

**THE FIFTEENTH ANNUAL REPORT
OF THE FIBRODYSPLASIA OSSIFICANS PROGRESSIVA (FOP)
COLLABORATIVE RESEARCH PROJECT**

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FOP research is neither a sprint nor a marathon, but an epic journey in a remote and formidable mountain range of science and medicine where massive peaks of ice and stone tower over the valley floor, and where every inch of rock and ravine pose unimaginable perils and promises. For fifteen long years, we have been searching in this desolate and dangerous aerial wilderness for the FOP gene - that elusive and metaphoric summit of the FOP range, whose discovery would create a beacon of hope that would instantly illuminate our world. For fifteen long years...

... It was late August, 2005. The air was thin and the climb difficult. The angle of the ridge was severe and our passage precarious. Every step was tedious, labored, exhausting. We picked our way from rock to rock across unexplored ledges, enveloped by clouds that obscured all visibility. We could not see the precipice beneath us, and stumbled - a heart-stopping lurch forward. As we struggled to regain our balance with a few quick steps, we emerged into an alien world.

For the first time in fifteen years in this unforgiving cirque of mountains, we were above the clouds. Every step was lit in brilliant sunshine that exploded from all directions, and beyond was a sky of limitless horizon. Vistas of smaller peaks poked through a solid carpet of clouds below. We were stunned by the revelation – as if transported by a dream. Could it possibly be? We rechecked our navigation, retraced our final steps, reviewed our crude maps, and stood in awe. The silence was reverential. Fifteen years in the high mountains; 15 years and finally, finally we had reached the summit. We had discovered the FOP gene.

The FOP gene – it is not just the most important FOP discovery of the year, of the decade, or of the century, but unquestionably the most important discovery in the history of FOP research. It is a discovery that belongs to no one scientist or physician or

laboratory, but to the entire FOP community worldwide and to the ages. It is a discovery that will change everything in the field of FOP research and likely far beyond.

In a special supplement to The Fifteenth Annual Report, we will share with you the details of this thrilling discovery, the pathways that led us to the summit, and the implications it has for FOP research from this day forward. Simply stated, this is the dawn of a new age.

Some have recently asked us to write a very brief version of The FOP Annual Report. Here it is in eight words, in headline form: **FOP Gene Discovered; Skeleton Key Offers New Hope.**

We have often said that when the real advances occur, we will not need books, but rather scraps of paper on which to scribble nature's most sacred secrets; guarded over geologic spans of time – in the case of the FOP gene, over nearly 500 million years – far longer metaphorically than these mountains have existed.

For those who relish the precision of mathematics, we offer this equation:

$$\mathbf{FOP = ACVR1(c.617G>A; R206H)}$$

That is about all the information you need to re-create FOP. That's it in a nutshell. More on that later (see supplement to the 15th Annual Report).

In a departure from tradition, we will devote nearly the entire annual report and supplement to one topic – the discovery of the FOP gene. First, a brief summary of the discovery and then a few of the year's other headlines.

THE DISCOVERY OF THE FOP GENE: A BRIEF SUMMARY

The FOP gene was discovered in the FOP Research Laboratory at The University of Pennsylvania, in Philadelphia in August, 2005. It was a breathtaking discovery. When we began our FOP research journey fifteen years ago, our area of exploration was the entire human genome which contains an amazing six billion basepairs of DNA in every cell – in other words, the human “genetic universe.” Using molecular DNA sequence markers from the human genome as a guide, along with information about these molecular markers on each person’s DNA (from the core multigenerational FOP families that we discovered over the past fifteen years), we narrowed the location of the FOP gene from the human genetic universe to the galaxy, from the galaxy to the solar system, from the solar system to the planet, from the planet to the hemisphere, from the hemisphere to the continent, from the continent to the country, from the country to the region, from the region to the state, from the state to the county, from the county to the city, and from the city to the neighborhood. In last year’s annual report, we indicated that we were “in the genetic neighborhood,” and what a big neighborhood it was!

During 2005, we obtained genetic mapping data from two additional multigenerational FOP families and further narrowed the location of the gene to a large street – a few hundred “houses” (candidate genes) on human chromosome two. A candidate gene for FOP is a gene that, if mutated (damaged) in some way, could plausibly cause FOP.

As in previous years, we checked many candidate genes (houses on our genetic street) and found nothing of interest for FOP. One house, however, looked particularly

interesting based upon molecular traffic we saw in the genetic neighborhood. So, we entered the house, searched each floor, each room, each piece of furniture, and found nothing. This can't be where the FOP gene is located, we thought, and we were ready to abandon the house and move on.

We were at the top floor of the house, in the very last room. We had checked every box, every piece of furniture, every drawer in every piece of furniture, and found nothing - not a single clue. Then, as we were about to leave, we spotted for the first time, a beautiful antique desk, hidden in a far and dimly-lit corner of the room. The desk was small and bare. We walked over to the desk and methodically opened every drawer. Again, we found nothing. The drawers were empty. Then, as we were closing the last drawer in the desk, it jammed. We removed the drawer to see what the problem was, and behind it, we found a loose panel. We removed the panel. Behind it was a small cubbyhole. Inside the cubby hole, was a miniature gold box. We removed the box and opened it. Inside, we found two tiny gold keys – the tiniest imaginable. The keys were identical except for one little notch. We turned the keys over. Inscribed on one key were the letters “**NORMAL**”; on the other key, the letters “**FOP.**” We looked inside the lid of the gold box, and engraved in the most ancient genetic code were these words: “**THESE ARE THE KEYS TO THE SKELETON.**” We had found the FOP gene and the keys to the FOP universe. We stood in awe, at the threshold of a new world.

THE FOP GENE

The FOP gene is known as ACVR1. The gene encodes a protein called ACVR1 or Activin Receptor Type 1A, a bone morphogenetic protein (BMP) receptor similar to BMPRIA and BMPRIB, the two BMP receptors that you have heard much about in

previous annual reports. ACVR1 was originally thought to be a receptor for the growth factor Activin, but recent studies have dramatically broadened that perspective. ACVR1 is, in fact, a receptor for BMP4, and other BMPs as well. ACVR1 is required for life, and plays an important role in the development of the heart, joints, spine, and limbs. ACVR1 is also highly expressed in skeletal muscle and connective tissue, but its normal functions in these cells and tissues are not known. The ACVR1 gene is located on the long arm of human chromosome 2. Each person has two copies of the ACVR1 gene. Those with FOP have one normal copy of the ACVR1 gene and one damaged copy.

We have so far examined the ACVR1 genes in more than 60 patients with classic features of FOP (typical congenital malformations of the great toes and progressive heterotopic ossification in characteristic anatomic patterns) and everyone has the exact same DNA sequence change in the exact same spot in the ACVR1 gene. The FOP mutation changes one DNA letter out of six billion DNA letters in the entire human genome, in exactly the same spot in everyone who has classic FOP. It does not matter whether someone inherited a damaged copy of the ACVR1 gene from an affected parent (as in one of the multigenerational families) or whether he/she has a new (spontaneous) DNA sequence change. The exact same mutation in ACVR1 is seen in affected individuals of every ethnic, racial, and geographic background.

The FOP mutation causes an amino acid (a building block of protein) called histidine (His) to be substituted for an amino acid called arginine (Arg) at position 206 of the ACVR1 protein in everyone who has classic FOP. That single amino acid substitution in ACVR1 causes FOP. As we said, ACVR1 is a BMP receptor, a molecular switch that determines the fate of the cells in which it is expressed. Position 206 of ACVR1 is in a part of the receptor that is inside of the cell – a component of the control switch that determines how and when and at what intensity the molecular signal will be transmitted

to the next molecule in the signaling pathway. In other words, position 206 of ACVR1 is the notch in the FOP key that is different from the normal key.

