

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

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While climbing mountains in Alaska some years ago, one of us scrambled to a ledge with a stunning view of the highest peak in the range – or so it seemed. Only by climbing further did the realization occur that the perceived summit was only one of many smaller peaks that obscured the real summit deeply shrouded in clouds. So, too, it is with FOP research. What seemed at the beginning like a high mountain - was actually one of many smaller peaks in a formidable range of unfathomable height.

Some day, a child with FOP will be born and everything about this daunting mountain range of FOP will be known – its genetic basis, its molecular origin, the nature of its pathways, the identity of its receptive cells and their downstream targets, the drugs to prevent it, and the therapies to cure it. That day is not yet at hand, but the journey and the climb towards the real summit continues unimpeded and uninterrupted.

Our journey over the years has given us great perspective, and we have learned an enormous amount about this massive mountain range called FOP. We are well above base camp where scientific instruments and a good sense of mountaineering tell us where to go next, but we must be humble, vigilant and alert. Despite an increasing reliance on clues at the cellular and molecular level, we can never ignore the bigger picture or the perspective we have gained on our climb. If there is a theme to this year's report, it is “that no clue is too small to ignore – not the shape of the clouds; not the sound of the wind; not even a whisper from a fellow FOP traveler – or a little mouse.”

This year's Annual Report might well be entitled, “Of Mice and Men” (not the account by the novelist John Steinbeck), but the less fictional account of scientists and physicians working on FOP – as the two seminal discoveries in FOP research in 2004 occurred on either side of the clinic door – one in a mouse and one in a man. One of the discoveries came from a unique patient with FOP who received a bone marrow transplantation for an

unrelated condition. The long-term follow-up of that patient has told us more about FOP in one hour in the clinic than one-quarter century of experiments could possibly have revealed. The second discovery, a serendipitous one as well, occurred on the other side of the clinic door - in a little transgenic mouse - developed by neuroscientists who were studying the role of bone morphogenetic proteins (BMPs) in brain development. The transgenic mouse, engineered to overproduce BMP4 in its nervous system, surprisingly developed heterotopic ossification. But, the most interesting thing of all was that the bone formation was progressive, and followed a pattern different from that seen in patients with FOP – but a pattern nonetheless.

Sir William Osler, one of the most famous 19th century physicians said, “*Clinics are laboratories – laboratories of the highest order.*” While there were very important discoveries made at the cellular and molecular level this past year, our most revelational insights came from the clinics – the patient clinics and the mouse clinics, and you will be hearing more about them in this report and in reports that follow.

The annual report is our chance to tell you what we have been doing all year, what we have discovered sometimes by accident, sometimes by plan, what we have been thinking about, what experiments we have done, which ones have worked, and which ones have not, what detours we have taken, what goldmines we have found, and which dead ends we have run into – what we expected to find, and what we found that no one could ever have anticipated because no one has ever been over this ground before. We will tell you what we used to think and what our new data compels us to think now – what we did last year, and what we plan to do in the year to come.

In order to keep the report fresh and crisp, informative and insightful, and of a size and magnitude that can be readily translated into seven or more languages in a timely manner,

we are experimenting with a new and shorter version for this year's annual report. Hopefully, there will be as much information as before, but it will be tightly compacted, sort of an "FOP Headline News." We are always trying to think of new ways to bring information to you – in *The Guidebook for FOP Patients & Families*, in the updated version of *The FOP Treatment Guidelines*, in *The FOP Connection*, and in bulletins from time to time on the FOP website. As one scientist said, "*Improved medical treatment depends on an unbroken path from creating knowledge, to disseminating it, to applying it.*"

Important highlights of FOP research during 2004 include the following discoveries and insights:

1. Evaluation of an FOP patient who received a bone marrow transplantation for an unrelated condition many years ago reveals that the immune system is a sensitive trigger, but not a specific trigger for FOP flare-ups.
2. Transgenic mice engineered to overexpress BMP4 in the nervous system develop progressive heterotopic ossification. However, there are vast differences in the rate of progressive heterotopic ossification even among genetically similar mice. (These mice, discovered by Dr. Lixin Kan at Northwestern University, have been affectionately named "THE FOPPY MICE" by IFOPA member, Louise Wedderburn of Rosehearty, Scotland).
3. The stimulus for heterotopic ossification in FOP patients may not simply be increased BMP4 delivery to tissues, as in the FOPPY mice, but more likely overactivity of the BMP4 pathway due to dysregulated BMP4 receptor signaling and trafficking across the cell membrane.
4. A "molecular speedometer" has been discovered for measuring the activity of the BMP4 pathway in FOP and control cells.

5. Despite widespread delivery of BMP4 to most of the body's tissues, only a small number of cell types in the body seem to be sensitive to chronically increased activity of the BMP4 signaling pathway.
6. We have added a new multigenerational family to the FOP genome-wide linkage analysis bringing the total now to 11 multigenerational families worldwide – four core families and seven supporting families. Using data from this new family, we have further narrowed the linkage interval for the FOP gene on the human chromosomes.
7. Adherent cells from the peripheral blood of FOP patients form bone *in vivo* and are derived from the bone marrow.
8. Inducible promoters (molecular on-off switches) have been developed for use in advanced pre-clinical studies on noggin gene therapy for the treatment of FOP.

In addition to pilot projects and continuing long-term studies, research efforts in 2005 will include:

1. Study of the mechanisms of heterotopic ossification in the FOPPY mice.
2. Use of the FOPPY mice to determine the efficacy of available drugs in slowing the progression of heterotopic ossification.
3. Study of the immune system in triggering FOP flare-ups.
4. Advanced molecular characterization of BMP4 receptor trafficking and signaling abnormalities in FOP cells in order to identify the underlying molecular defect in FOP.
5. Exploration of signal transduction inhibitor-approaches to block BMP4 signaling in FOP cells.
6. High resolution mapping of the FOP gene locus using genome-wide-linkage data and candidate gene loci from multigenerational families.

7. Advanced characterization of blood-derived adherent cells and their role in heterotopic ossification.
8. Pre-clinical studies for advancing noggin gene therapy using inducible promoters.

While the mission of FOP research is clear, the research projects and discoveries are not ends in themselves, but milestones along a difficult climb that will ultimately end with a cure for FOP.

This year's Annual Report will be divided into nine major sections that most clearly organize and highlight the vast amount of work and activity from the FOP core and collaborative laboratories. These sections are:

- I. Genes
- II. Pathways
- III. Cells
- IV. Models
- V. Triggers
- VI. Treatments
- VII. Presentations, Meetings, Reports, and Publications
- VIII. Your FOP Laboratory
- IX. Acknowledgements

We hope that you will find this year's annual report interesting, engaging, and hopeful. That is the attitude with which we approach our work every day. You have been with us on every step of this journey, and we want to share with you what we have found, what

we have seen, and what we have learned. Ultimately, you - the patients - will be the beneficiaries of this work.

