IFOPA’s FOP Drug Development Forum in Sardinia, Italy in October 2017 by Dr. Takenobu Katagiri from Saitama Medical University in Japan.

Signal Transduction Inhibitors (STIs)

STIs are the bullseye of all targets for FOP. As a class, these orally-available small molecules jam the ‘mouth’ of the FOP receptor, thus disabling it from transmitting its misdirected message to make more bone. STIs are important molecular tools for studying BMP signaling in FOP and have great potential for development into powerful therapeutic drugs for FOP. Selective STIs for FOP will inhibit ACVR1 rather than closely associated receptors and are concurrently being developed by at least four pharmaceutical or biotechnology companies. Broad spectrum STIs that target ACVR1 are also being considered for repurposing in clinical trials.

Allosteric Inhibitors

On a variation of the STI theme, known as allosteric inhibition, Dr. Jay Groppe from Texas A&M College of Dentistry and Dr. Alex Bullock from Oxford University highlighted alternative approaches to silence the mutant FOP receptor that hold promise for future drug development. Instead of targeting the mouth of ACVR1 that is nearly identical among related receptors, the allosteric approach capitalizes on the unique aspects of ACVR1, or even mutant ACVR1, specifically to work its magic.



PART 5 Notable Advances in FOP Research in 2017 – The Ignition Switch

If mutant ACVR1 is the rocket engine for FOP flare-ups and Activin A and BMP4 are the rocket fuel, then inflammation is the ignition switch that launches the rocket.

In 2017, we made great strides in understanding the ignition switch. Our progress is highlighted here in *Notable Advances in FOP Research in 2017*. We believe that these studies will inspire more work in this area of FOP research and will be crucial to identifying robust targets for therapy.

The search for immunological triggers for FOP flare-ups continues to dominate the frontier of FOP research. In all affected individuals, FOP is caused by an activating mutation in ACVR1 that results in dysregulated BMP pathway signaling. The mutant receptor appears to cause the myriad developmental features of FOP (such as the malformed toes, osteochondromas and cervical spine fusions), but may not be sufficient to induce the episodic flare-ups that lead to disabling heterotopic ossification. FOP flare-ups – both trauma-induced and spontaneous – strongly implicate the participation of an underlying immunological trigger.

1. MAST CELLS & MACROPHAGES

In individuals with FOP, episodes of HO frequently follow injury. The first sign of active disease is commonly inflammatory activity in tissues that precedes connective tissue degradation, progenitor cell recruitment and heterotopic ossification. In a major article (“Depletion of Mast Cells and Macrophages Impairs Heterotopic Ossification in an ACVR1 R206H Mouse Model of FOP”) published online in *The Journal of Bone and Mineral Research* in 2017, graduate student Michael Convente, Dr. Eileen Shore and colleagues from The Center for Research in FOP & Related Disorders used a mouse model of FOP to investigate the cellular and molecular inflammatory response in FOP lesions following injury.

The team found that the classic FOP mutation caused increased BMP signaling in post-traumatic FOP lesions and early divergence from the normal skeletal muscle repair program with elevated and prolonged immune cell infiltration. Various pro-inflammatory molecules were elevated and prolonged in FOP lesions and in FOP mast cells. Importantly, depletion of mast cells and macrophages significantly impaired injury-induced HO in FOP mice, reducing injury-induced HO volume by 50% with depletion of each cell population independently, and 75% with combined depletion of both cell populations. Together, the data showed that the immune system contributes to the initiation and development of HO in FOP.

The two immune cell populations previously implicated in HO development are mast cells and macrophages. Given the increased numbers and prolonged presence of mast cells and macrophages in FOP lesions, as well as their relevance in non-FOP HO disorders, the study investigated the

contribution of mast cells and macrophages to injury-induced HO by using immunodeficient FOP mouse lines. Together, the data show that both mast cells and macrophages contribute to HO development.

Inflammatory triggering events, such as intramuscular vaccinations, tissue trauma or viral infections, induce new episodes of HO in FOP patients with high frequency. The Convente study revealed that multiple immune cell populations that participate in a normal tissue injury response are increased in FOP lesions. Additionally, these cells persist at high levels during progression to heterotopic bone instead of returning to pre-injury levels as occurs during wound repair. This heightened immune cell response is accompanied by increased proinflammatory factors and, at least in mast cells, mutant ACVR1 alters expression of a subset of proinflammatory cytokines. Combined depletion of mast cells and macrophages in FOP mice further demonstrated key roles for both immune cells in promoting HO.

BMPs induce inflammatory cytokine production and edema, two features that frequently precede HO initiation in FOP patients. BMP signaling also contributes to inflammatory activation of multiple immune cell populations, including macrophages and T cells. In contrast to the normal repair program, the data in the Convente paper suggest that elevated BMP pathway signaling throughout lesion progression in FOP induces the altered inflammatory events that disrupt the normal skeletal muscle injury response and repair program, ultimately leading to HO.

The study highlights the immune system as an appealing target for therapeutic intervention in HO and FOP. Inflammation is the earliest recognized event in HO development. Therefore, inhibiting disease progression at this stage may limit or prevent HO. The study found that ablation of only mast cells or only macrophages reduced HO formation significantly, but not completely; whereas ablating both cell populations resulted in enhanced inhibition, indicating that a single target may be insufficient to completely prevent HO. Notably, the results show that although inflammatory upregulation in FOP is significant, not all inflammatory factors are elevated, suggesting that specific inflammatory pathways can be targeted while maintaining a functional immune system. Thus, an optimal treatment regimen may inhibit HO formation and maintain substantial immune function in patients.

Observations of an immune response that precedes HO in an FOP mouse model forming spontaneous (non-injury induced) HO suggests that an immune response could be a general feature of HO initiation and early progression. An inflammatory component is associated with almost all forms of HO, notably non-genetic HO disorders associated with severe trauma such as joint replacement surgery, combat blast injuries and other traumatic wounds.

Although there may be unique inflammatory contributions to FOP, such as a role for Activin A in disease pathology, common inflammatory mediators across all HO disorders establish the immune system as an appealing treatment target that may benefit large numbers of patients. The Convente study highlights the immune system as a major contributor to the initiation and development of HO in FOP and greatly expands the current knowledge on the immunological contributions to FOP progression. The compelling results provide a strong foundation for future studies on the immunological contributions to FOP. Given the shared immunological features of FOP and non-genetic HO disorders, the findings may provide insight into treatment for more common nonhereditary forms of HO, which could significantly benefit a wide range of patients.

In a highly related translational study (“Mast cell inhibition as a therapeutic approach in FOP”), published in *BONE* in 2017, Brennan, Kaplan, Pignolo and colleagues from The Center for Research in FOP & Related Disorders showed that episodic flare-ups of FOP are characterized clinically by severe, connective tissue swelling and intramuscular edema, followed histologically by an intense and highly angiogenic fibroproliferative reaction. This early inflammatory, angiogenic and fibroproliferative response is accompanied by the presence of abundant mast cells – far in excess of other reported

myopathies (inflammatory muscle diseases). Using an injury-induced, constitutively-active transgenic mouse model of FOP, the study showed that mast cell inhibition by cromolyn resulted in a dramatic reduction of heterotopic ossification. Cromolyn significantly decreased the total number of mast cells in FOP lesions. Furthermore, cromolyn specifically diminished the number of degranulating mast cells in pre-osseous lesions. This work demonstrates that FOP is a type of localized mastocytosis may offer new therapeutic interventions for treatment of this devastating condition.

Mast cells are found within connective tissue. Mast cell progenitors leave the bone marrow to enter the circulation then mature within target tissue (such as muscle). Mast cells are activated in response to any type of tissue injury, snake venom and vaccines. Their activation results in acute and chronic inflammation, vascular injury, cell recruitment and, ultimately, tissue remodeling and new blood vessel formation. Mast cells secrete preformed inflammatory mediators.

Frank Gannon and colleagues at The Center for Research in FOP & Related Disorders showed previously that mast cell density at the periphery of FOP lesions is as much as 150-fold greater than in normal skeletal muscle or in uninvolved skeletal muscle from patients with FOP and up to 40-fold greater than in any other inflammatory muscle disease. Those findings demonstrated that mobilization and activation of inflammatory mast cells are pathological hallmarks of FOP lesions and serve as potential targets for pharmacologic intervention in FOP. Mast cell involvement has also been demonstrated in non-hereditary HO, strongly suggesting the cell type is critical to the pathology of HO.

Although its existing FDA indication for asthma may suggest cromolyn as a potential option for FOP patients, its current routes of administration result in poor systemic distribution. Spray or aerosolized liquid forms for nasal or oral inhalation represent the best commonly used routes for systemic distribution. Cromolyn is currently used at the physician's discretion as a Class II medication in current FOP treatment guidelines. A liquid intravenous form of cromolyn has been reported; however, it undergoes rapid elimination with hypertension and nausea as potential adverse effects. An alternative option involves using the c-kit tyrosine kinase inhibitor imatinib to induce mast cell depletion, which has proven successful at reducing inflammation associated with rheumatoid arthritis and severe refractory asthma, as well as reducing HO in an Achilles tendon injury model, and importantly, HO in an FOP mouse model.

Considering FOP as a type of localized mastocytosis may offer new therapeutic opportunities for treatment. Cromolyn was safe and effective in abrogating HO in an injury-induced mouse model of FOP, but at doses in excess of those currently capable of being delivered in a safe and convenient manner to people. Improvement in the bioavailability of cromolyn, either by change in formulation or route of administration, may offer enhanced therapeutic benefits. Other mast cell inhibitors may offer similar and/or additional therapeutic benefits.

In an editorial on the mast cell study entitled "Problems with Mast Transit" in *Science Translational Medicine*, Dr. Ben Levi (University of Michigan) wrote: "Whereas excessive people in mass transit cause inflamed tempers, excessive mast cell transit, or mastocytosis can cause severe inflammation. Though mast cells have been known to play a key role in inflammatory responses, their role in heterotopic ossification and FOP is less well-defined. A recent study by Pignolo and colleagues characterizes the mastocytosis present in heterotopic ossification lesions.

