

## THE TWELFTH ANNUAL REPORT

# FIBRODYSPLASIA OSSIFICANS PROGRESSIVA COLLABORATIVE RESEARCH PROJECT

Presented by

Frederick S. Kaplan, M.D., David L. Glaser, M.D., Eileen M. Shore, PH.D.

**T**he mystery and misery of FOP are inextricably linked with the mission to solve and cure it. The goal of FOP research is to discover the molecular and genetic cause of FOP, and to use that knowledge to design effective preventions, treatments, and eventually a cure. We continue to make great progress in achieving those goals.

*Important advances in FOP research during 2002 included:*

Jack Sholund, of Big Fork, Minnesota, drew this antique microscope for the 2003 FOP T-shirts. Although a precursor to the technical marvels used in our FOP lab, it is symbolic of the distance FOP research has come.



© Sholund 2003

1. The discovery of abnormal BMP receptor biology in FOP cells.
2. The use of microarray technology to explore the simultaneous expression profile of thousands of genes in FOP cells vs. control cells.
3. The use of molecular cell lineage tracing experiments to determine the origins of some of the cells that participate in heterotopic ossification.
4. The development of new model systems of heterotopic ossification.
5. The recognition that the anti-angiogenic effects of cox-2 inhibitors and the newer aminobisphosphonates may provide a rationale for their use in FOP clinical trials.
6. The refinement of delivery vectors for noggin gene therapy in preclinical animal testing.

**I**n this year's Annual Report, we will discuss the progress in the war against FOP. We will describe discoveries in the laboratory and in the clinic as well as broad outlines of our research plans for this coming year. In addition, we will provide background where necessary to help elucidate how this work fits into the broader picture of developing effective preventions, treatments, and eventually a cure.

While the mission of FOP research is clear, the research discoveries are not ends in themselves, but mileposts along a journey that will ultimately end with a cure for FOP. Much of the work that we reported to you last April in the Eleventh Annual Report continues to

evolve.

Many of these projects are technically complex, and will take several years to complete. In some projects, valuable information emerges regularly and can be transmitted to you incrementally as it is verified and reviewed by our peers. In other projects, useful information emerges unexpectedly and serendipitously, and we are always excited about those clarifying insights as anyone involved in medical research can attest.

The process of scientific inquiry is a universally accepted standard and among the most structured of all human endeavors. Yet, the scientific discoveries that arise from these highly structured projects are often surprising and unexpected. Scientific discoveries rarely, if ever, move at a predictable pace. At times they creep forward, and at

times they leap forward with a verve that leaves everyone speechless. There is rarely, if ever, any advanced sign 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. Such was the case this past year when we discovered that a particular type of cell from skeletal muscle was caught in the act of becoming a bone cell; but more on that later.

Last year, we featured the proof-of-concept that a genetically engineered form of the noggin protein can, through a gene therapy approach, successfully prevent bone morphogenetic protein 4 (BMP4) - induced heterotopic ossification in a mouse model. That work con-

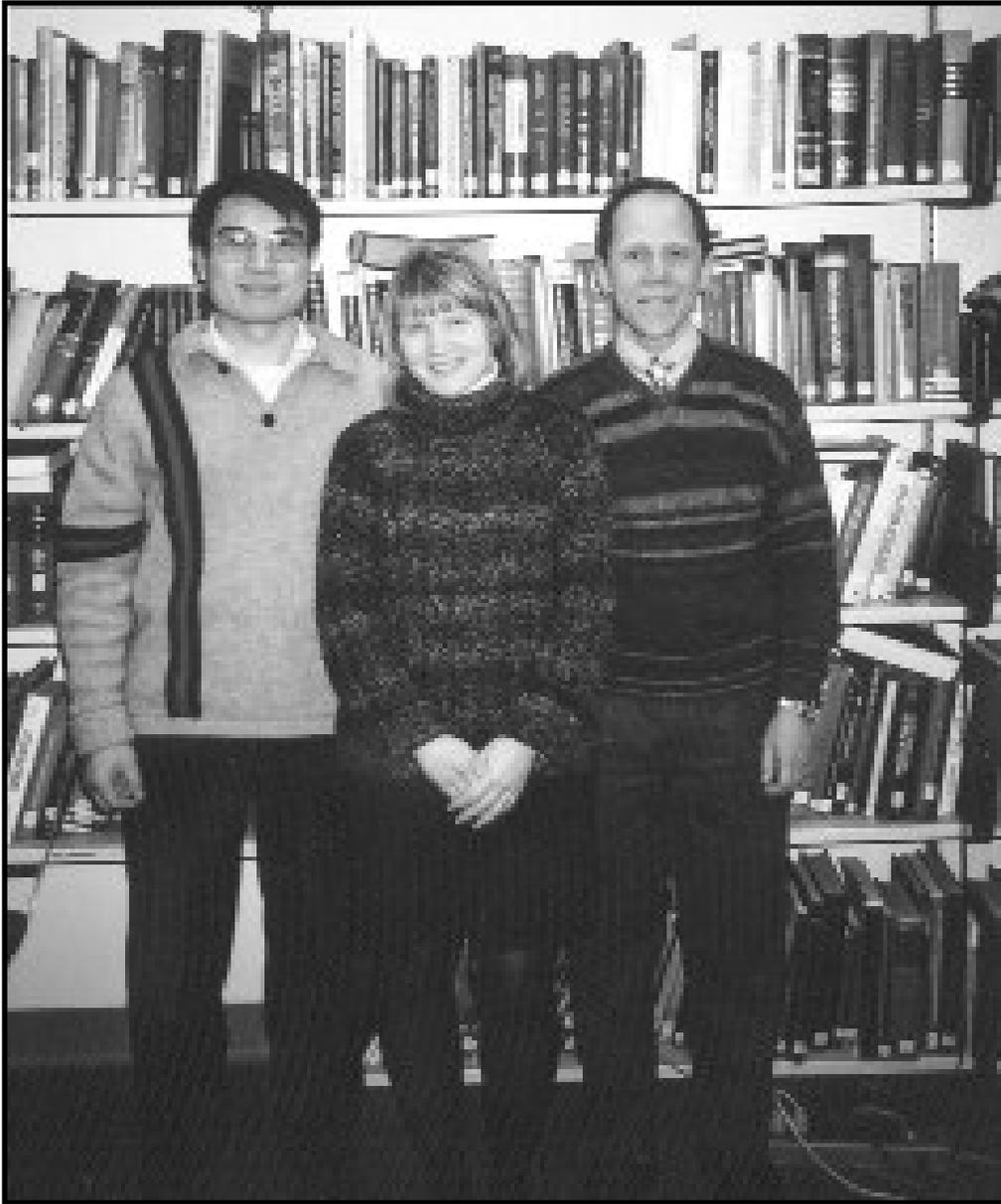
tinues to move forward and such approaches to inhibiting the overactive BMP4 pathway in FOP cells provide great hope for the future. A detailed scientific paper describing this work has been submitted for peer-review and publication.