I. GENES

Identification of the gene that (when mutated) causes FOP will be a key to understanding FOP as well as many more common conditions of skeletal development. The mutations responsible for most genetic conditions are identified by tracking the inheritance patterns of signature pieces of DNA (called markers) through large multigenerational families.

Genome-wide Linkage analysis

In the past several years, we have emphasized the importance of the genome-wide linkage analysis in locating and identifying the FOP gene. A detailed explanation of the principles of this analysis may be found in the 13th Annual Report at: www.ifopa.org.

Briefly, a genome-wide linkage analysis is a scientific tool that allows one to correlate the clinical features of a condition (such as FOP) with distinguishing molecular and genetic markers that are co-inherited with the damaged gene of interest through multiple generations of a family, and then to compare the information gathered from many such families to narrow the position on the human chromosomes where the gene is most likely located.

While the molecular aspects of linkage-analysis often receive most attention, the clinical aspects are equally important. Physicians who are extremely familiar with FOP must carefully examine each member of a multigenerational family to ascertain exactly who is, and who is not affected with FOP; and if someone is affected, to determine how severely

they are affected. This may seem trivial, but it is perhaps the trickiest part of the entire evaluation – and is as critical as the molecular analysis. If even one member of a multigenerational family is mis-assigned as incorrectly being affected or not affected with FOP (as sometimes the symptoms may be very mild in younger members - such as slightly malformed toes but no other signs of FOP), the integrity of the entire linkage analysis will be at risk. Therefore, well-coordinated clinical efforts are critical in evaluating each member of a multigenerational family as well as in obtaining DNA samples for genetic and molecular analysis.

Multigenerational Families & FOP Gene Localization Studies

There are presently four core multigenerational FOP families that are critical to mapping of the FOP gene, along with seven additional multigenerational families that support the genetic search for the FOP gene. Each of these 11 families is important to FOP research. However, the “golden” FOP family in terms of research would be a family that has multiple FOP patients and uninvolved siblings throughout three to four generations. No such multigenerational family has been discovered to date. While it is possible that one might be found, the chances are generally slim. One such large multigenerational family could, by itself, enable us to locate the FOP gene.

The eleventh multigenerational FOP family was located last year in South Korea. The patients have all been examined, DNA obtained for linkage analysis, and a genome-wide linkage analysis performed. Data from this new family has enabled us to narrow the locus for the FOP gene, although it still remains quite large – about one-half of one percent of the entire human genome. Imagine a 3000 mile road stretching from coast to coast in the United States representing the entire human genome. Two years ago, we had narrowed the location of the FOP gene to a 100 mile stretch; last year to a 60 mile stretch,

and this year, to a 30 mile stretch. We still have to find the street, the house, the room, and the cabinet where the FOP gene is hiding; but 30 miles is better than 3000 miles. We still have miles to go.

Presently, large scale, genome-wide linkage analysis is being performed both at The University of Pennsylvania and at Oxford University in a collaborative research effort. Additional multigenerational families will be very helpful in this effort and pilot recruitment programs are being conducted in India, Brazil, and Bangladesh at the present time.

Candidate Gene Analysis

Candidate genes are those genes that, if mutated, could plausibly cause a genetic condition. During the past year, we have continued searching for candidate genes for FOP within the narrowed linkage region as well as within functional candidate pathways. The candidates include genes that are involved in the bone morphogenetic protein signaling pathway, genes that control skeletal development, as well as genes involved in the regulation of either the innate or adaptive immune systems. We have, through our targeted DNA sequencing efforts, excluded many promising candidate genes, but we continue to evaluate many new ones each year in search of the true FOP gene. While targeted DNA sequencing of promising candidate genes is laborious, it is essential that we leave no potential FOP gene uninvestigated. This work is sponsored by The Whitney Weldon – Stephen Roach FOP Fellowship and by The Weldon Family Endowment.

Dripping Candle Wax Bone Disease

The power of linkage analysis and candidate gene searches is exemplified by an exciting discovery last year by colleagues in Belgium. Melorheostosis (which literally means “dripping candle wax bone disease”), is a rare genetic childhood condition that affects the

skeleton and soft tissues, mainly the limbs. Melorheostosis is characterized by new bone formation that appears to run like dripping candle wax on the outside of the long bones. Lesions are usually asymmetric and may involve only one limb or several limbs. They are often accompanied by stiffening of adjacent soft tissues associated with joint contractures, muscle atrophy, soft tissue swelling, and pain.

Using standard linkage analysis techniques (identical to the approach we are using to locate the FOP gene) in three large multigenerational families (we do not have such large and informative families with FOP), investigators from Belgium narrowed the location of the gene for melorheostosis to a very narrow interval on one of the human chromosomes. Their study was aided tremendously by one patient who additionally had a breakage in one of the chromosomes that enabled the scientists to pinpoint the location of the melorheostosis gene. Then, using a candidate gene approach in the linkage interval that encompassed the breakage region, the investigators discovered the gene for melorheostosis.

The most promising candidate gene in the linkage interval was a gene in the bone morphogenetic protein signaling pathway called SANE (Smad1 Antagonist Effector) that was also a candidate gene for FOP. In fact, the work that associated SANE with the BMP signaling pathway (long before it was discovered to be the gene for melorheostosis) was performed by Dr. Hui-Chuan Huang and Dr. Peter Klein at The University of Pennsylvania School of Medicine and was funded by a developmental grant from The Center for Research on FOP & Related Disorders! While SANE (also called MAN1 or

LEMD3) is not the gene for FOP, it is the gene for melorheostosis. Discovery of the melorheostosis gene highlights how research on FOP has provided a fundamental understanding for a related bone disease. Scientists can now begin to develop an animal model of melorheostosis using knowledge gained from the mutations in the SANE gene. With such an animal model, drug testing for melorheostosis will be greatly accelerated. Discovery of the gene for melorheostosis, a gene in the BMP signaling pathway, once again demonstrates the powerful connection between one branch of research and another in the pursuit of basic knowledge and the use of that knowledge to advance effective therapies that will help others.

II. PATHWAYS

The BMP4 Signaling Pathway

Establishing an effective treatment for FOP will result from identification of both the gene mutation and the dysregulated signaling pathway that causes FOP. For FOP, family pedigrees are scarce and genetic linkage and positional cloning is therefore difficult. A complementary approach to identify the primary pathology in FOP involves strategies to identify the dysregulated molecular pathway in FOP cells, and from there to trace back to the damaged gene. We have determined that the BMP4 signaling pathway is profoundly dysregulated in the cells of patients who have FOP, and plays an important role in the pathogenesis of heterotopic ossification in FOP.