The ACVR1 gene was high on our list of most likely candidate genes, but we never expected to find the type of mutation we did – a mutation like almost no other in the field of human genetics. It is incredible in its fidelity and uniformity throughout the entire FOP community worldwide. The mutation not only affects the same gene in every individual with classic FOP, but it affects the same segment (exon) of the gene, the same genetic word (codon) of the gene, and, in fact, the same genetic letter (nucleotide) of the gene in everyone with classic FOP. It is a breathtaking and thrilling finding.

Several years ago, scientists made artificially activated forms of ACVR1 (similar but not identical to those that we now know cause FOP), injected them into chicken and zebrafish embryos, and found that they upregulated BMP4, downregulated BMP antagonists, induced heterotopic ossification and stimulated joint fusions - clinical and molecular features of individuals who have FOP.

The substitution of the amino acid histidine for the amino acid arginine at position 206 of ACVR1 likely alters the structure of ACVR1 and its interactions with other proteins. Exactly how the mutation causes a change in the signal transmitted to the cell's nucleus (thus triggering a change in the fate of the cell) is presently unknown, but is already the subject of intense study at the FOP Laboratory, and must be better understood before adequate treatments can be designed. It's like discovering a very specific short circuit in a very specific switch (and in the case of FOP, in the switch that turns a light bulb into an atom bomb). We now know what the damaged switch looks like in FOP but we cannot safely defuse it until we know exactly how it works. And you can bet we're already working on it.

The presence of the amino acid arginine at position 206 of ACVR1 has been conserved throughout all of vertebrate evolution for nearly 500 million years. That means that nature cannot tolerate an amino acid substitution at that site without severe consequences. ACVR1 is an ancient protein “set in its ways,” and it doesn’t like to be tampered with. The FOP mutation, a substitution of the amino acid histidine for the amino acid arginine at position 206, therefore has bypassed one of the critical safeguards that nature has engineered into the ACVR1 gene and thus into BMP signaling pathway over hundreds of millions of years of evolution. It is a safeguard that exists in fish, chickens, and in humans, and has existed long before dinosaurs appeared on the face of the Earth. The FOP mutation thus disables an ancient safety switch in the ACVR1 gene and in the receptor it encodes.

The discovery of the FOP mutation in the ACVR1 gene confirms our original hypothesis about the cause of FOP, validates our previous work on the dysregulation of the BMP signaling pathway in FOP, and provides the first glimpse of how the broken switch may be stuck in the “ON” position. However, we do not yet fully understand how the broken switch activates the formation of bone or how it is influenced by injury or other immunologic triggers, and this knowledge will be critical in designing effective treatments to deactivate the switch. We have already begun to investigate these important questions.

The discovery of the FOP gene also provides the opportunity to produce genetically-engineered mice that have real FOP – a development that would open the door to designing and testing new therapies. While effective treatments will not be available immediately, there is no single discovery that has more suddenly expanded our horizon or given us more hope.

The discovery of the FOP gene is the brightest beacon we have on the journey to a cure. This discovery establishes a specific and robust link between a catastrophic human condition and a highly conserved cell signaling pathway in nature, and provides a tantalizing target for drug discovery for both FOP and for regenerative medicine. The discovery of the FOP gene will be meaningful to scientists worldwide and to physicians in many fields of medicine. Cause and cure have always been our guiding principles in FOP research. Today, we are halfway there.

THE YEAR'S OTHER HEADLINES

While the discovery of the FOP gene has understandably captivated our attention, other important highlights of FOP research during 2005 include the following discoveries and findings:

- **Two major branches of the BMP signaling pathway are dysregulated in FOP cells.** One branch of the pathway seems especially active during development and the other branch is active in response to physical stress, although there is a great deal of cross-talk between the two. Exactly how the newly discovered FOP gene mutation affects the two branches of the BMP pathway will be a focus of intense research in the FOP lab for some time to come, work that will likely reveal new opportunities for therapy. Two major papers on this subject were published by the FOP Laboratory in 2005; one in the **Journal of Bone & Mineral Research**, and the other in **Clinical Reviews in Bone and Mineral Metabolism**. Another manuscript has recently been accepted for publication.
- **Connective tissue stem cells isolated from baby teeth provide an important new tool for investigating BMP signaling pathways in FOP cells.** Until recently, all of our *in vitro* (cell culture) studies in FOP have been conducted in

blood-derived lymphoblastoid cells due to the ease and safety of obtaining these cells from routine blood tests. While some types of blood cells may play a role in the initial stages of FOP flare-ups and are present in early FOP lesions, they are not the primary cells that form heterotopic bone in FOP lesions. Thus, it is critical to study the BMP pathway in a cell type that may be more representative of the cells that comprise the early FOP fibroproliferative (pre-osseous) lesions. The connective tissue stem cells isolated from baby teeth provide such a resource, and one that is becoming invaluable in studying the two major branches of the BMP signaling pathway in FOP.

- **At least two distinct populations of adult stem cells are necessary to form an ectopic skeleton.** A central question in FOP is: What stem cells or progenitor cells are directly responsible for forming the second skeleton of FOP? We found that at least two populations of stem cells, one that gives rise to the bone marrow and another that gives rise to the skeletal scaffold, are necessary to form an ectopic skeleton. Interestingly, BMP pathway activation is sufficient to recruit and coordinately regulate both populations of stem cells in a dynamic pattern that cannot be reproduced by either stem cell population alone. This important discovery lays the groundwork for understanding how the highly specific FOP mutation in ACVR1 leads to the local tissue recruitment of discrete stem cell populations that can build an ectopic skeleton.
- **Abnormal BMP signaling in FOP affects joint formation and function.** For many years, we have observed that most young children with FOP have neck stiffness long before they form heterotopic bone in their neck. We therefore conducted an x-ray study of the neck bones and joints of 70 children with FOP in order to determine the exact site and severity of this problem. We observed

congenital abnormalities of the neck bones and joints in almost all young children who have FOP. Most interestingly, these abnormalities were present before heterotopic ossification occurred in the neck and were strikingly similar to those in mice that were genetically engineered to lack Noggin, a bone morphogenetic protein (BMP) antagonist. Since these mice could not make Noggin, they had too much active BMP. While we now know that the cause of FOP is a damaged BMP receptor (ACVR1) rather than too little Noggin, the end result is similar - overactive BMP signaling. Most importantly, overactive BMP signaling is implicated not only in heterotopic bone formation, but also in the malformation of the joints – especially the great toes, but also the joints of the neck (and maybe other joints as well, such as the joints that anchor the ribs to the spine). Thus, the mutation in ACVR1 that causes FOP has important implications for understanding joint disease as well as heterotopic bone formation. A detailed account of this work was published in the journal *SPINE* in 2005.

- **The FOPPY mouse continues to provide important clues about the mechanisms of heterotopic ossification.** Recent studies in the FOPPY mouse have revealed an important role for soft tissue injury and trauma in the induction of heterotopic ossification. Presently, we are working to develop a genetically-engineered mouse with real FOP based on knowledge of the FOP gene mutation. However, until such a mouse is developed, the FOPPY mouse will continue to be an important animal model for FOP research as well as for research on post-traumatic heterotopic ossification. Although FOPPY mice do not have real FOP, the development of progressive heterotopic ossification in any animal model provides an important dimension to our understanding of FOP.