This year's featured breakthrough is in the field of molecular signaling and is highlighted because it will help guide us to the point of engineering the most effective therapy for the treatment of FOP. Eventual treatments for FOP will be only as good as the basic knowledge supports, and that knowledge comes from a fundamental understanding of the abnormal activities of the FOP cells, their triggers, and their respective signaling pathways.

We know that some FOP cells produce too much BMP4, and not enough of the BMP4 antagonists, noggin and gremlin. As we



**Front row:  
Dr. Eileen Shore,  
Dr. Frederick Kaplan.  
Back row: Dr. David  
Glaser.**



Molecular Pathway Signaling Expert, Dr. Chen, from Memorial Sloan-Kettering Hospital, New York, visits the lab with Drs. Shore and Kaplan.

have said before, it is as if the gas pedal gets stuck at full throttle and the emergency brakes fail to engage. We have learned this past year that FOP cells also have an intrinsic inability to properly sense, monitor, and regulate the concentration of BMP4 in their environment. It is not just that FOP cells make too much BMP4 or too little of the BMP4 antagonists (such as noggin and gremlin), but that FOP cells in a very simple and pro-

found way are molecularly incapable of regulating the concentration of BMP4 in their immediate environment. It is as if the driver of the car doesn't know how far to depress the gas pedal to make the car move ahead appropriately and safely. A casual reader may ask why this is such an important discovery. The reason it is so important is that it points from two different directions to the

same region of cell signaling - to the BMP receptors and their associated molecules (essentially the driver of the car) - which sense, monitor, and regulate the levels of BMP4 in the environment of the cell.

In 2002, we confirmed that FOP cells have an over-abundance of one of the BMP4 receptor proteins on the cell surface, and that these proteins which should properly regulate the concentration of BMP4 are stuck in the on-position. We are now investigating how this occurs, and how the over-abundance and over-activity of this receptor may alter cell function to lead to bone formation. But, more too, on those exciting discoveries later.

This year's Annual Report will be divided into nine major sections that most clearly organize the vast amount of work and activity in 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

*Once again, we hope that you will find this year's annual report interesting, engaging, and hopeful. That is the attitude with which we approach this work every day.*

# I. GENES

## Multigenerational Families & FOP Gene Localization Studies

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 polymorphisms) through large multigenerational families. However, for FOP no such large families with many affected individuals have been identified anywhere in the world. During this past year, however, our work on FOP education is beginning to pay-off. In the area of multigenerational family identification, two fortuitous developments occurred in 2002, and each could prove to be a bonanza in the search for the



Microscopist and histologist Robert Caron.

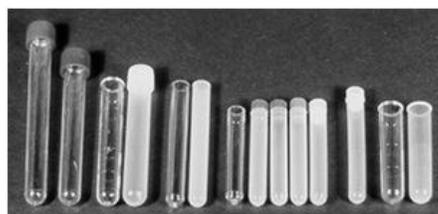
FOP gene. These developments were the unexpected discovery of two additional multigenerational families — one from the United Kingdom (thanks to the work of our valued colleagues and collaborators, Dr. Roger Smith & Dr. James Triffitt of Oxford University) and the other from the United States. Each of the families have been contacted. For one of the families, all relevant members have been seen and genetic and molecular studies are being conducted. For the other family, members will be seen very shortly and studies initiated following consent of the individuals. At this point, it is impossible to determine whether the addition of two multigenerational families will allow us to substantially narrow the genetic interval where the FOP gene is located and pursue positional cloning of the gene, but it will clearly bring us a big step closer.

Additional multigenerational families likely exist but may have had little contact with the medical community. The increasing amount of medical research on FOP and the growing international collaborative network of physicians and scientists who recognize FOP is changing that predicament. Also, we cannot underestimate the power of the Internet to disseminate new information and to attract the attention of physicians, scientists and the families themselves.

## Sequencing Candidate Genes

Last year, we reported the identification of four additional small FOP families whose DNA could be entered into the genetic mapping studies. While none of the families was a traditional multigenerational family, they

each possessed unique genetic characteristics that helped us to decipher clues about the chromosomal location of the FOP gene. The utility of these studies in further narrowing of the position of the FOP gene is important, and new genomic mapping data are being thoroughly analyzed. The analysis has suggested small regions on several chromosomes in addition to chromosome 4 that may also hold clues to FOP, and we are vigorously pursuing these leads. In addition, we continue to examine candidate genes both inside and outside of the linkage region; genes that if mutated, could plausibly cause FOP. The candidates include genes that are involved in the bone morphogenetic protein pathway, genes that control skeletal and/or bone marrow development, as well as genes involved in immune function and/or inflammation. We have, through our targeted DNA sequencing efforts, excluded many promising candidate genes, but there are still many more to evaluate. As work continues to annotate the human genome in laboratories around the world, the potential relevance of newly identified genes provides valuable information and perspective on genetic signaling networks relevant to FOP. While this targeted DNA sequencing effort for promising candidate genes is laborious, it is essential that we leave no potential FOP gene uninvestigated.