Last year, we provided considerable background on the BMP4 signaling pathway in normal cells and in FOP cells, and for those interested, we highly recommend the following background sections from the 13th Annual Report (www.ifopa.org):

1. What is a Morphogen?
2. Autoregulatory Negative Feedback Loops

3. Dysregulation of Autoregulatory Negative Feedback Loops in FOP cells
4. BMP4 Pathway Dysregulation in FOP
5. Elevated BMP Receptor Levels at the Cell Surface
6. BMP Receptor Traffic Jams at the Cell Membrane
7. Who are the BMP Traffic Cops at the Cell Membrane and What is Their Role in BMP Signaling?
8. Which Road to the Target?

FOP: The Lymphoblastoid Cell as a Useful Model System

Most of our studies on the BMP4 signaling pathway in FOP have been conducted in lymphocytes derived from the blood of FOP patients (lymphoblastoid cells) in comparison to lymphoblastoid cells from normal controls. While lymphocytes are involved in the earliest pathology of FOP lesions and are readily and safely available for study, their role as causative factors in the disease process remains uncertain. Nevertheless, the lymphoblastoid cell is a useful model system for investigating the BMP4 pathway in FOP patients.

FOP: The Runaway Car

Metaphors have great power in conveying complex ideas, especially in science, and it may be helpful to summarize several years of ongoing laboratory investigation on the BMP4 signaling pathway in FOP cells with an appropriate metaphor - the metaphor of the runaway car. Fasten your seat belts!

Imagine first, that a normal lymphocyte is like a well-functioning car (normal BMP4 signaling pathway) while an FOP lymphocyte is like a runaway car (dysregulated BMP4 signaling pathway). In the normal car, the accelerator pedal is depressed on demand and a switch that connects the accelerator pedal to the brake pedal limits how fast the car can

go (negative feedback control). In the FOP car, the accelerator pedal is stuck at full throttle (increased BMP4), and the switch between the accelerator pedal and the brake pedal is disconnected (loss of negative feedback control in the BMP4 signaling pathway).

In the normal car, the emergency brake (Noggin & Gremlin) engages when the accelerator pedal is fully depressed. In the FOP car, the emergency brake fails to engage when the accelerator pedal is fully depressed (insufficient BMP4 antagonist secretion in response to a BMP4 signal). To make matters worse, the driver of the FOP car cannot see the road because there is a partial blackout shade over the windshield (failure of FOP cells to properly sense ambient BMP4 concentrations in their environment). Thus, it is impossible to safely navigate down the road in the FOP car.

The state-of-the-art onboard computer (the cell's capacity to monitor its own activity) informs the driver of the normal car that the engine's eight cylinders (BMP receptors) are functioning properly, while the onboard computer in the FOP car surprisingly informs its driver that it has 48 functional cylinders (a six-fold increase in BMP receptors in FOP cells). As a result, the FOP car is not only going at break-neck speed, but begins to careen off of the road and down a ravine (increased and inappropriate signaling of downstream pathways in FOP cells).

In complete desperation, the driver of the FOP car tries to shift the car into neutral, but it will not shift (failure to downregulate and internalize the overactive BMP receptors in the presence of BMP4 ligand), and the engine keeps running on fumes (promiscuous receptor phosphorylation in the absence of ligand). In a final last-ditch attempt to turn-off the ignition key (the skeleton key), the driver of the FOP car finds that the key is stuck in the ignition switch and the car's electrical circuit breakers are not accessible (we have little present knowledge of receptor-associated molecules immediately upstream or

downstream of the BMP receptors in FOP and control cells). The car continues to race at deadly speed down a steep ravine (muscle cell injury), and the car tumbles and explodes (muscle cell death and resultant edema).

A fire extinguisher (prednisone) is used immediately to douse the flames and the local rescue squad and wreckage crew (the body's immune and repair system) immediately appear at the scene of the accident (forming scar tissue and bone). Once the accident scene is cordoned-off and the flames are extinguished, parts of the wreckage are taken back to the salvage shop (the laboratory) and the stages of the accident are reconstructed. The blueprints for the car (the human genome) are studied to ascertain which ones could, if damaged, cause the entire ill-fated journey of the runaway car. By studying the runaway car in a crash test laboratory (the FOP laboratory where studying the BMP signaling pathway is examined in lymphoblastoid cells), the broken part (damaged or insufficient protein encoded by a mutated gene) that leads to the catastrophic crash (flareup) will eventually be identified.

While the metaphor of the runaway car can be stretched only so far, it provides a novel perspective for understanding the integrated systems approach that must be used to decipher and understand the complex workings of the dysregulated BMP4 signaling pathway in FOP cells. It also illustrates how a complex series of events can self-magnify and lead to catastrophic results when critical negative feedback switches are broken and downstream backup systems are genetically disabled. Presently, we are attempting to decipher the beguiling array of dysregulated components of the BMP4 signaling pathway in FOP cells in order to identify the single component (protein) controlled by a master gene that sets the runaway car on its dangerous and fateful path.

A Speedometer for Monitoring the Runaway FOP Car

If FOP cells are like runaway cars - autonomous, independent, and failing to respond to normal feedback controls as in normal cells, then it would be helpful to have a simple “read-out” or speedometer to measure the direction and magnitude of that fateful drive. Moreover, such a speedometer would be helpful in measuring the responses of new therapies first investigated at the cellular level. This year, Jennifer Fiori, a graduate student in the FOP laboratory, discovered a reliable indicator or “molecular speedometer” of the BMP4 signaling pathway in FOP cells. The “speedometer” consists of two genes called ID1 and ID3 that function as an early response system for BMP activity. Jen has shown that the ID system provides a relevant, reliable and robust readout of downstream BMP4 pathway activity in control and FOP lymphoblastoid cells. This is an important discovery as it provides a means to monitor experimental and therapeutic manipulation of the BMP4 signaling pathway in FOP and control cells.

Present and Future Research on Understanding the Primary Engineering Problem with the Runaway Car

Our present hypothesis is that dysregulated BMP receptor activity and trafficking plays a key role in the pathogenesis of FOP. Our goal is to identify the specific perturbation in the BMP4 signaling pathway in FOP cells that leads to this dysregulated downstream signaling. Our specific aims are to:

1. Characterize the signal transduction pathways that are activated by overabundant BMP receptor signaling in FOP cells.
2. Determine the mechanism leading to BMP receptor overabundance on the surface of FOP cells.
3. Examine receptor-associated proteins that differentially interact with BMP receptors on FOP and control cells, and evaluate the potential role of these proteins in receptor internalization.

4. Determine whether BMP receptor signaling in FOP cells is BMP4-mediated or BMP-4 independent.

An analysis of the molecular pathology of BMP receptor over-abundance on the surface of FOP cells (why the FOP “engine” has six times more cylinders than the normal engine) will provide critical insight into the molecular mechanisms underlying the earliest events in the pathogenesis of FOP. These studies will foster our long-term goal of elucidating basic mechanisms of normal and disordered bone induction in FOP.