Recently, several studies have characterized the central role of inflammation on traumatic and genetic forms of heterotopic ossification. Specifically, several anti-inflammatory treatments have been shown to be effective in preventing HO in promising pre-clinical studies. Additionally, steroids have been a mainstay in attempts to combat HO flares in people living with FOP. Despite this known role of inflammatory cells, few studies exist characterizing which inflammatory cells are involved and what these cells do once they arrive at the HO site. The authors in this study used a ligand independent FOP



mouse model with hyperactivation of BMP signaling where adenovirus and cardiotoxin are injected to stimulate HO formation. The authors demonstrated that mast cell inhibitors/stabilizers such as cromolyn significantly diminished the number of mast cells and prevented HO formation in this model. Specifically, they demonstrated the most significant decrease in degranulating mast cells with cromolyn treatment. Conversely, the use of aprepitant which is a Substance P/neurokinin 1 receptor antagonist in mast cells did not decrease mast cell number in HO formation, further elucidating the specific mechanism by which mast cells decrease HO.

This study changes the previous focus of defining HO progenitor cells to better understanding the non-resident inflammatory cells that migrate to an injury site and how these cells alter the HO niche. Based on these findings, researchers can now consider targeting mast cells with therapeutics such as cromolyn in attempts to quell the inflammatory niche necessary for HO. Additionally, future studies to better understand what the mast cells may be secreting that exacerbates these HO lesions would lead to a significant step forward in our understanding of FOP.

2. FOP TAKES ITS TOLL

Last year, we unveiled pathways and targets involved in the amplification of inflammatory and hypoxic stages of early FOP lesions. This year, we explored cellular and molecular signaling at an even earlier stage – in essence, the launch codes of lesion formation.

Despite the occurrence of ACVR1 mutations in all FOP patients, individuals with FOP do not form bone continuously, but rather episodically and often following trivial injury or viral infection, a finding that suggests that the innate immune system contributes to the induction and evolution of HO.

The immune system must respond not only to external threats from invading organisms like bacteria and viruses, but also to damage signals from external injury and normal tissue maintenance and repair. Basically, the immune system has two interacting branches. One branch of the immune system is the acquired immune system that produces antibodies to very specific threats. For example, an antibody against one strain of influenza might not protect you against a different strain of influenza. The other branch of the immune system is the innate immune system. The innate immune system is far broader and general in its response to both internal and external threats and, from an evolutionary standpoint, is far more ancient than the adaptive immune system.

The innate immune system is like a giant alarm system that responds to generalized external danger signals from bacteria and viruses (called PAMPs) and internal danger signals from external injury and normal tissue maintenance (called DAMPs). The innate immune system is a first responder – a Paul Revere riding through town announcing “The British are coming; the British are coming,” without giving any specific details, but notifying and mobilizing the population that danger is on the way and a response is needed.

The receptors or molecular antennas of the innate immune system are called toll-like receptors (TLRs). They come in many different shapes and sizes – some tuned to bacteria, some to viruses, some to tissue injury signals and some to both. Some TLRs are on the cell surface and some are inside the cell, especially those that respond to viruses. These receptors are naturally expressed on cells of the immune system, but are also expressed on connective tissue cells (like those in muscle that respond to injury) since those cells also must respond to the damage signals (either external or internal) and generate a repair response. The overwhelming response of the innate immune system is not specific antibodies, but generalized inflammation. As we will see, this is a portion of the immune system that ignites the cascade of events leading to FOP flare-ups.

CTPCs, also known as Stem cells from Human Exfoliated Deciduous teeth (SHED) cells, are a type of mesenchymal stem cell (MSC) and have both differentiation and immunomodulatory properties.

In FOP, these cells serve as an *in vitro* model system for the pathophysiology of FOP. A central enigma in the pathophysiology of FOP is how CTPCs integrate inductive inflammatory signals in their microenvironment with the dysregulated BMP signaling that is a hallmark of the disease into a coherent output that influences cell fate and HO.

Importantly, we found that CTPCs express TLRs, the highly-conserved sentinels of the innate immune system. As we have explained, TLRs are activated by pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) in response to microbial and endogenous tissue injury – two key stimuli that activate FOP.

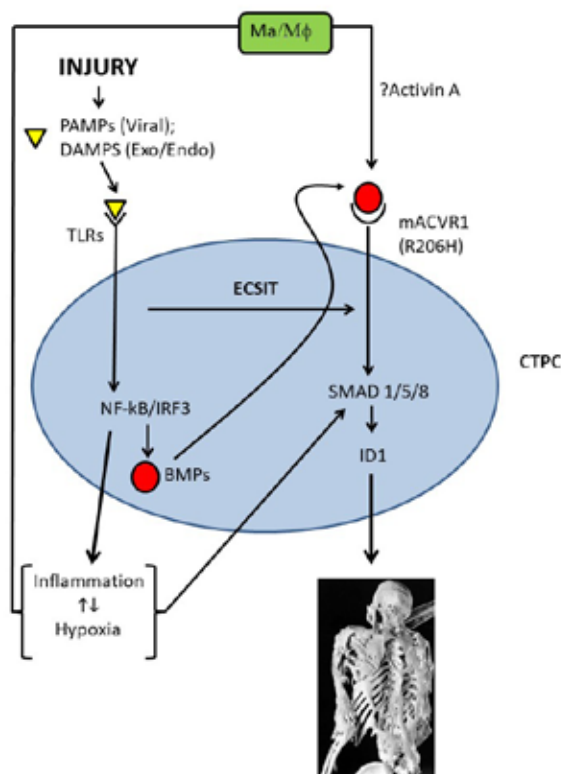
Previous studies have shown that BMP and TLR signaling are intimately co-regulated during development. It is plausible that in FOP, TLR signaling triggered by viruses, trauma or endogenous tissue injury stimulate BMP pathway signaling to alter cell fate and trigger progressive and disabling HO.

Our study identified a direct link between innate immune activation and dysregulated BMP signaling in FOP. Our data indicate that 1) TLR signaling amplifies dysregulated BMP signaling in FOP CTPCs and 2) that these critical signaling pathways in FOP are integrated in a cell-autonomous manner by the evolutionarily conserved signaling intermediate in the Toll pathway (ECSIT). ECSIT is an adaptor protein that links TLR signaling to the BMP pathway during development and is required for BMP signaling during embryogenesis. Importantly, ECSIT transduces and amplifies innate inflammatory signals to the BMP pathway, thus integrating damage signals to repair responses in the very cells that orchestrate FOP lesions.

What we discovered this year was that the TLR alarms of the innate immune system are hard-wired to the BMP pathway and dramatically amplify the BMP pathway response in FOP. But why should the ancient immune system whose output is inflammation be hard-wired to the BMP pathway? We think of the BMP pathway as primarily responsible for making bone, but in a much larger sense, the innate immune system is part of a highly conserved alarm network responsible for coordinating tissue repair.

A central challenge in FOP research is to construct a unified theory that explains both the inflammatory and repair features of the condition. Our findings allow us to construct a working hypothesis of the pathophysiology of flare-ups and resultant HO in FOP. It is intriguing to speculate that perhaps

all flare-ups, even those that appear spontaneously, are activated by the innate immune system and their stimulation of TLRs – in part through DAMPs and PAMPs. It is critically important to remember that the innate immune



No ECSIT: Hypothetical Schema for Interaction of the Innate Immune System and BMP Signaling in FOP

Activin A = Ligand that activates mACVR1

BMPs = Ligands that activate ACVR1

CTPC = Connective Tissue Progenitor Cell

DAMPs = Damage associated molecular patterns

ECSIT = Evolutionarily Conserved Signal Intermediate in the Toll Pathway

Exo/Endo = Exogenous or endogenous tissue injury signals

mACVR1 (R206H) = mutant ACVR1 (the FOP Gene)

Ma/MΦ = Mast cells/Macrophages

NF-κB/IRF3 = Inflammatory pathway intermediates between the TLRs and inflammation & hypoxia

PAMPs = Pattern associated molecular patterns

SMAD 1/5/8 & ID1 = Downstream mediators of heterotopic ossification in FOP

TLRs = Toll-like receptors



system is ubiquitously active and functional in vertebrates even in the absence of overt injury, especially in the context of normal tissue repair.

These seminal findings and their implications for FOP were described in a major study (“ECSIT Links TLR and BMP Signaling in FOP Connective Tissue Progenitor Cells”) by Haitao Wang, Edward Behrens, Bob Pignolo and Fred Kaplan published online in *BONE* in 2017.

One anonymous reviewer of the study wrote, “This is a seminal paper on FOP. It provides, for the first time, a key link and unifying hypothesis linking tissue injury and the innate immune system to dysregulated BMP pathway activity in FOP patients. The studies are thorough and the results compelling. This will lead to much important work on critical crosstalk between innate immune system and the BMP signaling pathway.” Another reviewer wrote, “This is a wonderful study from an exceptional group. It provides a wonderful hypothesis that a viral infection sensed by a FOP connective tissue progenitor cell could kick off the process of heterotopic ossification.”

In conclusion, this work establishes, for the first time, a direct link between innate immune activation and dysregulated BMP signaling in FOP and identifies a new target for pharmaceutical approaches to disrupt immune-related triggers of dysregulated BMP signaling in this catastrophic disease. These findings identify a new and unexpected target in the induction of FOP lesions – findings that will be further explored in *in vivo* studies – and will likely lead to new and bold therapeutic approaches to inhibit the onset of FOP lesions and disease progression.



PART 6 Infrastructure Development

Major infrastructure development occurred at The Center for Research in FOP & Related Disorders in 2017, as documented in the summaries of the following seven published papers. These studies, all undertaken at Penn, performed in collaboration with our colleague Dr. Bob Pignolo (now at The Mayo Clinic) and published online in 2017, cover the clinical landscape of physician-reported mobility assessments, patient-reported mobility assessments, biomarker development, qualitative and quantitative radiographic imaging analysis, and clinical staging of FOP.

BONE, 2017

Joint-specific Risk of Impaired Function in FOP

Pignolo RJ, Durbin-Johnson BP, Rocke DM, Kaplan FS

Using data from 500 FOP patients, we estimated age- and joint-specific risks of new joint involvement using advanced statistical methods. Compared to data from a 1994 survey of 44 individuals with FOP, these updated estimates of age- and joint-specific risks of new joint involvement are much more accurate with distinctly narrower confidence limits. Our new data are based on a wider range of ages and have less bias due to greater global comprehensiveness. The data set captures over three-fifths of the known FOP patients worldwide. At any given age and for any anatomic site, the data indicate which joints are at risk. This study of approximately 63% of the world's known population of classically-affected FOP patients provides a refined estimate of risk for new involvement at any joint at any age, as well as the proportion of patients with uninvolved joints at any age. Importantly, these joint-specific survival curves can be used to facilitate clinical trial design and to determine if potential treatments modify the predicted trajectory of progressive joint dysfunction in patients with FOP.