DNA sequencing effort for promising candidate genes is laborious, it is essential that we leave no potential FOP gene uninvestigated.

## Molecular Inheritance Patterns in FOP: Unusual Clues

In a promising new approach, which we began to use during this past year, we are

exploring targeted regions of the genome where the FOP gene may be located (identified through our multigenerational family studies) to look for loss of inheritance of one of the two copies of parental DNA. This type of investigation is called “a loss-of-heterozygosity (LOH) study.” In this context, “hetero” means different while “zygous” means parental origin.

For most genes, we have two copies – one copy from our mother and one copy from our father. Due to normal genetic variations between all individuals, it is possible to trace the father’s copy of the gene and the mother’s copy of the gene using specific markers associated with each copy of the gene. In some individuals who have FOP, it is possible that a small piece of DNA could be missing, and that the missing piece could contain one copy of the FOP gene. But that piece of DNA might be too small to notice microscopically and might only be identified by detailed molecular studies. Therefore, if one of the parental copies is missing, the genetic signature at a specific location along the chromosome would seem homogeneous (rather than heterogeneous) because one is able to identify only one of the two parental copies. Such homogeneous-appearing regions at the molecular level might provide a precise clue to where a small piece of DNA may be missing, and where the FOP gene might be located.

These studies were not possible for us even several years ago due to the fact that we had too many regions to search and too few molecular markers with which to search particular regions of interest. At the present time, however, such studies are possible and are being undertaken in collaboration with our esteemed colleague and collaborator, Dr. Michael Connor at The University of Glasgow in Scotland.



Yi-Chen Wu (standing next to Dr. Kaplan) and her family came all the way from Taiwan to visit the lab.

## The Mitotic Recombination Project

Last year we reported a novel approach to finding the FOP gene called *the mitotic recombination project*. The mitotic recombination project, an alternative genetic approach that completely circumvents the need to obtain DNA samples from large multigenerational families, could greatly expand our ability to identify the genetic cause of diseases like FOP for which large families are difficult to obtain. The idea behind the study is a remarkably simple and novel one that might allow us to capitalize on the rare shuffling of genetic information that occurs in our body’s cells from time to time. This novel idea proposes a method to use FOP lymphocytes, available to us in the laboratory from the blood samples you have given us, to generate “cellular families” that could be used to narrow the genetic interval where the FOP gene is located. One of the tantalizing aspects of this work, noted by one of the reviewers from the NIH is that, “the techniques to be developed in this

proposal would be applicable to a number of rare diseases for which pedigree analysis is impossible.”

While the strategy for this novel mitotic recombination project is simple and elegant, the logistics and tactics are fraught with numerous technical challenges. During the past year, we have devoted much attention to this project in an attempt to circumvent the technical obstacles. We have learned a great deal about the molecular genetics of FOP and about the behavior of FOP cells from this work, and we have a much better idea of the types of technical hurdles that must be overcome. Many of the techniques that we have been developing in the past year are applicable not only to this project, but to several other projects that we are pursuing in the FOP laboratory. This continues to be one of the most technically difficult projects in the laboratory, but regardless of its outcome, will shed a great deal of knowledge on the behavior of FOP cells.

## II. PATHWAYS

### A Disorder of Skeletal Regulation

Unlike in bone diseases such as fibrous dysplasia, osteogenesis imperfecta, or bone cancer where the bone formed is abnormal, there is nothing at all wrong with the extra bone in FOP except that it should not be there. FOP is not a disorder of skeletal structure or of skeletal composition, but of skeletal regulation.

The lessons of progressive osseous heteroplasia (POH), the sister disease to FOP, provide an important counterpoint to the next section of the report. For POH, we have discovered the causative gene, but have no knowledge yet of the pathways that the gene uses to make heterotopic (extra) bone. For FOP, we do not yet know the causative gene, but we are gaining increased knowledge about the signaling pathways used by the cells to make heterotopic bone. For both conditions, it is necessary to understand not only the causative gene, but also the regulatory pathways through which the genetic damage occurs.

Establishing an effective treatment for a genetically-based developmental disorder like FOP will result from identification of both the gene mutation and the dysregulated developmental pathway that causes FOP. For FOP, family pedigrees are scarce and genetic linkage and positional cloning are difficult. An alternative approach to identify the primary pathology in FOP involves strategies to isolate the dysregulated molecular pathway

and from there trace back to the damaged gene. During the past year, we have devoted much effort to this approach, and the results are beginning to pay-off.

In this section of the Annual Report, we will describe one of the major advances in FOP research during the past year: the discovery of abnormal BMP receptor biology in FOP cells. Before exploring this new avenue of research, it would be helpful to review some background

material, first described in last year's annual report, as this year's advances build directly upon it. We will, therefore, highlight the following three sections: "What is a morphogen?," "Autoregulatory negative feedback loops," and "Dysregulation of autoregulatory negative feedback loops in FOP cells." Following that introduction, we will describe some of our newest findings and the hypotheses that guide this seminal branch of our FOP research effort.

## What is a Morphogen?

**B**one morphogenetic protein 4 (BMP4) is a special type of protein referred to as a morphogen and is encoded by the gene that bears its name. BMP4 is highly conserved throughout evolution in the animal kingdom and has many different functions during development and following birth.

BMP4 is a morphogen, and a morphogen is a secreted signaling molecule that organizes a field of surrounding cells into patterns. In the case of the fruit fly (that has no bones), BMP4 organizes the patterns for the body plan, and for other structures such as the wing. In the case of a human being, BMP4 organizes the cellular pattern of various organs including the hair follicles, middle ear, and the skeleton itself. BMP4 forms a concentration gradient emanating from a localized group of cells, and the BMP4 protein determines the arrangement and fate of responding cells according to the concentration of BMP4 perceived by the cells in the environment. The idea of a morphogen gradient is intimately associated with the concept of positional information. A cell in the environment of a BMP4 molecule reads its position in a concentration gradient, and determines its developmental fate accordingly.