The FOP laboratory has presented important new findings on this subject at the recent annual meeting of The American Society for Bone & Mineral Research. A scientific paper describing this work has been accepted for publication in the **Journal of Bone & Mineral Research**. This work is being sponsored by The Roemex, Grampian and Allison Weiss Fellowships and by The National Institutes of Health (the people of The United States).

**The National Institutes of Health Research Grant
To Investigate Dysregulated BMP4 Signaling in FOP Cells**

The FOP laboratory received good news from the NIH last year when we were informed that a four-year competitive NIH grant was funded for expanding studies on the dysregulated BMP-4 signaling pathway in FOP cells. The NIH Review Committee felt that the accomplishments of the FOP laboratory were important and that there was a high likelihood of producing additional valuable information on the molecular pathology of FOP and related conditions. We are all extremely grateful for this important research grant.

Large Scale Microarray Gene Expression Studies

During the past several years, large scale microarray gene expression studies and high-speed computer analysis of comparative genome databases have made it possible for us to compare the expression patterns of thousands of genes in FOP cells vs. control cells. This remains an extremely important approach to deciphering the wiring diagram of FOP cells, essentially the onboard computer of the FOP runaway car. In microarray experimentation, the difficulty no longer resides with the technology, but rather with the enormity of the data analysis, as each experiment generates hundreds of thousands of individual data points.

During 2004, Gisela Melcon, M.D., a postdoctoral fellow with expertise in microarray experimentation and analysis has joined our laboratory. Dr. Melcon has discovered the expression of several clusters of genes that appear curiously different in FOP cells than in control cells. It is too early to determine exactly what these composite differences in gene cluster expression may mean, but they may provide important clues to functional differences in the activity of critical signaling pathways in FOP and control cells. FOP research is much like trying to decipher the wiring diagram of a runaway car built by a “molecular terrorist” (the mutated FOP gene) so that we can more effectively determine how to disable it before it causes harm.

III. CELLS

Tissue-Specific FOP Target Cells

In order to solve the intractable riddle of FOP, it is essential to determine not only the exact molecular cause of the runaway car (overactive BMP4 pathway in FOP cells), but also to determine the exact site where the car crashes (which cells receive and process the abnormal signals that leads to the formation of heterotopic bone). Defining the exact origin of FOP lesional cells would represent a major breakthrough in FOP research, as it would suggest precise targets and “stealth strategies” for therapeutic intervention.

During the past several years, our studies to examine the cellular origins of heterotopic cartilage and bone formation have advanced dramatically and have produced surprising and definitive insights. Important findings on the nature and identity of receptive cells in skeletal muscle were thoroughly discussed in last year’s Annual Report (Insights on the Identity of FOP Target Cells).

It appears now that there several different receptive cell types scattered through various connective tissues and skeletal muscle itself that have the capacity to respond to a BMP signal. The complete and unambiguous identity of these cells and how they interact in the true setting of FOP to cause heterotopic bone will continue to be a major focus of attention of the FOP laboratory. Our preliminary data suggest that there may be some big surprises ahead. Stay tuned!

Circulating Osteogenic Cells In Heterotopic Bone Formation

Last year, we reported an exciting new discovery in FOP adult stem cell research (An Historic Email And A Breakthrough In FOP Stem Cell Research). During the past year, we have devoted much effort to this project. Briefly, cells of bone-forming potential, including connective tissue precursors, can be found in a variety of tissues. Surprisingly, precursor cells have been identified in the circulation (blood-derived adherent cells or BdACs) that can produce bone *in vivo*.

To test the hypothesis that BdACs are more abundant in patients who are predisposed to heterotopic bone formation, we used peripheral blood samples from FOP patients and from unaffected individuals to determine relative circulating levels of these bone-forming precursor cells. BdACs were identified as a subpopulation of cells similar to circulating fibrocytes, cells that were initially described in the context of wound repair but subsequently found to participate in a variety of disorders related to exuberant scar tissue formation. FOP patients who had ongoing or recent flare-ups had significantly higher numbers of BdACs compared to either patients with stable disease or unaffected individuals. We further demonstrated by mineralization and bone-formation assays that BdACs from patients with FOP are capable of forming bone *in vivo*.

Using several different techniques, we have determined that BdACs are derived from bone marrow and migrate to sites of inflammation and tissue injury with resultant ectopic bone formation. The greater abundance and osteogenic potential of BdACs from FOP patients may portend a possible pathophysiologic role for these cells as osteoprogenitor

cells. These findings were reported in a major presentation at the annual meeting of The American Society for Bone and Mineral Research in Seattle, Washington in October 2004.

Continued Need For FOP Lesional Tissue

It is essential to reiterate that many of the important experiments, findings, and discoveries from the FOP laboratory in the past decade would have been impossible without the all-important FOP biopsy samples that you have so graciously provided to us. While many of the experiments described above used either blood samples or sophisticated animal models of heterotopic ossification, they were all based upon primary findings from FOP lesional biopsy specimens. While we realize that these biopsy samples were obtained prior to the definitive diagnosis of FOP, and should never be obtained prospectively, these biopsy specimens provide us with extraordinary insight into FOP that could not otherwise be reproduced by any other means currently available. To those of you in the FOP community who read this report and are reminded of biopsies that you may have had performed and whose samples we do not yet have in the FOP laboratory, we would ask you to please contact us so that we could help you obtain those samples for further investigation. Just as the multi-generational families are so important for the process of gene identification, and the blood samples are so important for the BMP pathway and stem cell studies, the FOP lesional biopsies are invaluable for determining the features of FOP at a cellular and tissue level.

If you contact us (email: Kamlesh.Rai@uphs.upenn.edu) we will provide you with the appropriate forms that will authorize the hospital or clinic (where a biopsy was performed), to release the specimens for review and study.

IV. MODELS

A New BMP4 Transgenic Animal Model for FOP

Genetic modification of mice is a powerful tool for the study of bone development and is beginning to contribute substantially to our understanding of the regulation of skeletal formation. The development of relevant animal models for FOP will be a major advance in the development of effective treatments.

One of the most important discoveries in FOP research in 2004 was the serendipitous finding that mice that are genetically programmed to overexpress and secrete BMP4 from nerve cells develop an FOP-like condition of progressive bone formation as they age. However, unlike the pattern of progressive bone formation in FOP which appears first in the trunk and moves out towards the limbs, the opposite pattern occurs in the BMP4 transgenic mice where heterotopic ossification first appears in the limbs and then moves centrally towards the trunk.