- **Severe harm is caused by diagnostic errors in FOP.** A collaborative research study conducted by Dr. Joseph Kitterman, Professor of Pediatrics at The University of California-San Francisco, showed that diagnostic errors and inappropriate medical procedures lead to permanent harm and alter the natural history of FOP. The study found that FOP is misdiagnosed 87% of the time, takes an average of four years to be accurately diagnosed, and often is inaccurately identified as cancer. The inaccurate diagnoses prompted painful and unnecessary biopsies and incorrect treatments that worsened FOP, speeding permanent loss of mobility in many. Unfortunately, harm resulting from diagnostic failures in FOP is common worldwide and has shaped the natural history of the disease for most affected individuals. Attention to easily identifiable signs and symptoms of FOP early in life can limit the disability and lifelong harm resulting from diagnostic errors and inappropriate invasive procedures. This study was published in the November, 2005 issue of *Pediatrics*. We hope that the study will have a substantial impact on the continuing education of pediatricians worldwide.

- **A study in identical twins showed that environmental factors play an important role in the progression of FOP.** Genetic and environmental factors each affect the clinical features of FOP. We studied three pairs of identical twins with FOP and found that within each pair, congenital malformations of the great toes were identical. However, the severity and rate of progression of heterotopic ossification varied greatly depending upon life history and environmental exposures. The study showed that genetic determinants strongly influence disease features during prenatal development, and that environmental factors, such as soft tissue injuries and viral infections, strongly influence postnatal progression of the disease. This study was published in a special FOP edition of *Clinical Reviews in Bone and Mineral Metabolism*.

- **Important advances in FOP translational research occurred in 2005 and included validation of regulated Noggin gene therapy in an animal model as well as the use of monoclonal antibodies (directed against a small subset of BMP receptors) to modulate overactive BMP pathway activity in FOP cells.**

The recent discovery of the FOP gene will dramatically refine thinking on the treatment and eventual cure of FOP. The specific nature of the FOP gene mutation provides a precise pharmacologic target in a key signaling pathway, and will become a prime focus of FOP research for the foreseeable future.

Approaches to blocking the activity of the abnormal BMP receptor in FOP might include the development of specific monoclonal antibodies against ACVR1, signal transduction inhibitors to neutralize the activity of the receptor, inhibitory RNA technology to block the formation of the renegade ACVR1 receptor, or gene therapy technologies to deliver BMP antagonists like Noggin and thus prevent BMPs from binding to an already overactive receptor. At present, it is impossible to determine which approach or combination of approaches will eventually be the most useful. But, the most important thing to keep in mind is that we now have a highly specific therapeutic target and the tools available to develop and test new treatments.

It is difficult to overestimate the extraordinary enthusiasm and hope that the discovery of the FOP gene brings to the future of FOP research and to the development of potential treatments for FOP. While the successful treatment of FOP will not be accomplished immediately and in fact will take a great deal of work by many, we are finally above the clouds, and the therapeutic horizon is visible and bright.

PRESENTATIONS, MEETINGS, REPORTS, AND PUBLICATIONS

During 2005, we were privileged to present major lectures on FOP at the:

- American Society for Bone & Mineral Research; Nashville, Tennessee
- Medizin Klinikum; Garmisch-Partenkirchen, Germany
- Mount Sinai Medical Center; New York, New York
- New York Academy of Sciences; New York, New York
- Northeastern Ohio Universities College of Medicine; Rootstown, Ohio
- Oxford University; Oxford, United Kingdom
- St. Louis University; St. Louis, Missouri

During 2005, we were honored to present highlights of FOP research at regional, national, and international FOP family meetings and gatherings in:

- Aberdeen, Scotland
- Bedminster, New Jersey
- Dingolfing, Germany
- Freyung, Germany
- Orlando, Florida
- Roscoff, France
- Santa Maria, California
- Sausalito, California
- Valbert, Germany
- Washington, D.C.

Papers Published

In 2005, there were twenty-five major peer-reviewed publications on FOP, 19 of which appeared in a special issue of *Clinical Reviews in Bone and Mineral Metabolism*

devoted entirely to FOP. In that issue, there were numerous articles on all clinical and basic science aspects of FOP. This special issue of *Clinical Reviews in Bone and Mineral Metabolism* was published in the fall of 2005 and is available on the IFOPA website at: www.ifopa.org or from Kamlesh Rai, Dr. Kaplan's assistant:

kamlesh.rai@uphs.upenn.edu. The table of contents of available articles includes:

1. **Introduction by Guest Editors**
2. **FOP: An Historical Perspective**
3. **The Phenotype of FOP**
4. **Immunological Features of FOP and the Dysregulated BMP4 Pathway**
5. **The FOP Lesion**
6. **The Genetics of FOP**
7. **Three Pairs of Monozygotic Twins with FOP: The Role of Environment in the Progression of Heterotopic Ossification**
8. **The Craniofacial Phenotype of FOP**
9. **Thoracic Insufficiency Syndrome in Patients with FOP**
10. **Dysregulation of BMP4 Receptor Trafficking and Signaling in FOP**
11. **Early FOP-Like Lesion Formation in Nude Mice Following Implantation of Lymphoblastoid Cells from FOP Patients**
12. **Animal Models of FOP**
13. **The Neuron-Specific Enolase-Bone Morphogenetic Protein 4 Transgenic Mouse: A FOP-Like Animal Model**
14. **Oral and Dental Healthcare and Anesthesia for Persons with FOP**
15. **Treatment Considerations for the Management of FOP**

16. **Rehabilitation for Individuals with FOP**
17. **FOP and POH: Two Genetic Disorders of Heterotopic Ossification**
18. **Nonhereditary Heterotopic Ossification: Implications for Injury, Arthropathy, and Aging**
19. **The International FOP Association**

YOUR FOP LABORATORY

During 2005, the research staff of the FOP Core Research Laboratory included as many as 16 researchers: four principal investigators, four research specialists, four post-doctoral fellows, two graduate students, one medical student, and one pre-medical student. In addition, two undergraduate college students - one from Rutgers University and one from Brown University volunteered to work in the FOP Laboratory during the summer of 2005.

Jennifer Fiori, a graduate student from The University of Pennsylvania, is finishing her Ph.D. thesis on BMP Signaling Pathways in FOP. She will present her groundbreaking work and defend her thesis in April 2006. Jen has made major contributions to FOP research and we are thrilled that she continues to be part of the FOP Laboratory.

Congratulations, Jen!

We are also delighted to have Michael O'Connell, a graduate student from The University of Southampton (England, U.K.), working with us in the FOP Laboratory for a second year. Michael is studying the role of cell surface heparan sulfate proteoglycans in modulating BMP4 signaling in FOP and control cells. Michael has extended his stay

with us through the fall of 2006, and will finish his Ph.D. thesis under the combined mentorship of Professor Trudy Roach in Southampton, U.K., and Drs. Kaplan and Shore in Philadelphia.

Pictures of the FOP children adorn the hallways of our core FOP Laboratory and are a constant reminder of our goals and our mission. As we tell the children and adults who visit the FOP Center and Laboratory, “This is really *your* Center and *your* Laboratory.” We love when you come and visit.

Working Groups

The core FOP Research Laboratory is organized into working groups. There are working groups on FOP genetics, cell signaling pathways, cell differentiation, immunology (triggers), and translational research (animal models and treatments). Our working groups and laboratory meetings reflect both the broad diversity and the integration of various scientific disciplines that are needed to accomplish our longterm goals.

Center for Research In FOP and Related Disorders

While the core FOP Laboratory occupies approximately 2000 square feet of space at The University of Pennsylvania, our virtual space has expanded during the past five years with the establishment of the intramural and extramural components of The FOP Research Developmental Grants Program. Through this remarkable program, sponsored by The Cali Family Endowment and administered through the Center for Research in FOP and Related Disorders, we have been able to expand collaborations with colleagues

in many departments and schools throughout The University of Pennsylvania, and now elsewhere.