BONE, 2017

A Cumulative Analogue Joint Involvement Scale (CAJIS) for FOP

Kaplan FS, Al Mukaddam M, Pignolo RJ

Assessment of functional mobility in FOP is essential to support clinical trials of investigational agents. Of necessity, we developed a simple, rapidly-administered, cumulative analogue joint involvement scale (CAJIS) for FOP based on assessments in 144 individuals worldwide with classic FOP. CAJIS scores correlated with patient age, activities of daily living (ADLs), and ambulatory status, with excellent inter-rater variability. We show that the CAJIS score provides an extremely accurate and reproducible snapshot of total body and regional mobility burden in FOP that correlates with age and functional status.

In fact, CAJIS score is not linked with flare-up frequency only. The reasons for this are that the CAJIS is a measure of global joint dysfunction due to all causes (e.g. HO due to flare-ups, non-flare-up progression of HO, accelerated arthritis, impingement of a joint due to osteochondromas, joint dysplasias and intra-articular synovial osteochondromatosis).

The CAJIS is not meant to replace detailed range-of-motion assessment for any particular joint. Rather, it is designed to enable a rapid assessment of total body mobility burden in any clinical setting. The central value of the CAJIS score for FOP is a rapid, comprehensive, cumulative analogue assessment of joint involvement that is independent of the rate, timing, order, or position of progressive disease activity.

Our evaluation of the average change in cross-sectional total CAJIS score over time, estimates that the score increases by about 0.5 units per year across all ages. This suggests that the CAJIS should be assessed at least every two years to detect an increase in the CAJIS score of one. However, younger patients can be expected to accumulate joint dysfunction more quickly than older patients and so annual assessment of CAJIS may be more appropriate. Presently, CAJIS evaluations have been incorporated into the design of three ongoing clinical studies and trials. More information can be found at clinicaltrials.gov. In conclusion, we developed and validated a simple, rapidly-administered, cumulative analogue joint involvement scale (CAJIS) for FOP. CAJIS scores correlate with patient age, ADLs, and ambulatory status, with excellent inter-rater variability. The CAJIS evaluation can be performed rapidly in any clinical setting.

BONE, 2017

Longitudinal Patient-reported Mobility Assessment in FOP

Kaplan FS, Al Mukaddam M, Pignolo RJ

In this study, we presented the first longitudinal patient-reported mobility assessment (PRMA) in FOP based on a simple x-question survey as an evaluation tool. At initial presentation and follow-up (1-11 year span; median: 6 year span), 64 patients (36 females; 28 males) with classic FOP completed the questionnaire, which was designed to rapidly assess mobility at 15 sites (three axial, six upper limb and six lower limb). In order to validate this approach, 21 of 64 patients (33%) underwent a cumulative analogue joint involvement scale (CAJIS) evaluation by two physicians within six months of the patients' second self-assessment. We found that: 1) mobility changes were episodic and regional, occurring first in the neck and trunk, followed by the upper limbs and finally the lower limbs; 2) interval improvements in mobility did occur, most notably in the lower limbs (18%) and less so in the upper limbs (12%) and trunk (3%) and 3) patient-reported mobility assessments correlate highly with physician-reported CAJIS evaluations.

Within a span of 1–11 years (median: 6 years), patients reported disease progression at almost all sites, but with great variability. Disease involvement was greater in the spine and chest than in the limbs at all ages, reflecting the earlier involvement of axial sites in nearly all individuals.

Axial (central body core, the spine, chest and jaw) and upper limb involvement preceded lower limb involvement at all ages. We found that more severe mobility loss occurred at axial sites and in the upper limbs during childhood, most likely due to the robust disease activity at those sites during childhood, coupled with the fact that anatomic targets were progressively removed from further functional involvement after a joint became ankylosed.

The findings in this study were consistent with those of previously reported cross-sectional natural history studies. However, in analyzing longitudinal data on individual patients, we were surprised to find improvements in PRMAs, most notably in the lower limbs in some individuals. The exact cause of this interval improvement in PRMA is unknown, but might possibly be related to resolution of edema

following acute flare-ups or functional adaptation to mobility restriction. Lower limb involvement most often occurs at or following skeletal maturity, so relative change in position of non-bridging heterotopic bone with growth is an unlikely explanation.

It is reasonable to conclude from data in cross-sectional natural history studies that patients with classic FOP have normal mobility at birth, with the possible exception of variably decreased mobility of the neck from congenital orthotopic fusions of the cervical vertebra. Our data on individuals less than two years of age strongly support this conclusion.

Importantly, our study reveals that while FOP is overwhelmingly progressive, there are periods when the disease is likely quiescent without additional flare-ups or mobility loss; during these periods, mobility lost during previous flare-ups may, in fact, improve. This is the first longitudinal PRMA in FOP and provides a simple and valid patient self-assessment tool that can be used in the design and evaluation of clinical trials in this progressively disabling disease.

BONE, 2017

Cartilage-derived Retinoic Acid-sensitive Protein (CD-RAP): A Stage-specific Biomarker of Heterotopic Endochondral Ossification (HEO) in FOP

Lindborg CM, Brennan TA, Wang H, Kaplan FS, Pignolo RJ

Genesis of a cartilaginous scaffold is an obligate precursor to bone formation in FOP. We tested the hypothesis that cartilage-derived retinoic acid-sensitive protein (CD-RAP) can serve as a plasma biomarker for the pre-osseous cartilaginous stage of heterotopic endochondral ossification (HEO), the obligate mechanism of ectopic bone formation in FOP.

Palovarotene, a retinoic acid receptor-gamma (RAR γ) agonist, is being studied in a Phase 3 clinical trial as a possible treatment for FOP and is a potent inhibitor of HEO in mouse models of FOP. Current drug development for FOP mandates the identification of stage-specific biomarkers to facilitate the evaluation of clinical trial endpoints.

We showed in an injury-induced, constitutively-active transgenic mouse model of FOP that CD-RAP levels peaked between day seven and day 10 during the zenith of histologically-identified cartilage-scaffold formation, preceded radiographically apparent HEO, and were diminished by palovarotene.

Cross-sectional analysis of CD-RAP levels in plasma samples from FOP patients demonstrated a statistically non-significant trend toward higher levels in the recent flare-up period (three weeks to three months within onset of symptoms). However, in a longitudinal subgroup analysis of patients followed for at least six months after resolution of flare-up symptoms, there was a statistically significant decrease of CD-RAP when compared to levels in the same patients at the time of active or recent exacerbations. These data support the further exploration of CD-RAP as a stage-specific biomarker of HEO in FOP.



ACADEMIC RADIOLOGY, 2017

Analog Method for Radiographic Assessment of Heterotopic Bone in FOP

Rajapakse CS, Lindborg C, Wang H, Newman BT, Kobe EA, Chang G, Shore EM, Kaplan FS, Pignolo RJ

Severe progressive multifocal heterotopic ossification (HO) is a rare occurrence seen predominantly in patients who have FOP and is difficult to quantify owing to patient, disease, logistical and radiation-related issues. The purpose of this study was to develop and validate a scoring system based on plain radiographs for quantitative assessment of HO lesions in patients with FOP.

First, we used a mouse model of FOP-like HO to validate a semi-quantitative analog scale for estimating relative heterotopic bone volume. Second, we used this validated scale to estimate the relative amount of HO from a retrospective analysis of plain radiographs from 63 patients with classic FOP. Finally, the scale was applied to a retrospective analysis of computed tomographic images from three patients with FOP.

In the FOP-mouse model, the observed rating on the analog scale was highly correlated to heterotopic bone volumes measured by microcomputed tomography ($R^2=0.89$). The scoring system that was applied to radiographs of patients with FOP captured the clinical range of HO typically present at all axial and appendicular sites. Analysis of computed tomographic scans of patients with FOP found that observed radiograph ratings were highly correlated with HO volume ($R^2=0.80$).

The scoring system that we developed could enable practical, quantitative assessment of HO in clinical trials to evaluate new treatment modalities, especially for FOP. The development of the six-point analog scale that we developed provides and validates a much-needed, reproducible and quantifiable method for describing and assessing HO in patients with FOP. This scale has the potential to be a key descriptor that can inform patients with FOP and clinicians about disease progression and response of HO lesions to interventions and treatments.

BONE, 2017

Imaging Assessment of FOP: Qualitative, Quantitative and Questionable

Al Mukaddam M, Rajapakse CS, Pignolo RJ, Kaplan FS, Smith SE

The progressive development of heterotopic bone and progressive arthropathy at many joints in FOP leads to significant limitation of mobility. We compared various imaging modalities used in evaluating early flare-ups, HO and skeletal anomalies in FOP. We concluded that different imaging modalities are required at different stages of the disease. Ultrasound and MRI can be useful for evaluating edema in early stages of a flare-up; MRI being superior to ultrasonography. Plain radiographs and computed tomography (CT) can evaluate heterotopic bone in later stages of HO, but CT scan was better at measuring the volume of heterotopic bone. Functional imaging demonstrated increased activity at sites of flare-ups. Its utility in determining disease progression needs to be further evaluated. Cost, radiation exposure, availability of various imaging modalities and the ability of FOP patients to fit into the scanners are all considerations when requesting advanced imaging in a patient with FOP. Future studies are needed to determine if early radiographic findings can determine disease progression and response to treatment in FOP.

Clinical Staging of FOP

Pignolo RJ, Kaplan FS

With recent reporting of a comprehensive global Natural History Study in FOP, scales to assess joint dysfunction and a more accurate prediction of joint survival, it is now possible to construct a conceptual framework for the clinical staging of FOP. Based on assessment of seven FOP features, it is possible to assign five clinical stages. FOP features evaluated were: flare-up activity, body regions affected, thoracic insufficiency, other complications, activities of daily living (ADLs), ambulatory status and the cumulative joint involvement scale (CAJIS) score. Assessments of these features assign an individual with FOP to early/mild, moderate, severe, profound, or late-stage disease. These criteria seek to be flexible enough to be used by clinicians without reliance on advanced imaging or specialized testing, as well as by investigators involved in research or clinical trial studies who could readily make these assessments. These staging measures for FOP assess the influence of HO and accelerated joint dysfunction (due to congenital abnormalities) on the ability to perform common functional activities, and thus a delay or lack of progression from one stage to the next represents the ultimate test of efficacy for drug trials. This framework will serve both as a prediction tool for FOP progression as well as a critical opportunity to substantiate therapeutic interventions that are targeted to alter the natural history of the disease. The staging system that we developed will permit an accurate assessment of disease severity to appropriately develop or revise clinical plans of care, define operational research criteria and identify the effectiveness of interventions. Ultimately, clinical staging will aid the field in moving toward earlier intervention at a stage where disease-modifying therapies may be most efficacious.