BMP4 is a morphogen, not an enzyme. Enzymes catalyze chemical reactions in the body. The more of an enzyme there is, the more of a chemical reaction there will be. If you have twice the concentration of an enzyme, you can have twice the number of chemical reactions taking place and therefore twice the product of those reactions. That is not the case for morphogens such as BMP4. Morphogens turn on different genes at different concentrations. For example, at low concentrations BMP4 turns on one set of genes, at intermediate concentrations, it turns on a different set of genes, and at a high concentrations, it turns on yet a third set of genes. This, of course, is an oversimplification, but the concept that morphogens have entirely different effects at different concentrations is an extremely important and relevant one for FOP.

How then are the gradients of a morphogen such as BMP4 established and maintained to allow the body to create exactly what it needs, exactly when it needs it, exactly where it needs it and absolutely nowhere else?

## **Autoregulatory Negative Feedback Loops**

The concept of autoregulatory negative feedback loops is central to attempts to understand the concept of morphogen gradients and the extraordinary fidelity of their control. During the past five years, numerous steps have been identified and elucidated in the molecular relay switches from secreted morphogens (such as BMP4) to downstream gene control at a molecular level. At each step in the molecular relay from the secreted morphogen to the downstream effects on target cells, nature has built-in negative feedback control switches. These negative feedback switches are a set of highly regulated brakes that sense the concentration of intermediate molecules in the pathway and work together as a molecular guidance system for the proper functioning of the pathway.

Negative feedback switches allow cells to operate with high fidelity within an extremely narrow range that never allows the morphogen gradient to waver far from its set-point in any particular tissue of interest. Negative feedback switches are implicit to the proper functioning of morphogen gradients, and their elucidation is central to the understanding of the functioning of those gradients.

The presence of autoregulatory negative feedback switches in gene circuits provide enormous stability, thereby limiting the range over which the concentration of network components fluctuate. The stability of a morphogenetic pathway served by autoregulation is far superior to that of an unregulated pathway. Autoregulatory negative feedback switches have been identified at every single step in the BMP4 pathway.

## **Dysregulation of Autoregulatory Negative Feedback Loops in FOP Cells**

With this background in mind, it will be easier to understand one of the most important basic science discoveries from the laboratory in the past several years – the finding that FOP cells are unable to properly regulate the BMP4 pathway in response to a BMP4 signal. The failure of FOP cells to appropriately upregulate expression of some secreted BMP4 antagonists in response to a BMP4 signal suggests a possible loss of negative feedback by which BMP4 expression levels and thus BMP4 activity may be markedly elevated and sustainable in FOP.

Heterotopic ossification in the setting of FOP begins in childhood, and can be induced by surgical trauma, soft tissue injury, intramuscular immunizations, or injections for dental procedures. BMP4 is produced by skeletal muscle and its expression can be upregulated at sites of soft tissue injury. Under normal circumstances, BMP4 dramatically stimulates the expression of at least several BMP antagonists. A blunted BMP4 antagonist response following soft tissue trauma would permit the rapid expansion of a BMP4 signal conducive to progressive bone formation. The growth of highly vascular preosseous fibroproliferative tissue seen locally in response to BMP4 overexpression would be magnified in the setting of a blunted BMP4 antagonist response, and could explain the explosive bone induction seen during an FOP flare-up. These findings from FOP illustrate the importance of a critical balance between an inductive morphogen (BMP4), and its secreted antagonists in the formation of an ectopic organ system and suggest the potential

for developing BMP antagonist based strategies for the treatment of FOP.

In addition, FOP cells have an intrinsic defect in the ability to regulate BMP4 levels across a wide range of metabolic and cell cycle events *in vitro*. In normal cells, the BMP4 levels are held tightly in-check throughout all phases of the cell cycle, while in FOP cells, the concentrations seem to vary dramatically. The inability of FOP cells to properly regulate the concentration of BMP4 throughout the cell cycle may reflect a basic defect in the regulation of the BMP4 pathway. The primary action of a damaged gene in the BMP4 pathway may affect one or more components of the BMP4 pathway, like a guard that regulates several checkpoints. Alternatively, a gene defect that affects only one aspect of the BMP4 pathway may have secondary repercussions that are widespread. This suggests that genes encoding proteins that regulate BMP4, BMP4 receptors, and perhaps proteins that degrade BMP4 or its cognate receptors may be dysfunctional in FOP cells.

## **BMP4 Pathway Dysregulation in FOP**

In order to test the hypothesis that the BMP4 pathway is dysregulated in FOP, we conducted a detailed examination of various autoregulatory negative feedback switches within the BMP4 pathway and found defects at multiple points. Such defects included not only the overexpression of BMP4 messenger RNA and protein, and the inability to upregulate multiple secreted BMP4 antagonists, but also the overexpression and the activation of BMP4 receptor proteins on the cell surface. These findings have been documented in numerous FOP cell lines and provide striking evidence that the

BMP4 pathway is intrinsically dysregulated in FOP. This work is presently the focus of intense research in the FOP laboratory. The first part of this work is the subject of a major scientific paper now in press at ***The Journal of Bone & Joint Surgery (JBJS)***.

The review of the article to be published in the **JBJS** stated, "This is an extremely interesting, well-written, and important article providing further evidence for the role of bone morphogenetic proteins in the pathogenesis (cause) of FOP. While prior reports have indicated that this protein's overexpression may be essential to the cause of FOP, the possibility that an impaired regulatory control loop involving secreted protein antagonists may be involved in the cause of this disease is potentially revealing and important. The ability of the FOP laboratory to obtain sufficient samples of lymphoblastoid cells from patients afflicted with this rare disorder makes this report a unique and valuable opportunity to learn much important new information. The concept that BMP function in humans is closely regulated by

an interplay between BMP-BMP receptor binding of morphogens and their antagonists certainly raises questions regarding its potential therapeutic role in orthopaedic surgery. That perspective, in addition to the findings of this report, make for a very important contribution on the role of osteoinductive proteins in human disease and therapeutics."