In 2005, the Developmental Grants Program funded its first extramural collaborative research grant on studies in the FOPPY mouse at the laboratory of Dr. Lixin Kan at Northwestern University in Chicago, Illinois. Projects involving the lineage of receptive cells for heterotopic ossification and the testing of experimental therapies for FOP in the FOPPY mouse are conducted collaboratively at Northwestern University and at The University of Pennsylvania.

Since its inception, the Developmental Grants Program of the Center for Research in FOP and Related Disorders has supported 12 novel projects relevant to our long-term mission. Many of these projects were highlighted in previous annual reports, and have produced important insights for FOP research.

A special luncheon and laboratory tour was held in November 2005 for donors and friends of the Cali Family Endowment, to honor their extraordinary generosity to FOP research over the past decade. More than 50 people visited the laboratory on that remarkable day, and heard comments from Dr. Arthur Rubenstein, Dean of the University of Pennsylvania School of Medicine; Dr. Frederick Kaplan, Isaac & Rose Nassau Professor of Orthopaedic Molecular Medicine; John Cali, Trustee of the University of Pennsylvania School of Medicine; and Amanda Cali, Chairman of the Board of the IFOPA.

ACKNOWLEDGEMENTS

FOP continues to be one of the most obstacle-ridden and perplexing quandries of the human condition, but with the discovery of the FOP gene in 2005, it is a much less perplexing quandry than it was a year ago. FOP research is the key to understanding not only FOP, but also many other common conditions that affect the formation of bone and the formation of the skeleton. In 2005, we discovered the skeleton key, a molecular switch that determines the fate of cells. That skeleton key will be used to unlock the secrets of FOP as well as the secrets of many common skeletal conditions in the years ahead.

As we have stated many times before, *cause* and *cure* are the two words that motivate us and provide the guiding principle for all we do: to discover the exact genetic and molecular cause of FOP and to use that knowledge to develop effective treatments and eventually a cure. This year, we have reached the summit of the FOP mountain. We discovered the genetic cause of FOP. But more difficult work lies ahead – the treacherous trip across the mountain range and down the mountain on the other side – eventually arriving at the clinic, with effective treatments and a cure. It will continue to be a thrilling adventure.

As we continue back down through the clouds and across treacherous terrain, we will be fortified with the clues that we have gained on our expedition to the summit – clues that we desperately need to solve the riddle of FOP. It is not a simple task to successfully treat or cure a genetic condition, and it will likely take many more years. It will be done. We will need the continued help and support of many scientists and many laboratories, and the continued generosity of patients, families, and communities throughout the world, more now than ever. We have entered a new era of FOP research. Hope is clearly on the

horizon. The FOP Center and Core Laboratory continue to be unique resources for FOP patients and for the medical community worldwide. As always, we strive for excellence and leadership in all areas vital to our mission: patient care, education, and the generation of new knowledge.

In summary, 2005 was a year of mind-boggling developments for FOP research - highlighted by the discovery of the FOP gene, the most important discovery in the history of FOP research. We are hopeful that 2006 will also be a year of great milestones in FOP research and that exciting advances and discoveries will highlight the year ahead.

The FOP research community has charted a long and difficult journey over the past 15 years, but it is amazing how far we have come. We continue to be a robust and vibrant community that spans the globe. We are united in our effort and we possess the momentum and verve to accomplish the goals we have set for ourselves. We are reminded each day that we have a long journey ahead to achieve those goals, but we are encouraged by our accomplishments and we are energized by our challenges.

As always, our heartfelt thanks go to the children, adults, and families who live with FOP every moment of their lives. Their equanimity and nobility provide the perpetual inspiration that dignifies this work and all who are privileged to participate in it.

This year, we wish to extend a special thanks to Jeannie Peeper, the founder of the IFOPA, who has retired as Chairman of the Board, but who remains in her lifetime role as President, spokesperson, and spiritual leader of our worldwide FOP community. We wish to extend special thanks as well to Amanda Cali, the new Chairman of the Board of the IFOPA, who has provided a seamless and magnificent transition during these challenging times. Jeannie's and Amanda's visionary devotion to FOP research and to the

FOP community worldwide brings clarity to our mission, and hope to future generations. The FOP Collaborative Research Project arose out of a mutual desire to find the cause and to establish a cure for this disabling condition. The words *care*, *compassion*, and *collaboration* are the working glue that link *cause* to *cure*. We are grateful for many colleagues and collaborators at medical offices, clinics, hospitals, research laboratories, centers and universities around the world without whose help and brilliance this ongoing effort would be even more difficult - if not impossible. Together, we have accomplished the goal of establishing the genetic cause of FOP, and together we will find a cure for this disabling condition. We will prevail. As David Ben-Gurion, the first Prime Minister of Israel said, “The difficult we do immediately; the impossible takes a little longer.” As always, finding an effective treatment and cure for FOP is not a job, it is a mission.

All of us at The FOP Center, in The Developmental Grants Program, and in the affiliated collaborative ventures around the world are extremely proud to be part of this mission, and are enormously grateful to those who support this vital research effort:

- The International FOP Association (IFOPA)
- The National Institutes of Health (The People of the United States of America)
- The Center for Research in FOP & Related Disorders
- 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 Allison Weiss Fellowship in Orthopaedic Molecular Medicine
- The Born-Lotke-Zasloff Fellowship in Orthopaedic Molecular Medicine
- The Whitney Weldon - Stephen Roach Fellowships in FOP Molecular Genetics

- The Roemex Fellowship in FOP Molecular Pathophysiology
- The Grampian Fellowship in FOP Molecular Pathophysiology
- The Medical Research Council and The University of Oxford (United Kingdom)
- The Association Pierre-Yves (France)
- FOPeV (Germany)
- The Brazilian FOP Association
- The Scandinavian FOP Association
- Members of the FOP International Research Consortium
- The People of Santa Maria (13 years of extraordinary service)
- And the many individuals, families, friends, and communities throughout the world who contribute generously and tirelessly to the FOP effort.

Thank you, as always, for your continued generous and heartfelt support of this vital and urgent effort.

SUPPLEMENT TO THE FIFTEENTH ANNUAL REPORT: THE PATIENT AND FAMILY GUIDEBOOK TO THE FOP GENE

Preface

The process of scientific inquiry is among the most structured of all human endeavors. Yet, progress in scientific and medical research rarely follows a straight path. At times, scientific discoveries creep forward, but at times they leap forward with a momentum that leaves everyone speechless. There is rarely, if ever, any advanced notice for such discovery. It happens, as any child trying to solve a puzzle will recognize, with an immediate sense that something new is happening – that pieces that previously seemed discordant begin to fit together in a completely new way. A breakthrough is an accomplishment so revolutionary that it allows one to enter a new world – an insight so spectacular that the entire horizon suddenly changes and everything is different from that moment forward. We recently had such a breakthrough – the discovery of the FOP gene.

QUESTIONS AND ANSWERS

- **Is the FOP gene involved in the bone morphogenetic protein (BMP) signaling pathway?**

Indeed, it is! We originally predicted that FOP was caused by a mutation of a gene in the BMP signaling pathway. That prediction was correct. After 15 long years, the FOP gene has been discovered and is a recently-recognized BMP receptor at the heart and soul of the BMP signaling pathway.

- **What is the FOP gene?
How does it work?**

The FOP gene is Activin Receptor Type IA or ACVR1. It is one of the three known BMP Type I receptors. The other BMP Type I receptors are BMPRIA and BMPRIB.