PART 7 Editor's Choice – Ten Highlights from the Literature



LOCATION, LOCATION, LOCATION

Most people with FOP have the same causative mutation in ACVR1. However, additional mutations within ACVR1 have been identified in a small number of FOP patients. While all FOP mutations in ACVR1 induce increased signaling through the BMP pathway, the molecular mechanisms underlying the different mutations remain undetermined. In a paper published in *BONE* (“Variable signaling activity by FOP ACVR1 mutations”), Julia Haupt, Meiqi Xu and Eileen Shore demonstrate that the exact genetic letter and location of the mutation within the gene determines its activity and sensitivity to ligand stimulation. Variant FOP mutations in the mouth of the ACVR1 receptor are more sensitive to low levels of BMP than mutations in the region of ACVR1 where the most common mutation occurs. The data confirm that cells with ACVR1 mutations are more responsive to both BMP and Activin A ligands. The study also showed that the mutant FOP receptors can function through ligand-independent mechanisms – in other words, without the action of BMPs or Activin A.

ZEBRAFISH MODELS OF VARIANT FOP

The early zebrafish embryo has proven to be an excellent assay system for examining the function of the BMP pathway. Knowledge of zebrafish BMP signaling during early development, together with the relative ease of genetic manipulation of zebrafish embryos make it a valuable *in vivo* tool to study the activities of the human ACVR1 mutant receptors in FOP.

In a 2017 publication in *BONE* (“Variant BMP receptor mutations causing FOP in humans show BMP ligand-independent receptor activation in zebrafish”), Mary Mullins, Eileen Shore and colleagues from the University of Pennsylvania used a zebrafish development assay to test the signaling of human ACVR1 in classic FOP and FOP variants. In the study, human FOP mutant receptors were expressed in zebrafish embryos to investigate their signaling activities. The study demonstrated that variant ACVR1 mutations, like the classic Arg206His mutation, increased BMP signaling activity. The study also showed that ACVR1 Gly328 variants can partially rescue the patterning abnormality of *bmp*-deficient zebrafish embryos, demonstrating that the mutant receptor is active in the absence of BMP ligand.

This study is the first *in vivo* examination of the effect of FOP-causing mutations in variant human FOP in a vertebrate animal model. The importance of this work is that it is among the first to explore the effects of variant FOP mutations in a vertebrate FOP animal model system that is well-suited for rapid screening of candidate drugs.



A ZEBRAFISH MODEL OF CLASSIC FOP

A variety of animal models are needed to study and understand the features of human FOP. In a paper in *Zebrafish* (“A Zebrafish Model of FOP”), Pam Yelick and her colleagues from Tufts University in Boston characterized the first adult zebrafish model for FOP. Zebrafish ACVR1 is nearly identical to human ACVR1, and has been studied extensively in the developing zebrafish embryo. However, FOP mutations do not permit zebrafish embryos to develop to adult stages. In order to study FOP mutations in adult zebrafish, the authors created fish in which they could heat-activate the FOP gene after the fish were hatched. Their study showed that adult zebrafish expressing the FOP mutation develop a number of human FOP-like features, including heterotopic ossification, spinal deformity, vertebral fusions and malformed pelvic fins. Together, these results suggest that transgenic zebrafish expressing the heat-shock inducible FOP mutation can serve as a model for human FOP.

FRUIT FLY MODELS OF FOP PROVIDE MECHANISTIC INSIGHT

All cases of FOP are caused by mutations in the ACVR1 gene that render the encoded ACVR1 receptor hypersensitive to ligands, resulting in the activation of BMP signaling at inappropriate times in inappropriate locations. The episodic or sporadic nature of heterotopic ossification (HO) associated with FOP rests with ‘triggers’ that push the hypersensitive ACVR1 receptor into full signaling mode. Identification of these triggers and their mechanism of action are critical for preventing HO and its devastating consequences in FOP patients.

Kristi Wharton from Brown University and her colleagues report in *BONE* (“Drosophila models of FOP provide mechanistic insight”) that models of FOP generated in *Drosophila* (fruit flies) activate the highly conserved BMP signaling pathway. The most common FOP mutation, R206H, in ACVR1 and its synonymous mutation in the fruit fly receptor Sax (same as ACVR1) lead to ubiquitous activation of the pathway, albeit with important differences. Although Sax and ACVR1 are almost identical, there are subtle differences in the way that mutations in the genes activate the BMP pathway. The differences exhibited by the *Drosophila* FOP model enables a valuable comparative analysis poised to reveal critical regulatory mechanisms governing signaling output from Sax and ACVR1. Modifier screens using FOP models in the fruit fly will be extremely valuable in identifying genes or compounds that reduce or prevent the hyperactive BMP signaling that initiates HO associated with FOP.

FRUIT FLY STUDY DISCOVERS COMPONENTS OF CHRONIC PAIN PATHWAY WITH IMPORTANT IMPLICATIONS FOR FOP

Nerve sensitization (a neurological phenomenon in which repeated administration of a nerve stimulus results in the progressive amplification of a neurological response) is a common feature in chronic pain, but its cellular mechanisms are shrouded in mystery. A fascinating study (“Drosophila Nociceptive Sensitization Requires BMP Signaling via the Canonical SMAD Pathway”) published in *The Journal of Neuroscience* by Follansbee and colleagues from the University of New England in Biddeford, Maine used the *Drosophila* (fruit fly) model system to identify genes required for modulation of an injury-induced chronic pain pathway. This study specifically identified a member of the BMP signaling pathway, Decapentaplegic (Dpp), as a component of that pathway. Dpp is the fruit fly equivalent of BMP4, a potent activator of the FOP receptor ACVR1. Furthermore, overexpression of Dpp in specific fruit fly nerve cells was sufficient to induce thermal hypersensitivity in the absence of injury. Importantly, the requirement of various BMP receptors including ACVR1 in chronic pain sensitization was also demonstrated.

Previous studies have shown that FOP is not only a disease of bones and joints, but also a systemic condition that involves many organ systems including the nervous system. The authors note that these



findings are consistent with the observation that ACVR1 hyper-activation causes bone abnormalities and pain sensitization in FOP. Because nerve sensitization is associated with chronic pain, these findings indicate that human BMP pathway components may represent targets for novel pain-relieving drugs.

In summary, the results show that the BMP pathway plays a crucial role in chronic pain sensitization. Because the BMP pathway is so strongly conserved between insects and humans, it seems likely that the BMP pathway components analyzed in this study represent potential therapeutic targets for the treatment of chronic pain in humans — perhaps in FOP.

ONE SIZE DOES NOT FIT ALL WHEN IT COMES TO HETEROTOPIC OSSIFICATION

Trauma-induced HO can occur following severe burns or trauma. Because previous studies have shown that FOP is caused by activating mutations of ACVR1, studies evaluating therapies for HO have been directed primarily toward drugs for ACVR1. However, patients with traumatic HO do not carry ACVR1 mutations. In an article in *Molecular Therapy* (“Strategic Targeting of Multiple BMP Receptors Prevents Trauma-induced Heterotopic Ossification”), Ben Levi from the University of Michigan and his colleagues show that, although BMP signaling is required for traumatic HO, no single BMP type I receptor (ACVR1, ALK3 or ALK6) alone is necessary, suggesting that these receptors have functional redundancy in the setting of traumatic HO. While very broad BMP inhibitors may be necessary for traumatic HO, more specific inhibitors will be needed for FOP, and they are being developed.

KIDNEY STONES ARE THREE TIMES MORE COMMON IN FOP PATIENTS THAN IN THE GENERAL POPULATION

Physicians at The Center for Research in FOP & Related Disorders at the University of Pennsylvania reported in *BONE* (“Prevalence and risk factors for kidney stones in fibrodysplasia ossificans progressiva”) that patients with FOP have an approximately three-fold greater prevalence of kidney stones than the general population. The study comprised two independent surveys of the worldwide FOP community. In both study populations, patients with kidney stones were more functionally impaired compared to those without kidney stones. The unusually high prevalence of kidney stones in FOP may be due to high bone turnover from chronic immobilization, or to unknown mechanistic effects of the FOP mutation, increasing the disease burden and morbidity in this already disabling condition. The study outlines preventative as well as therapeutic modalities, all of which have been incorporated into the [FOP Treatment Guidelines](#).

ACUTE UNILATERAL HIP PAIN IN FOP

Flare-ups of the hips are among the most feared and disabling complications of FOP and are poorly understood. In a study (“Acute unilateral hip pain in FOP”) published in *BONE*, Fred Kaplan, Mona Al Mukaddam and Bob Pignolo evaluated 25 consecutive individuals with classic FOP who presented with acute unilateral hip pain. All 25 individuals were suspected of having a flare-up of the hip based on clinical history and a favorable response to a four-day course of high-dose oral prednisone. Ten individuals (40%) experienced rebound symptoms of pain and/or stiffness within seven days after discontinuation of prednisone and all ten subsequently developed heterotopic ossification (HO) or decreased mobility of the affected hip. None of the 15 individuals who experienced sustained relief of symptoms following a single course of oral prednisone experienced HO or decreased mobility.

Incidental radiographic findings at the time of presentation were multifactorial and included osteochondromas of the proximal femur (18/25; 72%), degenerative arthritis of the hips (17/25; 68%), developmental hip dysplasia (15/25; 60%), previously existing heterotopic ossification (12/25; 48%), intra-articular synovial osteochondromatosis (8/25; 32%), or traumatic fractures through pre-existing heterotopic bone (1/25; 4%). Thus, developmental joint pathology may confound clinical evaluation of hip pain in FOP. The most useful modality for suspecting an ossification-prone flare-up of the hip was lack of sustained response to a brief course of oral prednisone. Evaluation of soft tissue edema by ultrasound or magnetic resonance imaging showed promise in identifying ossification-prone flare-ups and warrants further analysis in prospective studies.

CLINICAL-PATHOLOGICAL CORRELATIONS IN THREE PATIENTS WITH FOP

Although the most dramatic phenotype in FOP is the episodic and progressive heterotopic ossification, patients report a number of symptoms that affect other organ systems. Post-mortem examination of FOP patients may contribute to our understanding of the underlying causes and complications of FOP.