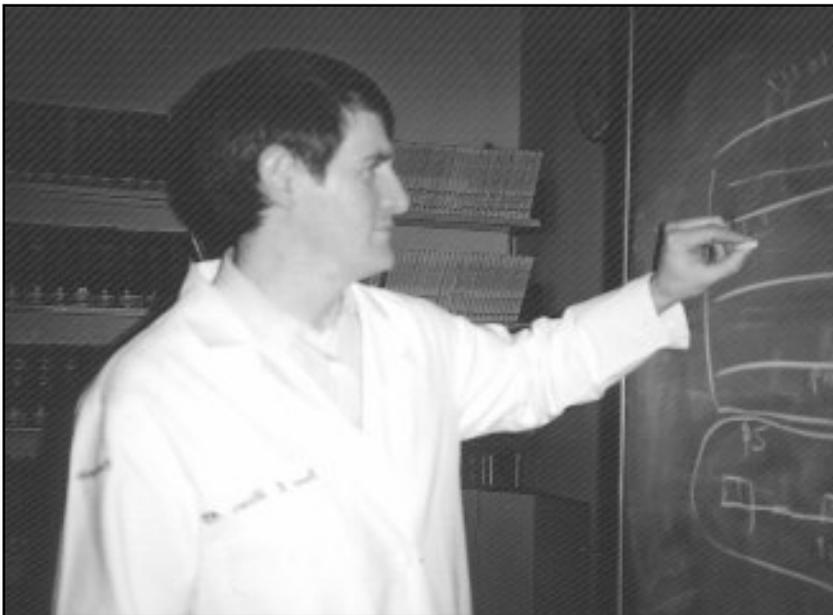
### **The BMP Receptors in FOP: One Up, One Down — The Basis of a Unified Theory**

As we have noted, the BMP4 signaling pathway is dysregulated in the cells of patients who have FOP. Recent studies suggest that FOP cells fail to properly regulate ambient concentrations of BMP4 and fail to appropriately regulate the transcription of BMP pathway target genes such as the BMP4 antagonists. Preliminary data (2002) indicate that one of the BMP receptors is present at very high levels on the surface of FOP cells while another BMP receptor is present at very low levels. These data are consistent with developmental studies showing that

postnatal overexpression of one of the BMP receptors (the one that is overabundant) can cause heterotopic ossification and that embryonic underexpression of the other BMP receptor (the one that is underexpressed in FOP cells) can cause toe malformations that are nearly identical to those seen in patients who have FOP. There are no mutations in the coding sequences of BMP4, multiple BMP4 antagonists, or the BMP receptors in FOP patients. Taken together, these data suggest that a primary defect exists in the BMP4 signaling pathway in FOP cells and that one of the BMP receptors may be constitutively active and unresponsive to normal signaling in FOP cells.

Cells derived from FOP patients provide a unique opportunity to gain insight into the role that altered BMP receptor signaling plays in induction of endochondral bone formation such as occurs ectopically in patients with FOP. This led to our current hypothesis that abnormal BMP signaling results from increased amounts of one of the BMP receptors on the cell surface and mediates the extra bone formation in FOP while the great toe malformations result from reciprocally decreased amounts of and/or activity of the other BMP receptor on the cell surface.

An analysis of the molecular pathology of BMP receptor activity on the surface of FOP cells is beginning to provide critical insight into the molecular mechanisms underlying the earliest events in the pathogenesis of FOP. A fundamental understanding of the molecular and genetic regulation of the BMP pathways in FOP cells will lead to a more rational therapeutic approach to FOP as well as to a wide variety of disorders involving the induction of bone formation in humans.



Dr. Glaser describing a genetic analysis at a lab meeting.

# Which Road To The Target?

The standard description of a cell signaling pathway goes something like this: A morphogen (such as BMP4) is made and secreted by a cell and binds to a cell membrane receptor causing activation (phosphorylation) of the receptor. This receptor activation leads to a cascade of events inside the cell that activates or represses downstream target genes that trigger events such as bone formation.

One of the major mysteries in biology concerns how cells regulate their downstream targets with such specificity and sensitivity. For the BMP pathway, there are multiple morphogens, multiple receptors, and multiple intermediate molecules. However, until recently, it was thought that there was only one major pathway through which BMPs could regulate downstream targets. During the past year, reports have emerged from several laboratories, including ours, suggesting that there are at least two (and perhaps even three) coordinate (or alternate) pathways through which a BMP signal may affect its downstream targets.

For the past decade, it has been thought that BMPs affect their downstream targets through the canonical SMAD pathway. During the past year, we have discovered that a coordinate or alternate pathway known as the p38 mitogen-activated protein kinase (MAPK) pathway also plays an important role in FOP cells. While the exact meaning of this finding remains unknown, it is quite clear that the more that can be learned about the pathways through which BMP4 signals its downstream target genes, the better able we will be to design treatment strategies that interrupt and correct any molecular imbalance in these pathways.

We are excited about our research progress in this area of molecular signaling (also known as signal transduction) in FOP cells, and are sure that there will be even more exciting developments in this area of research in the coming year. We will keep you apprised of them as they develop and are confirmed.

sis of comparative genome databases

Rapidly emerging DNA microarray technology now enables us to monitor thousands of genes simultaneously and constitutes a major technological advance that is beginning to give us unprecedented insights into gene expression in FOP.

Large-scale microarray gene expression studies, and the computer power and software necessary to analyze the overwhelming flood of data generated by such technology permits us now to mine the bounty of the human genome for the purpose of better understanding the damaged genetic pathways in FOP. Large-scale microarray analysis of gene expression patterns in FOP cells require the use of specialized core facilities and computing power outside of our FOP laboratory. Those facilities are now in place at The University of Pennsylvania and are available to us.

