

The Twenty-Fifth Annual Report of the Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project

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twenty-five years



DISCOVERIES

ACHIEVEMENTS

June 2016

Front Cover Photos.

Black and White Photos, Top to Bottom:

- 1) Dr. Michael Zasloff, Jeannie Peeper and Dr. Fred Kaplan, c. 1991
- 2) Dr. Eileen Shore, c. 1992
- 3) Dr. Fred Kaplan and Mrs. Diane Weiss, c. 1998
- 4) The Cali Family: (Left to Right) John, Ian and Amanda
- 5) The Weldon Family with Dr. Fred Kaplan:
(Left to Right) Bill, Whitney, Dr. Kaplan, William, and Hillary

Color Photo: Drs. Fred Kaplan, Robert Pignolo and Michael Zasloff observe while Dr. Vitali Lounev performs an experiment at The Center for Research in FOP & Related Disorders

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Clinics Are Laboratories; Laboratories of the Highest Order. - William Osler, M.D.



Photo Left: The First International FOP Symposium. The University of Pennsylvania. September, 1991.

Photos Below: The Opening Day of the FOP Laboratory at The University of Pennsylvania; May, 1992. In the bottom right panel: Drs. Michael Zasloff, Eileen Shore, and Fred Kaplan. Cutting the ceremonial ribbon, Ms. Carol Orzel



1992



Left Newspaper Article: "Finally, With Genetic Discovery, Hope for Escape From a Prison of Bone" – The New York Times; Tuesday May 9, 2006. Featured, Hayden Pheif of Mill Valley, CA

Part I: Introduction

A recent advertisement famously proclaimed: “The best way to predict the future is to create it.” For FOP, the future was created by two serendipitous encounters that occurred a year apart and that revolved around each other like binary stars – each gathering light from the other. The first “future-predicting encounter” occurred in September, 1987 at The National Institutes of Health - when Jeannie Peeper and Michael Zasloff exchanged information about the existence of 20 patients with FOP. That auspicious moment led to the establishment of the IFOPA by Jeannie on June 8, 1988 - to connect patients with FOP, to end the isolation of living with FOP, and to make a difference for future generations.

The second “future-predicting encounter” occurred a year later, on Wednesday September 28, 1988 in a windowless room of the Genetics Clinic at Children’s Hospital of Philadelphia where Michael Zasloff and Fred Kaplan, two physicians seeing patients there that morning (and who had never met) literally bumped into each other on their way to lunch. An unexpected conversation lasting over two hours about the profound ignorance surrounding a strange and rare condition called FOP led to a transformational exchange of ideas on that otherwise ordinary Wednesday morning.

From that prosaic beginning came the FOP Collaborative Research Project in September 1989; the First International Symposium on FOP, the First Annual Report of the FOP Collaborative Research Project, and the addition of Dr. Eileen Shore to establish a laboratory dedicated to FOP research in September 1991; the official dedication of the FOP Laboratory at the University of Pennsylvania in May, 1992; the sustained and generous support of the IFOPA; the establishment of The Cali and Weldon Endowments in 1995 (through the wonderful generosity of the Cali and Weldon families); the establishment of The Isaac & Rose Nassau Professorship of Orthopaedic Molecular Medicine for the study of FOP in 1997 (through the generous and visionary philanthropy of Mrs. Diane Weiss); the



Mrs. Diane Weiss (Boca Raton, FL; Benefactor of The Isaac & Rose Nassau Professorship of Orthopaedic Molecular Medicine at The University of Pennsylvania) visits the Perelman School of Medicine at Penn

creation of **The Center for Research in FOP and Related Disorders** at the University of Pennsylvania in 1997 (through the incredible generosity of the Cali Family and the foresight of William N. Kelley, M.D., then Dean of The University of Pennsylvania School of Medicine); and the establishment of The Cali-Weldon Professorship for FOP Research in 2012 (through the generosity of the Cali and Weldon families).

It has taken 28 years, generous benefactors, countless volunteers, hundreds of patients, scores of physicians and scientists, innumerable medical and scientific studies, a myriad of symposiums, meetings, conferences and clinics, the explosive interest of the pharmaceutical and biotechnology industry and the steady growth of a committed international community. Where we are today bears no resemblance to where we were 25 years ago when the first Annual Report was written in 1991.

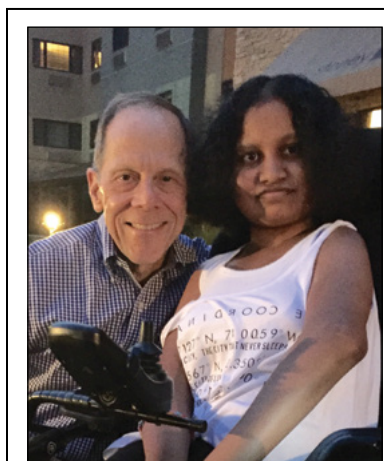
The report began, “The University of Pennsylvania Collaborative Research effort in FOP was established in September 1989... This effort arose out of a mutual desire to establish the cause and find a cure for this disabling disease.”

There was a time, not long ago, when the world ignored FOP, simply brushed it aside as an odd curiosity, and passed it by. Not so, any more. “The best way to predict the future is to create it.”

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 establish treatments and a cure for FOP. During the



Sivapriya Saravanakumaran (Woodbury, MN) visits with Dr. Kaplan

past twenty-five years, we have moved from the wastelands of a 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 of individuals with FOP.

In partnership with our benefactors, we have expanded the frontiers of discovery and drug development in this rare and catastrophically disabling condition, dismantled the physical and perceptual barriers that have impeded progress, and inspired global research in small molecules, antibodies, and gene therapy for FOP.

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

1. Clinical Care and Consultation Worldwide
2. Clinical Research and Infrastructure Development
3. Basic Research (Identification of Therapeutic Targets)
4. Translational Research (Preclinical Drug Testing & Biomarker Discovery Program)
5. Developmental Grants Program
6. Clinical Trial Development and Proof-of-Principle Investigation in Patients
7. 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 the following milestones in our FOP program:

Clinical Care and Consultation Worldwide

- Guided patients, families and doctors worldwide in their daily battles with FOP.
- Directed the world's current and historically largest FOP clinic and referral center, now in its new home at Penn Medicine - University City, Philadelphia, Pennsylvania.
- Coordinated medical management and dental care of FOP patients worldwide.
- Conducted international FOP clinics for patients and families in Rome, Italy; Valbert, Germany; Eskilstuna, Sweden; and Moscow, Russia.



Ursula and Laura Schaefer (Mainz, Germany) with Dr. Kaplan at the annual FOPeV meeting in Valbert, Germany

Clinical Research and Infrastructure Development

(Natural History Studies Highlighted Here)

- Published the findings of a global survey of FOP flare-ups that will inform the design and evaluation of ongoing and future clinical trials.

- Drafted a report of the first patient-reported Longitudinal Natural History Study in FOP.
- Drafted a report of the FOP Cumulative Analogue Joint Involvement Scale (CAJIS) that validates a rapid and clinically useful global evaluation of FOP.
- Consulted on the development and implementation of a patient-reported physical function outcome measure for adults and children with FOP.
- Consulted on the development and implementation of the Clementia Longitudinal Natural History Study.
- Advocated the prospective deposit of data from the Clementia Longitudinal Natural History Study into the IFOPA Registry Database.
- Championed one international registry for FOP by the IFOPA, and owned by the FOP community.

Basic Research

(Identification of Therapeutic Targets)

- Investigated molecular mechanisms of biochemical and immunologic triggers of FOP flare-ups in state-of-the-art knock-in mouse models of classic FOP.
- Studied an adult FOP patient with the classic FOP mutation who has minimal heterotopic ossification and initiated biochemical, immunologic, and genetic studies to determine why.
- Investigated molecular cross-talk between the bone morphogenetic protein (BMP) signaling pathways and immune-mediated pathways in connective tissue progenitor cells (surrogates for the development of heterotopic ossification) in FOP.
- Investigated cellular inflammatory triggers of early FOP lesions using novel triple knock-in

FOP mouse models in order to identify key immunologic targets for therapy.



Clementia Pharmaceuticals visits the FOP Center at Penn. (From left to right): Michael Harvey (Clementia), Dr. Eileen Shore (Penn), Dr. Maurizio Pacifici (Children's Hospital of Philadelphia & Penn), Dr. Donna Grogan (CMO, Clementia), Dr. Isabelle Lemire (Director, Preclinical Research, Clementia), Dr. Clarissa Desjardins (CEO, Clementia Pharmaceuticals) and Dr. Bob Pignolo (Penn)

- Investigated the role of innate immune cells in the induction of heterotopic ossification in FOP conditional knock-in mice.
- 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.
- Initiated studies in primary human FOP HO progenitor cells to test the veracity and strength of the Activin A discovery reported by Regeneron Pharmaceuticals.
- Initiated studies to investigate potential roles of Activin A in immune cells using a mouse model of FOP.
- Investigated the response of the dysregulated ACVR1 signaling pathway in FOP to physical cues in the early FOP lesional microenvironment.

- Developed an *in vivo* strategy in a conditional knock-in mouse model of FOP to express the FOP mutation in early development in order to induce spontaneous heterotopic ossification and to test drug efficacy.
- Applied this mouse model of early FOP mutation expression to examine the effects of the mutation on skeletal bone growth and endochondral ossification in growth plates.
- Showed in mouse models with the classic FOP mutation that Palovarotene prevents spontaneous heterotopic ossification (HO) as well as injury-induced HO.
- Demonstrated that Palovarotene restores normal structure and function to FOP growth plates, suggesting the possibility of safe and early intervention for HO in children - an observation with major implications for clinical trials
- Characterized the cellular and molecular phenotype of chondrogenesis (cartilage formation) at the growth plate and in heterotopic endochondral ossification in response to Palovarotene in an advanced conditional knock-in mouse model of classic FOP.
- Investigated the genetic and molecular mechanisms of abnormal joint formation and functional joint survival in FOP - fundamental knowledge that will be critical for identifying new therapeutic targets to treat and prevent disabling degenerative arthritis in FOP.
- Investigated molecular mechanisms by which ultra-rare FOP variants trigger promiscuous BMP signaling leading to FOP flare-ups.
- Continued to expand and develop the FOP SHED Cell Tooth Fairy Program. This substantial library of connective tissue progenitor cells is essential for ongoing and future studies in therapeutic target identification and drug discovery in FOP.
- Established mouse embryonic fibroblasts (MEF) *in vitro* cell assays for drug screening.
- Determined that mutant ACVR1 (mACVR1) alters the response of cells to their mechanical environment suggesting that mutant cells are predisposed to misinterpret signals from their tissue environment as instructions to form cartilage and bone.
- Initiated studies to identify mACVR1 modifier genes/factors in a man with the FOP mutation and characteristic toe malformations, but who is asymptomatic for heterotopic ossification. Exome sequencing was conducted on DNA samples from the patient, his unaffected brother, and both unaffected parents. The data were analyzed using multiple approaches and data filters, identifying candidate genes, and cellular pathways/processes of interest. To add strength to this analysis, DNA samples from three additional mild FOP patients have been prepared and are being processed for exome sequencing.



Timur Dautov and his mother Munira (from the Republic of Bashkortostan, Russia) meet with Dr. Kaplan at The Second International FOP Russia Family Meeting and Conference in Moscow, Russia

Translational Research

(Preclinical Drug Testing & Biomarker Discovery Program)

- Completed the third-year of a comprehensive pre-clinical drug-testing and biomarker discovery program in FOP mouse models.
- Screened new categories of compounds for efficacy in preventing HO in advanced FOP mouse models.
- Further characterized a key biomarker of heterotopic cartilage formation in FOP in mice and humans, continued to study the biomarker in an ongoing clinical trial, and submitted findings of pre-clinical and early clinical studies of the biomarker for publication.

Developmental Grants Program

- Awarded two new developmental research grants, one on a zebrafish model for FOP and another on a novel allosteric approach to inhibit mACVR1 in FOP.

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

- Continued Phase-2 clinical trials and proof-of-principle investigation with Palovarotene, an inhibitor of endochondral bone formation, in FOP patients. (The rationale and design of the clinical trials can be found on any or all of the following three websites):

www.ifopa.org

www.clinicaltrials.gov

www.clementiapharma.com

- Enrolled FOP patients in three major interventional clinical trials with Palovarotene.

- Advised 30 pharmaceutical and biotech companies on the development of novel drugs for clinical trials in children and adults with FOP.
- Advanced understanding of small molecule inhibitors in physiologic and pathologic chondrogenesis – knowledge and approaches that are vital to future clinical trials – involving children

Education

- 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.

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 to help us accomplish our ultimate mission.



The freshman medical students of The Perelman School of Medicine at The University of Pennsylvania honor Ian Cali after his captivating lecture on their first day of class



The scope of research in the FOP laboratory covers a range of investigations that are focused on basic science discoveries that lead to preclinical analysis of potential treatment strategies for FOP and on understanding the molecular and cellular basis of FOP disease onset and progression.

The collaborative activity and accomplishments of basic research at the FOP Laboratory are a key focus of the **Center** and focus on six major research areas:



Graduate students Niambi Brewer and Alexandra Stanley at The Center for Research in FOP & Related Disorders

Immunologic and microenvironmental mechanisms that induce and amplify HO and FOP flare-ups are conducted by graduate students, Michael Convente, Niambi Brewer, and Alexandra Stanley, and research scientists Haitao Wang and Vitali Lounev. Their projects investigate the cellular response to the immunologic, biochemical, physical, 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 repurposed drugs.

***In vitro* and *in vivo* FOP model development** is conducted by post-doctoral fellows and Salin Chakkalakal and Girish Ramaswamy, by graduate students Michael Convente, Robyn Allen, and Alexandra Stanley and by research scientists Vitali Lounev, Deyu Zhang, 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 treatment candidates. This work is a vital part of the infrastructure for drug discovery and development – the infrastructure for a cure.



Graduate students Michael Convente and Will Towler enjoy a round of Bingo at Joshua's Bingo for a Cure to Benefit FOP Research in Allentown, PA

FOP progenitor cell studies are conducted by Vitali Lounev, Michael Convente, Salin Chakkalakal, Haitao Wang, Will Towler, Alexandra Stanley, Andrew Chang, Ruth McCarrick Walmsley, and Robert Caron. These studies identify the specific cells and mechanisms that can be targeted to block heterotopic ossification.

Developmental biology studies of FOP are conducted by Will Towler, Salin Chakkalakal, and collaborator Adam Resnick and his team. These projects include two research areas:

1. Effects of the FOP mutation on skeletal development, joint formation, and degeneration.
2. Shared disease mechanisms of mACVR1 in DIPG brain tumors and FOP.

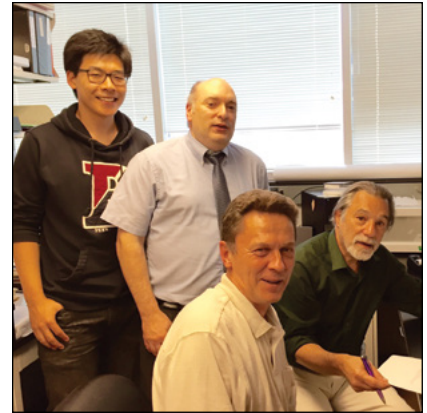
Molecular pathway studies in ACVR1 signaling in FOP are conducted by Meiqi Xu, Salin Chakkalakal, Michael Convente, Haitao Wang, and Alexandra Stanley, Robyn Allen, Will Towler, and Niambi Brewer. This vital research enables the development of drugs that target these pathways.

Pre-clinical drug testing in an FOP mouse model is conducted by Haitao Wang, 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 triggers FOP flare-ups?
- How does FOP progress in the absence of flare-ups?
- How do the immune system and the lesional 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 do the ultra-rare genetic variants of FOP (which affect only 2 to 3 per cent of FOP patients worldwide) teach us about the function of the genetic switch that drives heterotopic ossification in FOP?

These questions and more are under intense investigation in the FOP Laboratory, and their answers will help identify novel targets for drug discovery and development.



Penn Undergraduate student Andrew Chang, and Drs. Robert Pignolo, Vitali Lounev and Michael Zasloff plan an experiment at The Center for Research in FOP & Related Disorders



Joshua Scoble (Emmaus, PA) visits with Dr. Pignolo at The FOP Center



Joey Hollywood (Bridgewater, NJ) visits Dr. Kaplan at The FOP Center



Kay Rai, Casey Shechtman and Dr. Bob Pignolo at The FOP Center at Penn

"From Hopeless to Hopeful"

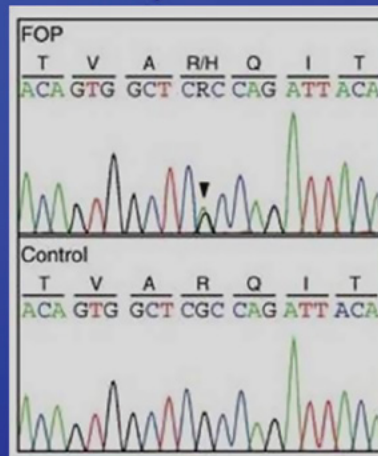
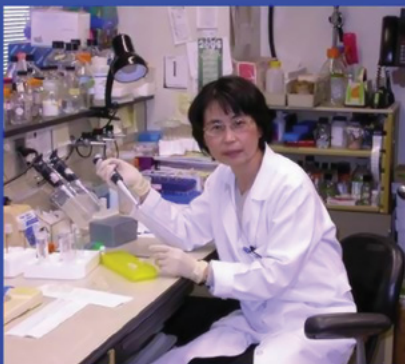


-Ian Cali

Photo Left: The FOP gene discovery marked the pivotal point in the history of FOP. Ian Cali described this as the moment when things went "From Hopeless to Hopeful."

Photo Right: Meiqi Xu at her lab bench where the FOP gene was discovered. April, 2006.

ACVR1 (c.617 G>A; R206H)



One misspelled letter in 6 billion

One of the most highly specific disease causing mutations in the human genome



**ACVR1
(R206H)**



**Inflammation;
Hypoxia;
Innate
Immunity**

Left: The FOP gene mutation [ACVR1(R206H)] turns a teddy bear into a sleeping grizzly bear. Inflammation, hypoxia and the innate immune system wake up the sleeping grizzly bear

Part II: Research Breakthroughs

Two notable discoveries this past year – one from the pharmaceutical industry and one from the FOP Center - are likely to transform the basic understanding and therapeutic landscape of FOP. In a nutshell, these discoveries showed that 1) the pathological activity of the mutant FOP receptor (mACVR1) is sensitive to an unsuspected extracellular hormone-like protein and immunological mediator, Activin A (Act A). Blocking the activity of Act A blocks heterotopic ossification (HO) in a mouse model of FOP; and 2) early FOP lesions are profoundly hypoxic (oxygen starved). The intracellular ‘alarm’ protein, HIF1- α which regulates the cellular response to hypoxia, retains mACVR1 in a signaling capacity when it should be destroyed, thus dramatically amplifying mutant BMP pathway signaling and stimulating HO. Blocking the activity of HIF1- α substantially diminishes HO in a mouse model of FOP.

Throughout the history of modern FOP research, transformative ideas have led to advances that have refined and deepened our understanding of FOP. The identification of the FOP gene in 2006 identified a prime target for therapy, now the focus of intense activity in the pharmaceutical and biotechnology industry worldwide. As Albert Einstein famously explained, “The mere formulation of a problem is often more essential than its solution, which may be merely a matter of mathematical or experimental skill. To raise new questions - to regard old problems from a new angle requires creative imagination and marks real advances in science.” This past year marked real advances in the deep understanding of FOP.

The ideas behind these transformative insights have the strange quality of an Escher painting - one is able to see something – and then to see something else. The broadest brushstrokes suggest a picture in which inflammation and the extracellular hormone-like molecules BMPs and Act A play a key role in initiating flare-ups – while the intracellular hypoxia alarm HIF1- α plays a key role in amplifying and fueling flare-ups. The first reaction

requires ligand; the second reaction is completely independent of ligand. Yet, both are true – much like a match may be needed to start a fire but is not needed to sustain it.

Thus, in the complexity of an FOP lesion, both ligand-sensitive and ligand- independent processes are paramount in amplifying BMP signaling from the mutant FOP receptor (ACVR1). That means that not only the ACVR1 receptor, but the Act A ‘fuse’ and the HIF1- α ‘amplifier’ (both denizens of the inflammatory response) are – along with mACVR1 itself - robust targets for therapy of FOP.

These discoveries subtend a deeper understanding of FOP. The elucidation of these strange and complimentary ideas have captivated our research world during the past year, and are the focus of Part II of the 25th Annual Report.

A. Lighting the Fire: Act 1 for Act A

In a landmark article published in September, 2015 in *Science Translational Medicine*, researchers at Regeneron Pharmaceuticals, Inc. in Tarrytown, New York announced that heterotopic ossification in FOP is caused by Activin A (Act A), a hormone-like factor not previously thought to play a role in the disease. In a series of *in vitro* analyses and *in vivo* studies using genetic animal models of classic FOP, the scientists showed that Act A stimulates the BMP signaling pathway when the mutated bone morphogenetic protein (BMP) receptor ACVR1 (R206H) (Activin A receptor, type I (R206H) is present, but does not stimulate cells with only the wild type receptor that lacks the FOP mutation. Thus, cells with the FOP mutation in ACVR1 are sensitized to a hormone-like factor (Act A) that normally does not stimulate the BMP signaling pathway. ACVR1 was originally named many years ago because it was thought to be responsive to Act A. However, subsequent studies showed that the wild type receptor was mute to Act A but responsive to closely-related hormone-like factors called bone morphogenetic proteins (BMPs) – a clue that led to the discovery of mutations in ACVR1 as the cause of FOP.

FOP is caused by mutations in the intracellular domain of the BMP receptor ACVR1 and was previously shown to drive inappropriate bone formation due to endogenous hyperactivity of the mutant receptor (the gas pedal partially stuck) as well as increased sensitivity of the receptor to BMPs (a very sensitive gas pedal, as well).

The Regeneron team, led by Dr. Aris Economides, Ph.D., found unexpectedly that the *ACVR1* gene mutation that causes FOP renders cells with the mutant ACVR1 protein responsive to a set of “non-canonical” (unusual and unexpected) ligands (locally acting hormone-like factors) including Act A, which are completely incapable of activating cells with the normal (undamaged) ACVR1 receptor that exists in most human beings. In the article, the scientists speculated but were unsure of the causes for this dramatic difference in sensitivity of cells with normal and mutant ACVR1 to Act A.