In an article published in *BONE* (“Clinical-Pathological Correlations in Three Patients with FOP”), Ed Hsiao from University of California, San Francisco (UCSF), and his colleagues from UCSF, University of Pennsylvania, Mayo Clinic, University of Wisconsin and University of Chicago present the autopsy findings from three patients with FOP. Autopsy findings in two of the three patients confirmed that the cause of death was cardiorespiratory failure in the setting of severe thoracic insufficiency from HO. Both of these patients also had evidence of right-sided heart failure likely due to thoracic insufficiency. The third patient died from complications of a traumatic head injury after a fall, but also had post-mortem evidence of thoracic insufficiency syndrome. All three patients had extensive, widespread HO and joint deformities consistent with FOP. There was extensive ossification of the spinal ligaments which likely contributed to cervical spine rigidity. One patient was diagnosed post-mortem with a brainstem malformation. No additional significant abnormalities were noted in the other organ systems. Finally, the study supported that cadaveric skin fibroblasts can be isolated for use as a potential source for future *in vitro* cell culture studies.

Thoracic insufficiency syndrome, right-sided heart dysfunction, widespread heterotopic ossification, spinal ligament ossification and central nervous system malformations were clearly evident; however, most other non-bone tissues appeared to be spared from gross malformations.

Finally, the ability to isolate live cells from cadaveric skin is an important technique that will facilitate future studies, particularly as induced pluripotent stem (iPS) cells and other cell-based technologies evolve. This case series highlighted the importance of post-mortem examinations and their contribution to our current knowledge of disease pathophysiology and co-morbidities

FOP CLUES FROM SALIVA

Heterotopic ossification (HO) occurs in the setting of persistent systemic inflammation. The identification of reliable biomarkers would serve as an early diagnostic tool for HO, especially given the current lack of effective treatment strategies. Although serum biomarkers have great utility, they can be inappropriate or ineffective in acute traumatic injuries and in patients with FOP. Reporting in *Frontiers of Endocrinology*, Ben Levi and colleagues from the University of Michigan collected serum and saliva samples from a mouse model of trauma-induced HO and a conditionally-induced FOP mouse model and identified several candidate biomarkers of inflammation for further study. The report “Evaluation of Salivary Cytokines for Diagnosis of both Trauma-induced and Genetic Heterotopic Ossification” is the first to explore easily obtainable salivary biomarkers in animal models of HO.

PART 8 Programs at The Center



A NEW FOP MOUSE

A new and improved conditional FOP mouse model has been developed at The Center for Research in FOP and Related Disorders. The new FOP mouse model has the classic FOP mutation knocked-in to the endogenous ACVR1 locus which means that the mutation is under the control of the endogenous FOP promoter. Additionally, the mutant copy of the gene is inactive until turned-on in any desired cell type or tissue by a medication administered to the animals at any stage of development. Importantly, a fluorescent reporter gene has been engineered into the mutant gene to allow investigators to determine in exactly which cells the mutant gene has been activated.

These new FOP mice are presently being characterized. These new mice exhibit all of the features of the currently available mouse models – and more! These new mice will be available for collaborative research by contacting Dr. Eileen Shore at shore@penndmedicine.upenn.edu. This work was made possible by a grant from an anonymous and generous donor from Caldwell, New Jersey. The donor, unrelated to but inspired by, the dedication of Angel R. Cali (Ian Cali's grandfather) to FOP research asked that the mice be designated as the "ARC Mice."

THE "TOOTH FERRY" PROGRAM AT THE FOP LAB: SHEDDING LIGHT ON FOP

The participation of so many patients and families who contribute blood/DNA samples to advance FOP research has been invaluable and is enormously appreciated. These samples were critical for discovering the FOP gene and for identifying the specific DNA sequence changes that occur in classic and variant forms of FOP. Although much FOP research is more rapidly and effectively conducted using mouse models of FOP, FOP patient cells and tissues will always be essential in order to confirm that the information that we learn from mice holds true in humans.

We relied on blood samples from patients for many years since blood can be safely obtained without risk of triggering a FOP flare-up. However, blood cells provide limited information about FOP lesion formation. Fortunately, recent advances have identified additional types of human cell and tissue samples that can be obtained safely and are vitally important to our work. One of these cell types is "SHED cells."

SHED stands for Stem cells from Human Exfoliated Deciduous teeth – a long name that describes the stem-like progenitor cells that are inside primary or baby teeth. When a baby tooth falls out naturally, we can recover the cells from inside the tooth. We have used baby teeth from FOP patients to show that these cells can be grown in our lab and treated in special ways to form cartilage and bone cells, providing us with an informative system to examine how the FOP mutation affects the differentiation potential of cells involved in an FOP lesion.

A few years ago, The FOP Center started a “Tooth Ferry” Program to encourage families to send FOP baby teeth to us so that cells from these teeth could be used for FOP research. These cells have already given us bountiful information about the effects of the FOP mutation on cartilage and bone cell formation. SHED cells were used in our recent studies to down-regulate the mutant (i.e. damaged) copy of the FOP gene by siRNA and are being used in our ongoing studies on the effects of microenvironment factors on FOP flare-ups and lesion formation. Thus, SHED cells continue to be extremely vital for many of our laboratory experiments. Because the cells have a limited lifespan and since multiple samples from a person are very informative, we continually need additional “donations” to continue to conduct our studies with SHED cells.

Anyone with a child who is losing teeth can participate in the Tooth Ferry Program. When your child loses a tooth or needs to have one pulled at the dentist’s office, you can send it to us in a preassembled kit that we will provide to you. Teeth from siblings and non-family members are also welcome for comparison. In addition to baby teeth, we are also happy to receive wisdom and other permanent teeth from people with FOP. Permanent teeth also contain stem cells and we are currently investigating their use and applicability in FOP research.

Patsy Hooker is heading up our effort to collect the teeth and study SHED cells. There is a brief window of opportunity for receiving the teeth with still-healthy cells, so we have developed specific instructions for their handling and shipping. If you decide to participate, we will send you a kit including the necessary return packaging (for several teeth), return FedEx labels, Patsy’s contact information, a tooth diagram to fill out and return, and a copy of instructions. We also provide information about the program on ifopa.org, however it is very important that you contact us before sending a tooth. If teeth arrive by surprise at the lab, we may not be able to prepare them optimally.

The Tooth Ferry kit is very simple to use. This is an IFOPA-supported program and there is no cost to you. If you have children with teeth still to lose or are being pulled, please contact Patsy at (215) 898-2330 or at phooker@pennmedicine.upenn.edu and a “Tooth Ferry Kit” will be on its way to you soon!

THE CALI DEVELOPMENTAL GRANTS PROGRAM

In 1997, the Cali Family, in consultation with William N. Kelley, MD, then Dean of the University of Pennsylvania School Of Medicine, established The Center for Research in FOP & Related Disorders at the University of Pennsylvania. This was and still remains the only such center of its kind in the world. Simultaneously, the Cali Family inaugurated the vanguard Extramural Developmental Grants Program which is administered by The Center. The mission of the Developmental Grants Program is to foster collaborative research between The Center and other research laboratories of excellence at University of Pennsylvania and at other universities in the United States and around the globe. The program has been in place for 20 years and has had vast outreach to the relevant basic and translational science community worldwide.

The Cali Developmental Grants Program is proudly one of the crown jewels of the FOP research world. Over the past 20 years, the Cali Developmental Grants Program has awarded 53 grants of \$50,000 each, for a total support of \$2.65 million. This innovative program has expanded horizons in FOP research well beyond the physical boundaries of the FOP laboratory at Penn into a true world-wide co-laboratory.

Research partners include other laboratories within the University of Pennsylvania as well as other universities and institutions including Baylor; Brown; Children’s Hospital of Philadelphia; Harvard; Mayo Clinic; Northwestern; Texas A&M; University of California, San Francisco; Vanderbilt and The Max Planck Institute for Molecular Biology in Germany.



1 Mr. John Cali & Mrs. Amanda Cali with Drs. Fred & Tina Kaplan at University of Pennsylvania; 2 Ian Cali and Penn Medical Student Ronald Obinna Nwumeh meet after Ian's lecture to the first year medical students at the Perelman School of Medicine of the University of Pennsylvania; 3 Ian Cali gave a captivating lecture to 150+ first year medical students on their second day of medical school at the Perelman School of Medicine of the University of Pennsylvania. Also, in attendance at the session and pictured here are: Michelle Davis (Executive Director, IFOPA), Mrs. Amanda Cali, Adam Sherman (Director of Research Development & Partnerships, IFOPA), Dr. Eileen Shore and Dr. Fred Kaplan

The Cali Family Fund and Developmental Grants Program has funded work that led to the discovery of new therapeutic targets for FOP and to the development of kinase inhibitors, antibodies, extracellular traps, cellular pathway inhibitors and inhibitory RNA for critical proof-of-principle studies in FOP.

Importantly, more than 80 percent of the scientists and researchers who participated or were represented at the 2014, 2016 and 2017 IFOPA FOP Drug Development Forums have been direct or indirect beneficiaries of a Cali Developmental Research Grant from The Center for Research in FOP & Related Disorders.

In 2017, the Cali Developmental Grants Program continued to support three highly innovative research projects:

1. “Molecular Basis of Pathogenic Signaling and High Throughput Testing of FOP Therapies in a Zebrafish Model System” is directed by Mary Mullins, PhD, University of Pennsylvania. Dr. Mullins describes the work:

“Zebrafish have been used extensively in the study of BMP signaling, and more recently have been recognized for their power as a model of human disease. Our lab is using zebrafish development as a model of the aberrant BMP signaling found in FOP, to develop high throughput screens for potential therapeutics and to elucidate the mechanism by which FOP progresses *in vivo*.

During zebrafish development, the head to tail axis is patterned by BMP signaling in a process conserved in human development. Altering BMP signaling causes distinct dose-dependent defects, with increased signaling leading to loss of head tissues and decreased signaling leading to loss of tail tissues in the zebrafish. Previously, we determined that injection of RNA from the human FOP mutation into zebrafish embryos causes a loss of tail structures demonstrating that the mutation activates BMP signaling. By developing a transgenic FOP zebrafish model, we can screen for compounds that mitigate this feature and may be used as potential treatments for FOP.

Using zebrafish deficient in BMP signaling components, we have begun to elucidate the receptors and ligands that are involved in mutant FOP ACVR1 signaling *in vivo*. In former times, the analysis of the FOP signaling mechanism has predominantly relied on cell culture systems lacking normal *in vivo* physiology. Our zebrafish system brings a relevant *in vivo* physiological context to the study of aberrant FOP signaling. We have determined the ACVR1 requirements for FOP mutant receptor signaling and are currently examining the Type II receptors. We have identified multiple ligands that enhance signaling of the human FOP ACVR1 receptor. Our results also indicate that the FOP mutant receptor can signal in the absence of BMP ligand. These experiments are revealing the receptors that cooperate with the FOP ACVR1 to signal, providing additional targets to test with potential therapeutic compounds.