A BMP Type I receptor is a protein “switch” that transmits BMP messages from the outside of a cell to the inside of a cell, and as a result, determines the fate of the cell. The ACVR1 receptor is 509 amino acids long (an amino acid is a building block of a protein). In FOP, the amino acid histidine is substituted for the amino acid arginine at position 206 of ACVR1, a substitution that is predicted to change the activity of the receptor, much like a short circuit in a light switch.

As is the case for most genes, every cell has two copies of the ACVR1 gene. In patients with FOP, one of the two ACVR1 gene copies harbors a mutation that causes the ACVR1 protein (that is manufactured by the cell) to be incorrectly made. It is extremely exciting that we no longer have to guess about the cause of FOP. We now know exactly what the problem is and where it occurs in the molecular switch. Our next goal is to determine exactly how the damaged (mutated) copy of the ACVR1 gene changes the fate of cells in which it is expressed.

- **If ACVR1 is a BMP receptor, why is it called an Activin receptor?**

ACVR1 was originally thought to be a receptor for the growth factor Activin which is involved in numerous bodily functions. However, it was recently discovered that the ACVR1 receptor is predominantly a BMP receptor, rather than an Activin receptor. Nevertheless, the original name ACVR1 remains. At the time of this writing, most research on BMP Type I receptors is focused on BMPRIA and BMPRIB, not ACVR1. With the identification of ACVR1 as the FOP gene, this state-of-affairs will rapidly

change, and scientists worldwide will begin to pay much more attention to ACVR1. In a sense, FOP has shined the spotlight on this much ignored BMP receptor, and catapulted it to scientific and medical infamy.

- **What functions does the ACVR1 gene perform in the body?**

ACVR1 is an important BMP signaling switch in cartilage cells of growing bones (especially in the hands and feet), in the jaw bone, in the middle ear, in the aortic arch, in the thymus, in the heart, and in skeletal muscle cells. A person cannot live without ACVR1 protein. A mouse embryo lacking both copies of the ACVR1 gene does not make ACVR1 protein and does not develop into a live mouse. In FOP, both copies of the ACVR1 gene (one from mother and one from father) are present. However, one copy is damaged in a very specific way. A damaged ACVR1 protein is made from the damaged or mutated copy of the gene.

When an overactive copy of the ACVR1 gene is genetically engineered into a growing chicken, the joints fuse, an indication of its role in joint formation. This observation, as well as its role as a BMP receptor, prompted us to consider ACVR1 as a very likely candidate gene for FOP. Development of genetically engineered mice with the exact same mutation in the ACVR1 gene that causes FOP in humans will allow us to better understand the roles that the ACVR1 protein plays in FOP and in normal development, and as a result will allow us to develop and test potential treatments.

- **How is the ACVR1 gene damaged in FOP?**

In FOP, the ACVR1 gene is damaged by the substitution of a single genetic letter at a specific location in the gene. That single nucleotide (or genetic letter) substitution changes the meaning of the genetic message encoded by ACVR1.

The human genome, which consists of 6 billion nucleotides or “letters” of DNA, is the genetic code for making the body’s proteins - the machinery of life. Genes are dispersed throughout the genome and are written in a four-letter code (A,T,C,G). Genes (the body’s cookbook) are copied by the genetic machinery of the cell (the scribes) into messenger RNAs (the recipes) which instruct the ribosomes inside the cells (the cooks) how to make the specific proteins (the soups).

Let’s suppose that a hypothetical gene called FRENCH ONION SOUP encoded a message that told the cook to add wine (VIN) to the soup. Genetic instructions are comprised of three-letter words, and the words of the genetic sentence run together without any punctuation marks. So, for example, one part of our hypothetical gene may read:

NOWADDTHEVIN

The “genetic cook” interpreting this message would have to separate the message into three-letter words, and so the message would be:

NOW ADD THE VIN

And the wine would be added to the soup!

Now, let’s suppose there is a mutation that removes the letter “A” from the word “ADD.” Remember, we said that all genetic words are comprised of three letters. So, the COOK decoding the message would read the following:

NOW DDT HEV IN

Well, this sentence makes no sense at all, and the cook would take the message, and throw it in the wastebasket, similar to what would happen in a real cell if such a mutation occurred.

Let's suppose, as another example, that an extra "A" was added to the message. Again, since the genetic code requires three-letter words, the message would be decoded by the cook as follows:

NOW AAD DTH EVI N

Again, this message is nonsense, and would be destroyed by the cook.

Now, let's suppose that instead of subtracting the letter "A" or adding the letter "A", the mutation involved a **substitution** for the letter "V." And, in fact, let's substitute a "G" for a "V" in the word VIN and see what happens. The message now read by the cook would be:

NOW ADD THE GIN

Now that sentence makes sense! But, it is a very different message than the original one, and would completely change the meaning of the recipe and the taste of the soup!

Now, that is exactly the kind of mutation that occurs in FOP - the substitution of one genetic letter for another that changes the meaning of the genetic message. So, whatever ACVR1 normally does in the body, when a single genetic letter is substituted in a specific genetic word (as in the case of the FOP mutation), it changes the genetic word, and thereby changes the genetic instructions. Instead of its normal function or level of activity, the change prompts the body to build a second skeleton.

- **Do only people who have FOP have the FOP gene?**

No. Every human being has two copies of the ACVR1 gene. We call ACVR1 the "FOP gene" because it is the gene in which the FOP mutation is found, but every human being

has two copies of the gene. When one copy is damaged at a very specific location in the gene, FOP results.

- **What does the following equation mean?:**

$$\text{FOP} = \text{ACVR1(c.617G>A; R206H)}$$

We have always said that when we really know something profound and fundamental about FOP, we will not need a full-length book to write it, we will need a scrap of paper. Some day, everything that is essential to know about the science and medicine of FOP will fit on an index card with a lot of room left over for baseball scores and shopping lists! The equation noted above is really a formula for creating FOP – a genetic blueprint that can be written on a scrap of paper.

The formula says that to make FOP, you need to take the ACVR1 gene and damage one of the two copies of the gene in a highly specific manner designated by the notations inside the parentheses. First, you have to isolate one copy of the ACVR1 gene. Then, you have to go to nucleotide (genetic letter) 617 and substitute a single genetic letter - an adenine (A) for a guanine (G) in the middle letter of the three letter codon (genetic word) number 206. Word 206 in ACVR1 is normally spelled **CGC** and signifies the amino acid arginine in the genetic code. When the letter “A” is substituted for the letter “G”, the three-letter word becomes **CAC**, and signifies the amino acid histidine in the genetic code. Thus, that simple substitution (of an “A” for a “G”) in the middle letter of the three-letter word at position 206 in the gene causes the substitution of the amino acid histidine (H) for the amino acid arginine (R), and FOP results. Thus, the substitution of one genetic letter for another, in one of six billion genetic letters that comprise the human genome – the smallest and most precise change imaginable – causes a molecular “short circuit” that turns a functioning set of muscles and connective tissues into a second skeleton - in essence turning a light bulb into an atom bomb.

Thus,

FOP = ACVR1(c.617G>A; R206H)

is the

E = mc²

for the skeleton.

- **How does the ACVR1 mutation in FOP cause the malformed toe to form in the embryo and the extra bone to form following birth?**

The short answer is that we do not yet know. The exact molecular mechanism by which the FOP mutation in ACVR1 specifically “short circuits” BMP signaling to cause the malformed toe before birth and the extra skeleton after birth is presently unknown but is the subject of intense investigation at the FOP Laboratory.

In order to really answer this question properly, it will be necessary to develop a genetically engineered mouse with real FOP so that these and other related questions can be studied in great detail. That work is currently underway.