With the help of the newly established Weldon Family FOP Research Endowment, we have begun this exciting new approach. Within the last year, a state-of-the-art core facility for microarray analysis was established at The University of Pennsylvania and has been a tremendous asset for the design and accomplishment of this approach to study FOP. An initial set of studies using FOP cell lines and control cell lines has been undertaken, and our work has been expanded to produce two experimental data sets that can be compared for similarities and differences. We are presently analyzing our first data sets comparing FOP and control samples and we are in the process of conducting supplemental experiments.

## Large-Scale Microarray Gene Expression Studies

Comparison of the expression pattern of multiple genes in the BMP4 pathway and related pathways in FOP cells vs. non-FOP cells is an extremely important approach to deciphering the wiring diagram of FOP cells. We have said in previous reports that FOP research is much like trying to decipher the wiring diagram of an atom bomb built by a "molecular terrorist" (the mutated FOP gene). A more comprehensive analysis of gene expression patterns in FOP cells will enable us to determine the rele-

vant wiring diagram of the BMP4 pathway and to more quickly determine how to effectively deactivate the bomb.

Two revolutionary developments in technology have recently provided the tools needed to probe deeper into the mysteries of the human genome, and have opened-up new and more productive avenues of inquiry for understanding the molecular basis of genetic diseases. These developments are highly relevant to our ongoing research in FOP and include:

- large-scale microarray gene expression studies

- high-speed computer analy-

An additional experimental strategy involves the comparison of individual RNA samples from affected and unaffected members of a family with inherited FOP. Since the individuals providing the samples used for this data set are related, inherent RNA expression variation is expected to be low (as compared to unrelated members of the general population). This data set allows comparison of individual persons with FOP to each other within the same family and to individual unaffected persons in various combinations.

Microarray experiments generate large and multivariate data sets that require computer-assisted evaluations to identify significant similarities and differences among compared samples. We are currently conducting the statistical analysis of the generated data sets in collaboration

with Dr. David Rocke, Professor of Statistics at The University of California, Irvine.

Preliminary analysis of our microarray data sets has revealed several potentially interesting differences between and FOP and control cells; verification of the significance of these differences is being pursued. In addition, these studies provide the foundation for more comprehensive and exploratory analyses and will provide the basis for ongoing research studies in this extremely important area of FOP research.

### **BMP-Interacting Pathways**

For reasons outlined above, most of our attention has focused on the BMP4 signaling pathway in FOP. However, it is likely that other signal transduction pathways may be involved in the FOP process. Nearly all investigations in developmental biology relevant to the study of FOP suggest the involvement of five major inter-related signal transduction pathways. These include the BMP signaling pathway, the hedgehog (HH) signaling pathway, the WNT signaling pathway, the NOTCH signaling pathway, and the fibroblast growth factor (FGF) signal-

ing pathway. Each of the four latter signaling pathways has critically important interactions with the BMP signaling pathway, and we have been investigating these interactions as they may relate to FOP.

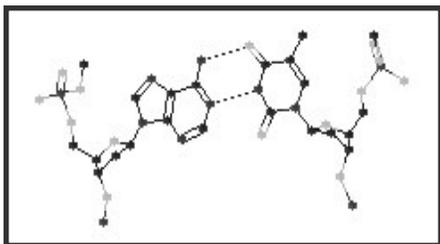
While we have, to date, found no primary pathology with the HH signaling pathway, the WNT signaling pathway, or the NOTCH signaling pathway, our discovery that basic fibroblast growth factor 2 (FGF2) is overexpressed in FOP lesions has provided a focus for several of our ongoing collaborative research efforts.

In a collaborative research project supported by the Developmental Grants Program of The Center for Research in FOP & Related Disorders, Dr. Hyun Duck Nah, Research Associate Professor in the Department of Biochemistry at the School of Dental Medicine at Penn, is investigating the interactions of FGF2 and BMP4 signaling in endochondral ossification as a potential pathogenic mechanism for FOP. Interestingly, her preliminary data shows that FGF2 significantly upregulates BMP4 gene expression in both cartilage precursor cells and differentiated cartilage cells. This has led to the hypothesis that FGF2 is an upstream transcriptional regulator of the BMP4 gene, and that integrated FGF2 and BMP4 signaling have essential roles in endochondral ossification. At the present time, Dr. Nah is investigating the potential role of integrated FGF and BMP signaling in endochondral ossification as well as FGF signaling in the transcriptional activation of the BMP4 gene in cartilage cells in an attempt to identify the region of the BMP4 promoter (regulatory DNA sequences of a gene) that confers FGF2 responsiveness.

In summary, these studies are carefully defining the interac-



**Eileen Shore, Ph.D.**



tions between FGF and BMP signaling in endochondral ossification, the type of bone formation seen in normal skeletal formation, fracture healing and FOP. These studies have provided new insights into potential mechanisms underlying the formation of early FOP lesions and have already influenced our thinking on the potential utility of anti-angiogenic agents in modulating the interactions between these pathways in controlling the growth of FOP lesions.

### **FOS & FOP: Important Clues from an FOP Patient**

Our attention recently has been redirected to work which we performed and reported several years ago, showing that embryonic overexpression of the FOS gene in the mouse leads to early postnatal heterotopic cartilage and bone formation with clinical features similar to those seen in children who have FOP. FOS is an important signaling protein that works inside the nucleus of cells and has important functions in turning-on and turning-off genes in almost every cell in the body. Curiously, however, the underexpression or overexpression of FOS seems to have particularly harsh effects on bone cells and muscle cells respectively. The overexpression of FOS during embryonic development of the mouse leads to heterotopic bone formation, at least in part through a BMP4-mediated signal transduction pathway.