Using genome editing techniques, we are working to produce a model of the human FOP ACVR1 mutations in the zebrafish, which may more closely mimic human FOP than the current mouse model, which causes embryonic lethality. We have developed an assay to characterize heterotopic ossification in this line and then use it to screen for potential therapeutics specific to heterotopic ossification. We have begun to screen small molecule inhibitors for their ability to inhibit FOP receptor function.

We recently published our studies analyzing several variant human FOP ACVR1 mutant receptors in a special issue of the journal *BONE* on heterotopic ossification, led by Drs. Fred Kaplan, Eileen Shore and Bob Pignolo. Our studies are among the first to examine the activity of these variant mutations in an *in vivo* system. We found that these receptors can also function in the absence of BMP ligand, although they may do so less effectively than the R206H FOP receptor.”

2. “Identifying Alternative Therapeutic Targets and Genetic Interactors in FOP” is directed by Ed Hsiao, MD, PhD, University of California, San Francisco. Dr. Hsiao describes the work:

“Although FOP is caused by an activating mutation in the ACVR1 gene, wide variation in clinical activity of FOP presents significant challenges for developing therapeutics that directly target ACVR1. This work seeks to identify alternate therapeutic targets through whole exome genetic analysis of FOP patients and their non-FOP family members. The work is focused on FOP patients with unusually mild or severe disease presentations. We anticipate finding modifier genetic variants that will help us understand what mitigates or worsens FOP presentations. These genes may also be useful alternative therapeutic targets if they display higher tissue specificity or can be targeted by drugs with lower toxicity. Together this study will establish a foundation for understanding the genetic contributors to the severity of FOP.

Using a collection of DNA samples generously donated from FOP patients seen at University of California, San Francisco and University of Pennsylvania, we have been performing exome sequencing analysis to identify how genes may interact with the classic mutation in FOP to change disease severity. We have been able to identify several potential key pathways that may interact together with the classic FOP mutation. Additional experiments will help reveal which of these genes and pathways are actually critical and may identify why some FOP patients have more aggressive disease while others are spared.”

3. “Novel Allosteric Destabilizers as Therapeutics for FOP” is directed by Jay Groppe, PhD, Texas A&M University College of Dentistry. Dr. Groppe describes the work:

“With support of an Ian Cali FOP Research Grant from The Center for Research in FOP & Related Disorders at the University of Pennsylvania (initiated in 2006), the effects on, and consequences of, FOP-linked mutations in the ACVR1/ALK2 bone morphogenetic protein receptor were carefully and systematically dissected in my protein structure-function laboratory in Dallas. By spring of 2013, the results of the studies pointed to alterations in the inherent conformational plasticity of the mutant proteins as the cause of dysregulated signal transduction in FOP. Hence, a novel means of dampening the effects was conceived and tested, i.e. with drug-like molecules that bind to another (so-called allosteric) place than those such as dorsomorphin.

After acquisition of preliminary data in support of the hypothesis, a one-year translational Ian Cali Grant was awarded in the summer of 2015. With the funding, the initial proof-of-concept was further validated through identification of a small family of related compounds that inhibited the over-active mutant proteins by changing their native shapes (conformations). Remarkably, targeted binding of the compounds destabilized the mutant proteins (ALK2), which depend on an intricately organized (albeit non-static) structure in order to function properly.



Over the last year and a half, we have thoroughly dissected the mechanism underlying the novel inhibitory effects of the novel compounds, finding the trigger of the destabilization and establishing the basis for the requirement for lower pH for activity of the compounds. This effect, a serendipitous property expected to be recapitulated at hypoxic pH in trauma-induced FOP flare-ups, imparts selective efficacy on the allosteric inhibitors, providing potential for safe prophylactic use over the long term.

With the award of an Accelerating Cures and Treatments (ACT) for FOP Grant from the IFOPA, in 2018 we will extend our investigations with the mutant proteins in test tubes (*in vitro*) to cell-based assays of efficacies of the compounds, while continuing to search for and develop more potent and specific derivatives. If successful, we will test the effects of the novel therapeutic lead compounds in animal models of FOP for inhibition of heterotopic bone formation, a major milestone toward advancing the Hypoxia-Selective ALK Allosteric Destabilizers and Degraders (H-SAAD/Ds) into clinic trials with FOP patients.”

Additional information on the Cali Developmental Grants Program can be obtained from Dr. Fred Kaplan at frederick.kaplan@uphs.upenn.edu or Dr. Eileen Shore at shore@pennmedicine.upenn.edu.

FOP Ashley's Cure Finding a Cure for FOP Charitable Event at The Wagner Hotel; New York City, N.Y.

1 Ashley Martucci and Dr. Kaplan; 2 Committee Chairs Gary & Donna Segal and Joanne & Anthony Maracic (Ashley Martucci's aunts and uncles)



ASHLEY MARTUCCI FOP RESEARCH FUND

Established in 2015, the Ashley Martucci FOP Research Fund supports FOP research at the University of Pennsylvania's Center for Research in FOP & Related Disorders – the world's first and only comprehensive program in FOP. The Ashley Martucci FOP Research Fund supports:

>> Basic Research

This effort seeks to identify novel therapeutic targets for FOP and investigates molecular mechanisms and immunologic triggers. The work is focused on investigating episodic bone growth (i.e. flare-ups) and understanding what triggers flare-ups; the molecular mechanisms in cells that amplify flare-ups; the role of the immune system in triggering this process; and how mechanical factors (e.g. growth, stress or mobility) and tissue hypoxia and inflammation induce and amplify flare-ups.

>> Ashley Martucci Fellowship

Scientists Haitao Wang, PhD and Vitali Lounev, PhD, along with Michael Convente, a PhD graduate student, have been investigating the immunological triggers of heterotopic bone formation in FOP.

Three major papers were published in 2017 as a result of the work of the Martucci Fellows.



FOP: The Spoken Word – 2017

During 2017, major lectures on FOP were presented at:

- Albany Medical Center; Albany, New York
- Canadian FOP Network; London, Ontario, Canada
- Cartilage Biology & Pathology Gordon Research Conference; Lucca, Italy
- FOPeV; Valbert, Germany
- FOP Latin America; Buenos Aires, Argentina
- FOP Russia; Moscow, Russia
- FOP Italia; Sardinia, Italy
- IFOPA's FOP Drug Development Forum; Sardinia, Italy
- IFOPA's FOP Family Gathering; San Francisco, California
- Johns Hopkins University School of Medicine; Baltimore, Maryland
- Mayo Clinic; Rochester, Minnesota
- Ministry of Health of the Republic of Argentina; Buenos Aires, Argentina
- National Organization for Rare Disorders; Washington, D.C.
- St. Luke's Medical Center; Sioux City, Iowa
- TGF-Beta Super-Family Signaling in Development & Disease FASEB Conference; Lisbon, Portugal
- Thomas Jefferson University; Philadelphia, Pennsylvania
- University of California, San Francisco; San Francisco, California
- University of Missouri – Kansas City; Kansas City, Missouri
- University of Pennsylvania; Philadelphia, Pennsylvania

We would like to acknowledge the extraordinary medical, scientific and patient meetings in 2017 that we were honored to attend and in which we were honored to participate in London, Ontario, Canada; Valbert, Germany; Moscow, Russia; Sioux City, Iowa; Buenos Aires, Argentina; Sardinia, Italy; and San Francisco, California. These meetings were a wonderful opportunity to meet with scientists, researchers, physicians, students and patients from around the world.

During 2017, highlights of FOP research were presented at local, regional, national and international FOP family meetings, gatherings and fundraisers in:

- Allentown, Pennsylvania
- Albany, New York
- Blackwood, New Jersey
- Buenos Aires, Argentina
- London, Ontario, Canada
- Moscow, Russia
- Mountainside, New Jersey
- New York City, New York
- Paris, France
- Philadelphia, Pennsylvania
- San Francisco, California
- Sardinia, Italy
- Sioux City, Iowa
- Valbert, Germany



1 At the crossroads of FOP research: Lincoln's Legacy (Sioux City, Iowa); **2** At Lincoln's Legacy for FOP Research (Sioux City, Iowa). Front row (left to right): Dr. Trisha Wheelock, Dr. Fred Kaplan and Robin Gambaiana. Back row (left to right): Hudson Wheelock, Lee Wheelock, Lincoln Wheelock, Dr. Doug Wheelock, Marilyn Wheelock and Mark Gambaiana; **3** Dr. Kaplan with his kids at BINGO (From left to right): Joey Hollywood (Bridgewater, N.J.), Ian Cali (Mountain Lakes, N.J.), Justin Henke (Middletown, Del.), Lindsay Ruiz (Somers, N.Y.), A.J. Gonzales (Bellmawr, N.J.), Kathy Ford (Somers Point, N.J.), Josh Scoble (Emmaus, Pa.), Bobby Johnson (Warren, Pa.) and Ethan Szumetz (Greencastle, Pa.)

FOP: The Written Word – 2017

In 2017, publications from numerous groups on FOP and FOP-related issues appeared in peer-reviewed journals including:

- Academic Radiology
- Biochem Biophys Acta
- BONE
- British Medical Journal Case Reports
- Cell Signaling
- Cytometry B Clinical Cytometry
- Developmental Dynamics
- Disability Rehabilitation
- European Journal of Medical Genetics
- Expert Opinion Orphan Drugs
- Frontiers in Endocrinology
- Human Pathology
- Intractable Rare Disease Research
- Journal of Bone & Mineral Research
- Journal of Clinical Investigation
- Journal of Neurosciences
- Journal of Oral and Maxillofacial Surgery
- Molecular Therapy
- Orphanet Journal of Rare Disease
- Science Translational Medicine
- Scientific Reports
- Stem Cells
- Zebrafish

In 2017, there were more than 80 papers published on FOP worldwide (the most in a single year) and a tribute to the newfound and broad interest and awareness of the disease.

More than 40 papers on FOP were accepted in the special issue of *BONE* on heterotopic ossification published online in 2017 and in print in 2018.

As of January 1, 2018, the classic paper in *Nature Genetics* (April 2006) describing the discovery of the FOP gene has been cited in 729 major scientific publications worldwide.