To test the implications of this finding in living animals, the scientists used engineered conditional “knock-in” mice with the classic FOP mutation (*Acrv1 R206H*) that they had previously developed in consultation with scientists at **The Center for Research in FOP and Related Disorders**. When *Acrv1 R206H* expression was induced (turned-on), the mice, as expected, developed heterotopic ossification resembling that in FOP, a finding originally established by scientists at **The Center for Research in FOP and Related Disorders** using an earlier genetic mouse model of classic FOP.

In this latest study, the Regeneron scientists demonstrated that the process of heterotopic ossification in the FOP mouse model could be inhibited by a highly specific (monoclonal) antibody that blocked Act A confirming previous studies that the process of heterotopic ossification in FOP was stimulated by ligand (hormone-like factor). Most importantly, the authors showed that Act A induced heterotopic ossification in mice expressing the mutant (*Acrv1 R206H*) receptor but not in mice expressing only the normal undamaged receptor. Astonishingly, inhibition of Act A with a fully humanized monoclonal antibody that the Regeneron



Alexey Lenskii, his mother Olga and his grandparents at The Second International FOP Russia Family Meeting and Conference in Moscow, Russia

scientists had developed for another, unrelated program, completely blocked formation of heterotopic ossification in the genetic mouse model of classic FOP. The surprising findings indicate that cells with mutant ACVR1 (but not normal ACVR1) are sensitive to an unexpected hormone-like factor (Act A), and that Act A can stimulate heterotopic ossification in this animal model of classic FOP.

The scientists believe that the fully humanized antibody to Act A may represent a potential near-term therapeutic option for FOP patients. Further laboratory work, extensive animal toxicity studies and human safety studies are necessary before this unexpected laboratory discovery can be translated into human clinical trials for individuals with FOP. However, the discovery clearly identifies a refined therapeutic target for FOP in the broad context of the mutant ACVR1 pathway and excavates a foundation for future clinical development.

In their paper, the authors caution that there are a paucity of data implicating Act A as a driver of heterotopic ossification in FOP patients, largely due to the inability to safely acquire relevant human tissues and cells for testing.

Nevertheless, due to the high degree of evolutionary conservation in this signaling system, together with the high fidelity of the genetically humanized mouse model,

the scientists were cautiously optimistic about eventually exploring therapeutic Act A blockade in FOP patients.

Presently, scientists at **The Center for Research in FOP and Related Disorders** are attempting to verify the findings of Economides and colleagues using human osteoprogenitor stem cells (SHED cells; see **The Tooth Ferry Program at the FOP Lab: SHEDding Light on FOP** – in The Twenty-Fourth Annual Report of the Fibrodysplasia Ossificans Progressiva Collaborative Research Project) that have been obtained directly from FOP patients and controls. Verification of the findings of this latest study in primary human stem cells directly from FOP patients will be critical before clinical development of this new antibody approach would be warranted.

Unlike small molecule inhibitors that can be ingested orally and absorbed through the gastrointestinal (GI) tract, antibodies (which are proteins, and are broadly known as biologics) must be administered by a non-GI route (such as intravenously or subcutaneously) in order to avoid being destroyed by protein-degrading enzymes in the stomach and intestines.

This recent discovery by scientists at Regeneron Pharmaceuticals underscores a rich historical background involving the FOP research community. The Penn-Regeneron affiliation, for example, dates back to 1996 - to the pre-clinical Noggin Gene Therapy Program in the era before the FOP gene discovery.

On August 21, 1996, the FOP laboratory at Penn published a landmark article in *The New England Journal of Medicine* describing the over-expression of BMP4 in patients with FOP and establishing dysregulation of BMP signaling as a keystone in the pathophysiology of FOP. The following day, Richard Harland and colleagues from the University of California-Berkeley, aided in part by reagents from Regeneron Pharmaceuticals, published an article in the journal *Cell*, describing that Noggin (the protein encoded by the *Nog* gene) binds and inactivates BMP4. The following week, Dr. Roy Vagelos, Chairman of the Board of the University of Pennsylvania and Regeneron



Bella Ostergren, her family and friends at The Scandinavian FOP Family Meeting in Eskilstuna, Sweden

Pharmaceuticals, connected the dots between the BMP4-FOP work at Penn and the Noggin-BMP4 work at the Harland and Regeneron labs, and brought the two research groups together – a collaboration that culminated in the publication of another landmark article, “*In vivo* somatic cell gene transfer of an engineered noggin mutein prevents BMP4-induced heterotopic ossification” in 2003 in the *Journal of Bone and Joint Surgery*. Following the historic discovery of the FOP gene in 2006, collaborations between scientists at **The Center for Research in FOP and Related Disorders** and Regeneron Pharmaceuticals led to the development of the conditional knock-in mouse model for FOP used in the Act A discovery reported here.

Kyosuke Hino and colleagues from Kyoto University in Japan, in a report in the **Proceedings of the National Academy of Sciences**, used FOP patient-derived iPS (induced pluripotent stem) cells to show that mACVR1 aberrantly stimulates BMP signaling in response to Activin A, a ligand that normally stimulates the TGF-beta signaling pathway (but not the BMP signaling pathway). Activin A enhanced chondrogenesis of mesenchymal stromal cells derived from FOP iPS cells

and induced endochondral ossification of FOP-induced mesenchymal stem cells *in vivo*. Their results support the findings of Hatsell on the role of Activin A in selectively inducing mACVR1.

The Act A discovery by scientists at Regeneron Pharmaceuticals and Kyoto University highlights that although ACVR1 is normally stimulated by signals from BMP ligands to activate the BMP pathway, a finding that led to its ultimate identification as “the FOP gene,” cells can also be triggered to activate the BMP pathway by Act A – but only in the context of FOP. Thus ACVR1 was initially misnamed - or was it? As Alfred Hitchcock demonstrated with clarity, the best way to conceal reality is to hide critical clues in broad daylight. The ironic twist in this saga is that although cells with wild type ACVR1 are mute to Act A, cells with mutant ACVR1 hear its call – and summon disabling heterotopic ossification. How? We do not yet know. Could the plot be more convoluted? Perhaps. Additional work is required to clarify this fascinating discovery and determine its significance for individuals with FOP. However, the implications could not be clearer – This is just Act 1 for Act A.



Aidyn Prescott (Hartsville, NC) visits with Dr. Kaplan at The FOP Center

Commenting on this discovery, Carl Zimmer (the author of **Mystery of the Second Skeleton**; **Atlantic Monthly**, June 2013) wrote on his blog, “There are still many challenges scientists will have to face before Act A can become an effective drug for FOP. For one thing, researchers have to see if it works as well in people as it does in mice. But because people with FOP are so sensitive to

injuries (even a muscle injection can trigger a new bone), regular human trials don’t work. Fortunately, Kaplan and his colleagues have discovered that they can harvest bone-generating stem cells out of baby teeth from children

with FOP. So they’re now trying to replicate the Act A studies with these cells. Setting aside the possible medical potential of this research, it drives home just how mysterious rare diseases can be. FOP might seem like it should be a simple disease to treat. After all, it’s just caused by a single mutation to a single gene. But it’s actually fiendishly complex, but because it disturbs an intricate web of chemical reactions that our bodies use to grow muscles and bones. The search for a cure for FOP has been going on for over a quarter of a century, and yet no one thought to consider Act A. A normal version of ACVR1 doesn’t relay Activin A’s messages. And, so no one even guessed that a mutant version would.”

B: Dousing the Flames: Silencing the Cellular Hypoxia Alarm Quenches FOP Flare-Ups

When English cleric Joseph Priestly, French chemist Antoine Lavoisier, and Swedish pharmacist Carl Scheele discovered oxygen c.1774, they showed that air was not inert and tackled the timeless question of why and how things burn. But, in the rare and disabling genetic disorder fibrodysplasia ossificans progressiva (FOP), it is ironically the lack of oxygen – or more precisely the cellular response to the lack of oxygen – that fans the flames of FOP flare-ups. What fuels those lesional fires and keeps them burning?

After extensive investigation beginning in 2009, nearly 280 years after John Freke first described FOP, scientists from **The Center for Research in FOP and Related Disorders** at the University of Pennsylvania recently provided the first answers to these elusive questions. Writing in *The Journal of Bone and Mineral Research*, University of Pennsylvania researcher Haitao Wang and colleagues announced a major breakthrough in understanding FOP, and identified new targets for possible treatment. In their paper, **Cellular Hypoxia Promotes Heterotopic Ossification by Amplifying BMP Signaling**, the authors examined the critical role of tissue hypoxia (oxygen starvation) and the cellular response to tissue hypoxia in the induction and amplification of FOP lesions, also known as “flare-ups.”

The research team, led by Drs. Robert Pignolo and Frederick Kaplan, showed that cells from FOP lesions in humans and in a genetic mouse model of FOP are markedly hypoxic (oxygen starved), that hypoxia triggers a molecular alarm called HIF-1 α (pronounced “hif one alpha”), that HIF-1 α amplifies bone morphogenetic protein (BMP) signaling in the oxygen starved cells and stimulates heterotopic ossification. Most importantly, by disabling HIF-1 α through genetic or pharmacologic means, BMP signaling is restored to normoxic levels in human FOP bone progenitor cells and profoundly reduces heterotopic ossification and resultant disability in a mouse model of FOP.

In 2006, researchers at **The Center** announced the discovery of the FOP gene and the recurrent mutation in the BMP type I receptor called Activin Receptor A type I (ACVR1) that occurs in all individuals who have classic FOP. The classic mutation in ACVR1 (mACVR1) causes the ACVR1 protein, a cell surface receptor, to be mildly overactive, thus stimulating the BMP pathway continuously, like a faucet that drips water when it should be turned-off. However, despite the presence of mACVR1 in all FOP patients, individuals with FOP do not form bone continuously but rather episodically during flare-ups, an important clue that suggested that “something else” could fuel the process of lesion formation. A tantalizing lead came from studying FOP lesions themselves. Importantly, all FOP flare-ups, whether spontaneous or triggered by trauma, are associated with inflammation, a well-known cause of oxygen starvation (hypoxia) to cells and tissues. Might oxygen – or the lack of it – be playing a role?

Every cell needs oxygen in order to generate energy and maintain viability. In health, blood carries oxygen to the body’s cells and tissues. Every cell generates energy from the nutrients and oxygen it receives, and uses that energy to maintain the cell membrane, make new proteins, and sustain viability.

Over eons, cells evolved an intricate alarm system to respond to hypoxic conditions. The cellular response to hypoxia is controlled by the hypoxia-inducing factor (HIF) family of proteins, mainly HIF1- α – a molecular



Elizaveta Kalishnikova and her mother Anastasia at The Second International FOP Russia Family Meeting and Conference in Moscow, Russia

alarm that allows a cell to respond instantaneously to the imminent danger of oxygen starvation. Every cell constantly produces HIF1- α but rapidly destroys it when the cell has an adequate supply of oxygen. When a cell is oxygen starved, the enzymes that inactivate HIF1- α instantly cease to function, allow HIF1- α to escape destruction, enter the nucleus of the cell, and trigger an alarm that instructs genes

to adapt to a low oxygen microenvironment and thus survive.

Hypoxia can occur for many reasons, but in early FOP flare-ups, we speculated that hypoxia might result from the edema-laden and inflammatory microenvironment where oxygen supply to the damaged tissue is impaired and oxygen-demand by the damaged cells greatly exceeds its supply.

To begin, we examined lesional biopsies of FOP flare-ups that had been obtained mistakenly from patients before the diagnosis of FOP had been correctly made. Additionally, we examined FOP lesions from mice genetically engineered to form FOP-like heterotopic bone. In humans and in mice, we found that the cells of early inflammatory FOP lesions were profoundly hypoxic (oxygen starved).

In order to understand the implications of oxygen starvation in FOP lesions, we examined the effects of hypoxia in human osteoprogenitor cells (SHED cells; see **The Tooth Ferry Program at the FOP Lab: SHEDding Light on FOP** – in The Twenty-Fourth Annual Report of the Fibrodysplasia Ossificans Progressiva Collaborative Research Project) directly from FOP patients and controls. These stem-like progenitor cells can be coaxed



Dr. Fred Kaplan and Gary Whyte at The 15th Annual Comedy Show to Benefit FOP Research (Mountainside, NJ)

to differentiate *in vitro* into fibrous cells, cartilage cells, or bone cells – the types of cells that progressively populate FOP lesions on their way to becoming bone.

When SHED cells from FOP patients were oxygen starved, they formed cartilage, an obligate precursor to bone

formation in FOP. Surprisingly, FOP SHED cells also exhibited a dramatic increase in both the intensity and the duration of BMP signaling under hypoxic conditions. Even if BMPs were blocked from reaching the FOP cells, mACVR1 remained extremely active in hypoxic conditions, far above its basal level in normoxic conditions (a normal oxygen microenvironment). This suggested that the increased BMP signaling observed under hypoxic conditions is the result of a change occurring inside the cell that affects the traffic and transit of the receptor itself, rather than from a change occurring outside the cell from BMPs or other ligands acting on the receptor. But why should the oxygen supply to an FOP cell be linked to the traffic and transit of the receptor or to the level of BMP signaling? And, if there was a link, what was it? We began to investigate.

An unexpected clue came from the field of cancer research from scientists who had recently discovered that hypoxia prolonged the activity of cell surface receptors by delaying their delivery to the cell's garbage disposal where they are normally destroyed after use. If a cell did not have ample oxygen to generate energy to make new proteins, it adapted by conserving its old proteins – like keeping an old car in tough economic times rather than discarding it and buying a new one. An old car may not run as well as a new car, but it would get you where you want to go – and you would not go broke if you broke down! Cancer researchers found that this adaptive response helped normal cells adapt to hypoxic conditions

but ironically stimulated cancer cells by conserving mutant receptors that were driving the cancer process. Could something similar be occurring in FOP?

ACVR1 is, in fact, a cell-surface receptor. We reasoned therefore that hypoxia, a microenvironmental condition that we had documented in early FOP lesions, might cause a disastrous retention of mACVR1 in FOP lesional cells - similar to the way that hypoxia caused cancer cells to retain their mutant receptors and thus fuel the cancer process.

To test this hypothesis, we examined the effect of hypoxia on ACVR1 activity in SHED cells from FOP patients and controls. We were astonished at what we found. Under normoxia (a normal oxygen microenvironment) in control and FOP SHED cells, ACVR1 was cycled, after use, to the cell's garbage disposal. However, under hypoxia, mACVR1 (in FOP SHED) cells was retained in endosomes (cellular vesicles), where it continued to signal for a longer duration and at elevated levels. But why should this be? We were determined to delve deeper and decipher the mechanism of this sabotage.

We returned to cancer research for more clues. We reasoned that both cancer (almost all types) and cartilage (the obligate pre-bone tissue that occurs in FOP lesions), thrive in hypoxic conditions (whereas most tissues suffocate under hypoxia). Cancer researchers found that HIF1- α , the molecular alarm that signals danger to the cell's genes in an hypoxic microenvironment, abruptly shuts-off Rabaptin-5 production, a critical protein that helps escort receptors to the cell's garbage disposal. As a result, the mutant receptors hung around longer, and continued to fuel the cancer. When we subjected FOP SHED cells to the same hypoxic conditions that existed in cancer cells (and that we had demonstrated in FOP lesions), we found exactly the same thing that the cancer researchers had found: HIF1- α disabled Rabaptin-5. As a result, mACVR1 was retained inside the cell's endosomes allowing hypoxic cells to transmit dangerous BMP signals from retained mACVR1 at higher levels and for a longer duration - in effect dramatically amplifying an already overactive signal from the mutation alone. Amazingly,

when we restored Rabaptin-5 levels in the cells to normal, the cells breathed a sigh of relief as elevated BMP signaling returned to normoxic levels, indicating that Rabaptin-5 was mediating the process.

Most importantly, when the HIF1- α alarm was silenced, either genetically or with drugs, Rabaptin-5 levels were restored to normal, mACVR1 was appropriately escorted to the cell's garbage disposal, and BMP signals fell to the baseline levels that existed in normoxia - indicating that HIF1- α was driving the entire process. So, while FOP is not cancer, the two conditions have something fundamentally in common. Both FOP and cancer have exploited the cellular hypoxia-HIF1- α alarm system to their advantage, by prolonging the activity of mutant receptors that dramatically amplify already overactive signaling pathways and drive their unique processes to their inexorable conclusions. Essentially, the hypoxic cells in FOP lesions trigger an alarm (HIF1- α) only to discover that the fire engine summoned to douse the fire, instead sprays gasoline on it! In essence, not only does mACVR1 alter the basal threshold for heterotopic bone formation, but the tissue microenvironment that it helps create triggers a cellular response that amplifies the damage.

Although many chemical compounds inhibit HIF-1 α in cells, only a few have been shown to effectively inhibit HIF-1 α *in vivo*. Could these same pharmacologic inhibitors that quenched HIF1- α in FOP cells *in vitro* do the same in FOP lesions *in vivo*, restoring amplified BMP signals to normoxic levels, and inhibiting heterotopic ossification and subsequent disability in FOP mice? To investigate, we pre-treated FOP mice with either a placebo or one of three pharmacologic inhibitors that quenched HIF1- α activity, and then induced FOP flare-ups with an intramuscular injection. We found that heterotopic ossification was greatly reduced and movement was preserved in the FOP mice receiving any of the three HIF1- α inhibitors compared to control mice that received a placebo.

The HIF-1 α inhibitors examined in both the SHED cells and the animal models were apigenin, imatinib and PX-478. Apigenin is a naturally occurring HIF-

1 α inhibitor found in parsley and other food sources. Imatinib, an inhibitor used to treat several cancers, is a potent HIF-1 α inhibitor. Although imatinib has off-target effects, it was selected because of its availability and low toxicity. PX-478 is a potent, experimental HIF-1 α inhibitor.

Our study showed that imatinib, apigenin and PX-478 potently inhibited dysregulated BMP signaling induced by HIF-1 α *in vitro*, as well as heterotopic ossification following tissue injury in a mouse model of FOP. In all cases, it is possible that these compounds also affect heterotopic ossification by mechanisms other than HIF-1 α -BMP pathway crosstalk. The implications for targeted clinical trials and for compassionate clinical use of HIF-1 α inhibitors are promising, but little data exist on critical issues of dosing, duration, timing, rebound, resistance or long-term safety of any of the HIF-1 α inhibitors. At the present time, caution is advised.

In a related study published in *The Proceedings of the National Academy of Medicine*, Agarwal and her colleagues from The University of Michigan showed that inhibition of HIF1- α prevents both trauma-induced and genetic heterotopic ossification. Using different approach, the study confirmed the veracity of HIF1- α as a target in HEO.



Stacy Scoble (Emmaus, PA), Kristi Gonzales (Bellmawr, NJ), Dr. Kaplan (Philadelphia, PA) and Suzanne Hollywood (Bridgewater, NJ) at Gary Whyte's 15th Annual Comedy Show to Benefit FOP Research (Mountainside, NJ)

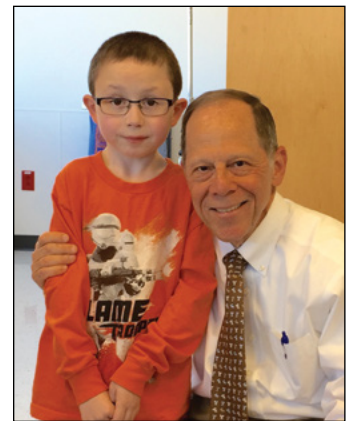
The groundbreaking studies described here support that FOP lesions thrive in a hypoxic microenvironment – not simply due to oxygen deprivation, but due to a

maladaptive response to hypoxia by the HIF1- α molecular alarm, similar to events that occur in cancer. Importantly, by silencing HIF1- α , the amount of heterotopic ossification and the resultant functional disability are greatly reduced in a genetic mouse model of FOP.

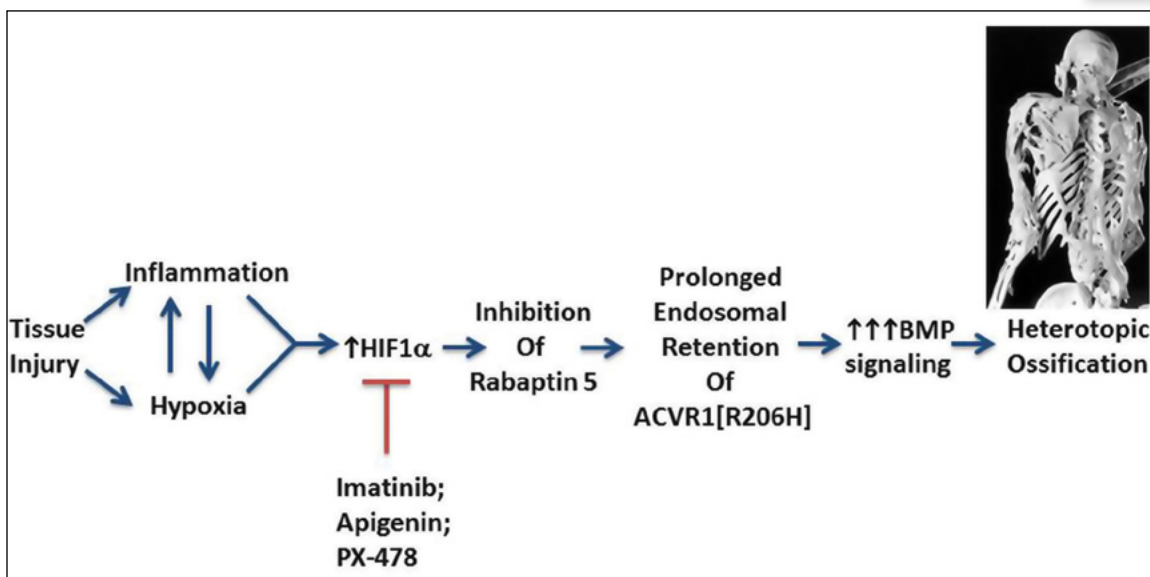
Generation of a hypoxic microenvironment in injured skeletal muscle appears to be a critical step in the formation of heterotopic bone. Our studies demonstrate that BMP signaling in FOP lesions is BMP ligand-independent under hypoxic conditions in both an injury-induced animal model of FOP and in spontaneous lesions from FOP patients. However, injury-induced inflammation is a complex physiologic response to tissue damage involving immune cells, progenitor cells, and secreted hormone-like factors. Thus, while hypoxia mediates ligand-independent BMP signaling, other responses in the local microenvironment could be ligand-dependent and result in a more complex regulation of heterotopic ossification in FOP.