- **Are there racial, ethnic, or geographic variations in the FOP mutation?**

No. The mutation in ACVR1 that causes classic FOP appears to be identical in all racial and ethnic groups and in all patients from all geographic locations around the globe.

- **Does everyone with FOP have the exact same mutation?**

So, far, we have found that every person with classic FOP has the exact same mutation in ACVR1. As more people are tested however, it is very likely that some people with variations of FOP may not have this exact mutation. We already found one small multigenerational family whose affected members had ambiguous features of FOP. One

affected individual in the family had only toe malformations without heterotopic ossification while another had no toe malformations but had very mild heterotopic ossification. This family was not included in the linkage analysis that we used to locate the FOP gene since every member did not fulfill the most stringent diagnostic criteria for classic FOP. And in fact, the FOP mutation was not detected in any member of this family. So, in all of our analyses, and in all future studies, it is very important to carefully identify who has classic FOP and who has a variation of classic FOP. We define classic FOP as those individuals who have a typical congenital malformation of the great toes and progressive heterotopic ossification in characteristic anatomic patterns. All clinical variations from this classic picture of FOP must be noted with great care. In patients who have unusual forms of FOP (including other clinical features not normally seen in FOP), it would not be surprising if we find genetic variability.

It is interesting to note that the FOP mutation at codon 206 in ACVR1 was found even in the person with a previously reported but unverifiable mutation in the noggin gene. We have known for a long time that the reported noggin mutations in FOP were erroneous.

- **What is the relationship between the FOP gene mutation and other features of FOP such as hearing loss, angiogenesis, etc?**

This is an important and intriguing question, but we do not yet know the answer. We speculate that the ACVR1 protein plays a direct role in all of these processes, and it will be important to study these particular aspects of ACVR1 function. This is yet another example where an animal model of real FOP would be enormously helpful, and we are presently working to develop such an animal model.

- **Where is the ACVR1 gene (the FOP gene) located in the human genome?**

ACVR1, an average size gene, is located 158,418,469 basepairs (genetic letters) to 158,557,131 basepairs from the beginning of human chromosome two. If you were addressing a letter, the envelope would read:

FOP GENE MUTATION

CGC → CAC

ARGININE TO HISTIDINE

CODON 206

GS DOMAIN (THE SWITCH)

ACVR1

CHROMOSOME 2

ZIP CODE: 158,418,469-158,557,131

THE HUMAN GENOME

If you started at one end of chromosome two, and took a walk along a very long street (chromosome two is the second longest chromosome in the human genome), you would have to take 158,418,469 steps before you were in front of ACVR1. That's the house in which we found the FOP mutation - on the third floor, in the antique desk, behind the drawer, in the cubby hole, in the box marked "these are the keys to the skeleton."

- **For several years, it was thought that the FOP gene was on chromosome 4. How did it end up on chromosome 2?**

Our initial linkage analysis, that led us to examine chromosome 4, used four small families that showed the classic autosomal dominant inheritance pattern of FOP, although not all affected individuals in each small multigenerational family had malformation of

the great toes. With the experience of examining many more FOP patients over time, we became less certain that patients who did not have both of the classic features of FOP (typical malformations of the toes and heterotopic ossification) had true FOP. Thus, in our definitive linkage study that we used to identify the FOP gene, we decided to use only a subset of families in whom all affected individuals had unambiguous features of progressive heterotopic ossification as well as malformed great toes. The strict adherence to stringent diagnostic criteria for FOP, the additional multigenerational families, and the refined maps of the human genome allowed us to clarify the true location of the FOP gene which is on human chromosome two, at the exact address previously noted.

- **How was the FOP gene discovered?**

The discovery of the FOP gene was the result of painstaking work by many physicians and research scientists over many years. It involved the identification and clinical examination of multigenerational families, the performance of genome wide linkage analysis, the identification of candidate genes, and finally the DNA sequencing and analysis of those candidate genes.

A detailed description of genome wide linkage analysis and candidate gene screening can be found online (www.ifopa.org) in the 13th Annual Report of the FOP Collaborative Research Project; May 2004.

- **When was the FOP gene discovered?**

The FOP gene mutation was discovered in August 2005 in the FOP Research Laboratory at The University of Pennsylvania School of Medicine in Philadelphia.

- **Who discovered the FOP gene?**

The FOP gene mutation was discovered by physicians and scientists at the FOP Research Laboratory at The University of Pennsylvania School of Medicine. The first person to recognize the FOP gene mutation was Meiqi Xu, a highly respected senior scientist who has been working passionately and tirelessly in the FOP Laboratory since 1995. Meiqi had noted some irregularities in the ACVR1 gene, a highly interesting candidate gene selected for screening. These findings were unusual enough to mandate further testing. A possible disease-causing mutation was suspected by Meiqi after comparing DNA sequence data from FOP patients and from normal controls.

In an email dated August 17, 2005, Meiqi wrote to Dr. Kaplan (who was attending the Annual Meeting of The FOPeV, the German FOP Organization, in Valbert, Germany):

Dear Dr. Kaplan:

I want to knock on your door in the middle of the night, but I know you are not home this week. I think the news from my data of ACVR1 is very exciting!!!

More than 10 FOP patients and more than 20 normal controls have been screened and the sequencing data show that all FOP patients have the same exact heterozygous mutation at the same exact position in the ACVR1 gene and that all normal controls do not have this mutation. The results indicate that the single

basepair change may relate to FOP. I am screening more FOP patients now to reconfirm, and I have told Dr. Shore about the data this afternoon because I am so excited about it. I am waiting for you to come back from your trip to Germany to discuss this with you. Let us pray this is the gene we are looking for. Have a good trip home.

Meiqi.”

- **Were we surprised by the discovery of the FOP gene?**

Yes and no. No, because we had singled-out the ACVR1 gene for analysis as an excellent candidate gene in the chromosome-2 linkage region. Yes, because of the amazing specificity of the mutation. In other words, we were not surprised to find the gene in the house we found it in. But, we were surprised to find it in the specially marked box in the antique desk (see 15th Annual Report; The Discovery of the FOP Gene: A Brief Summary).

- **Why did it take so long to discover the FOP gene?**

In reality, it was not a long time considering the small number of multigenerational families we had available to help track down the location of the gene. The major limiting factor was the paucity of multigenerational families, and it took time to establish the medical and scientific infrastructure that made successful identification of those families possible. Multigenerational families are the fuel that power the search for the causative gene of a human genetic disease through a complex process called genome wide linkage analysis (explained in depth in the 13th Annual Report of the FOP Collaborative

Research Project; May 2004 - available online at: www.ifopa.org). The discovery of additional multigenerational families, the use of more stringent clinical criteria for defining classic FOP, the establishment of more refined genetic maps of the human genome (made possible by the human genome project), and the discovery and characterization of candidate genes in the linkage interval have accelerated the search for the FOP gene during the past several years.

▪ **How can we be sure that ACVR1 is really the FOP gene?**

The support for ACVR1 as the FOP gene is overwhelming:

1. ACVR1, a gene encoding a BMP receptor, is a superb candidate gene for FOP.
2. ACVR1 is on chromosome 2, at the exact location where FOP is linked by genetic analysis in all classically-affected multigenerational families.
3. The FOP mutation alters the protein-coding region of ACVR1 and substitutes an amino acid that has been highly conserved over hundreds of millions of years of evolution. This substitution predicts a change in the function of ACVR1.
4. The mutation in ACVR1 is seen in all FOP patients but not in any normal controls.
5. The same mutation in ACVR1 is seen in all affected individuals from multigenerational families and in all sporadically-affected individuals with classic FOP.
6. ACVR1 is expressed in many tissues including skeletal muscle and cartilage cells, and an activated or “trigger happy” copy of ACVR1 causes muscle cells to behave like bone cells, upregulates BMP4, downregulates BMP antagonists,

expands cartilage elements, induces heterotopic ossification, and causes normal joints to fuse - clinical and molecular features nearly identical to those seen in individuals who have FOP.