PART 9 The International Clinical Council on FOP



The International Clinical Council on FOP (ICC) is an autonomous and independent group of 19 internationally-recognized physicians who are clinical experts in FOP from 13 nations (Argentina, Australia, Brazil, China, France, Germany, Italy, Japan, Republic of Korea, South Africa, The Netherlands, United Kingdom and the United States) and six continents (Africa, Asia, Australia, Europe, North America and South America). The ICC was established to coordinate and consolidate a global voice for the best practices for clinical care and clinical research for people who suffer from FOP. The Council was officially established, and its Constitution unanimously ratified, on June 21, 2017.



The ICC independently established its rules, committees, and criteria for membership and meets at least twice annually, either in-person and/or by teleconference. The ICC has a very proactive agenda. Formal announcements, updates and activities will be presented at relevant meetings and on websites, including ifopa.org.



The International Clinical Council on FOP meets in Sardinia, Italy. From left to right: Dr. Genevieve Baujat (Paris, France), Mrs. Amanda Cali (Ex-officio, Mountain Lakes, N.J., USA), Dr. Edward Hsiao (San Francisco, Calif., USA), Dr. Matthew Brown (Brisbane, Australia), Dr. Frederick Kaplan (Philadelphia, Pa., USA), Dr. Bob Pignolo (Rochester, Minn., USA), Dr. Patricia Delai (São Paulo, Brazil), Dr. Zvi Grunwald (Philadelphia, Pa., USA), Dr. Marelise Eekhoff (Amsterdam, The Netherlands), Dr. Maja DiRocco (Genoa, Italy), Dr. Carmen De Cunto (Buenos Aires, Argentina), Dr. Mona Al Mukaddam (Philadelphia, Pa., USA), Dr. Nobuhiko Haga (Tokyo, Japan), Dr. Christiaan Scott (Cape Town, South Africa), Dr. Tae-Joon Cho (Seoul, Republic of Korea), Dr. Rolf Morhart (Garmisch-Partenkirchen, Germany) and Dr. Coen Netelenbos (Amsterdam, The Netherlands). Not pictured: Dr. Richard Keen (London, England, United Kingdom), Dr. Michael Zasloff (Philadelphia, Pa., USA) and Dr. Keqin Zhang (Shanghai, China)

The detailed background and rationale for the ICC is described in the Preamble of the Constitution of the ICC:

“During the past 25 years, the fibrodysplasia ossificans progressiva (FOP) community has moved from the wastelands of a rare disease to the watershed of clinical trials. Together, we identified the genetic cause of FOP and used that knowledge to spearhead worldwide research efforts to develop therapies that will transform the care of individuals with FOP. We have expanded the frontiers of discovery and drug development, dismantled the physical and perceptual barriers that have impeded progress, and inspired global research in small molecules, antibodies, and gene therapy for FOP. We have formulated best practices and assembled teams of experts to optimize ambulatory and in-patient care of the FOP patient.

Research scientists at university laboratories, pharmaceutical companies, biotechnology firms and government agencies are racing to create effective treatments and to cure FOP. Presently, at least 30 independent laboratories, pharmaceutical or biotechnology companies are working on the development of kinase inhibitors, target cell inhibitors, ligand traps, antibodies, small inhibitory RNA technology, gene editing – all propelled by the historic discovery of the FOP gene and by the identification of its robust therapeutic target – the mutant ACVR1 kinase, its upstream activators, downstream targets and side-stream modulators.

The recent seismic activity in FOP basic and clinical research presents exciting challenges for clinicians caring for FOP patients worldwide. Importantly, ongoing clinical care and emerging clinical trials present medical and logistical challenges for individuals with FOP. Additionally, the pharmaceutical-biotechnology complex continually seeks expert advice from our ranks on clinical trial development – all of which hinges on critical clinical studies on the natural history and biomarkers of disease activity.

There is clearly an urgent unmet need to consolidate and coordinate clinical knowledge and advice on clinical care, symptomatic treatment and clinical trial development into a framework that best serves the needs of FOP patient community worldwide. In the past, this function has been performed informally by the various members of the International Clinical Consortium – the authors of the popular [FOP Medical Treatment Guidelines](#). It is time now to formalize this emergent opportunity as leaders in the care of FOP patients and in the robust clinical activities of the FOP community internationally.”

The mission of the ICC is:

1. **To educate** on best practices for the care of individuals with FOP
2. **To advise** on the design and conduct of interventional trials in FOP patients
3. **To publish** from time-to-time the FOP Clinical Guidelines
4. **To advocate** for a robust infrastructure for data sharing and collaboration on vital and emerging matters of clinical concern to the FOP community
5. **To identify** less explored areas of FOP patient care and issues that may drive insight into research
6. **To share** valuable clinical experiences from the care of patients with classic and variant FOP
7. **To better** understand the variable phenotype of FOP and the systemic nature of FOP pathology

The ICC has five standing committees that meet regularly in person and by teleconference:

» **Governance & Membership Committee**

Function: To establish the ICC governing rules, membership terms, auditing processes, bylaws

» **Ethics Committee**

Function: To guard the health and safety of FOP patients by supporting transparency and compliance with good clinical practices

» **Communications & Relations Committee**

Function: To provide the external communications to the public

» **Publications Committee**

Function: To revise and publish the clinical guidelines and provide the resource for all materials published on behalf of the ICC

» **Clinical Trials Committee**

Function: To provide guidelines for clinical trials in support of safe and transformative treatments for FOP



The Governance & Membership Committee of the ICC meets in Sardinia, Italy. From left to right: Dr. Tae-Joon Cho (Republic of Korea), Dr. Frederick Kaplan (USA), Dr. Coen Netelenbos (The Netherlands), Dr. Zvi Grunwald (USA), Dr. Mona Al Mukaddam (USA)

THE FOUNDING MEMBERS OF THE ICC ARE:

Mona Al Mukaddam, MD

Philadelphia, Pennsylvania, USA

Genevieve Baujat, MD

Paris, France

Matthew Brown,

MBBS, MD, FRACP, FAHMS, FAA
Brisbane, Australia

Tae-Joon Cho, MD

Seoul, Republic of Korea

Carmen L. De Cunto, MD

Buenos Aires, Argentina

Patricia L. R. Delai, MD

São Paulo, Brazil

Maja Di Rocco, MD

Genoa, Italy

E. Marelise W. Eekhoff, MD, PhD

Amsterdam, The Netherlands

Zvi Grunwald, MD

Philadelphia, Pennsylvania, USA

Nobuhiko Haga, MD

Tokyo, Japan

Edward Hsiao, MD, PhD

San Francisco, California, USA

Frederick S. Kaplan, MD

Philadelphia, Pennsylvania, USA

Richard Keen, MD, PhD

London, United Kingdom

Rolf Morhart, MD

Garmisch-Partenkirchen, Germany

J. Coen Netelenbos, MD, PhD

Amsterdam, The Netherlands

Robert J. Pignolo, MD, PhD

Rochester, Minnesota, USA

Christiaan Scott, MBChB

Cape Town, South Africa

Michael Zasloff, MD, PhD

Washington, DC, USA

Keqin Zhang, MD, PhD

Shanghai, China



PART 10 Awards & Honors

AWARDS & HONORS

In 2017, the National Organization for Rare Disorders (NORD) honored Dr. Kaplan with its **Rare Impact Award**, which recognizes those working to make a difference in the lives of people with rare diseases. In accepting the award on May 18, 2017 at a ceremony in Washington, D.C., Dr. Kaplan delivered the following address and tribute to the FOP community:

“Thank you NORD for this truly magnificent honor and thank you to our FOP community – those who are here tonight and those who are watching at home and around the world – who share in this tribute, including my lifelong professional partner Dr. Eileen Shore. Thank you to my loving wife Tina, Clementia Pharmaceuticals, the IFOPA, my friends and colleagues in academia and industry, and most especially, my patients and their families.

This award is about impact. You have spoken kindly about the impact I have had on your lives. But personal impact is a mutual process – the action of one soul on another. Without mutuality, there is no impact.

I pay tribute tonight to the tremendous impact that the FOP community – and that still, quiet and powerful patient voice – has had on me.

One extraordinary person, in particular, is Jeannie Peeper – a FOP patient and dear friend who used a magic loom to first imagine and then create the IFOPA to end the isolation of an ultra-rare disease, to stimulate monumental scientific progress, and to weave together a scattered global community into a single indelible tapestry from the golden thread of committed patients and families.

Jeannie did this not from a tried formula of what other nonprofits do, but from a unique vision of empathy, compassion, commitment and need, based on patients and their families.

Diseases like FOP don’t just affect individuals, they affect entire families – and Jeannie mobilized that faction in her vision and in her work. The tremendous accomplishments of this global community are due, in no small part, to that shared vision and voice – the patient voice. Patients are the reason, the strength and the inspiration that allow us all to care, to do research, to work tirelessly for a better future. Jeannie is that voice.

The resilience of the global village that Jeannie created, with the towering guidance of Dr. Michael Zasloff, and fostered by the incredible generosity of Diane Weiss, the Cali’s, the Weldon’s, and by the tireless work of FOP families around the globe has stretched to the ends of our human universe and to the limits of our medical and scientific imagination and has kindled hope with dedicated purpose and relentless determination.



1 Drs. Tina & Fred Kaplan with Dr. Larry Jameson, Dean of the Perelman School of Medicine of the University of Pennsylvania at The Distinguished Graduate Award Ceremony (Philadelphia, Pa.); 2 Jeannie Peeper (Winter Springs, Fla.), President Emeritus and Founder of the IFOPA; 3 Mrs. Diane Weiss (Boca Raton, Fla.) – Benefactor of The Isaac & Rose Nassau Professorship of Orthopaedic Molecular Medicine at the University of Pennsylvania with Dr. Fred Kaplan

The impact that Jeannie and the global FOP family have had on me is unfathomable. You are a chorus of angels who have invited me into your homes, into your hearts and into your lives.

You have taught me what every doctor should know and relearn every day – that diseases are not just physical processes, but human experiences. You have endowed my life with purpose and meaning.

That's the kind of impact we all want to have.

Thank you NORD and our entire FOP family for allowing me to make an impact on your lives.

And, most importantly; thank you for making an indelible impact on mine."



PART 11 FOP: “What Can We Do to Help?”

As this annual report opened in the twilight zone on the maiden moon voyage, it is entirely fitting to recount this story.

On a visit to the launch complex at Cape Canaveral in 1961, President Kennedy is said to have asked a man who was sweeping the floor what he was doing. “Mr. President,” he said, “I am helping to put a man on the moon.” What is the message of that parable? Every job is important and teamwork is essential to achieve complex goals.

Patients, families, friends, even casual visitors to The Center for Research in FOP & Related Disorders often ask: “What can we do to help?” The answer is simple. “Anything you can.”