Although this study focused on the cellular response to hypoxia in the development of FOP flare-ups, it also verified the existence of cellular hypoxia in non-genetic forms of FOP – thus opening the door to modulating the cellular response to hypoxia in more common forms of heterotopic ossification.

Our study provides critical insight into the role of cellular hypoxia in the episodic induction and amplification of FOP flare-ups and establishes that cellular oxygen sensing through HIF1- α is a critical regulator of the BMP signaling pathway and heterotopic ossification in FOP. Most importantly for individuals with FOP, our study identifies HIF1- α as a robust therapeutic target for FOP – vital new knowledge that will likely contribute to the development of more effective treatments for FOP and related common disorders of heterotopic ossification. Usually, when the oxygen is low, the fire goes out. For FOP lesions, as with cancer, when the oxygen is extinguished, the fire grows. Quenching the cellular hypoxia alarm quells FOP flare-ups.



Bobby Johnson (Warren, PA) visits with Dr. Kaplan at Penn



Schematic summary of the discovery that cellular response to hypoxia promotes heterotopic ossification by amplifying BMP signaling. The response is blocked in FOP mice by Imatinib, Apigenin, and PX-478. Courtesy, *Journal of Bone and Mineral Research*, 2016.



Dr. Burton Nussbaum and Mrs. Diane Nussbaum at the IFOPA Retirement Dinner for Dr. Nussbaum. The plaque reads: "In Appreciation to Dr. Burton Nussbaum for twenty years of faithful service and dedication to the International FOP Association. FOP Dental Consultant (1995-2015). Your skill and dedication to the health and welfare of FOP patients has been instrumental in helping us educate and support our FOP members and their families."



Adnan Hai (Queens, NY) visits with FOP researcher Andrew Chang and Nurse Casey Shechtman at The FOP Center



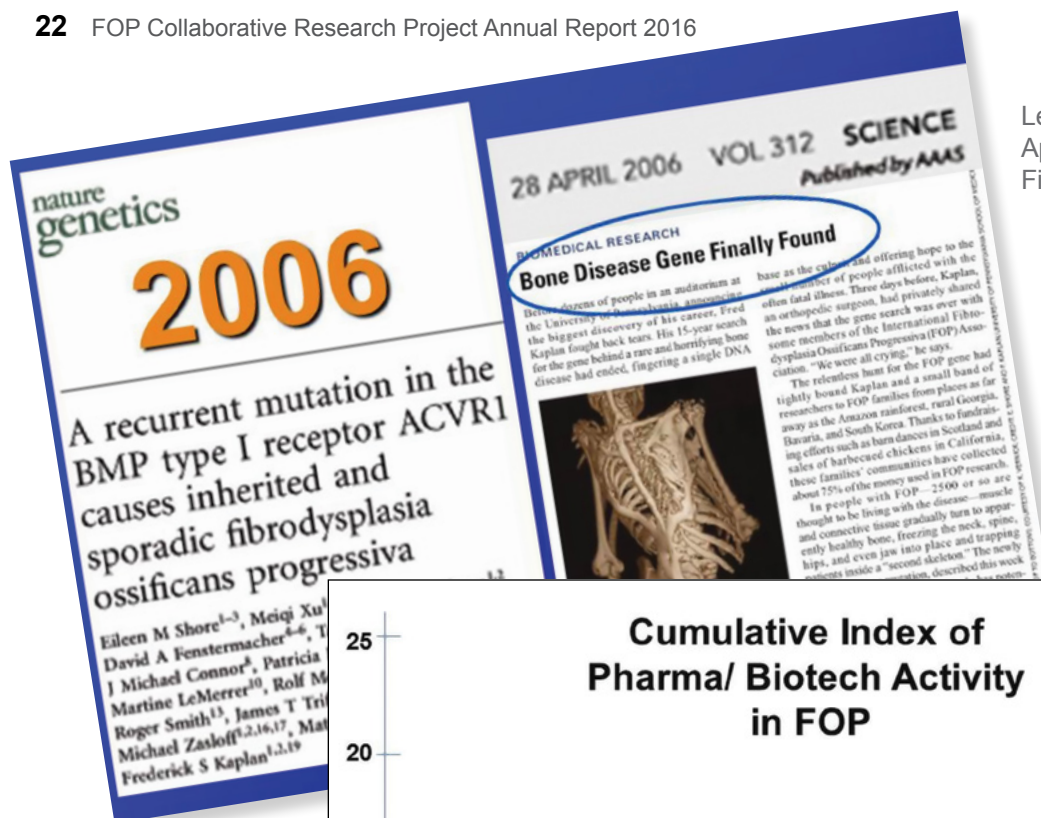
Ashley Kurpiel (Peachtree City, Georgia) and Oliver Collins (Brisbane, Australia) in 2015 (left) and in 1994 (right).



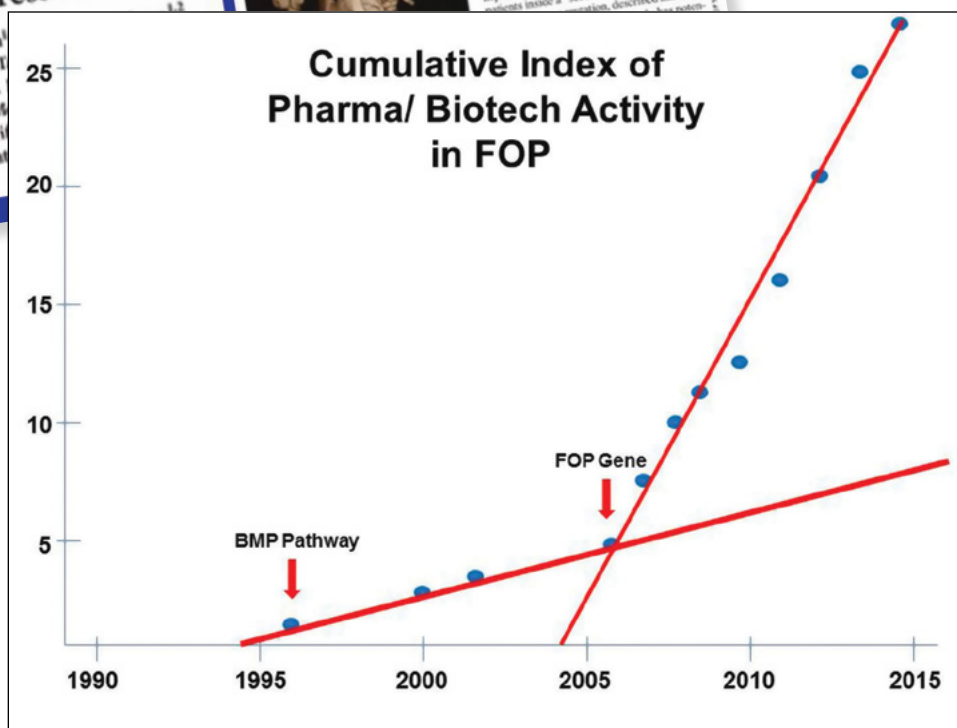
Pontius Camelfelt (seated, center), his mother Brigitta Camelfelt (seated, left) and his sister Sara Camelfelt (standing, right) from Tyreso, Sweden with friends from the FOP medical community at The Scandinavian FOP Family Meeting in Eskilstuna, Sweden



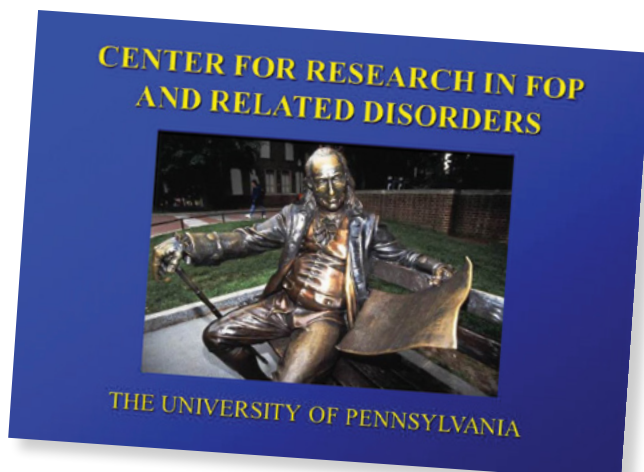
Igor Zahvatov, his mother Nadezhda meet with the Russian, Ukrainian, American and German medical staff at The Second International FOP Russia Family Meeting and Conference in Moscow, Russia



Left: Nature Genetics and Science, April 2006. Bone Disease Gene Finally Found



Cumulative Index of Pharmaceutical Industry/Biotechnology Company Interest & Activity in Drug Discovery & Development for FOP. The slope of interest/activity changed dramatically immediately after the FOP gene discovery in 2006 – a seminal event in the history of FOP which gave the world a robust central target for drug discovery. X-axis = years; Y-axis = cumulative # of Pharma/biotech interest and activity.



Right: Center for Research in FOP and Related Disorders; the University of Pennsylvania. Benjamin Franklin, Founder of the University of Pennsylvania.

Part III: Therapeutic Horizons

Worldwide interest in FOP research skyrocketed in the wake of the historic discovery of the FOP gene in 2006. FOP research is now a worldwide enterprise.

The concept of orphan drugs for treatment of orphan diseases has been widely-embraced in just the past several years – a concept that is leading to massive research investment on the part of industry. Pharmaceutical and biotechnology companies have expressed keen interest in FOP and are engaged in an arms race to create better treatments and a cure for FOP. Science writer Carl Zimmer wrote “Pharmaceutical companies have increasingly turned their attention to rare diseases in recent years, because, paradoxically, the rare disease market may turn out to be very profitable.”

At least eight pharmaceutical or biotechnology companies are working on the development of kinase inhibitors for FOP, at least six are working on the development of antibodies or ligand traps, at least four are pursuing the development of small inhibitory RNA technology, and at least two are working on alternate cell and microenvironmental approaches – all propelled by the discovery of the FOP gene and by the identification of its robust therapeutic target – the ACVR1 receptor kinase and its interacting molecules and pathways.

The discovery of the FOP gene in 2006 was a transformative event in the history of FOP and immediately revealed at least four approaches to the treatment and/or prevention of FOP. All four approaches were reviewed in detail in last year’s 24th Annual Report of the FOP Collaborative Research Project.

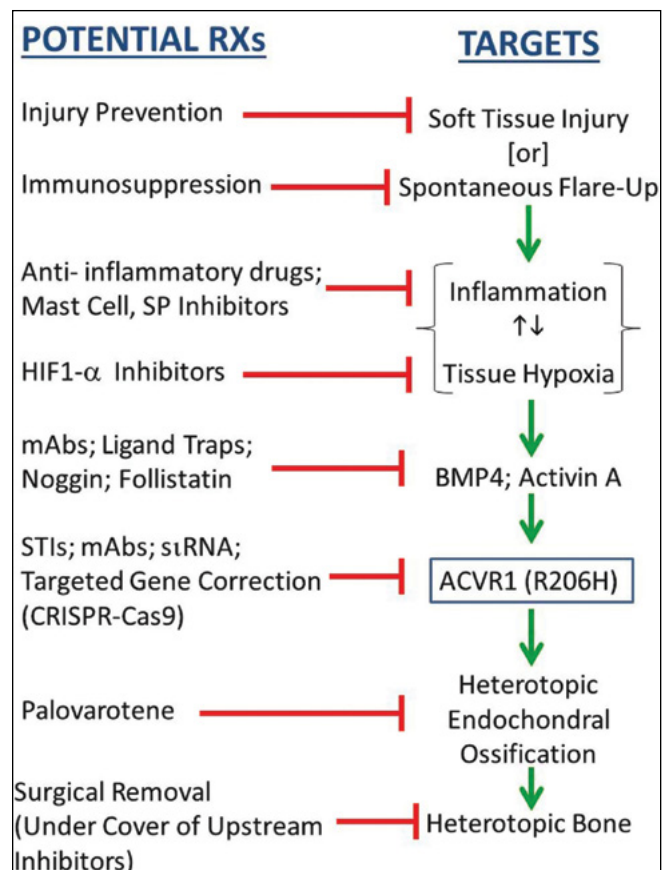
These approaches include:

1. Blocking activity of the mutant receptor (ACVR1/ALK2) that causes increased BMP pathway signaling (through four possible strategies which include: monoclonal antibodies, signal

transduction inhibitors (STIs), inhibitory RNA, and secreted antagonists).

2. Inhibiting inflammatory triggers of FOP flare-ups.
3. Directing FOP stem cells to an alternate tissue fate other than cartilage or bone.
4. Blocking the body’s response to microenvironmental signals that promote the formation and/or amplification of FOP lesions.

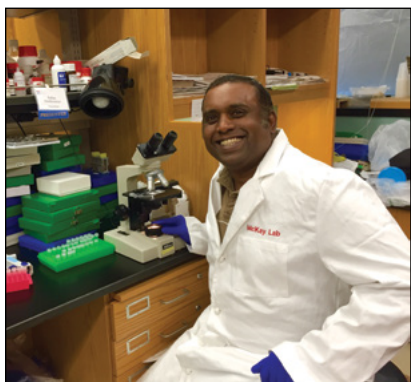
A contemporary view of therapeutic possibilities for FOP places the mutated receptor at the center of the therapeutic horizon and is summarized in the accompanying figure below (Hypothetical Schema for Drug Development in FOP).



Potential Treatment Strategies for FOP Based on Identified Targets. Key: SP= substance P; mAbs= monoclonal antibodies; STI= small molecule signal transduction inhibitors; siRNA= small interfering (inhibitory) RNA; CRISPR-Cas9= novel gene editing approach.

In this year's annual report, we focus on major new findings reported recently in a published study from **The Center for Research in FOP & Related Disorders**.

The findings of this pre-clinical study have important implications for ongoing Phase II clinical trials of Palovarotene in FOP.



Dr. Salin Chakkalakal at The Center for Research in FOP & Related Disorders at Penn

Recently, scientists from **The Center for Research in FOP and Related Disorders** at the University of Pennsylvania, writing in *The Journal of Bone and Mineral Research*, announced a major breakthrough in understanding the

role of Palovarotene in FOP. In their paper, **“Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human ACVR1R206H FOP fibrodysplasia ossificans progressive (FOP) mutation”**, lead author Dr. Salin Chakkalakal, senior scientist Dr. Eileen Shore and their colleagues Masahiro Iwamoto, Maurizio Pacifici and Fred Kaplan reported that Palovarotene prevented spontaneous HO in a novel conditional knock-in mouse model carrying the classic FOP mutation. In addition, Palovarotene restored long bone growth, maintained growth plate function, and protected growing newborn FOP mice when given to lactating mothers. Importantly, Palovarotene maintained joint, limb and body motion, providing clear evidence for its encompassing therapeutic potential as a treatment for FOP.

In an explanatory editorial, “Learning more about Palovarotene,” the authors write:

Heterotopic ossification (HO) in fibrodysplasia ossificans progressiva (FOP) is a multi-step process that includes an intermediate stage of cartilage formation

and concludes with the formation of mature mineralized bone in soft tissues such as skeletal muscles. This cartilage-to-bone process is known as endochondral ossification. The primary goal of research for FOP is to identify effective treatments to prevent the extra-skeletal endochondral bone formation that begins early in childhood and progresses throughout life. A milestone was reached in July 2014 with the beginning of a multicenter phase 2 clinical trial for FOP. The drug that is being evaluated in the phase 2 trials is palovarotene, a retinoid agonist that activates a specific component of the retinoic acid signaling pathway (RAR γ) in cells and tissues.

The clinical trial for FOP was initiated based on the strength of previous studies by Drs. Iwamoto and Pacifici and their coworkers demonstrating that RAR γ agonists are particularly powerful to prevent HO at the cartilage stage and were effective in blocking it in injury and genetic mouse models. Palovarotene is a drug that was previously tested for emphysema. Although the drug was not developed as a treatment for emphysema, it was shown to produce few side effects in phase 2 clinical trials in adults.

However, many questions remained unanswered about palovarotene and its use for treating FOP, including whether the drug would be effective in preventing HO triggered by the most common FOP *ACVR1^{R206H}* human mutation, whether it would also improve skeletal function and joint movement, and whether it may actually impair the normal process of endochondral ossification required for skeletal growth and elongation during childhood.

These and related questions were investigated in a study that was recently published online in the *Journal of Bone and Mineral Research*: **Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human *ACVR1^{R206H}* Fibrodysplasia Ossificans Progressiva (FOP) mutation**. This study was a joint effort and the result of a highly productive collaboration between researchers at the Children's Hospital of Philadelphia (Kenta Uchibe, Maurizio Pacifici, Masahiro Iwamoto) and those at the



At Lincoln's Legacy for FOP Research (Sioux City, Iowa). Front row (left to right): Lee Wheelock, Hudson Wheelock, Lincoln Wheelock. Standing (left to right): Marilyn Wheelock, Dr. Doug Wheelock, Fred Kaplan, Dr. Trisha M. Wheelock, Robin Gambaiana and Mark Gambaiana

Center for Research in FOP and Related Disorders at the University of Pennsylvania School of Medicine (Salin Chakkalakal, Michael Convente, Deyu Zhang, Frederick Kaplan, Eileen Shore).

The results of this study provide strong and clear support for palovarotene as an effective potential treatment to not only prevent HO in FOP, but also protect and sustain skeletal growth and joint function.

The authors previously created and described transgenic mice in which they inserted the R206H mutation into the *ACVR1* gene. The mice mimicked human FOP and developed HO and the characteristic toe malformations, but the model did not allow the initiation of the HO process at specific times as is often the case in FOP patients following a flare-up. In the current study, we used a new 'conditional' mouse model that enabled activation of the mutation at will, allowing the adjustment of timing and location as well as the cell types expressing the mutation depending on the question to answer. The conditional FOP mouse model was created by Aris Economides and his colleagues at Regeneron Pharmaceuticals in collaboration with researchers at Penn. Dr. Economides is also a co-author on the current study.

In a first set of experiments, the FOP mutation was activated in all cells of young mice and demonstrated that injury to skeletal muscles induced local formation of HO, just as in children with FOP. When these mice were treated with palovarotene, however, very little HO formed and the HO process was halted at the cartilage formation stage, as expected. Importantly, palovarotene elicited additional beneficial effects at the early stages of HO formation, specifically a reduced immune cell response.

A severe functional consequence of HO during FOP is that it may eventually impair or completely block skeletal movement and joint function. Very promisingly, palovarotene-treated mice retained good skeletal function and could move and function well.

The *ACVR1*^{R206H} mutation is likely to affect cells during embryonic development since most FOP patients display the great toe malformation at birth. In order to determine whether the new conditional mouse line was able to mimic this characteristic feature of FOP, the *ACVR1*^{R206H} mutation was activated in the cells of the developing embryonic limbs by using a genetic approach (Prrx1+ cells). Note that the mutation had to be restricted to those cells because we had learned from our previous studies that general activation of the mutation throughout the whole body in developing mouse embryos was too aggressive and resulted in premature death.

When the Prrx1-*ACVR1*^{R206H} mice (Prrx1-R206H mice) were examined right after birth, the first digits of the hind feet (the mouse equivalent of human great toes) were in fact malformed. In a manner analogous to FOP children, the newborn mice did not display HO, but began to develop HO spontaneously starting around 2 to 4 weeks of age. The extraskeletal bone formed consistently and in the absence of injury. Because the mutation was active only in limb cells, the mice did not develop HO in other parts of their body that are often affected in FOP patients such as the back and neck.

The Prrx1-R206H mice were slightly shorter than siblings lacking the mutation. Measurements of the

lengths of several limb bones determined that, indeed, the bones were on average shorter in FOP mice. Bones, such as the femur in the leg and the humerus in the arm, normally grow in length through the expansion and development of cells in ‘growth plates’ located at each end near the joints. This expansion, like HO in FOP, occurs through the process of endochondral ossification. When we examined the growth plates of *Prrx1-R206H* mice, we found that there was a significant reduction in the region of the growth plate that contains the more mature cartilage cells and is responsible for long bone elongation.

HO in the *Prrx1-R206H* mice forms in the absence of injury, and this feature provided us with the opportunity to test the efficacy of palovarotene for spontaneous HO. Since HO in these mice does not begin until after birth, we began treatment with palovarotene at birth. Palovarotene was administered to nursing mouse mothers for the first two weeks, with the expectation that the drug would be passed to the pups through breastfeeding. It was then given directly to the two week-old mice for another two weeks until about one month of age. At the end of this period, *Prrx1-R206H* mice that had not been treated with palovarotene had extensive HO, but those treated with the drug had substantially reduced amounts of it.

We also examined the effects of palovarotene on long bone elongation and the growth plates. When mice without the FOP mutation were treated with palovarotene, skeletal growth was slightly reduced and there was some impairment of growth plate cartilage function, consistent with known effects of other retinoid agonists on skeletal growth. However, when *Prrx1-R206H* mice were treated with palovarotene, long bone growth and elongation were significantly improved and were similar to those in untreated mice without the FOP mutation. In addition, and very importantly, the growth plates of palovarotene-treated *Prrx1-R206H* mice were restored to a near normal appearance, and the mice remained mobile and functioned well over time.

Individually, both the treatment with palovarotene and the increased basal BMP signaling activity by the

ACVR1^{R206H} mutation can impair normal skeletal bone growth. Because palovarotene treatment blocked HO and reversed the skeletal defects in *Prrx1-R206H* mice, our data indicate that there is a fine balance between BMP and retinoid signaling - and in particular between *ACVR1* and *RAR γ* - that is critical for growth plate function and skeletal growth. Further characterization of *ACVR1* and *RAR γ* interactions will not only increase our understanding the action and mechanisms of such key regulators, but may also identify additional druggable targets and even more effective potential treatment regimens.

In summary, the goals of the study were to determine whether palovarotene can block HO caused by the human *ACVR1^{R206H}* mutation and whether the drug could also correct other skeletal defects caused by the mutation. The results clearly demonstrate that palovarotene can do so and, quite significantly, appears to be able to act on neonates via the mother’s milk. While the cellular mechanisms through which palovarotene and *ACVR1^{R206H}* interact remain to be elucidated, our findings are a major step in establishing whether palovarotene is an effective and preventative treatment for FOP, possibly administrable from infancy.