Our attention was first directed to the FOS/JUN family of genes because of their involve-

ment in cartilage and bone development. FOS is present at extremely high levels in most human osteosarcomas (bone cancers) and was first recognized by its ability to induce bone tumors in rodents. In addition, increased expression of the FOS protein was noted in bone from patients with fibrous dysplasia, a condition whose name sounds a lot like FOP, but which is very different. Bones affected with fibrous dysplasia have incomplete maturation of their bone forming cells, and have a radiographic appearance of expansile “soap bubble” type lesions.

These bones are often extremely painful, and are easily fractured.

The genetic mutations that cause fibrous dysplasia are not carried or passed from one generation to another through the germ cells (sperm cells or egg cells), but arise spontaneously during early embryogenesis and affect the cells of one or more bones in the body of those who have the condition. Ironically, the gene responsible for fibrous dysplasia happens to be *GNAS1*, the same gene that causes POH, the sister disease to FOP! However, in fibrous dysplasia, the gene is overactive, whereas in POH, the gene is underactive.

To make the story even more interesting, we had previously met an older woman who had classic features of severe FOP as well as features of severe fibrous dysplasia. While an immediate connection between the two conditions is not obvious, it struck us as extremely interesting and extremely unusual that one individual would have two rare conditions that happen to affect the skeleton, and both with a connection to FOS! To summarize:

1. When FOS is overexpressed in bones, mice and people develop bone lesions that look like fibrous dysplasia.

2. When FOS is overexpressed in muscles, mice develop lesions that look like FOP.
3. Mice that overexpress FOS in their muscles, overexpress BMP4.
4. People with FOP have too much BMP4.
5. A patient has features of both fibrous dysplasia and FOP.

What does this all mean?

While we strongly doubt that the FOS gene is the cause of FOP, it is very likely that FOS is involved in the BMP4 pathway, and that overexpression of FOS may play a role in the development of FOP.

Although it is too early to determine the exact molecular significance of the occurrence of FOP and fibrous dysplasia in the same individual, it is not too early to pay attention to such potentially important clues. We have begun work in 2003 to investigate these relationships and these molecular pathways. It was, in fact, such a clue from a single patient that led us to decipher the exact genetic cause of POH. Whether this clue will eventually prove useful or will lead to a blind alley is too early to determine.

William Harvey, the physician who discovered the circulation of the blood in the late 1600s, arguably one of the most important discoveries in the history of medicine, wrote: “*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.*” In a similar comment, Sir William Osler, the famous 19<sup>th</sup> century physician said: “*Clinics are laboratories; Laboratories of the highest order.*” Very often it is a clue from the clinic that changes the ways we think in the laboratory and point us in an exciting and unanticipated new direction.

## III. CELLS

### Insights on the Identity of FOP Target Cells

An important piece of the FOP puzzle that must be solved in order to effectively prevent the complications of FOP is the identity of the target cells involved in heterotopic bone formation. It is essential to determine not only which molecules activate the BMP4 pathway in FOP, but also which cells receive and process the message that leads to the formation of heterotopic bone. Defining the 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. Knowledge of the identity of the cells that incorrectly become bone cells during the process of heterotopic ossification will be an important component in developing treatments and therapies not only for FOP, but also for a plethora of common disorders of heterotopic ossification as well as for FOP’s “sister condition”, progressive osseous heteroplasia (POH). During the past year, our studies to examine the cellular origins of heterotopic cartilage and bone formation have advanced dramatically producing definitive and surprising results.

The Developmental Grants Program of The Center for Research in FOP & Related Disorders, established by the Cali Family Research Endowment, has funded collaborative work for the past several years with the laboratory of Dr.

David Goldhamer to identify the responding cells in FOP lesions. This work, which will be explained below, represents a major breakthrough in FOP research, as it begins to identify targets for therapeutic intervention. But first, we must review some background information.

The original focus of the work was to test whether satellite cells (the adult stem cells that regenerate injured muscle) within skeletal muscle tissue are precursors of BMP4-induced heterotopic bone. The impetus to address this question was based upon two observations:

1. FOP lesions most often occur in skeletal muscle tissue.
2. Under certain cell culture conditions, muscle cells exhibit characteristics of bone cells.

Two other cell types that reside in skeletal muscle tissue - perivascular cells (smooth muscle cells that surround the small blood vessels), and endothelial cells (cells that form the inside of the blood vessel walls) — have also emerged as possible contributors to heterotopic bone formation. Both cell types are found in early FOP lesions, and both cell types exhibit bone forming characteristics in other settings. Therefore, the experimental scope of the original work has expanded to include the investigation of these additional cell types. The experiments use a sophisticated molecular cell lineage tracing approach in mice to follow the fate of satellite cells, smooth muscle cells, and endothelial cells after stimulating heterotopic bone formation with recombinant human BMP4 protein.

Until recently, cell lineage tracing experiments were not technically possible due to the inability to stably mark specific cell populations. This technical hurdle was overcome by methods

that allow permanent genetically-based marking of specific cell types using a sophisticated genetic labeling approach called the Cre-Lox recombination system. The fundamental requirement in this approach is the development of transgenic mice that express an enzyme called Cre-Recombinase in a cell specific manner (for example, in satellite cells, smooth muscle cells, or endothelial cells). Breeding Cre-Lox mice to a marked mouse strain (the Rosa-26 reporter strain) results in the stable expression of the marker gene (that causes the genetically-marked cells to have a blue color when exposed to a particularly enzyme) exclusively in the cells in which the Cre enzyme is present.

We have previously shown that the injection of BMP4 into the muscle of mice causes heterotopic bone formation that is histologically identical to that seen in FOP lesions. By injecting BMP4 into the muscles of mice whose satellite cells, perivascular cells or endothelial cells are genetically-labeled, it is now possible for us to determine both the presence and the magnitude of the contribution to heterotopic bone formation of each cell type.

The first part of the study completed last year identified two precursor cell populations that contribute to BMP4 induced heterotopic cartilage and bone formation. Results with the MyoD-Cre transgenic mice (which label satellite cells — the cells that regenerate muscle after an injury) show participation of satellite cells in the induced heterotopic ossification consistent with their previously identified osteogenic potential, although they did not seem to be the major cell type involved.