Studies from fruit flies to mice have shown that BMPs play an important role not only in the formation and repair of the skeleton (both the exoskeleton of the fly and the internal skeleton of vertebrates), but also in the development of other organ systems including the brain. Ironically, it is the exclusion of the BMPs from a specific region on the head and back of the embryo (orchestrated by BMP antagonist proteins such as Noggin, Gremlin, Chordin, Folistatin, DAN, Cerberus, and others) that is primarily responsible for the development of the central nervous system. Later in development, the BMPs re-enter the picture as important promoters of growth and development of various supporting cells in the nervous system. So, it is not at all strange that developmental neurobiologists might be interested in studying the role of the BMPs in nervous system development. One such prominent group, led by Dr. Jack Kessler, Professor of Neurosciences at Northwestern

University in Chicago was interested, in fact, in the specific role of BMP4 in the development of the nervous system.

The complete lack of BMP4 is a well-known lethal mutation early in embryogenesis. Animals, like mice and humans, simply cannot develop without any BMP4 protein. The effect of excessive BMP4 on the maturation of the nervous system, however, was not known and Dr. Kessler's group made a transgenic mouse that was genetically engineered to overexpress and secrete BMP4 primarily from mature nerve cells in order to examine that question. The initial results were interesting. On examining microscopic sections of the animal's brains, Dr. Kessler's group noted some subtle changes in various populations of glial cells that support the growth and development of the nerve cells. The animals, however, did not manifest any overt changes in behavior. The authors published their findings in the journal **Development** and were essentially finished with their work. That's when the story really became interesting!

A young postdoctoral fellow by the name of Lixin Kan joined the Kessler Laboratory. One day, while he was visiting the laboratory's animal colony, he noted that something appeared unusual with the aging BMP4 transgenic mice that were no longer being used for neurological studies. He noted that they had developed hard swellings of their hindlimbs and abdomen. He also noted that the mice seemed unusually stiff. He brought these findings to the attention of other members of the laboratory who were less excited about these "tumors" as they were not associated with any obvious neurological findings. Furthermore, the veterinarians in charge of the animal colony strongly suggested that the mice be sacrificed as they had "enlarging tumors." However, Dr. Kan decided to pursue the analysis further and obtained x-ray pictures of the animals. The x-rays suggested that the "tumors" were not cancer, but rather heterotopic bone! Microscopic studies confirmed that the lesions were indeed heterotopic bone and were forming by an

endochondral process, nearly identical to the stages of FOP lesion formation and to the stages of the previously reported BMP4 implant-induced heterotopic ossification.

The most interesting finding, however, was that the heterotopic ossification was progressive, and different from the pattern of progression observed in FOP patients. It was also noted that the animals did not develop heterotopic ossification in the tongue, diaphragm, or extraocular muscles, muscles that were also spared in FOP.

Interestingly, most of the lesions seemed to be quite superficial and only secondarily involved the deep muscular structures. The rate of progression varied dramatically between animals even of similar genetic background, suggesting an environmental or immunologic component or both might be responsible for the differences. In all cases, early lesions were accompanied by massive swelling and soft tissue edema, very similar to that seen in early FOP lesions. In all cases where the heterotopic ossification progressed into the skeletal muscle, evanescent infiltration of lymphocytes was noted, much like in FOP lesions. When the BMP4 transgenic animals were mated with transgenic animals that overexpressed the BMP antagonist Noggin, the heterotopic ossification was prevented, indicating the specific role of BMP4 in the pathogenesis of the FOP-like disease in these animals.

While the BMP4 transgenic animals do not have a naturally occurring mutation, they are genetically engineered to overproduce and secrete BMP4 from nerve cells. Thus, the nervous system can act as a vehicle for delivering BMP4 into the local tissues. That bone formed under these circumstances was not a great surprise, but that it progressed in a specific anatomic pattern was indeed a great surprise to all. Also, the pattern of progressive heterotopic ossification in the transgenic mice was quite different from the

pattern seen in patients with FOP, a finding that raises many questions about the dynamics of BMP delivery, the nature of the receptive cells in the local tissues, and the developmental differences between mice and humans.

It is particularly instructive to note that the BMP4 transgenic animals do not have any obvious abnormalities in their normal skeleton. Specifically, the toes are normal. We suspect that the reason for this is that the cartilage model of the normal skeleton is completely formed before the extra production of BMP4 is switched on in the transgenic mice. Also, the pattern of post-natal heterotopic ossifications in the BMP4 transgenic mice is almost certainly showing us the field of receptive progenitor or adult stem cells that reside in the body's tissues and that respond to chronically elevated levels of BMP4.

Robust collaborations have been established between the neurobiology research group at Northwestern and the FOP Research Group at The University of Pennsylvania.

Collaborative research will begin by exploring the source of receptive cells contributing to bone formation in the transgenic mice, the sensitivity of heterotopic ossification to intramuscular immunizations, the variability in the rates of disease progression among animals, and the testing of existing drugs which might have a beneficial impact on the progression of heterotopic ossification. The potential for additional research using this animal model is enormous and the serendipitous discovery of the model is extremely exciting for everyone in the FOP community.

This new collaborative research effort is being supported by The Developmental Grants Program of the Center for Research in FOP and Related Disorders.

V. TRIGGERS

Autoimmune Features of FOP & The Dysregulated BMP4 Pathway

While the causative mutation of FOP remains unknown, an overactive and dysregulated BMP4 signaling pathway has been implicated in the pathophysiology of the disease and provides a rational basis for understanding both the postnatal heterotopic ossification and the congenital skeletal malformations that are signatures of the disease.

Despite satisfactory concordance of these developmental and postnatal features of FOP with abnormalities in the BMP4 signaling pathway mounting, evidence from all levels of investigation strongly implicates involvement of the immune system in the pathogenesis of the disorder. The presence of lymphocytes and mast cells in early FOP lesions, lymphocyte-associated death of skeletal muscle, flare-ups following viral infections and immunizations, the erratic timing of flare-ups and the beneficial response of early flare-ups to corticosteroids are all important pieces of evidence that implicate the immune system in the pathogenesis of FOP flare-ups. Furthermore, these clinical features strongly suggest an autoimmune component to the conditions, perhaps an autoimmune trigger. Autoimmune clinical features of FOP are not presently accounted for by hypotheses that focus solely on dysregulation of the BMP4 signaling pathway, although such possibilities certainly exist.

A central hypothesis suggests that the immunological features of FOP may be due to a disturbance in the regulation of the immune system in FOP patients, plausibly related to a mutation of a gene in the BMP4 signaling pathway. Numerous studies demonstrate a key role for the BMP4 signaling pathway in the development and regulation of the adaptive immune system, and recent studies provide strong evidence of a link between the downstream pathways that regulate BMP signaling and the pathways that regulate signaling in the innate immune system - the ancient limb of the immune system that regulates inflammation and wound repair.