HO is highly damaging to the well being of FOP patients because it progressively interferes with, and limits, multiple body functions including walking, bending, breathing, mastication, and swallowing. Since palovarotene inhibits the cartilage stage of heterotopic endochondral ossification, palovarotene treatment would be needed at each flare-up to reduce or possibly prevent each new round of HO, possibly starting from a young age. Once formed, HO is permanent in FOP patients; the ectopic bone cannot be removed by surgery because the resulting tissue damage triggers additional episodes of HO. Given the potency of palovarotene to prevent spontaneous as well as injury-induced HO, it has the potential to suppress HO initiation following surgery in FOP patients, a therapeutic feat if indeed possible. Notably, the near complete recovery of growth plate structure and function that we observe in palovarotene-treated FOP mice supports the possibility that drug treatment of skeletally-immature patients might enable

suppression of HO during childhood while restoring skeletal growth, an attainment that originally seemed counter-intuitive with a retinoid agonist.

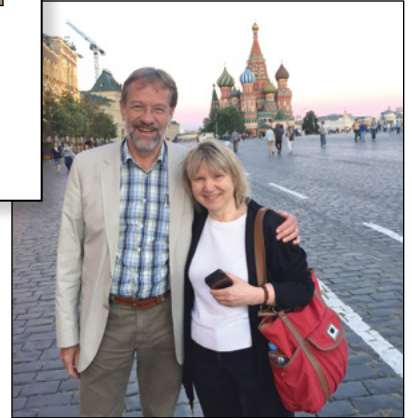
For the first time we show that palovarotene:

- Prevents HO that is caused by a mutation that occurs in human patients. The ACVR1 (R206H) mutation examined in our study is the most common mutation in patients with FOP;
- Rescues defects in mutant bone growth and preserves joint function;
- Can be given to lactating mothers to inhibit HO in nursing pups, raising the possibility of safe and early, or even pre-natal, intervention for HO in children;
- Restores growth plate function in mutants, in contrast to the predicted detrimental effects on wild-type growth plates – a well-known side-effect of retinoids. This remarkable observation indicates that mutant and wild-type cells and tissues respond differentially to this drug, an observation with major implications for clinical trials and drug testing, and drug safety and use.

The data in this study provide strong support for palovarotene to not only prevent HO, but also to restore long bone growth and maintain joint function and mobility. Our data demonstrate that palovarotene is not only highly effective, but could also be safe for use in children with FOP, offering the first possibility of a chronic preventive treatment for FOP.



Natalya Siteva & Andrey Darovansky at The Second International FOP Family Meeting and Conference in Moscow, Russia



Dr. Rolf Morhart (Garmisch-Partenkirchen, Germany) and Dr. Eileen Shore (Philadelphia) in Red Square; Moscow, Russia



At the Second International FOP Family Meeting and Conference in Moscow, Russia. (Left to right): Dr. Fred Kaplan (Philadelphia, USA), Dr. Irina Nikishina (Chairman, Pediatrics; Federal Research Institute of Rheumatology; Russian Academy of Medical Sciences; Moscow), Dr. Vladimir Vitiaz (Kiev, Ukraine), Elizaveta Matrosova and her mother Elena (Central Russia), Dr. Svetlana Arseneva (Moscow), Dr. Eileen Shore (Philadelphia, USA), and Dr. Rolf Morhart (Garmisch-Partenkirchen, Germany)



Infrastructure for A Cure

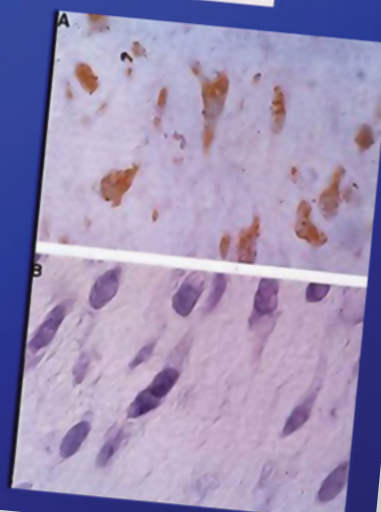
April 14, 1736

“ There came a boy
of healthy look,
and about 14 years of age,
to ask us at the hospital,
what should be done to cure him
of the many large swellings
on his back...”

-John Freke,
St. Bartholomew's Hospital, London
Philos. Trans. Royal. Society

**Bone Morphogenetic Protein 2/4 in Early
Fibromatous Lesions of Fibrodysplasia
Ossificans Progressiva**

FRANCIS H. GANNON, MD, FREDERICK S. KAPLAN, MD,
ELIZABETH OLMSTED, PhD, GERALD C. FINKEL, MD,
MICHAEL A. ZASLOFF, MD, PhD, AND EILEEN SHORE, PhD



Part IV: An Update – Infrastructure for a Cure

While ongoing clinical trials have been a major milestone of the past year, a commitment to clinical trial **infrastructure**, necessary for any and all clinical advances in FOP, continued to be a major focus of attention.

In order for the FOP community to fulfill the promise of better treatments and eventually a cure, we will need an **infrastructure for a cure** – similar in concept to the highway networks, mass transit systems, power grids, and communication networks that allow societies to function.

Great scientific and medical enterprises, like great nations need a stable and robust **infrastructure**. The **infrastructure for a cure** is a vital priority of the **Center**, and will facilitate the discovery and development of drugs and the conduct of clinical trials that will allow the entire FOP community to navigate better treatments and a cure. Like a large public works project, it will take a committed effort of the entire international FOP community to accomplish this goal and we fully embrace that concept.

The infrastructure for a cure is composed of five essential components:

1. Natural history infrastructure
2. Biomarker infrastructure
3. Mouse model infrastructure
4. Communications infrastructure
5. Global Registry infrastructure

In this section of the annual report, we will articulate our vision for the **infrastructure for a cure** and what we have done at the **Center for Research in FOP &**

Related Disorders during the past year to facilitate its development.

1. Natural History Infrastructure

The Natural History of Flare-ups in Fibrodysplasia Ossificans Progressiva (FOP): A Comprehensive Global Assessment

Comprehensive natural history studies in FOP are needed to determine outcome measures for the design of informative clinical trials of potential disease-modifying agents. These measures should be clinically meaningful milestones and predictors of outcome, such as the occurrence of HO or change in function due to HO. Clinically important differences, such as preservation of joint function, need to be understood in the context of a comprehensive, global assessment.

Previous natural history studies on FOP are more than two decades old, evaluated a limited number of subjects (approximately 40), and were conducted before the era of symptomatic treatment of exacerbations (flare-ups) with corticosteroids. Furthermore, these early studies were neither global nor comprehensive as the FOP community was small and regional. In the past twenty years, the organized FOP community has grown tremendously, largely due to the efforts of interested patients, doctors, and families and due to the explosive growth of the internet and social media.

We are proud to report the results of a massive world-wide, prospective, cross-sectional survey of flare-ups in FOP patients - episodic exacerbations that over time result in disabling HO. The study by Pignolo and colleagues from **The Center for Research in FOP & Related Disorders** was recently published in *The Journal of Bone and Mineral Research*. The entire article can be viewed on the IFOPA website and is summarized below:

A 78-question survey on FOP flare-ups, translated into 15 languages, was sent to 685 classically-affected patients in 45 countries (six continents). Five hundred patients or knowledgeable informants responded (73%; 44% males, 56% females; ages: 1 to 71 years; median:



Graduate student Niambi Brewer and senior research scientist Meiqi Xu support Joshua's Bingo for a Cure to Benefit FOP Research in Allentown, PA

23 years). The most common presenting symptoms of flare-ups were swelling (93%), pain (86%), or decreased mobility (79%). Seventy-one percent experienced a flare-up within the preceding 12 months (52% spontaneous; 48% trauma-related). Twenty-five percent of those who had

received an intramuscular injection reported an immediate flare-up at the injection site, 84% of whom developed HO. Axial flare-ups most frequently involved the back (41.6%), neck (26.4%), or jaw (19.4%). Flare-ups occurred more frequently in the upper limbs before 8 years of age, but more frequently in the lower limbs thereafter. Appendicular flare-ups occurred more frequently at proximal than at distal sites without preferential sidedness.

Seventy percent of patients reported functional loss from a flare-up. Thirty-two percent reported complete resolution of at least one flare-up and 12% without any functional loss (mostly in the head or back). The most disabling flare-ups occurred at the shoulders or hips. Surprisingly, 47% reported progression of FOP without obvious flare-ups. Worldwide, 198 treatments were reported; anti-inflammatory agents were most common. Seventy-five percent used short-term glucocorticoids as a treatment for flare-ups at appendicular sites. Fifty-five percent reported that glucocorticoids improved symptoms occasionally whereas 31% reported that they always did. Only 12% reported complete resolution of a flare-up with glucocorticoids. Forty-three percent reported rebound symptoms within 1 to 7 days after completing a course of glucocorticoids. This study is the first comprehensive global assessment of FOP flare-ups and establishes a critical foundation for the design and evaluation of future clinical trials.

In summary, the results from this first world-wide survey of flare-ups in patients with FOP provided a comprehensive perspective on the presentation, initiating factors, anatomic location, and resolution of flare-ups. These results will form the basis for design of future clinical trials of drugs for episodic and chronic treatment of FOP flare-ups.

2. Biomarker Infrastructure

Biomarker discovery and assessment is a vital component of the infrastructure for a cure. Biomarkers are objectively measured indicators of biological processes or pharmacologic responses to an intervention. For example, blood cholesterol levels, blood pressure, and tumor size are biomarkers for heart disease, hypertension, and cancer respectively. In FOP, useful biomarkers will be ones that indicate whether an individual is having a flare-up (even perhaps before a flare-up is clinically apparent), and which lesional stage of a flare-up an individual is experiencing. Biomarkers could also be helpful in assessing progression of FOP in the absence of a flare-up, and biomarkers will likely be used to assess effects of treatment. Importantly, biomarkers will enable faster, more efficient monitoring of clinical trials.

There are three compelling reasons why biomarkers are needed for successful clinical trial design and evaluation in FOP:

1. To measure and monitor the variability and progression of FOP in each individual and between individuals.
2. To measure and monitor the stages of disease activity during and between flare-ups and the absence of flare-ups.
3. To measure and monitor each individual's response to the drug being studied.

Unlike in cancer, for which biomarkers may be the same or similar throughout the course of the disease, biomarkers will likely vary in FOP based upon the stage of the disease and the phase of a flare-up. For example,

biomarkers at the earliest inflammatory phase of an FOP flare-up may be very different from those in the later cartilage and bone formation phases of a flare-up.

Knowing when a flare-up is imminent would be vital in guiding the administration of preventative medications. For example, a drug might be effective for treating FOP but not recognized because the dose, duration of treatment, or stage-specific use of the drug was incorrect during evaluation. Monitoring biomarkers during a flare-up might reveal such critical information. Without biomarkers, it would be impossible to know if a drug being studied might be useful, but not quite reach optimal timing, dosage, or potency. Thus, disease-specific, stage-specific, and drug-specific biomarkers will be essential in assessing the results of any clinical trial.

In 2014, we completed collection of serial urine samples over three months on 24 FOP patient volunteers for the comprehensive evaluation of biomarkers of disease activity. The study was sponsored by Novartis Institute for Biomedical Research (NIBR) in order to identify stage-specific biomarkers associated with FOP flare-ups. This work is vital to the design and implementation of future clinical trials.

The Center for Research in FOP & Related Disorders has a large and valuable biobank of plasma samples on FOP patients from various stages of FOP flare-ups. In 2015, we completed a comprehensive annotation of these samples and began a detailed analysis of biomarkers using these samples under a sponsored research agreement. When completed, the results of this study will be published and will be widely available to researchers worldwide. **Biomarker infrastructure** is a vital component of the **infrastructure for a cure**.

3. Mouse Model Infrastructure

Mouse model development, characterization and access are a critical part of the **infrastructure for a cure**. Mouse models of FOP are necessary for pre-clinical drug testing in FOP. Presently, five types of mouse models are in use: implantation of recombinant BMP into skeletal muscle, transgenic regulation of BMP expression under

the control of various gene promoters, a constitutively active ACVR1 mutation (that does not exist in any known FOP patient but can lead to heterotopic bone formation), a chimeric knock-in animal model of genuine FOP with the classic R206H ACVR1 mutation, and mice that conditionally express the classic FOP mutation.

Of these five mouse models of heterotopic ossification, the most relevant animal model for FOP research is the conditionally active knock-in mouse model of FOP. There are presently two such animal models known to exist and both are being fully characterized while even more advanced, informative models are being developed.

These newer FOP mouse models will be important in order to answer a myriad of clinically-relevant questions: Is a drug of interest effective once a flare-up occurs? Can the drug be stopped without a rebound effect? Does resistance to the drug occur over time? What is the proper window for treating an FOP flare-up? What is the correct dosage and duration of the drug for treating an FOP flare-up? Is the drug effective for spontaneous as well as induced flare-ups?



Josh Scoble (Emmaus, PA) with his aunt Chrissy Flexer, Director, Joshua's Bingo for a Cure to Benefit FOP Research (Emmaus, PA), his mother Stacy Scoble and aunt Susan O'Neill (Emmaus, PA) during a visit to Philadelphia

What are the short-term and long-term side-effects of drug treatment in the context of the classic mutation? How do spontaneous flare-ups differ fundamentally from trauma-induced flare-ups? What is the mechanism by which disability in FOP progresses in the absence of flare-ups?

The design of alternative mouse models for classic



Rare Disease Day – February 29, 2016. Fred Kaplan (Philadelphia, PA), Lindsay Ruiz (Somers, NY), Holly LaPrade (Madison, CT) and Jasmin Floyd (Woodstock, CT) gather for a special celebration “Join Us in Making a Rare Disease Heard” at The Frank H. Netter School of Medicine at Quinnipiac University; Quinnipiac, CT

FOP was initiated by the **Center for Research in FOP & Related Disorders** in 2014. Progress on one model, using a new gene-editing approach is highly promising and we anticipate the development of a colony in 2016. Conditional knock-in mouse models of FOP must be refined, characterized, and shared. We

intend to do so. We hope that other laboratories will do so as well. Mouse models of FOP are a necessary component of the **infrastructure for a cure**.

4. Communications Infrastructure

Open communication channels between FOP patients, clinicians and researchers are paramount to progress, a longstanding tradition in the FOP community, and an integral part of the **infrastructure for a cure**. The tradition of open communications in the FOP community harkens back to the very beginning of modern FOP research with the start of the FOP Collaborative Research Project in 1989. Even before the **Center for Research in FOP & Related Disorders** was officially established in the mid-1990s, seminal scientific, medical, and patient symposiums were held at the University of Pennsylvania in order to bring patients, physicians, and scientists together to educate each other about the questions driving FOP clinical and basic research and to stimulate clinical and basic research collaborations. International symposia and

scientific workshops at the University of Pennsylvania in 1991, 1995 and 2000 set the stage for modern scientific exploration of FOP and heralded the discovery of the FOP gene in 2006. These international symposia, and an international scientific workshop held at Penn in 2011 supported in part by the IFOPA, set the standard for developing regional international patient and scientific meetings worldwide that further expanded the scope and depth of FOP research internationally.

In advancing this longstanding tradition of open communication channels among patients, clinicians, and researchers, the IFOPA conducted the first **FOP Drug Development Forum (DDF)** in November 2014. As described by the IFOPA, “The forum was a groundbreaking event that brought together researchers from universities and pharmaceutical companies from around the globe to discuss the challenges of developing a treatment for FOP. The goals of the meeting were to address questions and knowledge gaps that exist in FOP Drug Development; stimulate new ideas to help advance development of potential therapies as quickly and efficiently as possible; and facilitate dialogue, foster collaboration, and form connections among interested researchers.”

The DDF was an important milestone in the history of the FOP community and a vital part of the communications infrastructure for a cure. **The Center for Research in FOP & Related Disorders** was proud to advise the IFOPA in planning this important event and will again work with the IFOPA on the next DDF, planned for the fall of 2016.

5. Global FOP Registry Infrastructure

A global FOP registry owned and operated by the FOP patients is a keystone in the **infrastructure for a cure**.

Mrs. Betsy Bogard, the global research director at the IFOPA, remarked: “Registries play a critical role in the development of therapies for rare diseases. One of the biggest challenges in rare diseases is limited patient and disease data. Building an international registry helps us overcome that challenge and takes an important step

to support therapeutic development. Over time, and with the participation of the entire FOP community, we believe this registry will provide valuable insights into the prevalence, diagnosis, symptoms, impact, and treatment of FOP.”

In December 2014, the IFOPA announced that it would sponsor the development of a global FOP registry called **The FOP Connection Registry**. “There will be two portions to the registry: the patient portal where patients can share their own experience with FOP, and the medical portal: where doctors can enter data about FOP patients under their care. The registry will be available to the international FOP community and to researchers as well as drug developers and regulators, and will help identify trends and provide new insights about FOP.”

The goals of the global registry, as described by the IFOPA are to:

- Empower patients
- Improve collective understanding of FOP
- Organize the community for clinical studies
- Advance the development of treatments

The patient portal of the global FOP registry was launched in 2015. **The Center for Research in FOP & Related Disorders** was proud to advise the IFOPA on developing this global FOP registry. “The international FOP community is small but mighty and speaks with one voice in one language understandable to all: we want a cure and we need one international FOP patient registry owned and operated by the FOP patients to help make that happen. It is one critical goal we can accomplish together and one critical way we can change our world,” said Dr. Fred Kaplan.



Charlie Boyer-White (Chesterfield, UK) sports a new hat



Jordyn Bugarin (Baltimore, Maryland) visits with Dr. Kaplan



FOP Ashley's Cure – Finding a Cure for FOP. FOP Charitable Event at The Ritz Carlton Hotel; Battery Park, NY. With the Martucci Family the morning after the gala event. (From left to right): Mr. Joseph Martucci (Member, IFOPA Board of Directors), Mr. Joseph Martucci, Mrs. Mildred Martucci, Mrs. Diane Martucci, Ms. Ashley Martucci, Dr. Fred Kaplan, Mr. Gary Segal (Ashley's Uncle and Event Co-Chair), Mrs. Donna Segal (Ashley's Aunt and Event Co-Chair) and Ms. Megan Martucci

Greetings from the Center for Research in FOP and Related Disorders at the University of Pennsylvania.



(Seated left to right): Drs. Frederick Kaplan, Eileen Shore and Robert Pignolo.

(Standing left to right): Kamlesh (Kay) Rai, Renee Jurek, Patsy Hooker, Dr. Deyu Zhang, Casey Shechtman, Will Towler, Dr. Haitao Wang, Niambi Brewer, Alexandra Stanley, Dr. John Fong, Dr. Vitali Lounev, Ruth McCarrick-Walmsley, Dr. Girish Ramaswamy, Dr. Salin Chakkalakal, Michael Convente, Katherine Toder, Dr. Sun Peck, and Bob Caron



Maria Wray of Rochester, NY and her parents Felicia and Taylor, and brothers Taylor and Philip visit with Drs. Kaplan and Pignolo at Penn



Dr. Fred Kaplan visits with Kyle McWilliams and his parents Margie and Curtis McWilliams of Victor, Iowa

Part V: Ongoing Programs at the Center

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 can now be 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 an 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 **S**tem cells from **H**uman **E**xfoliated **D**eciduous 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. These cells were used in our recent studies to down-regulate the mutant (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.

Ruth McCarrick-Walmsley 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 all of the necessary return packaging (for several teeth), return FedEx labels, Ruth’s contact information, a tooth diagram to fill out and return, and a copy of instructions. We are also providing information about the program on the IFOPA website, 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 ready and 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 Ruth by phone (610-513-4470) or email (rwalmsle@mail.med.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 Dr. William N. Kelley, M.D., 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 Penn, and at other universities in the United States and around the globe. The program has been in place for 19 years and has been extremely successful in fulfilling its mission of dismantling the physical and conceptual barriers that impede progress in this compelling area of human need.



The Cali Family (Jason, Ian, Amanda and John) meet with Fred Kaplan during a visit to Penn

Over the past 19 years, the Cali Developmental Grants Program has awarded 20 grants of 100,000 dollars each for a total support of 2 million dollars. This innovative program has expanded horizons in FOP research well beyond the physical boundaries of the FOP laboratory at Penn into a true worldwide co-laboratory.

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

The Cali Family Fund and Developmental Grants Program has supported 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 per cent of the scientists and researchers who participated or were represented at the **2014 IFOPA Drug Development Forum** in Boston have been direct or indirect beneficiaries of a Cali Developmental Research Grant from the **Center for Research in FOP & Related Disorders**.

During the past year, the Cali Developmental Grants Program funded research on novel ACVR1 inhibitors in the laboratories of Dr. Charles Hong at Vanderbilt University and Dr. Jay Groppe at Texas A&M University, induced pluripotent stem cell models for FOP in the laboratory of Dr. Ed Hsiao at the University of California – San Francisco, and zebrafish models for FOP in the laboratory of Dr. Mary Mullins at The University of Pennsylvania.

Additional information on the Cali Developmental Grants Program can be obtained from Dr. Kaplan at: Frederick.kaplan@uphs.upenn.edu, or Dr. Eileen Shore at: shore@mail.med.upenn.edu.

Cherish Your Exceptions

One of our vital ongoing programs at The Center is to study, decipher and interpret how variations in ACVR1 mutations cause phenotypic variations in the clinical manifestations and progression of FOP. In 2015, we reported on multi-organ system involvement in two children with a novel and severe variant of FOP

in a major research article in *The Journal of Medical Genetics*.

Individuals classified as FOP variants are broadly distributed into two groups: 1) those who have minimal or no obvious malformations of the great toes and/or adult-onset progressive heterotopic ossification (<2% of all FOP patients), and 2) those who have severe malformations of the great toes and/or wide-spread reduction deficits of the digits of the feet and hands (<2%). Like those with classic FOP, all individuals classified as FOP variants have germline heterozygous activating mutations of ACVR1/ALK2. However instead of carrying the ACVR1 R206H mutation, FOP variants have mutations that cluster at other places in the gene, in either the GS domain or the downstream kinase domain of the receptor.



Drs. Ed Hsiao (San Francisco), Fred Kaplan and Bob Pignolo (Philadelphia) meet to discuss FOP

developmental delays, facial dysmorphology, small malformed teeth, and abnormal hair development. One child had hydrocephalus, sensorineural hearing loss, severe anemia, and a tethered spinal cord; and the other had a congenital heart defect and gonadal dysgenesis with sex reversal (karyotype 46, XY female). Both children had the identical mutation in ACVR1 mutation at c.772A>G; p.Arg258Gly (R258G), not previously described in FOP.