As Kate Griffo and John Glick at the University of Pennsylvania’s Perelman School of Medicine said, “In philanthropy, as in medicine, even brief inaction can do harm. A hiatus in research funding may mean that a promising treatment or a new line of inquiry may come to an untimely and devastating end. A break in efforts could halt progress toward finding a treatment that could relieve suffering or save lives.”

Research is laborious, time consuming, often frustrating, and costly, and is filled with false starts, blind alleys, glimmers of hope and the fog of frustration, but so too is the FOP we are trying to cure. Formidable enemies require formidable opponents, and teamwork requires resources. When seminal discoveries are made and ignorance is extinguished, the fog lifts and the summits and the paths between them become clear. When knowledge advances, it illuminates the next horizon. It is a powerful beacon that changes the world like nothing else can. The feeling of accomplishment for all who contribute to this endeavor lights a fire of personal fulfillment and brings knowledge that they have contributed something important and enduring for other human beings for generations to come.

When modern FOP research began 27 years ago in a small laboratory at the University of Pennsylvania, there was little knowledge about this terrible disease and little hope outside an infinitesimally small circle of believers who knew in their heart that something could be done to change it. Hope prevailed – hope fueled by the faith and commitment of a dedicated and persistent few who year after year funded studies to create and sustain a team devoted to make a difference. Over the years, that team has grown and expanded, and its reach now extends around the world.

Through a sustained effort at The Center for Research in FOP & Related Disorders, research is eradicating the stifling ignorance that was prevalent just two decades ago. Barrier after barrier has fallen and achievable goals are in reach. FOP research holds real promise of preventing, treating



1 More than 1,700 FOP supporters play BINGO at the 11th Annual Joshua's Future of Promises Bingo for a Cure in Allentown, Pa.; **2** The Center for Research in FOP & Related Disorders at Bingo for a Cure (Seated): Renee Jurek, Katherine Toder and Fred Kaplan (Standing): Rob Burgese, Kay Rai, Ruth McCarrick-Walmsley, Will Towler and Salin Chakkalakal; **3** Josh Scoble (Emmaus, Pa.) delivers opening remarks at Bingo for a Cure while the Star Wars Storm Troopers look on; **4** Joe Hollywood, Kay Rai, Ruth McCarrick-Walmsley and Will Towler at Bingo for a Cure; **5** Kathy & Chuck Ford (Somers Point, N.J.) at Bingo for a Cure

and curing FOP. It is no longer an imaginary dream. We need your help now more than ever to make this a reality.

The often-heard comment, “Call us when you have a treatment or a cure,” is an option, but not one that will help us find a cure. Everyone has a stake in this effort. We need your help in getting there: bake sales, swimming events, Burns’ Suppers, barn dances and bingo; chicken barbeques and spaghetti dinners, garage sales and silent auctions; country fairs and benefit concerts at the Metropolitan Opera; raffles and rodeos, sales of holiday cards and embroidered quilts, 5K runs and ice fishing contests; chamber music benefits and hard rock concerts; horse-plowing contests and competitive swims; golf tournaments and bowling parties; wine tasting events and lemonade stands on busy street corners.

No idea or endeavor is too small or too outlandish to help. Every second counts.
Please help cure FOP.



PART 12 Many Thanks to You

MANY THANKS TO YOU

The members of The Center for Research in FOP & Related Disorders at the University of Pennsylvania and at collaborating laboratories around the world are extremely proud to be a part of this mission; and are enormously grateful to all of those who support this vital research effort to find better treatments and a cure.

Much has been accomplished, thanks in large part to the many benefactors and partners who have supported our work. The Center for Research in FOP & Related Disorders identified the genetic cause of FOP in 2006 and used that knowledge to spearhead worldwide research efforts to develop therapies that will transform the care of individuals with FOP. In 2014, clinical trials for FOP began – a major step forward. Now, as a comprehensive center, we manage and coordinate care for FOP patients, not only at University of Pennsylvania, but globally, and also engage in vital clinical, basic science and translational research that can change the course of this rare and debilitating condition. We are vitally committed to education; we want to ensure that the next generation of physicians and scientists is as passionate about FOP research as we are.

Despite the progress we have made, there are still many unanswered questions and more monumental discoveries on the horizon that will improve treatment and bring us closer to ultimately finding a cure. Our work is broad and focuses on several areas of major activity, including: clinical care and consultation worldwide; clinical research and infrastructure development; basic research to identify therapeutic targets; translational research for preclinical drug testing and biomarker discovery; clinical trial development; and education.

The generous support of our benefactors has led to new therapeutic targets for FOP, new drug discoveries and a rich research pipeline with diverse approaches to treatment of FOP. Our lifelong goal is to propel the development of therapies and eventually a cure for children and adults with FOP. This year, new clinical trials were launched, and more are anticipated in the year ahead. We envision the day when FOP patients no longer hear the words “no treatment, no cure.”



We acknowledge the generous support of:

- The International FOP Association
- The National Institutes of Health (The people of the United States of America)
- The Penn Center for Musculoskeletal Disorders (PCMD)
- The Cali Family Endowment for FOP Research
- The Weldon Family Endowment for FOP Research
- Ashley Martucci Fund for FOP Research
- The Isaac and Rose Nassau Professorship of Orthopaedic Molecular Medicine
- The Cali-Weldon Professorship of FOP Research
- The Roemex Fellowship in FOP Research
- The Jesse David Hendley Foundation
- The Morgan Fund for FOP Research
- The Canadian FOP Families & Friends Network
- FOP Australia
- FOPeV (Germany)
- FOP Italia
- Michael & Donna Gordon
- Gary Whyte
- The people of Santa Maria, California
- A generous and anonymous donor from Caldwell, New Jersey
- And the many individuals, families, friends and communities throughout the world who contribute generously and tirelessly to the FOP effort



PART 13 The Last Word – A Tribute

“To make an end is to make a beginning.
The end is where we start from.” — T.S. Eliot

The last word belongs not to the donors and benefactors, not to the physicians, scientists, researchers, journalists, or historians, but to the patients who struggle valiantly and who look to us for a better way. The last word always belongs to them. The following tributes were written by Jeannie Peeper, President Emeritus and Founder of the IFOPA, on the passing of two of our dear friends, Sarah Steele and Stephanie Snow.



Sarah Steele (1984-2017)

“With deep sadness, I share with you that our dear friend, Sarah Steele passed away peacefully. Sarah was 32 years old, just shy of her 33rd birthday. We have lost a friend and gained an angel.

Sarah joined the IFOPA in 1991 and I first met her and her parents that same year at the First Symposium on FOP in Philadelphia. She was six years old at the time and with only eight people with FOP attending that first event, she was one of the adorable children that drove us to work harder at the IFOPA to find a treatment.

Sarah’s list of achievements and impact within the FOP community are still felt today. She co-authored with her mom, Marilyn Hair, and illustrated the first book for children: “What is FOP? Questions and Answers for the Children.” She loved to make pencil holders and sell them to raise money for FOP. She developed the “theme” and the illustration for the 1995 Symposium “Accentuate the Positive” – a motto Sarah lived by. She also added the patient voice when speaking to scientists and researchers at this Symposium. Her impact did not stop there, she translated many IFOPA print materials over the years into Spanish, continued to advocate for FOP, most recently at the 2nd Drug Development Forum on the patient panel. Sarah was also a volunteer for the IFOPA’s Quality of L.I.F.E Awards committee. However, she may be most remembered by the many people she reached out to in the community offering a kind or supportive word to their life journey with FOP. And in song – Sarah loved to sing in the choir at her church. Her kindness radiated for so many of us.

Sarah's generous spirit lives on with her extraordinary gift to research, and all of us, as a donation of her tissues to the biobank at University of California, San Francisco. Sarah's legacy will live on and teach us so much.

Sarah will be in my heart forever. We have been on this journey together, spending fun and memorable times at different IFOPA events. Her kindness, support and generosity will be forever remembered."



Stephanie Snow (1991-2018)

"My heart is broken, and I am truly at a loss for words – another FOP angel and another loss for the FOP community. My close friend Stephanie Snow, age 26, passed away yesterday, January 2, 2018. She fought a very hard battle against not only FOP, but another rare condition known as myoclonus.

Stephanie is one of my heroes. Her strength, bravery and courage always amazed me, and I so admired her. Her kind smile and beautiful spirit will be deeply missed by all that knew and loved her.

I have known Stephanie and her family since 1994, when they joined us as pioneers of the IFOPA. She started sharing her FOP story at age four – appearing on local TV interviews with her parents to spread FOP awareness and to promote their fundraising efforts.

The Snow family held many grassroots events, including the Find-a-Cure Bar-B-Que, which was the longest running annual event in IFOPA history – 17 years and raised more than a million dollars.

Throughout her young life, Santa Maria has raised funds for a cure and cheered Stephanie on in her battle.

In early December, Stephanie wrote on Facebook:

"I wanted to let everyone know that my health has severely declined in the last eight months. Unfortunately, FOP has taken my physical mobility away for me. I am trying to accept the fact that I have been faced with a fight that I can't win. I just want to say thank you to all my family and friends because it wasn't for the money raised in the 16 years of fundraising, we wouldn't be where we are in regards to research and the clinical trials."

Stephanie was deeply loved and supported by her local community. She touched many lives in a profound way. Stephanie and I had the privilege to participate together in the FOP gene discovery at the University of Pennsylvania in 2006. We both spoke at this event and Stephanie graced the front page of the *Philadelphia Inquirer* and other newspapers highlighting this life-changing announcement. We were both very proud to be a part of this historic and monumental milestone.

Stephanie had a passion for animals. They always brought her great joy! She raised rabbits and lambs for 4H and showed them at fairs, fostered many kittens that she loved, had a variety of chickens, crabs and fish too. She especially loved her faithful service dog, Elliott, and her three cats – Tanner, Hunter and Lexi.

In 2012, Stephanie was awarded the Emerging Leader Award by the IFOPA for her many years of dedicated work for the FOP cause – 20 years of fundraising and funding research, awareness-building in the early years that highlighted the FOP fight for both the community and the public, and her passion for bringing awareness to neurological issues surrounding FOP.



Stephanie's motto since the time of the gene discovery was "Hope is Alive!" And as in life, her motto carries forward with her extraordinary legacy gift to the FOP community and research at the biobank at University of California, San Francisco. There is no doubt that there is much to be learned about FOP and the neurological connections. It is comforting to know that Stephanie's motto "Hope is Alive" carries on in all of us. We will all miss you."



Penn Medicine