Taken together, a large body of data strongly implicate the BMP signaling pathway in the development and regulation of both the innate and adaptive immune systems. We hypothesize that the immunological features of FOP are due to a disturbance in the regulation of the innate and/or adaptive immune system in FOP patients. Several scenarios could plausibly lead to the striking immunological features of FOP and are now the subject of intense investigation in the FOP laboratory.

Extensive work at this new frontier of FOP research is beginning to reveal unanticipated new insights into the pathophysiology and variability of this disabling disorder. This work is generously supported by The Born-Lotke-Zasloff Fellowship, The Ian Cali Fund, and by The Center for Research in FOP and Related Disorders.

FOP: Insights From Bone Marrow Transplantation

One of the most important discoveries in FOP research in 2004 arose from ongoing investigations on the role of the immune system in FOP. A unique insight into the cause of FOP flareups was provided by an FOP patient who received a bone marrow transplantation many years ago for an unrelated condition. While the bone marrow transplantation did not cure the FOP, observations from this unique patient support continuing studies on the role of the immune system in triggering FOP flare-ups and provide a rationale for testing immunomodulators to suppress disease activity in FOP. This approach might be too dangerous to try in FOP patients without first experimenting in an appropriate animal model. The availability now of a genetically relevant animal model (the FOPPY mice) for testing drugs that might interrupt the progression of heterotopic ossification makes such an approach feasible in the near future.

The novel and serendipitous insights gained from this unique patient will obviate the need for performing additional bone marrow transplantation experiments in mice and humans, and will allow us to bypass years of expensive, dangerous and unnecessary experimentation.

Data from this patient is currently being evaluated and a full-length report will follow.

We will keep you updated on the progress of this analysis and its very important implications for understanding the pathophysiology and treatment of FOP.

VI. TREATMENTS

The ultimate goal of FOP research is the development of treatments that will prevent, halt, and eventually reverse the progression of the condition.

The therapeutic horizon for patients with FOP is infinitely brighter than it was a decade ago. Through the efforts of a collaborative international FOP research team dedicated to these goals, fundamental advances continue to be made in understanding the molecular basis of the condition, and in understanding the detailed genetic, cellular, molecular, physiologic, and developmental changes that characterize FOP. From that horizon, better solutions will emerge.

In the meanwhile, work continues in parallel on the basic science and treatment fronts to advance the therapy of FOP. The discovery this past year of a BMP4 transgenic animal that develops progressive heterotopic ossification opens the door to testing a myriad of currently available medications to dampen the cellular and tissue response to a chronically active BMP signaling cascade.

The Medical Management of FOP: Current Treatment Considerations

The third edition of **The Medical Management Of FOP: Current Treatment Considerations**, (also known as **The FOP Treatment Guidelines**) was completed in December 2004, and posted on the IFOPA website in January 2005.

The document has been updated to specifically address relevant new data on the non-steroidal anti-inflammatory medications, the cox-2 inhibitors, the aminobisphosphonates,

and the influenza vaccine. Due to the generous help of friends and translators, the document is being translated into seven languages. We urge all of you who have not yet seen this document, to read it, and download it from the IFOPA website (www.ifopa.org). A hard copy can be obtained by contacting Dr. Kaplan's assistant, Kamlesh Rai at: kamlesh.rai@uphs.upenn.edu.

Each physician caring for a patient with FOP must constantly review evolving scientific information and chart the safest and most responsible course for the patient until definitive medications are available and their safety and efficacy is verified. The optimal treatment for FOP will be based upon integrated knowledge of the cellular and molecular pathophysiology of the condition and will change as new discoveries emerge.

Four Advances In Research On FOP Treatments In 2004

There have been four major advances in research on FOP treatments in 2004. They are:

1. The discovery of a genetically-based animal model of progressive heterotopic ossification (FOPPY mice) that will enable us to test currently available and experimental medications.
2. Research to identify signal transduction inhibitors (STIs) that interfere with molecular targets in the BMP4 signaling pathway.
3. Development of inducible promoters for pre-clinical testing of regulated noggin gene therapy.
4. Discovery of the role of the immune system as a very sensitive but non-specific trigger for FOP flare-ups. This finding will stimulate pre-clinical testing of a new class of drugs called immunomodulators in the prevention of FOP flare-ups.

The FOPPY Mouse: A New Genetically-Based Animal Model for Drug Testing

The need to carefully test new drugs in animal models before embarking on clinical trials in patients has been recognized for nearly one hundred years. Nevertheless, there are situations where animal tests fail to accurately predict whether a drug will work on people and the only accurate testing can be done in patients and normal controls. Many drugs that have powerful effects in an animal model are useless or dangerous in humans and many drugs that have no effects whatsoever in an animal model have powerful therapeutic effects in human diseases. Nevertheless, it is important, when possible for safety and ethical reasons, to test major new categories of drugs in animal models before testing them in patients.

One of the most important advances in FOP research in 2004 was the discovery of a transgenic animal model, the FOPPY mouse, for testing currently available and experimental medications. Even though FOPPY mice do not have FOP and the pattern of disease progression is different than that seen in real FOP, the development of progressive heterotopic ossification in any pattern provides an enormous improvement on currently existing animal models. As such, the FOPPY mice will enable us to test currently existing drugs such as prednisone, anti-inflammatory medications, mast cell inhibitors, cox-2 inhibitors, aminobisphosphonates, signal transduction inhibitors, anti-angiogenic agents, and immune modulators. FOPPY mouse colonies will be expanded at Northwestern University and at The University of Pennsylvania in order to perform such studies.

Signal Transduction Inhibitors

Signal transduction inhibitors (STIs) are drugs that inhibit signal transduction pathways. This new class of medications received much attention several years ago in a popular television show, *The West Wing* when President Bartlett considered a campaign to

eliminate cancer based on new evidence that some signal transduction inhibitors can cure some forms of cancer.

Among all of the STIs, the one that has received the most attention is Imatinib (Gleevec), a small molecule drug that inhibits a specific class of transmembrane receptors called protein tyrosine kinases. Imatinib can be administered orally with few side-effects and has become the standard of care for patients who have chronic myelogenous leukemia. While Imatinib may not be applicable for treating FOP, other drugs that inhibit the serine-threonine kinase pathway (the pathway involved in BMP4 signal transduction) may be extremely relevant for FOP. For example, the BMP antagonist Noggin is a signal transduction inhibitor as it affects the extracellular portion of the signal transduction pathway by blocking the ability of BMP to bind to its receptor. Several of the discoveries during this past year on the BMP4 signal transduction pathway in FOP cells (the runaway car) have identified new drug targets in the BMP4 signaling pathway that might eventually prove to be even better than Noggin.