The high fidelity clinical-genetic relationship in these two unrelated and severely affected children suggests that the shared phenotypes are due to the dysregulated activity of the mutant FOP receptor during development and postnatally, and provides vital insight into the structural



Hugo Fahlberg (Eskilstuna, Sweden) with his grandfather Per Hallbert

biology and function of ACVR1 as well as aiding the design of small molecule inhibitors.

Ultra-rare FOP variants, such as the R258G mutation, harbor a unique opportunity to probe phenotype-genotype (clinical-molecular) relationships that have revelational implications for deciphering the role

of the BMP signaling pathway in normal physiology and for understanding how single amino acid substitutions in proteins like ACVR1 alter the function of a highly conserved BMP receptor. Studies on FOP variants have shown that seemingly small variations in the FOP gene can give rise to large variations in clinical findings that provide important insight into the molecular mechanisms of FOP and BMP signaling. Additionally, understanding the effect of a specific mutation on ACVR1 function could help guide the design of pharmacologic agents that will modify or prevent the postnatal consequences of the disease.

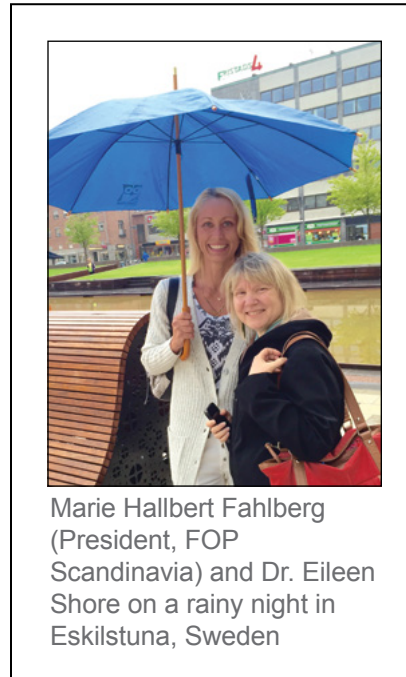
The two unrelated children that we evaluated were the most severe FOP variants ever reported. These two children shared the common features of severe reduction deficits of the hands and feet with absence of nails, progressive heterotopic ossification, under-development of the brain stem, motor and cognitive developmental delay, facial dysmorphology, small malformed teeth, and abnormal hair development. All of the affected tissues have been implicated as affected by dysregulation of the BMP pathway.

Mutations at codon 258 are not the only ones that provide parallax on clinical and genetic features in FOP. Severe phenotypes have been described in FOP variants

with codon 328 mutations that are distinct from the less severe mutations that occur at the same codon in ACVR1. It therefore appears that ACVR1 is particularly sensitive to mutations at codon 328 and 358, suggesting their importance in regulating receptor function and BMP signaling during embryonic development in many tissues including the central nervous system, specifically the brainstem. Although both R258S and R258G germline mutations are causative of FOP, only the latter, associated with the more severe phenotype, has been identified as a somatic mutation at residue 258 in diffuse intrinsic pontine gliomas (DIPGs), a rare childhood brainstem tumor. Somatic activating mutations in ACVR1 presumably function as oncogenes in DIPG, consistent with different outcomes of BMP signaling during brain development. This finding is suggestive of a higher gain-of-function threshold for DIPG, which also requires a second site mutation in a histone gene contributing epigenetic effects.

Our data and those of others support that all identified ACVR1 germline missense mutations influence the promiscuous postnatal induction of cartilage and bone cell differentiation. Progressive postnatal heterotopic ossification is the common feature shared by all patients with FOP. Although the rate of progression and the severity of heterotopic ossification vary among individuals with classic FOP, there appears to be a correlation between the severity of heterotopic ossification and specific mutations among the ACVR1 mutations identified in FOP variant patients.

Enhanced expression of BMP pathway transcriptional targets is observed in FOP cells. Overactive BMP signaling in FOP cells may lead paradoxically to orthotopic ankylosis of the joints and early degenerative joint disease as seen in FOP patients and in animal models of promiscuous BMP signaling. Aberrant ACVR1 signaling may also be relevant to the pathogenesis of degenerative joint disease, as seen in early orthotopic degenerative changes of the great toe, thumb, cervical spine, and in the costovertebral joints before the appearance of FOP flare-ups and subsequent heterotopic ossification. All the classic and common variable features of FOP as well as many, if not all, of the atypical features



Marie Hallbert Fahlberg
(President, FOP
Scandinavia) and Dr. Eileen
Shore on a rainy night in
Eskilstuna, Sweden

evaluated in our study could plausibly be ascribed to dysregulation of the BMP signaling pathway.

Further studies of BMP signaling in animal models of classic and variant FOP will be critical to address these questions. Mouse models containing the ACVR1 R206H mutation successfully mimic the classic FOP phenotype

and a knock-in mouse containing the ACVR1 R258G mutation is expected to provide additional insight into the consequences of this mutation; such a model is under development.

Identification of disease-causing mutations in ACVR1 has important diagnostic and therapeutic implications. Presently, there is no definitive treatment for patients with FOP or its variants and the identification of heterozygous missense mutations in ACVR1 reveals pharmaceutical targets for the development of signal transduction inhibitors (STIs) as well as other therapeutic strategies.

However, in addition to treating FOP, postnatal inhibition of ACVR1 could have a significant role in treating common acquired disorders of orthotopic and heterotopic ossification and, conversely, the mutation(s) of FOP and its variants could be harnessed for tissue engineering to form new bone for therapeutic applications. Clinical-genetic correlations of ACVR1 mutations in FOP will help elucidate ACVR1 signaling mechanisms and *in vivo* functions to further these goals.

Editor's Choice – a Few Highlights from the Literature

Two transformative technologies that have recently burst onto the FOP research scene are worthy of attention: induced pluripotent stem cells and CRISPR-Cas9 genome editing. The first enables the production of patient-specific stem cells from almost any cell type in the body, while the latter enables the rapid development of genetic animal models as well as the permanent correction of genetic mutations.

1. Engineering Stem Cells

Jie Cai and colleagues from Leiden University Medical Center in the Netherlands reported in *Stem Cell Reports*, “Induced pluripotent stem cells to model FOP.” The researchers generated human induced pluripotent stem (iPS) cells from somatic cells of FOP patients who had the classic ACVR1 (R206H) mutation. Mutant ACVR1 changed the efficiency of iPS cells to differentiate into potential FOP bone-forming progenitor cell types: endothelial cells and pericytes. Endothelial cells derived from FOP iPS cells showed reduced expression of vascular endothelial growth factor receptor-2 and could transform into mesenchymal cells through an endothelial-mesenchymal transition. Increased mineralization of pericytes from FOP iPS cells could be partly inhibited by an ACVR1 signal transduction inhibitor. Thus, differentiated FOP human iPS cells recapitulate some aspects of the FOP disease phenotype *in vitro*, and might be useful in further elucidating underlying mechanisms of FOP and development of therapeutic drug candidates.

In a paper reported in *Stem Cells*, Yoshihisa Matsumoto and colleagues from Kyoto University in Japan reported successful *in vitro* FOP “disease recapitulation” using patient specific iPS cells. However, the *in vitro* technique was demanding and required two technical adjustments: appropriate control cells and robust differentiation protocols. Using their method of inducing pluripotent stem cells, the scientists identified two genes responsible for accelerating chondrogenesis (cartilage formation). The findings suggest that the

method of inducing mesenchymal stromal cells from iPS cells may be useful for investigating the molecular mechanisms of FOP and associated drug discovery.

In a feature article in *Methods in Molecular Biology*, Emily Barrouet and Ed Hsiao from the University of California-San Francisco reported “using human induced pluripotent stem cells to model skeletal diseases.” In their major review article, Barrouet and Hsiao state that, “The recent advent of human iPS cells provide an unparalleled opportunity to create human-specific models of human skeletal diseases. iPS cells have the ability to self-renew allowing us to obtain large amounts of starting material, and having the potential to differentiate into any cell type in the body. In addition, they can carry one or more mutations responsible for the disease of interest or be genetically-corrected to create isogenic controls. Our work has focused on modeling rare musculoskeletal disorders including FOP.” In the review, Barrouet and Hsiao discuss their experiences and protocols differentiating human iPS cells towards the osteogenic lineage and their application to model skeletal diseases. A number of critical challenges and exciting new approaches are discussed which will allow the skeletal biology field and specifically the FOP field to harness the potential of human iPS cells as a critical model system for understanding abnormal skeletal formation and bone regeneration.

2. Engineering the Genome

Few discoveries transform a discipline overnight. Recent accomplishments in gene editing across biological disciplines have been so remarkable in the past year that the method known as clustered regularly interspaced short palindromic repeats (or CRISPR-Cas9) was reported as the scientific breakthrough of 2015 in the journal *Science*. And, well it should have, as it has the power to change our world.

Less than a generation ago, we learned how to read the human genome. With CRISPR-Cas9, we have the tools to edit it – permanently. That’s right – not just the likelihood of developing drugs to silence the activity of the renegade ACVR1 gene – but the possibility of

permanently correcting the one ill-fated letter in every cell that could give rise to FOP. The CRISPR-Cas9 technology has yet to win a Nobel Prize, but is likely to do so in the near future. More importantly, the dream of Manuel Robert, a young man with FOP from Buenos Aires, Argentina, may come true someday – a true cure for FOP. Shortly after the FOP gene discovery in 2006, Manuel then 10 years old asked, “Why can’t you just correct the one genetic letter that is misspelled in FOP and cure the condition?” Well, with CRISPR-Cas9, it can now be done for some conditions, and maybe someday for FOP.

As described recently in *Nature*, “Three teams of researchers have used CRISPR-Cas9 gene editing to treat mice that have the most common and severe form of muscular dystrophy. Duchenne muscular dystrophy (DMD) is a fatal disease caused by mutations that disable the gene encoding dystrophin, a vital muscle protein. Teams led by Charles Gersbach of Duke University, Amy Wagers of Harvard University and Eric Olson of the University of Texas Southwestern Medical Center in Dallas used the CRISPR-Cas9 gene editing technique to repair the dystrophin gene in mice that have DMD. The three teams used viruses to shuttle the components of the CRISPR-Cas9 system into the muscle cells of infant and adult mice. Treated mice made functional dystrophin and showed improvements in cardiac and skeletal muscle function.”

Presently, three technical hurdles to applying CRISPR-Cas9 for FOP are 1) to unambiguously identify the molecular bar codes on FOP target cells, 2) to devise a safe, non-viral method (viruses can trigger FOP) of shuttling the CRISPR-Cas9 into the target cells, 3) to identify high-fidelity CRISPR-Cas9 molecular editing tools with no detectable genome-wide off-target effects. Once solved, two viable approaches would be to correct the single gene mutation that causes FOP or simply inactivate the mutant allele. Either approach, in enough cells to accomplish the permanent gene correction with high enough efficiency, could lower the threshold for inducible heterotopic ossification to a clinically insignificant level.

How did CRISPR-Cas9 emerge? Researchers have long sought better ways to edit the genetic code in cultured cells and laboratory organisms to silence, activate, or change targeted genes to gain better understanding of their roles.

This CRISPR-Cas9 technology was conceived in 2007 after a yoghurt company identified an unexpected defense mechanism that its bacteria used to fight viruses. Now, CRISPR-Cas9 has matured into a molecular marvel and much of the world is taking notice of this genome editing method. It is only a slight exaggeration to say that if scientists can dream of a genetic manipulation, CRISPR can now make it happen.

Jennifer Doudna, one of the discoverers of this new technology from the University of California-Berkeley noted in a perspective in *Molecular Cell*, “The remarkable speed at which the CRISPR-Cas9 technology has spread throughout the biological community attests to its substantial impact in transforming our ability to manipulate cells. Genome engineering with CRISPR-Cas9 has become so routine that soon the CRISPR-Cas9 method for editing chromosomal sites in model organisms will require no more attention in research articles than accorded to PCR and molecular cloning. Indeed, the ease with which this technology can be practiced, and its tremendous utility suggests that CRISPR-Cas9 will increasingly become a tool of choice for the next generation of biologists.”

In her successful application for a **Cali Developmental Grant**, Mary Mullins, Ph.D. Professor of Cell and Developmental Biology at the University of Pennsylvania noted, “A new advance that allows genes to be modified in their normal location on a chromosome will allow us to generate a state-of-the-art model of FOP in the zebrafish. The new technology is called CRISPR-Cas9 and it functions very effectively in the zebrafish to modify genes. We have already used this technology to make specific mutations in the type II BMP receptors. We will use this method in a more sophisticated manner to insert the human FOP mutation gene segment into the zebrafish ACVR1 gene to generate a robust model of FOP. This will then allow us to rapidly test compounds

for their ability to block FOP receptor activity using our efficient embryonic assays.”

Importantly, the CRISPR-Cas9 technology is being used at our **FOP Center** to generate new conditional FOP mouse models.

A Profile in FOP Research: A Brief Statement from Haitao Wang, the First Author of the Landmark Study on Hypoxia and Heterotopic Ossification in FOP

“People don’t care what you know until they know that you care.” – Anonymous



Research scientist Haitao Wang at The Center for Research in FOP & Related Disorders

In 1996, driven by the inspiration to help improve people’s lives and to help discover the mystery of diseases, I entered a Ph.D. program at Peking Union Medical College. In 2002, I came to the U.S. to pursue postdoctoral education on innate immunity and liver regeneration. During my fellowship, I solved several basic mechanism questions and published several papers in major research journals. I became well-trained in biomedical research and acquired new knowledge and bench techniques. Now, I am even more enthusiastic to integrate my scientific knowledge to decipher mechanisms of disease and to enhance human health.

I believe I found the lab to discover treatment targets and to ultimately improve patients’ health when I joined the prestigious FOP laboratory. This is truly an amazing environment to perform translational studies. Dedicated researchers, supported by the International FOP Association, have made tremendous advances in finding the gene mutation and deciphering the cause FOP. All of our research is dedicated to finding better answers for patients with FOP. All of our research conditions try to mimic patient’s real exposures during daily life. I never worked in a lab that integrated the concept of bench to bedside so perfectly. Because of the close interaction among physicians, scientists, and patients, I have opportunities to interact directly with patients, attend fundraising events, talk with FOP families during their lab visits, explain how the gene mutation causes FOP, and importantly investigate strategies to develop treatments. At the same time, these direct patient-scientific interactions motivate our basic research tremendously.

During my post-doctoral fellowship in the FOP lab, I obtained excellent training and made important progress in FOP research. Dr. Kaplan, Dr. Pignolo, and Dr. Shore always have insightful and intelligent viewpoints on my projects. I benefit tremendously from this interaction and have gained confidence in pursuing an innovative project from clinical observation, conceiving a hypotheses, designing experiments, analyzing results, and writing drafts of manuscripts. Moreover, I have enjoyed and helped create a collaborative and creative environment in the FOP laboratory.

In my work, I found that the cellular response to hypoxia through HIF-1 α can contribute to heterotopic ossification, and I figured-out the molecular mechanism by which this pathological adaptation occurs. I also showed that HIF-1 α inhibitors could block heterotopic ossification in a mouse model of FOP, which could potentially be developed into clinical drugs for patients.

As a seasoned FOP scientist, I have been well-trained by three top FOP professors. I have acquired solid knowledge and all the necessary techniques to continue to perform my FOP research, and I have the ability to

contribute even more to the FOP community. I will continue to clarify how microenvironmental factors such as hypoxia, temperature, and inflammation affect the initiation and progression of FOP flare-ups. Reversing the exacerbating factors that cause FOP flare-ups can lead to clinical treatments.

Sometimes, I ask myself “What is my life for? How can I make my life more meaningful?” As the father of two children, I got the answer after seeing the hopeful outlook of FOP kids and their families as they anticipate a treatment and perhaps a cure. All ask the same question: “How do the laboratory studies lead to a potential therapy or cure for FOP?” What I do in exploring these answers is meaningful for patients, and also it has become the very significance of my life.



Drs. Robert Pignolo, Fred Kaplan and Haitao Wang at The FOP Center

FOP: The Spoken Word – 2015

During 2015, major lectures on FOP were presented at:

- Biocryst Pharmaceuticals; Birmingham, Alabama
- Children’s Hospital of Philadelphia; Philadelphia, Pennsylvania
- Dapping Hospital and Third Military Medical University; Chongqing, China
- FOPEv Annual Meeting; Valbert, Germany
- FOP Italia Annual Meeting; Rome, Italy

- FOP Netherlands; Amsterdam, Netherlands
- FOP Russia; Moscow, Russia
- FOP Scandinavia; Eskilstuna, Sweden
- International Musculoskeletal Research Conference; Changsha, China
- Lorentz Center Program in FOP; Leiden, The Netherlands
- Northwestern Polytechnical University; Xi’an, China
- Regeneron Pharmaceuticals; Tarreytown, New York
- Sanofi-Penn Center for Innovation - Penn Orphan Disease Center Conference; Philadelphia, Pennsylvania
- Shanghai University of Traditional Chinese Medicine, Shanghai, China
- The University of Hong Kong; Hong Kong, China



Dr. Fred Kaplan, Mr. Mikhail Belyaev (Director of The Northwest Federal District of FOP Russia; St. Petersburg, Russia), Dr. Rolf Morhart (Garmisch-Partenkirchen, Germany), and Dr. Eileen Shore at the Second International FOP Family Meeting and Conference in Moscow, Russia.



Dr. Kaplan at the
FOP Patient and
Family Clinic;
Moscow, Russia

We would like to acknowledge the extraordinary medical, scientific, and patient meetings in 2015 that we were honored to attend and in which we were honored to participate in Amsterdam, The Netherlands; Eskilstuna, Sweden; Rome, Italy; Moscow, Russia and Valbert, Germany. These meetings were a wonderful opportunity to meet with scientists, researchers, physicians, students, and patients from around the world.



Drs. Bob Pignolo and Eileen Shore enjoy a summer picnic along the Autobahn with Ralf Fischer (Oberursel, Germany; President, FOPeV) and his wife Regine Fischer on their way from Frankfurt to Valbert, Germany for the Annual FOPeV meeting

During 2015, highlights of FOP research were presented at local, regional, national, and international FOP family meetings and gatherings in:

- Allentown, Pennsylvania
- Amsterdam, The Netherlands
- Eskilstuna, Sweden
- Moscow, Russia

- Mountainside, New Jersey
- Philadelphia, Pennsylvania
- Rome, Italy
- Valbert, Germany



Augusta Josefsson
Carlsson
(Johanneshov,
Sweden) at The
Scandinavian FOP
Family Meeting in
Eskilstuna, Sweden

FOP: The Written Word – 2015

In 2015, publications from numerous groups on FOP and FOP-related issues appeared in peer-reviewed journals including:

- American Journal of Medical Genetics
- Annals of Human Genetics
- Clinical Gynecology and Obstetrics
- Current Biology
- Current Osteoporosis Research
- Cytokine Growth Factor Reviews
- European Journal of Human Genetics
- Journal of Bone & Mineral Research

- Methods in Molecular Biology
- Proceedings of the National Academy of Sciences
- Rheumatology International
- Science Translational Medicine
- Seminars in Cell and Developmental Biology
- Stem Cells
- Stem Cell Reports
- Trends in Biochemical Science

As of January 1, 2016, the classic paper in *Nature Genetics* (April 2006) describing the discovery of the FOP gene has been cited in 546 major scientific publications worldwide.

FOP: What Can We Do to Help?

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.



Hudson and Lincoln Wheelock (Des Moines, Iowa) with their grandfathers Mark Gambaiana (Chairman Emeritus, IFOPA) and Dr. Doug Wheelock (Sioux City, Iowa) at Lincoln’s Legacy for FOP Research

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 25 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, 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 bring us closer to 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.

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 Penn, 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 meeting therapies and eventually a cure for children and adults with FOP. In 2015, new clinical trials were launched (Clementia's Natural History Study and Palovarotene Phase 2 trial for children) 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 Cali Family Endowment for FOP Research
- The Weldon Family Endowment for FOP Research
- The Isaac and Rose Nassau Professorship of Orthopaedic Molecular Medicine
- The Cali-Weldon Professorship of FOP Research
- The Ian Cali Distinguished Clinician-Scientist Scholarship
- The Roemex Fellowship in FOP Research
- The Jesse David Hendley Foundation
- The Ashley Martucci Fund for FOP Research
- The Gary Whyte FOP Research Fund

- The Brinkman Family
- The McGuire Fund for FOP Research
- The Canadian FOP Families & Friends Network
- FOP France
- FOP Germany (FOPeV)
- FOP Italia
- 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.



Dr. Eileen Shore and Dr. Clarissa Desjardins
(CEO, Clementia Pharmaceuticals)



Patrick Doerr (King of Prussia, PA) and Ashley Kurpiel (Peachtree City, Georgia) visit with Dr. Kaplan in Philadelphia



Anthony Barbera (Elmont, NY) and his father Joe Barbera visit Dr. Kaplan at Penn

The Last Word

*“What we call the beginning is often the end.
And to make an end is to make a beginning.
The end is where we start from.” – T.S. Eliot*

For twenty-three years, we concluded the Annual Report by acknowledging the incredible generosity of our donors and benefactors without whom there would be no research, no progress, and no hope for the future. This year, as last, the acknowledgements are the penultimate word. This year, the last word belongs not to the donors and benefactors, not to the physicians, scientists, researchers, journalists, or historians – but to the founder and creator of this entire enterprise – Jeannie Peeper – whose deep personal struggle with FOP has inspired generations of followers to seek a better future:

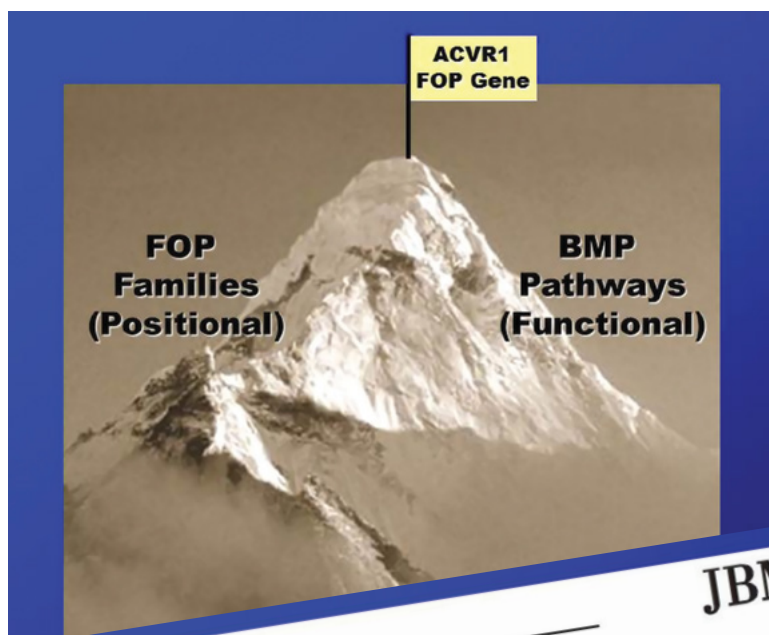
“Since the inception of the IFOPA in 1988, Dr. Kaplan's interest in FOP and the launch of dedicated research at the University of Pennsylvania was comparable to the feeling when the first man walked on the moon. Euphoric!