The ultimate confirmation of the FOP gene mutation will rest on the demonstration of a functional change in BMP signaling by ACVR1(R206H). Those studies are underway.

- **Do we need to find additional multigenerational families?**

Multigenerational families were the key to identifying the FOP gene. They allowed us to dramatically narrow the region in the human genome where the FOP gene is located and thus make a house-to-house search possible using the candidate gene approach. Without the multigenerational families, the work could not have been accomplished. This is a perfect opportunity to thank all of the multigenerational families who have contributed to the search for the FOP gene. Without their help, their cooperation, and their generosity, the identification of the FOP gene would not have been possible.

Now that the FOP gene has been identified, we no longer need to find multigenerational families in order to localize the gene. However, we are still vitally interested in finding multigenerational families for even more important reasons – for welcoming them into the international FOP community, for providing help and information to families who are exceptionally challenged with more than one affected individual, and for making available the wide variety of resources of a vibrant and generous international community.

From a research perspective, we will always want to verify the genetic findings in all new multigenerational families (as in sporadically affected individuals) and to be vigilant about the possibility of new clinical and genetic variations of FOP. Such variations, should they exist, would certainly broaden and deepen our understanding of FOP and provide important perspectives on treatment.

- **ACVR1 is described as a highly conserved gene, and the specific portion of the gene where the FOP mutation is found is described as extremely highly conserved throughout all of vertebrate evolution. What does that mean and what is the functional significance of such evolutionary conservation?**

The ACVR1 gene and protein became encoded in the molecular machinery of the vertebrate genome (DNA) and the vertebrate cell more than two hundred million years before the earliest dinosaurs appeared on the face of the Earth. The genetic “word” that encodes the amino acid arginine at codon 206 of the ACVR1 protein has been preserved throughout this entire time span, suggesting that nature needs to maintain an arginine at position 206 of ACVR1 to support the normal functions of cells, tissues and organs.

- **Will it be possible now to make a mouse with real FOP? What are the obstacles? What will the mouse be called? What will the mouse enable us to do? How will it help us find better treatments and eventually a cure?**

Now that the FOP gene mutation has been identified, it is very important to develop a genetically engineered mouse with the exact same mutation in ACVR1 that is found in people who have FOP. The ACVR1 gene is highly conserved throughout vertebrate evolution, from fish to mice to humans, and the specific codon that is mutated in FOP

(codon 206) is exactly the same in everyone who has classic FOP. Whether or not the mouse will actually develop FOP remains to be seen. Even though the ACVR1 gene in the mouse is nearly identical to that in humans (and the specific codon of the gene mutated in FOP is truly identical), it is possible that the mouse may not develop FOP, since other genes in the mouse genome might modify the development of FOP. We cannot predict what will happen, but we will make such a mouse and watch it carefully. We will examine the digits very carefully for malformations, and we will, of course, look for any speck of heterotopic bone.

It is technically difficult to make a mouse with real FOP, but the knowledge needed to make such a mouse is presently available. Development of FOP in such a genetically engineered mouse would herald another tremendous advance in FOP research. It would enable us to study the process of FOP lesion formation and would enable us to test medications and therapies that might otherwise be extremely difficult or dangerous to test without such a model. In many ways, a genetically engineered mouse with real FOP will help dramatically advance the search for better treatments and a cure. But, we will have to wait and see if the mouse genome and the mouse physiology cooperate with our scientific attempts to duplicate FOP. If a real FOP mouse is developed, we will have a contest to name it. Stay tuned!

- **The FOP gene mutation affects ACVR1, a BMP Type I receptor. What other human genetic conditions are associated with mutations in BMP receptors?**

The FOP gene mutation is the only genetic mutation in humans that has, to date, been associated with the ACVR1 gene. However, the FOP mutation in ACVR1 is among a

growing list of human disease-causing mutations in BMP receptors that includes mutations of BMPRIA (that cause a syndrome of colonic polyps), BMPRIB (that cause a syndrome of congenital malformations of the limbs and digits), and BMPR2 (that cause primary pulmonary hypertension, a fatal condition affecting the blood vessels in the lungs). The mutation in ACVR1 that causes FOP adds to this list and establishes a specific link between a catastrophic disorder of renegade bone formation and the highly conserved BMP signaling pathway.

Although ACVR1 has previously been recognized as a receptor for BMPs, investigations of its functional role in embryonic development and in regulating cell differentiation have been extremely limited. Identification of a specific disease-causing mutation in ACVR1 has critical diagnostic and therapeutic implications for FOP and identifies a novel investigative focus for skeletal biology and regenerative medicine.

- **Are there any other human genetic conditions where the mutation is as specific and precise as it is in FOP?**

We know of two other human genetic conditions in which the vast majority of patients have a mutation at a specific site in the causative gene. One condition is Timothy syndrome, a complex multiorgan disorder, and the other condition is achondroplasia - a common form of dwarfism. In achondroplasia, the affected gene is also a receptor, called fibroblast growth factor receptor 3 (FGFR3). But, instead of a receptor for BMPs, FGFR3 is a receptor for another very important group of signaling molecules called the fibroblast growth factors (FGFs). The mutation in achondroplasia changes a very specific amino acid in the portion of the receptor that is anchored in the cell membrane. The normal

function of FGFR3 is to slow down the growth of cartilage cells in the growth plates of growing bones. The mutation in achondroplasia makes FGFR3 more active, thus slowing down growth even more than it is supposed to - and dwarfism results. As with FOP, the mutation in achondroplasia affects only one copy of the responsible gene. Although most mutations that cause human genetic diseases affect a single gene, the mutations are usually scattered throughout the gene. That is not the case with Timothy syndrome or achondroplasia and it is not the case with classic FOP. Those are the only known genetic disorders in humans where the mutation in most all affected individuals is caused by the substitution of a single genetic letter in the entire human genome of six billion genetic letters.

- **How will the discovery of the FOP gene affect FOP research?**

We now know the cause of FOP at the genetic level, and it will not be long before we understand the mechanism at the molecular level - in other words, how the mutation affects the function of the ACVR1 receptor, and as a result, alters the fate of cells. That knowledge can perhaps be applied - not just for understanding and treating FOP, but for understanding and treating many common disorders that affect the skeleton - conditions such as non-genetic forms of heterotopic ossification (following hip replacements, head injuries, spinal cord injuries, sports injuries, and blast injuries from war), heterotopic ossification in heart valves in endstage valvular heart disease and even osteoarthritis. Perhaps, some day 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 for those children born with congenital malformations of the spine and limbs.

▪ **Why is there such excitement over the discovery of the FOP gene?**

The excitement over the discovery of the FOP gene is summarized in a letter written by Drs. Shore and Kaplan to the editors of **Nature Genetics**, the most prominent genetics journal in the world, and the journal in which the FOP gene discovery is published (online version: April 23, 2006).

“Identification of the mutation that causes FOP is highly significant for patients with this condition since it will re-focus efforts to develop desperately needed therapies. However, we strongly believe that this discovery will be of broad general interest to physicians and scientists worldwide for several reasons:

1. The study identifies the causative gene mutation for FOP, a severely debilitating disorder in humans, and thereby identifies this gene, ACVR1, as a key regulator of cellular differentiation.
2. FOP is the first human genetic disease ascribed to ACVR1.
3. Although FOP is a rare disorder, understanding the genetic cause of the misregulated bone formation that occurs in FOP patients has important implications for a wide range of more common skeletal disorders.
2. The FOP gene discovery is one the most specific genetic mutations to be associated with a human disease. The unusual finding of a disease caused by a mutation of a specific amino acid has been identified in only two other human

genetic disorders - achondroplasia, a form of dwarfism, and Timothy syndrome, a rare genetic disorder affecting multiple organ systems.