Just as a relevant animal model is necessary for testing the *in vivo* effects of currently existing and newly developed drugs, a high-throughput drug testing system at the cellular level is necessary to screen compounds that might be developed as drugs. Much of our work during the past several years on the study of BMP signal transduction pathways is based on the identification of such tangible targets. New *in vitro* test systems will be based upon clear and dramatic differences in the molecular phenotype between FOP cells (the runaway car) and control cells (measured for example by the “molecular speedometer” of the ID genes, discussed previously in **Section III** of this report). We will continue to devote much attention to identifying appropriate therapeutic targets in the BMP4 signal transduction pathway and to identifying signal transduction inhibitors to block those targets.

Gene Therapy: Development of Safer Viral Vectors

In 2003, we described the publication of a milestone “proof-of-concept” paper using noggin gene therapy in an animal model relevant to FOP. This study demonstrated that the delivery of a modified Noggin protein through a systemic gene therapy approach successfully prevented BMP4-induced heterotopic ossification in a mouse model.

This major breakthrough proved the concept that the noggin gene can be modified to permit systemic delivery of the biologically engineered protein, that the gene can be inserted into a viral delivery vector, that the viral vector carrying the modified noggin gene can be introduced into the bloodstream, that it can travel to the liver where it is introduced into liver cells, that the liver can act as a factory for producing an active form of the modified Noggin protein, that the modified Noggin protein can circulate systemically as a hormone, and that at therapeutic concentrations, the modified Noggin protein can effectively bind and inactivate ambient levels of recombinant human BMP4 and completely block the formation of even the most rudimentary FOP-like lesion.

While the possibility of using a form of noggin gene therapy to treat patients with FOP remains promising, there are numerous challenges - some specific to FOP and some general to the field of gene therapy – that need to be solved before gene therapy can become a reality. The major challenge of gene therapy can be summarized in three words – delivery, delivery, delivery! The development and use of safer viral vectors for noggin gene delivery will be critical in considering whether the noggin gene or a modified noggin gene can be administered via a viral-based delivery system for human use.

Our colleagues in The Division of Human Genetics at Penn have isolated new adeno-associated viruses and are developing them as vectors for human gene therapy. In general, adeno-associated viruses are less toxic to the body than adenoviruses (but still

have some toxicity) and elicit a less robust reaction from the immune system. The hope was that these novel adeno-associated viruses could be developed as vectors for human gene therapy, that they would have improved efficiencies of gene transfer, and that they would not be recognized by antibodies generated to adeno-associated virus infections in humans. During the past two years, we have successfully used these new delivery vectors in our modified noggin gene therapy experiments in animal studies.

Toxicity studies in the mice using the novel adeno-associated virus (AAV) vector delivery system indicate that although the adeno-associated virus vector induces less inflammation in the liver than the adenovirus, the Noggin protein and modified Noggin protein themselves may be toxic at high-sustained dosages. These findings are compatible with studies showing that BMPs are important in the development and maintenance of the liver, the blood, the ear, and the eye. Thus, if Noggin is to be considered for human use, it must be precisely regulated and it must be kept at the lowest effective levels possible for the briefest periods of time to inhibit heterotopic ossification. As a result, it is essential to develop regulated and inducible systems for the production of Noggin and then target the gene therapy to cells that are either direct targets of BMP action or to cells in the vicinity of the BMP targets.

Gene Therapy: Inducible & Regulated Production of Noggin

In last year's Annual Report, we noted that we were working to apply inducible gene promoters to regulate the timing and dose of noggin gene therapy. We are thrilled to

announce that in late 2004, we have these highly-sophisticated inducible promoters to regulate the noggin gene.

Since Noggin is an antagonist to multiple BMPs, and BMPs subserve important roles outside of the skeletal system, too much Noggin may impair the function of other organs. In essence, while too much BMP is bad for the body, too much Noggin is also bad for the body. The body does not tolerate excessive amounts of anything, and requires its critical pathway-regulating molecules to be exquisitely balanced. The challenge, therefore, will be to understand the exact cause of the imbalance in BMP trafficking and signaling in FOP and to restore it in the most safe and effective manner without delivering any molecule in greater concentration than the body needs, wants, or can tolerate. Only with meticulous studies using an inducible promoter of noggin (such as that developed in 2004), in conjunction with the serendipitous discovery the FOPPY mice, can we move forward to determine the safest and most effective regimens for blocking FOP-like lesion formation.

It is not yet possible to determine when or even if noggin gene therapy will become a practical clinical reality for children and adults who have FOP, but it is presently our best hope and we will continue to pursue it relentlessly until it either becomes a reality or until better solutions emerge.

Cox-2 Inhibitors & Non-Steroidal Anti-inflammatory Drugs

The recently emerging problems with the cox-2 inhibitors (drugs like rofecoxib, celecoxib, and valdecoxib) highlight just how difficult it is to effectively develop and

safely use medications that specifically target disease-causing molecules and leave all healthy molecules completely undisturbed. While the benefits of the cox-2 inhibitors likely outweigh the risks in young FOP patients, the concern about their cardiovascular side-effects prompts a cautious approach, especially in older individuals. The new and emerging data on the cox-2 inhibitors, their problems and their promises - are clearly noted and thoroughly discussed in the updated **FOP Treatment Guidelines** that is now available on the IFOPA website: www.ifopa.org. We will keep you updated throughout the year as new data emerge on these important classes of drugs for FOP therapy.

Immune Modulators

The discovery of the immune system as a powerful trigger for FOP flare-ups provides the opportunity to test a whole new array of immunomodulators in the prevention of FOP flare-ups. However, there are not enough FOP patients in the world to try all of the combinations of medications that we might want to test. The FOPPY mouse will allow us to test the effectiveness of various combinations of immune modulators to inhibit progressive heterotopic ossification in a much safer way than we could test them in patients. Such testing will begin as soon as the animal colonies have been expanded, and as soon as the natural variability of disease progression in the FOPPY mice is better understood. Knowledge gained from the animal testing will then enable us to better assess which medications and/or combinations of medications might be used to prevent and slow down the progression of FOP in patients. As Jeri Licht, the mother of Daniel Licht, stated so eloquently and passionately in the **BBC** documentary, *Skeleton Key*, “They need to slow down the progression of this condition and slow down or stop the formation of the bone once the flare-up starts. Then they’ll have the time, and we’ll have the luxury to have them look for a cure for the condition completely.”

VII. PRESENTATIONS, MEETINGS, REPORTS, AND PUBLICATIONS

During 2004, we were privileged to present major lectures on FOP at:

- Alfred I. Dupont Hospital for Children; Wilmington, Delaware
- American Society for Bone & Mineral Research; Seattle, Washington
- Johnson & Johnson Research Foundation; New Brunswick, New Jersey
- Michigan State University; East Lansing, Michigan
- Orthopaedic Research Society; San Francisco, California
- University of California-Irvine; Irvine, California
- University of Maine; Orono, Maine
- University of Pittsburgh; Pittsburgh, Pennsylvania

During 2004, we were honored to present highlights of FOP research at regional, national, and international FOP family meetings and gatherings in:

- Aberdeen, Scotland
- Iowa City, Iowa
- Los Angeles, California
- Orlando, Florida
- Santa Maria, California
- Sausalito, California
- Solbacka, Sweden
- Valbert, Germany
- West Orange, New Jersey

Six major presentations on FOP and POH were made by members of the FOP Laboratory at The American Society for Bone and Mineral Research (ASBMR) in Seattle, Washington in October 2004. Several of these papers were presented at major Plenary

Sessions of the meeting which were well-attended by physicians and scientists worldwide.