Beginning, sustained and anchored by the FOP team at the University of Pennsylvania, the past twenty seven years has seen an explosion of progress in the landscape of FOP. The FOP gene discovery in 2006 laid the ground work for researchers, scientists and pharmaceutical companies everywhere to eagerly engage in the discovery of the mysteries of FOP and to quickly navigate possible treatments and a cure.

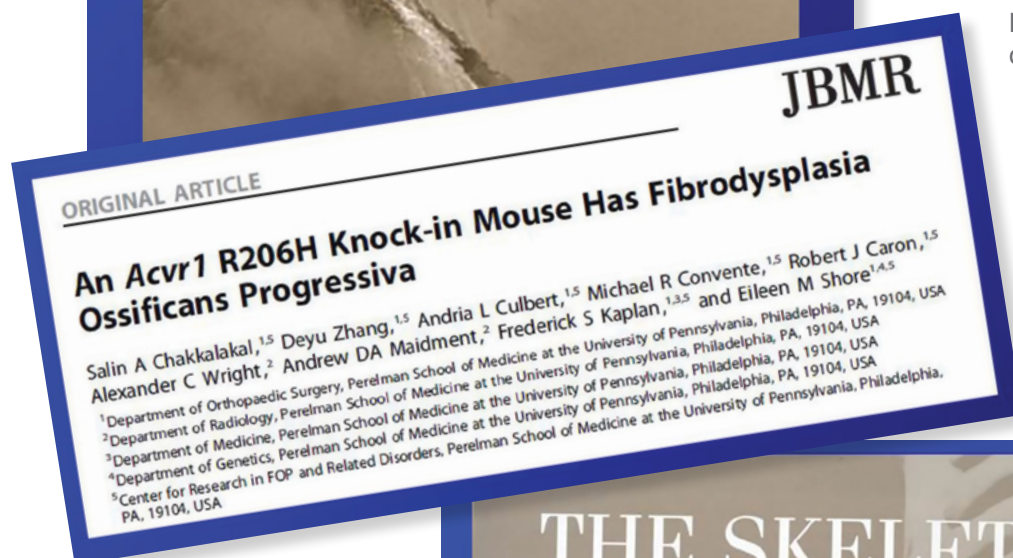
Today, work on FOP is expansive - a global effort in great part due to the inclusive and diverse interdisciplinary atmosphere encouraged by Dr. Fred Kaplan and Dr. Eileen Shore. The FOP community continues to join together, as the possibilities for the future are bright that a treatment for FOP is close at hand.”



Jeannie Peeper, President and Founder of the IFOPA with Annie Peeper, Robert Anderson, and Mrs. Marie Peeper.



Left: Positional genetic mapping studies on small multigenerational FOP families and functional studies on the dysregulation of the BMP pathway in FOP coordinately led to the definitive discovery of ACVR1 as the FOP gene at the Center for Research in FOP & Related Disorders in April, 2006



Left: The definitive mouse model of FOP; 2012.



Right: The Skeleton Key.
The University of Pennsylvania

Part VI: From Time to Time

*“Nature uses only the longest threads to weave her patterns, so each small piece of her fabric reveals the organization of the entire tapestry.”
– Richard Fineman, Nobel Laureate in Physics.*

“The University of Pennsylvania Collaborative Research Effort in FOP was established in September 1989....This effort arose out of a mutual desire to establish the cause and find a cure for this disabling disease.”

Thus began the first Annual Report of the Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project in August 1991 – a quarter of a century ago.

In 1991, after two very productive years investigating clinical and laboratory clues to FOP and fostering the growth of the FOP patient community, we planned and organized the First International Symposium on FOP at the University of Pennsylvania using the funds that an FOP family had raised. We felt that the best use of funds was to bring the most brilliant scientific minds in the fields of skeletal biology and genetics together with FOP patients and families so that the scientists could see and learn first-hand about the magnitude of the problem that needed to be solved. As one prominent scientist noted at the conclusion of the meeting, “I think war was just declared on FOP.” But, the battle was just beginning.

Several weeks before the symposium, Dr. Eileen Shore, PhD joined the mission to establish the FOP Laboratory devoted to this effort. The unique and complex mission was to understand FOP at the cellular, molecular and genetic levels and to use that knowledge to develop effective treatments and eventually a cure for FOP. That work continues to this day.

Over the past 25 years, we have worked to unravel this puzzle at a fundamental level and to stimulate clinical and laboratory investigations on FOP and

related disorders at Penn, other Universities, research institutes, small biotech companies, and pharmaceutical corporations worldwide. Together with generous support from FOP patients and families and a highly supportive university administration, we established **The Center for Research in FOP and Related Disorders** at Penn and a flexible funding structure to expand the geographic boundaries of our work in order to accomplish our goals. We have worked diligently with students, physicians, scientists, and colleagues worldwide to build international collaborations that foster hope and improve lives.

Notable achievements in FOP research from **The Center for Research in FOP & Related Disorders** have been the definition of the clinical and biological basis of genetic disorders of heterotopic ossification, the discovery of the FOP gene, the discovery of the molecular and cellular pathophysiology of FOP, the development of animal models of FOP, the stimulation of drug development for FOP, and the institution of clinical trials for FOP based, in part, on these discoveries. In addition, we have fostered and organized the medical, scientific and patient community with FOP in order to improve the lives of patients worldwide.

First, we will provide a brief historical background for FOP research followed by an annotated bibliography on FOP – highlighting a selected group of major achievements and discoveries in this mission from our group and others over the past 25 years.

I. Historical Background

In 1740, **John Freke**, a London surgeon, published the first case report of FOP (Philos. Trans. Royal Soc. London) describing the disabling heterotopic ossification in this rare disease. Description of the ubiquitous malformations of the great toes came more than 130 years later (Frankel B. Ein Fall von Erblicher Deformatat, Berlin Klin. Wschr. 8:418, 1871). For more than two centuries, little progress was made in understanding FOP.

Following the Second World War, the computer genius and cryptographer **Alan Turing** (who deciphered

the German Enigma and Lorenz codes and was cited by Winston Churchill as making “the single biggest contribution to the Allied victory”) turned his scientific attention to mathematical models of development. In his classic paper, *The chemical basis of morphogenesis* (Philos. Trans. Royal Soc. London. 237:37-72, 1952), Turing coined the word “morphogen” and proposed a diffusible chemical mechanism for pattern formation in living organisms.

In the early 1960’s, **Marshall Urist**, a UCLA orthopaedic surgeon, working with the US Atomic Energy Commission to investigate the skeletal deposition of radioisotopes following atmospheric tests of nuclear weapons discovered that a fraction of demineralized bone matrix could induce new skeletal elements when placed at extraskeletal sites (**Bone formation by autoinduction**. Science 150: 893-899, 1965). Urist called this fraction, “bone morphogenetic protein (BMP),” and showed that BMP acted as a morphogen-like substance during skeletal formation and regeneration.

In 1988, **John Wozney, Vicki Rosen** and colleagues cloned the human genes for BMP1-7 and showed that the recombinant BMPs induced heterotopic ossification by an endochondral process (**Novel regulators of bone formation: molecular clones and activities**. Science 242: 1528-1534). Others rapidly demonstrated that the BMPs acted as gradient morphogens during vertebrate development.

In 1988, **William Gelbart**, Professor of Molecular and Cellular Biology at Harvard University, identified the *Drosophila* protein Decapentaplegic (Dpp) as a morphogen in the development of the exoskeleton of the fruit fly. Gelbart quickly recognized that Dpp was the dipteran homologue of human BMP2/4, and that the BMPs were highly conserved over more than a half billion years of evolution (**The decapentaplegic gene: a TGF-beta homologue controlling pattern formation in Drosophila**. Development 107 (S):65-74, 1989).

These milestones provide the historical context for the studies that follow.

II. Annotated Bibliography

1. Kaplan FS, Tabas JA, Zasloff MA. Fibrodysplasia ossificans progressiva: A clue from the fly? **Calcif Tiss Int** 47: 117-125, 1990

In a first attempt to make sense of this enigmatic childhood disease, we evaluated the published data on the natural history of FOP and discovered that characteristic patterns of disease progression in FOP patients were similar to developmental abnormalities of the exoskeleton induced by mutations of the decapentaplegic (dpp) locus in the fruit fly, *Drosophila melanogaster*. But flies don’t have bones and people don’t have wings. What could be the basis of the similarity? We were shocked to find that the dpp locus in the fly encodes a protein nearly identical to BMP2 and BMP4. Thus, the same blueprints that are used to build the exoskeleton of the fly are used to build the internal skeleton of humans. This startling finding suggested that FOP might not only be a bone disease but rather a skeletal disease and that the responsible molecular pathways might be similar in humans and *Drosophila* regardless of whether the mutations encoded endo- or exoskeleton. Thus, we proposed that FOP was a skeletal (not bone) disorder characterized by a disturbed developmental expression of the BMP signaling pathway resulting in a gain of function. The developmental similarities between the decapentaplegic abnormalities in the fly and FOP in man suggested a useful model for the study of FOP – both linked to the ancestral blueprints of the BMP pathway. Such a model might be especially fruitful in suggesting a molecular basis for FOP. Stimulated by observations in the fruit fly, this first, brief paper grappled with the enigmatic pathogenesis of FOP and proposed the hypothesis that a bone morphogenetic protein (BMP) pathway abnormality was the cause of FOP. Although broadly misunderstood and highly controversial, the paper served as the cornerstone for establishing the FOP Collaborative Research Project, the **Center for Research in FOP and Related Disorders**, the meteoric expansion of the newly-established International Fibrodysplasia Ossificans Progressiva Association (IFOPA) and all the studies that followed over the next twenty-five years. Ultimately, the

disease-causing mutation discovered in people with FOP would prove to be in a BMP type I receptor, a keystone of the BMP signaling pathway (Nat Gen 38:525-527, 2006) and a receptor for Dpp. Introduction of the FOP point mutation into the *Drosophila* genome (by Kristi Wharton and colleagues) would produce a fly with FOP (Dev Dynamics 241:200-214, 2012).

2. Tabas JA, Zasloff M, Wasmuth JJ, Emanuel BS, Altherr MR, McPherson JD, Wozney JM, Kaplan FS. Bone morphogenetic protein: Chromosomal localization of human genes for BMP1, BMP2A and BMP3. **Genomics** 9: 283-289, 1991
3. Hahn GV, Cohen RB, Wozney JM, Levitz CL, Shore EM, Zasloff MA, Kaplan FS. A bone morphogenetic protein subfamily: chromosomal localization of human genes for BMP5, BMP6, and BMP7. **Genomics** 14: 759-762, 1992
4. Tabas JA, Hahn GV, Cohen RB, Seaunez HN, Modi WS, Wozney JM, Zasloff M, Kaplan FS. Chromosomal assignment of the human gene for bone morphogenetic protein 4. **Clin Orthop Rel Res** 293: 310-316, 1993

These three studies (2-4), mapped the chromosomal locations of the human genes that encode BMP 1, 2, 3, 4, 5, 6, and 7 and provided the scientific basis for future linkage and linkage exclusion studies of the BMP genes in FOP.

5. Cohen RB, Hahn GV, Tabas J, Peeper J, Levitz CL, Sando A, Sando N, Zasloff M, Kaplan FS. The natural history of heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. **J Bone Joint Surg** 75-A: 215-219, 1993

This study identified the temporal and spatial patterns of progressive heterotopic ossification in FOP and together with the following paper on the histopathology of FOP, changed the outlook on the pathogenesis of FOP from a dysregulation of bone formation to a dysregulation of skeletal formation (the formation of normal skeletal elements at ectopic locations).

6. Kaplan FS, Tabas J, Gannon FH, Finkel G, Hahn GV, Zasloff MA. The histopathology of fibrodysplasia ossificans progressiva: an endochondral process. **J Bone Joint Surg** 75-A: 220-230, 1993

This important study revealed that the histopathology of extraskeletal bone formation in FOP was due to heterotopic endochondral ossification (HEO) at ectopic sites, thus suggesting that dysregulation of genes associated with skeletogenesis were intimately involved in the pathophysiology of the disease.

7. Kaplan FS, McCluskey W, Hahn G, Tabas J, Muenke M, Zasloff MA. Genetic transmission of fibrodysplasia ossificans progressiva. **J Bone Joint Surg** 75-A: 1214-1220, 1993

This seminal report established that FOP was, in fact, a genetic disease. The genetic transmission of FOP from an affected father to all three children (two daughters and a son), established the autosomal dominant Mendelian basis of genetic inheritance in FOP and excluded X-linked inheritance. Prior to the publication of this report, it was assumed that FOP was a genetic disease, but that reproductive fitness was low, thus accounting for the lack of documented inheritance and the existence of only sporadic cases. The identification of this small, multigenerational family prompted a worldwide search for multigenerational families. Over the next 15 years, with the help of a network we established of FOP physicians around the globe, we were able to identify a total of seven small multigenerational families (rural Georgia, suburban Michigan, London, Paris, rural Bavaria, Seoul, and the Amazon) that served as the cornerstone for a genome-wide linkage analysis that definitively identified the chromosomal location of the FOP gene.

8. Rocke DM, Zasloff M, Peeper J, Cohen RB, Kaplan FS. Age and joint-specific risk of initial heterotopic ossification in patients who have fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 301: 243-248, 1994

This study described Kaplan-Meier survival curves of joint function for all major axial and appendicular sites of involvement in FOP and established “the standard model” for all future natural history studies and clinical trials in FOP.

9. Kaplan FS, Strear CM, Zasloff MA. Radiographic and scintigraphic features of modeling and remodeling in the heterotopic skeleton of patients who have fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 304: 238-247, 1994

This was the first comprehensive report of congenital and post-natal radiographic features of FOP, established that normal bone remodeling occurred in the heterotopic skeleton of individuals with FOP, and suggested that heterotopic bone in FOP was perfectly normal bone but at an ectopic location.

10. Shah PB, Zasloff MA, Drummond D, Kaplan FS. Spinal deformity in patients who have fibrodysplasia ossificans progressiva. **J Bone Joint Surg** 76-A: 1442-1450, 1994

This clinical research study established the dangers of scoliosis surgery in FOP and established new guidelines for the management of progressive spinal deformity in the condition.

11. Einhorn TA, Kaplan FS. Traumatic fractures of heterotopic bone in patients who have fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 308: 173-177, 1994

This brief report documented accelerated healing of fractures of heterotopic bone in patients with FOP.

12. Janoff HB, Tabas JA, Shore EM, Muenke M, Dalinka MK, Schlesinger S, Zasloff MA, Kaplan FS. Mild expression of fibrodysplasia ossificans progressiva. **J Rheumatology** 22: 976-978, 1995

This brief report documented the variable progression FOP and advanced our understanding of the genetics of the condition.

13. Lanchoney TF, Cohen RB, Rocke DM, Zasloff MA, Kaplan FS. Permanent heterotopic ossification at the injection site after diphtheria-tetanus-pertussis immunizations in children who have fibrodysplasia ossificans progressiva. **J Pediatrics** 126: 762-764, 1995

This seminal study established that intramuscular immunizations caused permanent and disabling heterotopic ossification in FOP, changed the routine care of children with FOP, and provided the rationale for future basic science studies investigating the role of the innate immune system and inflammation in the pathophysiology of FOP.

14. Luchetti W, Cohen RB, Hahn GV, Rocke DM, Helpin M, Zasloff M, Kaplan FS. Severe restriction in jaw movement after routine injection of local anesthetic in patients who have fibrodysplasia ossificans progressiva. **Oral Surg Oral Med Oral Pathol Oral Radiol Endod** 81: 21-25, 1996

This important clinical study established that routine mandibular blocks for dental care triggered permanent and disabling heterotopic ossification in FOP, altered the medical/dental recommendations for routine dental care of patients with FOP, and provided the basis for future basic science studies on the response to soft tissue injury in FOP.

15. Janoff HB, Muenke M, Johnson LO, Rosenberg A, Shore EM, Okereke E, Zasloff M, Kaplan FS. Fibrodysplasia ossificans progressiva in two half-sisters: evidence for maternal mosaicism. **Am J Med Genetics** 61: 320-324, 1996

This report established the existence of gonadal mosaicism in FOP and changed the basis for genetic counseling in the disease.

16. Janoff HB, Zasloff MA, Kaplan FS. Submandibular swelling in patients with fibrodysplasia ossificans progressiva. **Otolaryngol Head Neck Surg** 114: 599-604, 1996

This important clinical study documented submandibular flare-ups as a potentially fatal complication of FOP, and established guidelines for prevention and treatment of this problematic regional flare-up.

17. Shafritz AB, Shore EM, Gannon FH, Zasloff MA, Taub R, Muenke M, Kaplan FS. Over-expression of an osteogenic morphogen in fibrodysplasia ossificans progressiva. **N Engl J Med** 335: 555-561, 1996

This seminal paper presented the first compelling evidence that an abnormality in BMP signaling was associated with FOP and that the immune system was involved in the pathogenesis of the disease.

18. Moriatis JM, Gannon FH, Shore EM, Bilker W, Zasloff MA, Kaplan FS. Limb swelling in patients who have fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 336: 247-253, 1997

This study documented the pathophysiology of limb swelling in patients with FOP and established guidelines for prevention and treatment of this complication.

19. Gannon FH, Kaplan FS, Olmsted E, Finkel G, Zasloff MA, Shore EM. Bone morphogenetic protein 2/4 in early fibromatous lesions of fibrodysplasia ossificans progressiva. **Hum Pathol** 28: 339-343, 1997

This laboratory study demonstrated the presence of BMP4 in the early pre-osseous lesions of FOP. Previously, pathologists could not distinguish between aggressive juvenile fibromatosis and pre-osseous fibroproliferative lesions of FOP. This study showed that lesional cells produce robust amounts of BMP4 that fuel the explosive growth of FOP lesions.

20. Gannon FH, Valentine BA, Shore EM, Zasloff MA, Kaplan FS. Acute lymphocytic infiltration in an extremely early lesion of fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 346: 19-25, 1998

This critical study established the presence of lymphocytic infiltration in the earliest stages of FOP lesion formation. The observation elucidated the histopathology of FOP by defining the skeletal metamorphosis in two phases – an early inflammatory and catabolic (tissue destruction) phase and a subsequent anabolic and endochondral (tissue formation) phase.

21. Kaplan FS, Sawyer J, Connors S, Keough K, Shore E, Gannon F, Glaser D, Rocke D, Zasloff M, Folkman J. Urinary basic fibroblast growth factor: a biochemical marker for preosseous fibroproliferative lesions in patients with FOP. **Clin Orthop Rel Res** 346: 59-65, 1998

This collaborative research study identified the first stage-specific biomarker in FOP (in the highly angiogenic pre-osseous fibroproliferative lesion) and served as a benchmark for future research studies on disease- and stage-specific biomarkers in FOP.

22. Kussmaul, WG, Esmail AN, Sagar Y, Ross J, Gregory S, Kaplan FS. Pulmonary and cardiac function in advanced fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 346: 104-109, 1998

This important prospective clinical research study of cardiopulmonary function in patients with FOP established the role of restrictive chest wall disease in the evolution of right-sided heart failure and pulmonary hypertension in advanced FOP.

23. Glaser DL, Rocke DM, Kaplan FS. Catastrophic falls in patients who have fibrodysplasia ossificans progressiva. **Clin Orthop Rel Res** 346: 110-116, 1998

This important epidemiological study documented the causative role of falls in FOP flare-ups and subsequent life-threatening heterotopic ossification and established international guidelines for prevention of falls and life-threatening head injuries in FOP.

24. Zasloff MA, Rocke DM, Crofford LJ, Hahn GV, Kaplan FS. Treatment of patients who have fibrodysplasia ossificans progressiva with isotretinoin. **Clin Orthop Rel Res** 346: 121-129, 1998

This open-label clinical study repurposed an available retinoid to prevent heterotopic endochondral bone formation in FOP and was based on the known teratogenic effect of retinoids on embryonic endochondral ossification. This study re-focused attention on retinoids as a class of molecules that might inhibit the disabling post-natal heterotopic endochondral bone formation in FOP.

25. Shore EM, Xu M-q, Shah PB, Janoff HB, Hahn GV, Deardorff MA, Sovinsky L, Spinner NB, Zasloff MA, Wozney MA, Kaplan FS. The human bone morphogenetic protein (BMP4) gene: molecular structure and transcriptional regulation. **Calcif Tissue Int** 63: 221-229, 1998

This laboratory research study determined the molecular structure and transcriptional regulation of the

human BMP4 gene, a critically important gene in the pathogenesis of FOP.

26. Levy CE, Lash AT, Janoff HB, Kaplan FS. Conductive hearing loss in individuals with fibrodysplasia ossificans progressiva. **Am J Audiol** 8: 29-33, 1999

This study defined the prevalence and pathogenesis of hearing loss in FOP and established guidelines for detection and treatment.

27. Mahboubi S, Glaser DL, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva. **Pediatr Radiol** 31: 307-314, 2001

This report provided a comprehensive description of the classic radiographic features of FOP.

28. Kaplan FS, Glaser DL, Shore EM, Mitchell D, Emerson S, and the International Clinical Consortium of the Third International Symposium on Fibrodysplasia Ossificans Progressiva. The medical management of fibrodysplasia ossificans progressiva: current treatment considerations. **Clin Proc Third Intl Symp FOP** 1: 1-52, 2001

Physicians and scientists worldwide assembled at the Third International Symposium on FOP in Philadelphia in 2000 to draft the first evidence-based guidelines for the management of FOP. The Third International Symposium on FOP, as with its predecessors in 1991 and 1995, was organized for physicians, scientists, patients and families to educate each other about FOP, provide a forum for clinical consultation and clinical research, and catalyze future research collaborations.