5. ACVR1 encodes a Type I receptor for bone morphogenetic proteins (BMPs). BMP signaling is one of the most highly conserved signaling pathways in nature with important roles not only in bone formation, but also in the formation of other organ systems during early embryonic development.
6. The BMP signaling field has focused on two other type I receptors (BMPRIA and BMPRIB) as regulators of cartilage and bone cell differentiation. The identification of ACVR1 as a critical regulator of endochondral bone formation during embryogenesis and in muscle and connective tissues following birth will undoubtedly re-focus thinking and stimulate major new research directions in bone biology. This discovery will have a major impact on skeletal biology and regenerative medicine.”

- **How long will it take to use this new knowledge to develop treatments and a cure?**

This is the most difficult question of all to answer and only a fool would put a time limit on it. There is no doubt that the FOP gene mutation is the most valuable piece of information in the FOP puzzle, but it is not the only piece. We will need to understand how normal and mutated ACVR1 functions and interacts with other BMP receptors, how it is regulated under normal conditions and in FOP, how it affects and alters the fate of cells in which it is expressed, how it is affected by trauma, and how it functions during embryogenesis and following birth. Such seminal knowledge will help us determine what types of drugs we will need to develop and how we will need to administer them.

We have often said that FOP research is like trying to figure-out the wiring diagram of an atom bomb so that the bomb can be safely defused before it explodes. We have identified the FOP mutation, the trigger of the atom bomb. Now, we have to figure-out how to safely inactivate it. This will take time. We have a lot more to learn about that trigger, and how it is wired-in to the bomb. That is the sobering news. But the great news is that we now have an extremely specific target for drug development that will immediately focus an enormous amount of medical and scientific attention on this gene and on FOP.

- **Why is ACVR1 considered to be the skeleton key?**

ACVR1 is the skeleton key because it harbors the secrets to the formation of a second skeleton. It is more of a key than we ever even imagined. ACVR1 is one of a few dozen genes and proteins that has been used over the past half billion years to build both the external and internal skeleton of living organisms from fruit flies to humans. If you remove ACVR1 from an organism, it is lethal. If you tamper with it, there is trouble. The FOP mutation in ACVR1 is a vital key to unlocking some of Nature's most secret mysteries.

- **How important is the discovery of the FOP gene?**

“Nature is nowhere more likely to show you her secret mysteries than in cases where she shows you traces of her workings apart from the beaten path.” Thus, wrote William Harvey, the discoverer of the circulatory system in 1657, in a letter that could have very well prefaced the discovery of the FOP gene, nearly 350 years later. ACVR1(R206H) is

not simply the mutation for a rare and disabling genetic condition, but the molecular key to an intriguing biological process - the formation of an ectopic organic system, a second skeleton.

As the author Thomas Maeder said in an article about FOP in *The Atlantic Monthly* (“A Few Hundred People Turned to Bone,” February 1998), “FOP and its problems lie at the crossroads of several seemingly unrelated disciplines. Answers to questions that FOP poses will also address grander issues of how the body first creates its shape, and then knows where to stop; how tissues decide to become what they are, and why they don’t turn into something else.”

The discovery of the mutation in ACVR1 that causes FOP illustrates how the smallest possible mutation in a component of a critical signaling pathway can lead to the formation of a second or ectopic skeleton. ACVR1 (R206H) will have to be disabled, neutralized, or bypassed to treat and cure FOP, therapeutic challenges that lie ahead – but it might also be harnessed to create bone and skeleton for those who desperately need it – for those who have congenital skeletal malformations, for those who have catastrophic bone loss from severe trauma or amputations and, of course, for those who have more common conditions such as osteoporosis that affect millions. The discovery of the FOP gene is the most important discovery in the history of FOP research, but it is also an extraordinarily important discovery for all of skeletal biology.

- **Who contributed to the discovery of the FOP gene?**

The discovery of the FOP gene has been a worldwide effort by many individuals over the past fifteen years. We thank the members of the FOP International Research Consortium who have identified the multigenerational families and/or made important contributions to this collaborative effort. We thank the FOP patients and families for providing blood samples for these studies and for their courage and faith during this long journey. We thank Jeannie Peeper and Amanda Cali for their inspiration and leadership of the international FOP community; many colleagues for valuable discussions over the course of these investigations, and many physicians worldwide who referred patients and helped in sending samples for analysis. We acknowledge with appreciation the contributions of scientists at our own university for their extraordinary technical help and for valuable discussions during the course of this discovery. We thank the people of many communities around the globe for their faith and generosity over many years. We also thank the many present and past members of the FOP Research Laboratory for their dedication and invaluable technical support, particularly those who have participated in candidate gene screening over many years.

The discovery of the FOP gene was supported by The International Fibrodysplasia Ossificans Progressiva Association, by endowments from The Weldon and Cali families and their associates and friends, by the Isaac & Rose Nassau Professorship of Orthopaedic Molecular Medicine, by the Stephen Roach-Whitney Weldon Fellowships, by The Roemex and Grampian Fellowships, by The University of Pennsylvania and The University of Oxford, by grants from The Association Pierre Yves, The FOPeV, and The National Institutes of Health, by the People of Santa Maria and by the many individuals,

families, friends, and communities throughout the world who contribute generously and tirelessly to the FOP effort. We dedicate this discovery to the memory of the children and adults with FOP who have given us the inspiration and determination to reach this extraordinary milestone in FOP research.

▪ **What will happen next in FOP research?**

Ironically, we recently received this exact question from Hugo Fahlberg, a seven-year old child with FOP from Eskilstuna, Sweden. Hugo's mother, Marie, wrote the following email:

“Dear Fred:

Hugo is eating dinner and planning for his future – his hockey and soccer future – and he knows that you must find the appropriate gene first before he can play real hockey - and until then, he must play at home carefully with his friends on the grass. But, today, he has a question that I was unable to answer, so I will let him write it to you by himself, and here it is: When you find the gene, what are you going to do with it?

Signed, Hugo.”

Well, Hugo, that is a wonderful question, and it could not have come at a better time! We actually just found the FOP gene! The first thing we should all do is have a big party and celebrate!! Now, we finally know the cause of FOP, and that is a reason to celebrate because it gives us the first real clue about how to fix it.

Even before the party, we did some homework and we wrote a description of the FOP gene discovery for other scientists and doctors. We think that many doctors and scientists around the world will be interested to know what the FOP gene is, and perhaps do research on it as well. Some scientists may even want to use that information to help some people make more bone, like those who have broken bones from playing soccer and other sports, or some children who are born without enough bone in certain parts of their body, for example.

We are now trying to make a mouse that has real FOP, and we have already started working on that. Mouse genes are a lot like people genes, so discovering the FOP gene gives us a big clue about how to make an FOP mouse. If we can make a mouse with real FOP, we'll be able to test new medicines in the mouse before we test them in you. Now that we know the FOP gene, we have a better idea about the types of medicines that might stop FOP so that kids with FOP can play hockey and soccer and not form extra bone if they get bumped. We don't have those medicines yet, but we already started working on them.

The discovery of the FOP gene is a very important discovery that will help us solve the puzzle of FOP. Thanks for asking such a great question!

Love to you and to your whole family,

Dr. Kaplan.