Papers In Press

In 2004, there were five major peer-reviewed publications on FOP and POH. Several papers are currently in press, and many others in preparation. Recently, the directors of the FOP Center were invited to edit an issue of **Clinical Reviews In Endocrinology And Metabolism** devoted entirely to FOP. There will be numerous articles on all clinical and basic science topics related to FOP. The articles are due at the publisher by July, 2005, and the special issue is scheduled for publication in December, 2005.

VIII. YOUR FOP LABORATORY

While the core FOP laboratory occupies approximately 2000 square feet of space, our virtual space has expanded during the past five years with the establishment of the intramural and extramural components of The 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 are able to expand collaborations with colleagues in many departments and several schools throughout The University of Pennsylvania, and now elsewhere.

The pictures of the FOP children adorn the hallways of our core 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 Laboratory.” We love when you come and visit.

During 2004, 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, four undergraduate college students and a high school student volunteered to work on FOP projects in the laboratory during the summer of 2004.

We are delighted to have Michael O’Connell, a graduate student from The University of Southampton (England, U.K.), visiting us in the FOP Laboratory this year. Michael is studying the role of cell surface heparan sulfate proteoglycans in modulating BMP4 signaling in FOP and control cells. You will be hearing more about this exciting work as conclusions emerge. Michael will be with us throughout the summer of 2005, and then will return to Southampton to finish his Ph.D. thesis under the direction of his mentor and our collaborator, Professor Trudy Roach.

Working Groups

The core FOP Research Laboratory is organized into working groups in a similar manner to the organization of our Annual Report. There are working groups on FOP genetics, signaling pathways, cell differentiation, immunology (triggers), translational (models and treatments), and POH. Our working groups and laboratory meetings reflect the division and integration of these various areas of scientific research.

Center for Research In FOP and Related Disorders

The Center for Research in FOP and Related Disorders was established in the late 1990s by the Cali Family Endowment with the goal of expanding multidisciplinary research on FOP to other laboratories in the University, and most recently to collaborative research groups at other universities.

In 2005, the Developmental Grants Program will fund its first extramural collaborative research grant at the laboratory of Dr. Lixin Kan at Northwestern University in Chicago, Illinois. Collaborative projects involving the lineage of receptive cells for heterotopic ossification and the testing of currently available and experimental therapies for FOP in the new BMP4 transgenic animal model will be conducted collaboratively between Northwestern University and 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. Several of these projects have been highlighted in previous sections of the report (or in previous reports), and have already produced important results and insights for FOP research. Work sponsored by the Developmental Grants Program over the past several years has included studies to identify target cells in BMP-induced heterotopic ossification, studies to characterize the role of angiogenesis during skeletogenesis, work to develop safer and more effective adeno-associated viral vectors for use in the noggin gene therapy experiments, work to develop an inducible promoter for regulated delivery of noggin gene therapy, studies to define the role of the hedgehog family of morphogens and the fibroblast growth factors in BMP4 signaling, studies to define the role of glucocorticoids in modulating the BMP antagonist response to ambient BMP4 levels, efforts to use microarray gene expression studies to determine the response of chondrocytes to fluoroquinolone-stimulated apoptotic signals in pre-osseous cartilage

cells, a unique project to evaluate a patient with FOP who received a bone marrow transplantation for an unrelated condition many years ago, and novel studies to understand the role of blood-derived adherent cells in the formation of heterotopic ossification.

IX. ACKNOWLEDGEMENTS

FOP continues to be one of the most obstacle-ridden and perplexing quandries of the human condition. Despite its rarity, we truly believe that FOP research is the key to understanding not only FOP, but also many other more common conditions that affect the formation of bone and the formation of the skeleton. It is fundamentally the skeleton key.

As we have mentioned many times before, *cause* and *cure* are the two words that motivate us and provide the guiding principle for all that we do: to discover the exact genetic and molecular cause of FOP and to use that knowledge to develop effective treatments and some day a cure. The FOP Center and core laboratory continue to be unique resource for both patients and the medical community world-wide. We strive for excellence and leadership in all areas vital to our mission: patient care, education, and the generation of new knowledge.

In summary, 2004 was a year of major developments for FOP research and was highlighted by the discovery of a new transgenic animal model for FOP research that will enable us to test currently available and experimental drugs, by the clarification of the role of the immune system in triggering FOP flare-ups, by the discovery of a new multigenerational FOP family that has enabled us to narrow the chromosomal region where the FOP gene is located, by continued seminal discoveries on the dysregulation of

BMP receptor signaling and trafficking in FOP cells, by new and important insights from the powerful FOP microarray experiments, by a more comprehensive understanding of the role of blood-derived adherent cells in heterotopic ossification, by the identification of potential therapeutic targets in the BMP signal transduction pathway, by the development of safer adeno-associated viral delivery systems for noggin gene therapy in FOP, and by the very exciting and long-awaited development of an inducible gene promoter for regulated noggin gene therapy in FOP. As before, we have much work yet to do and a summit yet to reach. We are hopeful that 2005 will be a year of even greater 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 14 years but it is amazing how far we have come. We continue to be, in fact, a robust and vibrant community that spans the globe. We are united in our effort and we possess a momentum and verve to accomplish the goals we have set for ourselves. We are reminded each day that we have a long way yet to go 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 is retiring as Chairman of the Board, but will remain as President, spokesperson and moral leader of our FOP community. Her visionary devotion of the support of FOP research 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 links *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. Without their unselfish help and support, this would not be possible. Together, we will accomplish the goal of establishing the cause and finding a cure for this disabling condition and we will prevail. As always, finding an effective treatment and cure for FOP is not a job, it is a mission.

All of us in the core FOP Laboratory, 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 Fellowship in FOP Molecular Genetics
- The Roemex Fellowship in FOP Molecular Pathophysiology
- The Grampian Fellowship in FOP Molecular Pathophysiology

- The Progressive Osseous Heteroplasia Association
- The Hartford Foundation
- The Medical Research Council and Oxford University (United Kingdom)
- The Association Française Contre Les Myopathies (France)
- Members of the FOP International Research Consortium
- Johnson & Johnson, Inc.
- Regeneron Pharmaceuticals
- The People of Santa Maria (12 years of extraordinary service)
- And the many individuals, families, and friends 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 effort.