29. Gannon FH, Glaser D, Caron R, Thompson LD, Shore EM, Kaplan FS. Mast cell involvement in fibrodysplasia ossificans progressiva. **Hum Pathol** 32: 842-848, 2001

Lymphocytes and macrophages had been identified in early FOP lesions, but the intense and rapidly appearing edema of early FOP flare-ups suggested that other cells and factors are also at work. This paper documented

the previously unrecognized and surprising presence of intense mast cell infiltration at every stage of FOP lesion formation, and stimulated much additional experimentation on the role of mast cells in the pathophysiology of the disease.

30. Ahn J, Serrano de la Peña L, Shore EM, Kaplan FS. Paresis of a bone morphogenetic protein-antagonist response in a genetic disorder of heterotopic skeletogenesis. **J Bone Joint Surg** 85-A: 667-674, 2003

Following the discovery of over-expression of BMP4 in cells of patients with FOP, studies in our lab excluded BMP4 as the causative gene in FOP. We therefore explored the myriad feedback switches in the BMP signaling pathway that might be dysregulated in the disease. Our rationale was: If BMP4 expression was elevated in FOP cells, but the BMP4 gene was not mutated, perhaps a secreted BMP inhibitor was under-expressed, and if so, perhaps that was the cause of FOP. In this important study, we found that expression of multiple BMP antagonists (Noggin, Gremlin, etc) was reduced in FOP cells and that the genes encoding those antagonists were not mutated in FOP patients. This curious finding led to the discovery that FOP cells (in contrast to cells from unaffected individuals) were not able to regulate the concentration of BMP4 in their environment. Taken together, these findings strongly suggested that if FOP cells over-expressed BMP4, under-expressed multiple BMP antagonists, and could not regulate the concentration of BMP4 in their environment, then there must be a primary abnormality in one of the cell surface receptors for BMP4. At the time, two such receptors were known, BMPRIA and BMPRII. We examined both receptors, but could not identify a mutation in either one. Through many studies such as this, a great deal of extremely valuable insight was gained about the dysregulation of the BMP signaling pathway in FOP cells. Such insight eventually led to the definitive identification of the FOP gene.

31. Hegyi L, Gannon FH, Glaser DL, Shore EM, Kaplan FS, Shanahan CM. Stromal cells of fibrodysplasia ossificans progressiva lesions express smooth muscle lineage markers and the osteogenic transcription factor Runx2/Cbfa-1: clues to a vascular origin of heterotopic ossification. **J Pathol** 201: 141-148, 2003

This basic science study provided evidence of a vascular origin of heterotopic endochondral ossification in FOP and stimulated further investigation into the cellular origins of the heterotopic skeleton of FOP.

32. Glaser DL, Economides AN, Wang L, Liu X, Kimble RD, Fandl JP, Wilson JM, Stahl N, Kaplan FS, Shore EM. In vivo somatic cell gene transfer of an engineered noggin mutein prevents BMP4-induced heterotopic ossification. **J Bone Joint Surg** 85-A: 2332-2342, 2003

This landmark study demonstrated that it was possible to chemically modify a potent locally-acting BMP antagonist (Noggin) and convert it into a circulating hormone for systemic delivery. That accomplished, the circulating factor inhibited BMP-induced heterotopic ossification in a surrogate mouse model of FOP.

33. Scarlett RF, Rocke DM, Kantanie S, Patel JB, Shore EM, Kaplan FS. Influenza-like viral illnesses and flare-ups of fibrodysplasia ossificans progressiva (FOP). **Clin Orthop Rel Res** 423: 275-279, 2004

We showed here that viral infections, specifically influenza, could trigger FOP flare-ups and lead to heterotopic ossification. As a result, this study led to international guidelines on influenza immunization for FOP patients, measures to prevent and mitigate influenza infections in FOP patients, and more recently to laboratory investigations on the role of the innate immune system and toll-like receptors in triggering viral-associated flare-ups in FOP.

34. Serrano de la Peña L, Billings PC, Fiori JL, Ahn J, Shore EM, Kaplan FS. Fibrodysplasia ossificans progressiva (FOP), a disorder of ectopic osteogenesis, misregulates cell surface expression and trafficking of BMPRIA. **J Bone Miner Res** 20: 1168-1176, 2005

This laboratory study provided strong support that a dysregulated BMP receptor was the proximate cause of FOP.

35. Schaffer AA, Kaplan FS, Tracy MR, O'Brien ML, Dormans JP, Shore EM, Harland RM, Kusumi K. Developmental anomalies of the cervical spine in patients with fibrodysplasia ossificans progressiva are distinctly different from those in patients with Klippel-Feil syndrome. **Spine** 30: 1379-1385, 2005

Clinical examination of children with FOP revealed impairment of cervical spine motion in infancy and early childhood, even before heterotopic ossification appeared at that site. This investigation revealed fusions of the cervical vertebra identical in scope and distribution to those seen in Noggin knockout mice (Science 280: 1455-1457, 1998). Although Noggin is not a causative gene for FOP, this study provided unequivocal support for the hypothesis that the underlying molecular pathology of FOP activates the BMP signaling pathway both pre and post-natally.

36. Hebel N, Shore EM, Kaplan FS. Three pairs of monozygotic twins with fibrodysplasia ossificans progressiva: the role of environment in the progression of heterotopic ossification. **Clin Rev Bone & Miner Metab** 3: 205-208, 2005

Genetic and environmental factors affect the phenotype of FOP, but their relative effects were unknown. We studied three pairs of monozygotic twins with FOP and found that, within each pair, congenital toe malformations were identical. However, post-natal heterotopic ossification varied greatly between each twin depending on life history and environmental exposure. This study showed that genetic determinants strongly influence disease features during prenatal development

and that environmental factors more strongly influence postnatal progression of the disease.

37. Kitterman JA, Kantanie S, Rocke DM, Kaplan FS. Iatrogenic harm caused by diagnostic errors in fibrodysplasia ossificans progressiva. **Pediatrics** 116: 654-661, 2005

To document the frequency of diagnostic errors in FOP and the complications resulting from misdiagnoses, we conducted a worldwide survey of FOP patients. Incorrect diagnoses were given initially to 87% of individuals with FOP. This astonishing rate of diagnostic errors occurred worldwide, regardless of ethnicity, geographic background, or physician's specialty. The most common incorrect diagnosis was cancer (32%). The mean period from the onset of symptoms to correct diagnosis was four years, and the median number of physicians consulted before the correct diagnosis of FOP was six. In 67% of patients, unnecessary invasive procedures (biopsies) were performed; 68% received inappropriate therapies. Forty-nine percent of all patients reported permanent loss of mobility resulting from invasive medical interventions that caused post-traumatic heterotopic ossification. This study showed dramatically that diagnostic errors and inappropriate medical procedures lead to permanent harm and alter the natural history of FOP.

38. Shore EM, Xu M, Feldman GJ, Fenstermacher DA, Cho T-J, Choi IH, Connor JM, Delai P, Glaser DL, Le Merrer M, Morhart R, Rogers JG, Smith R, Triffitt JT, Urtizberea JA, Zasloff M, Brown MA, Kaplan FS. A recurrent mutation in the BMP type I receptor ACVR1 causes inherited and sporadic fibrodysplasia ossificans progressiva. **Nature Genetics** 38: 525-527, 2006

This is the study that changed everything; it identified the FOP gene, defined the canonical genetic mutation in all individuals with classic FOP and identified the central molecular target for therapeutic intervention and eventual cure of the condition.

Associated Press: Researchers Discover Gene That Creates Second Skeleton

Pinpointing Cause of Fibrodysplasia Ossificans Progressiva (FOP) Will Accelerate Development of Treatments for FOP and Common Bone Disorders

(Philadelphia, PA) – Researchers at the University of Pennsylvania School of Medicine have located the “skeleton key,” a gene that, when damaged, causes the body’s skeletal muscles and soft connective tissue to undergo a metamorphosis into bone, progressively locking joints in place and rendering movement impossible. Identifying the gene that causes fibrodysplasia ossificans progressiva (FOP), one of the rarest and most disabling genetic conditions known to humans and a condition that imprisons its childhood victims in a “second skeleton,” has been the focus at Penn’s Center for Research in FOP and Related Disorders for the past 15 years. This important discovery is relevant, not only for patients with FOP, but also for those with more common skeletal conditions.

Senior authors Eileen M. Shore, PhD, and Frederick S. Kaplan, MD, both from the Penn Department of Orthopaedic Surgery, and their international consortium of colleagues, report their findings in the April 23 advanced online edition of *Nature Genetics*. “The discovery of the FOP gene is relevant to every condition that affects the formation of bone and every condition that affects the formation of the skeleton,” says Kaplan.

The discovery of the FOP gene was the result of painstaking work by the Penn scientists and their colleagues in the International FOP Research Consortium over many years. It involved the identification and clinical examination of multigenerational families, often in remote regions of the world; genome-wide linkage analysis; identification of candidate genes; and finally, the DNA sequencing and analysis of those candidate genes. The team found that FOP is caused by a mutation of a gene for a receptor called ACVR1 in the bone morphogenetic protein-signaling pathway.

Kaplan describes FOP as the “Mount Everest” of genetic skeletal disorders. His lifelong ambition, as he puts it “is to conquer the summit of this daunting mountain range and see this emerging knowledge turned into novel therapies that can dramatically improve the life of these children. This is nothing less than a campaign for physical independence and personal freedom for these kids. If the knowledge helps us to see farther to help others, that will be great, but this work is for and about the children.”

The Penn team originally surmised that FOP was caused by a mutation of a gene in the bone morphogenetic protein (BMP) signaling pathway, one of the most highly conserved signaling pathways in nature. BMPs are regulatory proteins involved in the embryonic formation and post-natal repair of the skeleton.

Indeed, the FOP gene encodes a BMP receptor called Activin Receptor Type IA, or ACVR1, one of three known BMP Type I receptors. BMP receptors are protein switches that help determine the fate of the stem cells in which they are expressed. The ACVR1 protein is 509 amino acids long, and in FOP the amino acid histidine is substituted for the amino acid arginine at amino acid position 206 in all affected individuals.

FOP is the first human genetic disease ascribed to ACVR1. “Our identification of ACVR1 as a critical regulator of endochondral bone formation during embryogenesis and in post-natal tissues will undoubtedly re-focus thinking and stimulate new research directions,” says Shore. “This discovery will have a major impact on the study of skeletal biology and regenerative medicine.

“This single amino acid substitution is predicted to change the sensitivity and activity of the receptor,” continues Shore. “As is the case for most genes, every cell has two copies of the *ACVR1* gene. In FOP patients, one of the two *ACVR1* gene copies harbors a mutation that causes the ACVR1 protein to be incorrectly made.”

In FOP, the *ACVR1* gene is damaged by the substitution of a single genetic letter at a specific location in the gene. The single nucleotide substitution changes

the meaning of the genetic message encoded by the *ACVR1* gene. “Thus, the substitution of one genetic letter for another out of six billion genetic letters in the human genome – the smallest and most precise change imaginable – is like a molecular terrorist that short circuits a functioning set of muscles and connective tissues and transforms them into a second skeleton – in essence turning a light bulb into an atom bomb,” says Kaplan.

The researchers have found that every person with classic FOP has the identical mutation in the *ACVR1* gene. “We now know the cause for FOP at the genetic level, and we expect that it will not be long before we understand the mechanism at the molecular level,” says Kaplan. “That knowledge may someday be used, not just for understanding and treating FOP, but for treating many common disorders that affect the skeleton – conditions such as non-genetic forms of extra bone growth that may occur following total hip replacement, head injuries, spinal cord injuries, sports injuries, blast injuries from war, and even osteoarthritis and damaged heart valves.

Perhaps someday we will be able to harness the gene mutation that causes the renegade bone formation in FOP and make bone in a controlled way – for patients who have severe osteoporosis, for those with severe bone loss from trauma, for those with fractures that fail to heal or spinal fusions that are slow to heal, or for those with congenital malformations of the spine and limbs. We have reached a summit on our epic journey to understand FOP – knowledge we desperately need to help the kids and that will likely help many others. We still have a long way to go, but finally we can see a therapeutic horizon above the clouds, and the view is promising.”

39. Kaplan FS, Glaser DL, Shore EM, Pignolo RJ, Xu M, Zhang Y, Senitzer D, Forman SJ, Emerson SG. Hematopoietic stem-cell contribution to ectopic skeletogenesis. **J Bone Joint Surg Am** 89: 347-357, 2007

This remarkable study established that that bone marrow transplantation does not cure FOP, that the immune system plays a seminal role in triggering flare-ups of FOP, that immunosuppression inhibits flare-ups of FOP, and importantly that even a normal immune system can trigger FOP in a genetically susceptible host. This study showed that therapeutic regulation of immune and connective tissue cell populations involved in FOP lesions holds promise for treatment of FOP and possibly other disorders of heterotopic ossification.

40. Kaplan FS, Glaser DL, Pignolo RJ, Shore EM. A new era of fibrodysplasia ossificans progressiva (FOP): a druggable target for the second skeleton. **Expert Opinion on Biological Therapy** 7: 705-712, 2007

The discovery of the FOP gene established a critical milestone in understanding FOP, revealed a highly conserved druggable target in the TGFβ/BMP signaling pathway, and stimulated therapeutic approaches for the development of small molecule signal transduction inhibitors for ACVR1. Effective therapies for FOP, and possibly for a vast array of more common conditions of heterotopic ossification, will be based on blocking ACVR1, a critical node in the BMP signaling pathway.

41. Groppe JC, Shore EM, Kaplan FS. Functional modeling of the ACVR1 (R206H) mutation in FOP. **Clin Orthop Rel Res** 462: 87-92, 2007

Protein modeling predicted that substitution with histidine, and only histidine, at codon 206 in ACVR1 (the classic FOP mutation seen in 97% of individuals with FOP) creates a pH-sensitive switch within the activation domain of the receptor that leads to ligand-independent activation of ACVR1 in FOP. This study has important implications in developing kinase inhibitors of mutant ACVR1 in FOP.

42. Kaplan FS, Groppe J, Pignolo RJ, Shore EM. Morphogen receptor genes and metamorphogenesis: skeleton keys to metamorphosis. **Ann NY Acad Sci** 1116: 113-133, 2007

Metamorphosis, the postnatal transformation of one normal tissue or organ system into another, is a biological process rarely seen in higher vertebrates or mammals, but exemplified pathologically by FOP. The recurrent missense mutation in ACVR1, a morphogen receptor, is one of the most specific disease-causing mutations in the human genome and the first identified human metamorphogene. The study of skeletal metamorphosis in FOP provides profound insight into the molecular mechanisms that ensure phenotypic stability following morphogenesis and that ordinarily lay deeply hidden in the highly conserved signaling pathways that regulate cell fate. Such insight is applicable to a broad range of human afflictions.

43. O'Connell MP, Billings PC, Fiori JL, Deirmengian G, Roach HI, Shore EM, Kaplan FS. HSPG modulation of BMP signaling in fibrodysplasia ossificans progressiva cells. **J Cellular Biochem** 102: 1493-1503, 2007

Cell surface heparan sulfate proteoglycans (HSPGs) play important roles in morphogen gradient formation and BMP signaling. This study supports that HSPG modulation of BMP signaling is altered in cells from patients with FOP and that altered HSPG-mediated BMP signaling may play a role in the pathogenesis of the disease.

44. Deirmengian GK, Hebela NM, O'Connell M, Glaser DL, Shore EM, Kaplan FS. Proximal tibial osteochondromas in patients with fibrodysplasia ossificans progressiva. **J Bone Joint Surg Am** 90: 366-374, 2008

Proximal tibial osteochondromas are a common phenotypic feature of FOP, a finding that expands the recognized consequences of recurrent activating mutations in ACVR1 to include not only congenital skeletal malformations and heterotopic ossification but also benign osteochondral neoplasms or orthotopic

lesions of skeletal modeling. This study provides insight into the genetic basis of osteochondroma formation in patients with FOP and into more common conditions in which these lesions occur.

45. Billings PC, Fiori JL, Bentwood JL, O'Connell MP, Jiao X, Nussbaum B, Caron RJ, Shore EM, Kaplan FS. Dysregulated BMP signaling and enhanced osteogenic differentiation of connective tissue progenitor cells from patients with fibrodysplasia ossificans progressiva. **J Bone Miner Res** 23: 305-313, 2008

The study of FOP is hampered by the lack of readily available primary connective tissue progenitor cells. We isolated such cells from discarded primary teeth of patients with FOP and from controls and discovered dysregulation of BMP signaling and rapid osteoblast differentiation in FOP progenitor cells compared with control cells. This is the first study of BMP signaling and osteogenic differentiation in primary connective tissue progenitor cells from patients with FOP. Our data strongly support both basal and ligand-stimulated dysregulation of BMP signaling from mutant ACVR1 in FOP. This study revolutionized our understanding of dysregulated BMP signaling in FOP, dramatically expanded the repertoire of in vitro studies that could be performed in a primary cell type relevant to the pathophysiology of FOP and provided a cellular basis for primary drug screening in FOP.

46. Kaplan FS, Xu M, Glaser DL, Collins F, Connor M, Kitterman J, Sillence D, Zackai E, Ravitsky V, Zasloff M, Ganguly A, Shore EM. Early diagnosis of fibrodysplasia ossificans progressiva. **Pediatrics** 121: e1295-e1300, 2008

Most patients with FOP are misdiagnosed early in life before the appearance of HO and undergo diagnostic procedures that can cause lifelong disability. With the genetic cause of FOP identified, genetic testing for FOP is now possible, even before the appearance of HO. We evaluated seven children for diagnosis of FOP before the onset of HO. All seven children had congenital malformations of the great toes, but none had

radiographic evidence of HO at the time of evaluation. DNA sequence analysis found that all seven children had the classic FOP mutation. Clinical suspicion of FOP early in life on the basis of malformed great toes can lead to early clinical diagnosis, confirmatory diagnostic genetic testing, and the avoidance of additional harmful diagnostic and treatment procedures. This was the first report of genetic confirmation of FOP before the appearance of HO. Pediatricians should be aware of the early diagnostic features of FOP, even before the appearance of HO. This awareness should prompt early genetic consultation and testing and the institution of assiduous precautions to prevent iatrogenic harm.

47. Yu PB, Deng DY, Lai CS, Hong CC, Cuny GD, Bouxsein ML, Hong DW, McManus PM, Katagiri T, Sachidanandan C, Kamiya N, Fukuda T, Mishina Y, Peterson RT, Bloch KD. BMP type I receptor inhibition reduces heterotopic ossification. **Nat Med** 14:1363-1369, 2008

This pre-clinical study in a surrogate mouse model for FOP supported the role of dysregulated ACVR1 activity in the pathogenesis of FOP and suggested that small molecule inhibition of BMP type I receptor activity may be useful in treating FOP.

48. Kaplan, FS, Xu M, Seemann P, Connor JM, Glaser DL, Carroll L, Delai P, Fastnacht-Urban E, Forman SJ, Gillessen-Kaesbach G, Hoover-Fong J, Köster B, Pauli RM, Reardon W, Zaidi S-A, Zasloff M, Morhart R, Mundlos S, Groppe J, and Shore EM. Classic and atypical fibrodysplasia ossificans progressiva (FOP) phenotypes are caused by mutations in the bone morphogenetic protein (BMP) type I receptor ACVR1. **Hum Mutat** 30 (3): 379-390, 2009

This classic study identified the clinical and genetic spectrum of FOP and defined the spectrum of FOP variants.

49. Fukuda T, Kohda M, Kanomata K, Nojima J, Nakamura A, Kamizono J, Noguchi Y, Iwakiri K, Kondo T, Kurose J, Endo KI, Awakura T, Fukushima J, Nakashima Y, Chiyonobu T, Kawara A, Nishida Y, Wada I, Akita M, Komori T, Nakayama K, Nanba A, Yoda T, Tomoda H, Yu PB, Shore EM, Kaplan FS, Miyazono K, Matsuoka M, Ikebuchi K, Ohtake A, Oda H, Jimi E, Owan I, Okazaki Y, Katagiri T. Constitutively activated ALK2 and increased SMAD 1/5 cooperatively induce bone morphogenetic protein signaling in fibrodysplasia ossificans progressiva. **J Biol Chem** 284: 7149-7156, 2009

This study identified 19 sporadic Japanese patients with classic FOP, examined pathologic downstream events that enhance BMP signaling in the presence of the mutant receptor and showed that selective kinase inhibition may represent a tangible strategy for blocking the activity of mutant ACVR1 in FOP.

50. Lounev V, Ramachandran R, Wosczyzna MN, Yamamoto M, Maidment ADA, Shore EM, Glaser DL, Goldhamer DJ, Kaplan FS. Identification of progenitor cells that contribute to heterotopic skeletogenesis. **J Bone Joint Surg Am** 91: 652-663, 2009

This study identified a critical population of progenitor cells that contributes to specific stages of BMP-induced heterotopic ossification. The data strongly suggest that dysregulation of the BMP signaling pathway and an inflammatory microenvironment are both required for the formation of FOP-like lesions. An understanding of the cellular basis of heterotopic ossification will aid in the development of targeted, cell-specific therapies for the treatment and prevention of heterotopic ossification.

51. Shen Q, Little SC, Xu M, Haupt J, Ast C, Katagiri T, Mundlos S, Seemann P, Kaplan FS, Mullins MC, Shore EM. The fibrodysplasia ossificans progressiva R206H ACVR1 mutation activates BMP-independent chondrogenesis and zebrafish embryo ventralization. **J Clin Invest** 119(11): 3462-3472, 2009

This study showed that mutant ACVR1 activated BMP signaling in the absence of BMP ligand and mediated

BMP-independent chondrogenesis (cartilage formation) that is enhanced by BMP. Importantly, the study also showed that the classic FOP mutation in ACVR1 impaired the binding of an inhibitory protein called FKBP1A/FKBP12, thus accounting, in part, for the elevated basal activity of the mutant receptor. Consistent with these findings, *in vivo* analyses of zebrafish embryos showed BMP-independent hyperactivation of BMP signaling in response to the mutant receptor, resulting in increased embryonic ventralization. Thus, the mutant ACVR1 receptor in FOP patients is an activating mutation that induces BMP signaling in a BMP-independent and BMP-responsive manner to promote chondrogenesis, consistent with the ectopic endochondral bone formation in FOP.

52. Kaplan FS, Pignolo RJ, Shore EM. The FOP metamorphogene encodes a novel type I receptor that dysregulates BMP signaling. **Cytokine Growth Factor Reviews** 20:399-407, 2009.

The ability of mature organisms to stabilize tissue differentiation patterns has enormous selective advantage across all phyla, but the mechanisms have been largely unexplored. Individuals with FOP undergo a pathological metamorphosis in which one normal tissue is transformed into another through a highly regulated process of tissue destruction and phenotype reassignment. This disabling metamorphosis is mediated by the FOP metamorphogene, which encodes a mutant bone morphogenetic protein (BMP) type I receptor that exhibits mild constitutive activity during development and severe, episodic, inflammation-induced, ligand-dependent dysregulation postnatally. The discovery of the FOP metamorphogene reveals a highly conserved target for drug development and identifies a fundamental defect in the BMP signaling pathway that, when triggered by injury and inflammation, transforms one tissue into another.

53. Kaplan FS, Zasloff MA, Kitterman JA, Shore EM, Hong CC, Rocke DM. Early mortality and cardiorespiratory failure in patients with fibrodysplasia ossificans progressiva. **J Bone Joint Surg Am** 92: 686-691, 2010

This important paper established clinical guidelines for the cardiorespiratory care of patients with advanced FOP. Little is known about the lifespan or causes of mortality in FOP patients. Sixty deaths (thirty male and thirty female patients) were reported in the FOP community during a thirty-three-year-period. The median age at the time of death was forty years (range, three to seventy-seven years). The most common causes of death in patients with FOP were cardiorespiratory failure from thoracic insufficiency syndrome (54%; median age, forty-two years) and pneumonia (15%; median age, forty years). Thus, FOP is not only an extremely disabling disease but also a condition of early mortality. The most common cause of death in patients with FOP was cardiorespiratory failure from thoracic insufficiency syndrome.

54. Shore EM, Kaplan FS. Inherited human diseases of heterotopic bone formation. **Nat Rev Rheumatol** 6: 518-527, 2010

In human disorders of hereditary and nonhereditary heterotopic ossification, the resulting extraskeletal bone is normal. The aberration lies within the mechanisms that regulate cell-fate determination, directing the inappropriate formation of cartilage or bone, or both, in tissues such as skeletal muscle (FOP) and adipose tissue (POH). In FOP, activating mutations in ACVR1, a bone morphogenetic protein type I receptor, induce heterotopic endochondral ossification, which results in the development of a heterotopic organ system that includes bone and bone marrow. In POH, heterotopic ossification leads to the formation of bone tissue through an intramembranous in response to inactivating mutations in the GNAS gene. Patients with these diseases variably exhibit malformation of normal skeletal elements, identifying the causative genes and their associated signaling pathways as key mediators of skeletal development in addition to regulating cell-fate decisions by adult stem cells.

55. Kaplan FS, Seemann P, Haupt J, Xu M, Mullins M, Shore EM. Investigations of activated ACVR1/ALK2, a bone morphogenetic protein type I receptor that causes fibrodysplasia ossificans progressiva. **Methods Enzymol** 484: 357-373, 2010

Bone morphogenetic protein (BMP) type I receptors are serine-threonine kinase transmembrane signal transduction proteins that regulate a vast array of ligand-dependent cell-fate decisions with temporal and spatial fidelity during development and postnatal life. The single nucleotide mutation in ACVR1 that causes FOP is one of the most specific disease-causing mutations in the human genome and to date the only known inherited activating mutation of a BMP receptor that causes a human disease. Thus, the study of FOP provides the basis for understanding the clinically relevant effects of activating mutations in the BMP signaling pathway.

56. Medici D, Shore EM, Lounev VY, Kaplan FS, Kalluri R, Olsen BJ. Conversion of vascular endothelial cells into multipotent stem-like cells. **Nat Med** 16: 1400-1406, 2010

Mesenchymal stem cells can give rise to several cell types. This study showed that vascular endothelial cells can transform into multipotent stem-like cells by an ACVR1-dependent mechanism. In lesions from individuals with FOP or from transgenic mice expressing constitutively active ACVR1, chondrocytes and osteoblasts expressed endothelial markers. Expression of constitutively active ACVR1 in endothelial cells caused endothelial-to-mesenchymal transition and acquisition of a stem cell-like phenotype. These stem-like cells could be triggered to differentiate into osteoblasts, chondrocytes or adipocytes. This report highlighted conversion of endothelial cells to stem-like cells under the influence of mutant ACVR1 and may provide a new approach to tissue engineering and FOP therapeutics.

57. Kaplan FS, Shore EM, Pignolo RJ. (eds), and The International Clinical Consortium on FOP. The medical management of fibrodysplasia ossificans progressiva: current treatment considerations. **Clin Proc Intl Clin Consort FOP** 4:1-100, 2011.

These evidence-based medical guidelines provide an international standard-of-care for clinical consultation and clinical management of FOP, and suggest promising directions for both basic and clinical research.

58. Groppe JC, Wu J, Shore EM, Kaplan FS. *In vitro* analysis of dysregulated R206H ALK2 kinase-FKBP12 interaction associated with heterotopic ossification in FOP. **Cells Tissues Organs** 194 (2-4): 291-295, 2011

A single recurrent mutation in the regulatory subdomain of ACVR1 is the cause of classic FOP. The substitution of arginine for histidine, led to the hypothesis of an aberrant, pH-sensitive switch for the ligand-independent activation of BMP signaling through the mutant receptor. To test the putative pH-dependent mechanism, *in vitro* interaction analyses with purified wild-type and mutant ACVR1 and inhibitory FKBP12 protein were performed. The study found that substitution with histidine led to partial loss of inhibition of the mutant ACVR1 through diminished binding of FKBP12.

59. Kan L, Lounev VY, Pignolo RJ, Duan L, Liu Y, Stock SR, McGuire TL, Lu B, Gerard NP, Shore EM, Kaplan FS, Kessler JA. Substance P signaling mediates BMP-dependent heterotopic ossification. **J Cell Biochem** 112: 2759-2772, 2011.

This collaborative study showed that sensory nerves regulate the innate immune system and amplify heterotopic ossification (HO) in animal models of FOP. The study found that the expression of the neuro-inflammatory factor Substance P (SP) is dramatically increased in early lesional tissue in patients who have either FOP or acquired HO, and in three independent mouse models of HO. These observations established a potent neuro-inflammatory induction and amplification circuit for BMP-dependent FOP-like lesion formation, and identified novel molecular targets for prevention of HO in FOP and related disorders.

60. Kaplan FS, Lounev VY, Wang H, Pignolo RJ, Shore EM. Fibrodysplasia ossificans progressiva: a blueprint for the metamorphosis. **Ann NY Acad Sci** 1237: 5-10, 2011

The most important milestone in understanding a genetic disease is the identification of the causative mutation. However, such knowledge is often insufficient to decipher the pathophysiology of the disorder or to effectively treat those affected. While activating mutations of the ACVR1/ALK2 receptor are necessary for FOP, they are not sufficient. Disease activity and progression also depend on altered cell and tissue physiology. Recent findings identify inflammatory and immunological factors, receptive mesenchymal stem cells, and a hypoxic lesional microenvironment that triggers, promotes, and enables episodic progression of FOP in the setting of mutant ACVR1. Effective therapies for FOP will consider these seminal pathophysiologic interactions.

61. Shimono K, Tung WE, Macolino C, Chi AH, Didizian JH, Mundy C, Chandraratna RA, Mishina Y, Enomoto-Iwamoto M, Pacifici M, Iwamoto M. Potent inhibition of heterotopic ossification by nuclear retinoic acid receptor- γ agonists. **Nat Med** 17:454-460, 2011

This study from The Children's Hospital of Philadelphia and The University of Pennsylvania indicates that RAR- γ agonists are potent inhibitors of heterotopic endochondral ossification in mouse models and thus, may also be effective against injury-induced and congenital heterotopic ossification in humans.

62. Kaplan FS, Shore EM. Derailing heterotopic ossification and RARing to go. **Nat Med** 17:420-421, 2011

Retinoic acid receptors inhibit chondrogenesis, but their ability to block the cartilaginous scaffold of heterotopic endochondral ossification has not been explored. A study in mice shows that agonists of retinoic acid receptor- γ potentially inhibit heterotopic endochondral ossification, suggesting therapeutic potential in people with FOP.

63. Kaplan J, Kaplan FS, Shore EM. Restoration of normal BMP signaling levels and osteogenic differentiation in FOP mesenchymal progenitor cells by mutant allele-specific targeting. **Gene Therapy** 19: 786-790, 2012

This study established proof-of-principle that allele-specific small inhibitory RNA (ASP-siRNA) has potential therapeutic efficacy for the treatment of FOP. The study showed conclusively that ASP-siRNA duplexes were capable of suppressing the expression of the mutant ACVR1 allele in mesenchymal progenitor cells from FOP patients and that this ASP-RNAi approach decreased the elevated BMP signaling of patient cells to levels of control cells and restored enhanced osteogenic differentiation in FOP cells to control levels.

64. Chakkalakal SA, Zhang D, Culbert AL, Convente MR, Caron RJ, Wright AC, Maidment AD, Kaplan FS, Shore EM. An *Acvr1* Knock-in mouse has fibrodysplasia ossificans progressiva. **J Bone Miner Res** 27: 1746-1756, 2012

Gene targeting was used to develop a unique *Acvr1* knock-in model for FOP. This chimeric mouse model of FOP established the first direct *in vivo* evidence that the R206H mutation in ACVR1 causes FOP. Histological analysis of FOP lesions in the mouse demonstrated inflammatory infiltration and death of skeletal muscle followed by robust formation of heterotopic bone through an endochondral pathway, identical to that seen in patients. Importantly, both wild-type and mutant cells were present within the ectopic bone tissue in this chimeric mouse model of FOP, an unexpected finding that indicated that although the mutation is necessary to induce the bone formation process, the mutation is not required for progenitor cell contribution to bone and cartilage.

65. Hammond P, Suttie M, Hennekam RC, Allanson J, Shore EM, Kaplan FS. The face signature of fibrodysplasia ossificans progressiva. **Am J Med Genet** 158A: 1368-1380, 2012

Anecdotal observations of facial similarity in FOP patients have been made by clinicians and parents,

but no objective quantitative analysis of the faces of FOP patients has ever been undertaken. This study showed that the classic FOP mutation affected the morphogenesis of the cranial skeleton in the upper midface and mandible and may have important diagnostic and clinical implications for the dental care and anesthetic management of patients with FOP.

66. Kitterman JA, Strober JB, Kan L, Rocke DM, Cali A, Peeper J, Snow J, Delai PLR, Morhart R, Pignolo RJ, Shore EM, Kaplan FS. Neurological symptoms in individuals with fibrodysplasia ossificans progressiva. **J Neurol** 259: 2636-2643, 2012

Neurological problems have not been associated with FOP, but neurological symptoms are commonly reported by FOP patients. This worldwide survey indicated that neurological symptoms were common in FOP and that these symptoms were likely related to effects of dysregulated BMP signaling on the central and/or peripheral nervous systems.

67. Kan L, Kitterman JA, Procissi D, Chakkalakal S, Peng C-Y, McGuire TL, Goldsby RE, Pignolo RJ, Shore EM, Kaplan FS, Kessler JA. CNS demyelination in fibrodysplasia ossificans progressiva. **J Neurol** 259: 2644-2655, 2012

A variety of atypical neurologic symptoms are reported by FOP patients. This study showed that dysregulated BMP signaling disturbs normal regulation of target tissues, including central nervous system (CNS) where focal demyelination may manifest as the neurologic symptoms frequently observed in FOP. These findings support previous studies that showed that dysregulated BMP signaling stimulated astrocytes and inhibited oligodendrocytes and have important implications for the diagnosis and management of neurological problems in FOP patients.

68. Kaplan FS, Chakkalakal SA, Shore EM. Fibrodysplasia ossificans progressiva: mechanisms and models of skeletal metamorphosis. **Dis Model Mech** 5: 756-762, 2012

The FOP knock-in mouse and other FOP models in *Drosophila*, zebrafish, chickens and mice provide an arsenal of tools for understanding BMP signaling and addressing outstanding questions of disease mechanisms that are relevant not only to FOP but also to a wide variety of disorders associated with regenerative medicine and tissue metamorphosis.

69. Chaikuad A, Alfano I, Kerr G, Sanvitale CE, Boergermann JH, Triffitt JT, von Delft F, Knapp S, Knaus P, Bullock AN. Structure of the bone morphogenetic protein receptor ALK2 and implications for fibrodysplasia ossificans progressiva. **J Biol Chem** 287:36990-36998, 2012

This study determined the crystal structure of the cytoplasmic domain of ACVR1 and offers a valuable template for the further design of specific inhibitors of BMP signaling.

70. Le VQ, Wharton KA. Hyperactive BMP signaling induced by ALK2(R206H) requires type II receptor function in a *Drosophila* model for classic fibrodysplasia ossificans progressiva. **Dev Dyn** 241:200-214, 2012

In *Drosophila*, human ACVR1/ALK2 (R206H) induces hyperactive BMP signaling. The study found that a key determinant for ACVR1 hyperactivity was a functional type II receptor, and that like its *Drosophila* ortholog, Saxophone (Sax), wild-type ACVR1 can antagonize, as well as promote, BMP signaling. The dual function of ACVR1 is of tremendous interest given the heterozygous nature of FOP. This amazing study provides a compelling rationale for *Drosophila* as a model organism to study the molecular basis of FOP.

71. Kaplan FS. The skeleton in the closet. **Gene** 528: 7-11, 2013

The origins of fibrodysplasia ossificans progressiva (FOP) in human history are unknown but the condition has been well described since Freke's account in 1740. Important contributions by physicians and scientists in the past two and a half centuries have converged on the

remarkable skeleton of Harry Eastlack at The Mutter Museum of The College of Physicians in Philadelphia.

72. Zhang W, Zhang K, Song L, Pang J, Ma H, Shore EM, Kaplan FS, Wang P. The phenotype and genotype of fibrodysplasia ossificans progressiva in China: A report of 72 cases. **Bone** 57: 386-391, 2013

This study identified 72 patients with FOP in China comprising the world's largest ethnically homogeneous population of FOP patients. The study found that the clinical and genetic profile of individuals with FOP from the Han nationality in China are similar to those reported elsewhere and support the fidelity of this ultra-rare disorder in the world's most highly populated nation and across wide racial, ethnic, gender and geographic distributions.

73. Kaplan FS, Pignolo RJ, Shore EM. From mysteries to medicines: drug development for fibrodysplasia ossificans progressiva. **Expert Opinion on Orphan Drugs** 1: 637-649, 2013

Despite germline mutations of ACVR1 in FOP, episodic disease activation is induced by soft tissue injury and resultant inflammatory triggers that are dependent on responding progenitor cells and a tissue microenvironment that supports heterotopic ossification. This review outlines opportunities and challenges for the development of effective therapies for FOP. The long-term treatment of FOP is likely to involve not one, but several concomitant approaches that acknowledge molecular mechanisms involved in the induction and progression of the disease. High fidelity animal models of FOP have been developed and pre-clinical and clinical testing is underway.

74. Culbert AL, Chakkalakal SA, Theosmy EG, Brennan TA, Kaplan FS, Shore EM. Alk2 regulates early chondrogenic fate in fibrodysplasia ossificans progressiva heterotopic endochondral ossification. **Stem Cells** 32: 1289-1300, 2014.

This study established that ACVR1 is a direct regulator of cartilage formation and mediates chondrogenic

commitment of progenitor cells. At least one effect of ACVR1 gain-of-function mutations in FOP patients is enhanced chondrogenic (cartilage) differentiation which supports formation of heterotopic endochondral bone. This study established ACVR1 as a therapeutic target for preventing heterotopic bone formation in FOP during early chondrogenic stages of lesion formation. Human clinical trials are now underway with one such compound, Palovarotene.

75. Haupt J, Deichsel A, Stange K, Ast C, Bocciardi R, Ravazzolo R, Di Rocco M, Ferrari P, Landi A, Kaplan FS, Shore EM, Reissner C, Seemann P. ACVR1 p.Q207E causes classic fibrodysplasia ossificans progressiva and is functionally distinct from the engineered constitutively active ACVR1 p.Q207D variant. **Hum Mol Genet.** 23: 5364-5377, 2014

This study explores the pros and cons of the various genetic mouse models for FOP in the context of the classic and exceedingly rare variant mutations in ACVR1 seen in humans with FOP. The study demonstrates that the Q207E FOP variant mutation, thought likely to function similarly to the constitutive active Q207D mutation, in fact has mild gain-of-function activity similar to R206H.

76. Kaplan FS, Kobori JA, Orellana C, Calvo I, Rosello M, Martinez F, Lopez B, Xu M, Pignolo RJ, Shore EM, Groppe JC. Multi-system involvement in a severe variant of fibrodysplasia ossificans progressiva (ACVR1 c.772G>A; R258G): A report of two patients. **Am J Med Genet A.** 167A: 2265-2271, 2015

Severe variants of fibrodysplasia ossificans progressiva (FOP) affect <2% of all FOP patients worldwide, but provide an unprecedented opportunity to probe the clinical-genetic relationships that propel the pathology of this disabling disease. The high fidelity clinical-genetic relationship in two unrelated children with the most severe FOP phenotype reported to date suggests that the shared features of their remarkable phenotype are due to the dysregulated activity of mutant ACVR1 during development and postnatally, and provides vital insight

into the structural biology and function of ACVR1 as well as the design of small molecule inhibitors.

77. Convente MR, Wang H, Pignolo RJ, Kaplan FS, Shore EM. The immunological contribution to heterotopic ossification disorders. **Curr Osteoporos Res.** 13: 116-124, 2015

Heterotopic ossification (HO) is a significant clinical event, rendering affected individuals with immobility and a diminished quality of life, can appear in patients following invasive surgeries and traumatic injuries, as well as progressively manifest in several congenital disorders including FOP. A unifying feature of both genetic and non-genetic forms of HO is immune system involvement at the early stages of disease. Activation of the immune system sets the stage for the downstream anabolic events that eventually result in HO, rendering the immune system a particularly appealing site of early therapeutic intervention for optimal management of disease. This paper outlines the immunological contributions to HO with specific focus on contributing cell types, signaling pathways, relevant animal models, and potential therapeutic targets.

78. Hatsell SJ, Idone V, Wolken DM, Huang L, Kim HJ, Wang L, Wen X, Nannuru KC, Jimenez J, Xie L, Das N, Makhoul G, Chernomorsky R, D'Ambrosio D, Corpina RA, Schoenherr CJ, Feeley K, Yu PB, Yancopoulos GD, Murphy AJ, Economides AN. ACVR1(R206H) receptor mutation causes fibrodysplasia ossificans progressiva by imparting responsiveness to activin A. **Sci Transl Med** September 2, 2015

This study led by scientists at Regeneron Pharmaceuticals suggests that ACVR1(R206H) causes FOP by gaining responsiveness to the normally antagonistic ligand activin A, demonstrating that this ligand is necessary and sufficient for driving HO in a genetic mouse model of classic FOP; and that a blocking antibody to activin A represents a potential therapeutic approach for FOP.

79. Kaplan FS, Pignolo RJ, Shore EM. Granting immunity to FOP and catching heterotopic ossification in the Act. **Semin Cell Dev Biol.** 49: 30-36, 2016

The progressive transformation of one organ system into another is a fundamental signature of FOP. Loss of autoinhibition of the mutant receptor (mACVR1) results in dysregulated BMP pathway signaling, and is necessary for the myriad developmental features of FOP, but does not appear sufficient to induce the episodic flare-ups that lead to disabling post-natal heterotopic endochondral ossification (HEO) and that are a hallmark of the disease. Post-natal FOP flare-ups strongly implicate an underlying immunological trigger involving inflammation and the innate immune system. Recent studies implicate canonical and non-canonical TGF β /BMP family ligands in the amplification of mACVR1 signaling leading to the formation of FOP lesions and resultant HEO. BMP and Activin ligands that stimulate mACVR1 signaling also have critical regulatory functions in the immune system. Cross-talk between the morphogenetic and immunological pathways that regulate tissue maintenance and wound healing identifies potential robust therapeutic targets for FOP. This article outlines a novel hypothesis for an immunological trigger for flare-ups and HEO in FOP, propose a working schema for the pathophysiology of observed phenomena, and highlight outstanding questions under investigation.

80. Chakkalakal SA, Uchibe K, Convente MR, Zhang D, Economides AN, Kaplan FS, Pacifici M, Iwamoto M, Shore EM. Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human ACVR1 (R206H) fibrodysplasia ossificans progressiva (FOP) mutation. **J Bone Miner Res.** 2016.

The retinoic acid receptor γ (RAR γ) agonist Palovarotene has previously been shown to effectively inhibit HO in injury-induced and genetic mouse models of the disease. Here we report that the drug additionally prevents spontaneous HO, using a novel conditional-on knock-in mouse line carrying the human ACVR1^{R206H} mutation for classic FOP. In addition, Palovarotene restored long bone growth, maintained growth plate

function, and protected growing mutant neonates when given to lactating mothers. Importantly, Palovarotene maintained joint, limb and body motion, providing clear evidence for its encompassing therapeutic potential as a treatment for FOP.

81. Pignolo RJ, Bedford-Gay C, Liljestrom M, Durbin-Johnson BP, Shore EM, Rocke DM, Kaplan FS. The natural history of flare-ups in fibrodysplasia ossificans progressiva (FOP): A comprehensive global assessment. **J Bone Miner Res** 31: 650-656, 2016.

This study is the first comprehensive global assessment of FOP flare-ups and establishes a critical foundation for the design and evaluation of future clinical trials.

82. Wang H, Lindborg C, Kim J-H, McCarrick-Walmsley R, Mangiavini L, Groppe J, Shore EM, Schipani E, Kaplan FS, Pignolo RJ. Cellular hypoxia promotes heterotopic ossification by amplifying BMP signaling. **J Bone Miner Res**, 2016.

Despite the occurrence of germline activating mutations of ALK2 in all FOP patients, individuals with FOP do not form bone continuously, but rather episodically and often following tissue injury. FOP flare-ups are predictably associated with inflammation, a well-known cause of tissue hypoxia. Hypoxia and inflammation are implicated in the induction of heterotopic endochondral ossification (HEO); however, the molecular mechanisms are unknown. This study explores the molecular basis of oxygen sensing in early FOP lesions, supports that FOP lesions are induced by a maladaptive molecular response to local tissue hypoxia that amplifies and prolongs promiscuous BMP signaling. This study has identified a new therapeutic target for FOP and has important implications for the development of transformative therapies.



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