A numerically more significant contribution to heterotopic cartilage and bone was observed for the cells tagged by Tie-2 gene expression (from the Tie-2-Cre

mice). Although blood-forming cells express Tie-2, bone marrow transplantation experiments ruled-out a direct bone marrow contribution to BMP4-induced heterotopic cartilage or bone in the early lesion. The most likely source of these Tie-2 positive cells are therefore endothelial (blood vessel) cells of the local vasculature within skeletal muscle or Tie-2 stem cells resident in muscle tissue.

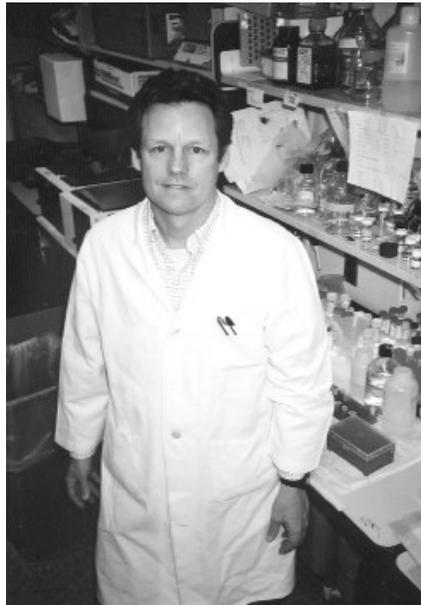
Experiments with smooth muscle myosin heavy chain-Cre mice in which the Cre recombinase enzyme is expressed exclusively in smooth muscle, showed no labeling of cartilage cells in the BMP4 implant studies.

These data exclude mature smooth muscle cells as progenitors to BMP4-induced heterotopic ossification in this model system. This result was unexpected as smooth muscle cells express osteogenic markers in cell culture and occur in abundance amongst fibroproliferative cells in FOP lesions.

These data, along with those from the FOP lesions themselves, strongly suggest that the FOP lesional cells are not likely derived from a smooth muscle cell lineage, but rather express smooth muscle cell markers along their path of differentiation into cartilage and bone. However, despite its similarities to FOP, it is important to keep in mind that the BMP4-induced model system for FOP may differ in critical ways from those of the FOP lesions, although current interpretation seems more likely.

The results of these experiments demonstrate the incredible and unexpected plasticity of stem cells derived from the muscle and local vasculature in the evolution of the FOP lesion and their ability to express various markers during their transformation towards a cartilage and bone phenotype.

Thus, an important and



Dr. Paul Billings

novel project in cell biology has produced extraordinary insight into the origin of cells in BMP-induced FOP-like lesions. Our research has identified at least two sources of progenitor cells that contribute to heterotopic bone formation in mice. A minor contribution was observed by presumed muscle satellite cells that expressed MyoD at some point in their developmental history. Future work will investigate whether these precursor cells represent quiescent or activated satellite cells or whether a resident stem cell population that expresses the MyoD-Cre transgene is responsible. A more significant contribution is provided by Tie-2 expressing cells.

Endothelial cells represent a major Tie-2 positive cell type resident in skeletal muscle. Our working hypothesis is that mature Tie-2 positive endothelial cells from skeletal muscle, in the presence of abundant BMPs, can undergo a phenotypic conversion to cartilage and bone cells. Ongoing experiments will investigate the precise identity of this Tie-2 positive precursor and the mechanism by which vascular markers are expressed during the evolution of BMP-induced

FOP-like lesions.

In summary, we have found that BMP4-induced lesions contain cellular contributions from muscle satellite cells as well as endothelial cells resident within the skeletal muscle. Taken together with the findings of the collaborative work from Catharine Shanahan of Cambridge University, FOP lesions express smooth muscle markers during the course of evolution although it is unlikely that the cartilage and bone cells in FOP lesions arise from smooth muscle cells. Furthermore, our cell lineage tracing experiments indicate that additional cell populations contribute to the fibroproliferative cells, cartilage cells and bone cells in the evolving FOP-like lesions. The origin of these cells is presently undetermined, but other precursors and stem cell populations are being actively pursued using the Cre/lox lineage tracing technology that has been so successful in our hands to date. Suffice it to say, that an extremely powerful molecular cell lineage tracing technology has been developed and refined by the FOP laboratory and its collaborators to determine the definitive origin of cells in the BMP4-induced FOP-like lesions.

The results of the cell lineage tracing study (in collaboration with The Goldhamer laboratory at The University of Pennsylvania) and the results of the FOP lesional cell study (in collaboration with The Shanahan laboratory at The University of Cambridge) strongly suggest that the early lesional cells arise from blood vessels within skeletal muscle. A detailed scientific paper describing the findings of the Shanahan study has been submitted for publication to a major peer-reviewed research journal. The results of the Goldhamer study are currently being prepared for publication.

## **The Role of the Vasculature in Endochondral Ossification: Insights from Developmental Biology**

In an important and related Developmental Grants project undertaken by the Pacifici laboratory at the University of Pennsylvania, a developmental biology approach has been used to study the role of endothelial cells (blood vessel cells) in the formation of the normal skeleton. The long-term goal of the project was to understand the role of blood vessels in initiation, progression and completion of bone formation. It is well-established that blood vessels are required for replacement of cartilage with bone during both fetal and early postnatal life. Blood vessels are also required for fracture repair as well as for ectopic bone formation. However, several lines of experimental evidence suggest that blood vessels may have additional and previously unsuspected roles in much earlier stages of skeletal formation and heterotopic ossification. To test these hypotheses, the Pacifici lab carried out two sets of studies:

In the first study, they asked whether experimental inhibition of angiogenesis (new blood vessel formation) in and around a developing skeletal element would cause inhibition of bone development. To answer this question, they made use of developing chick embryos. In the chick experimental system, skeletogenesis can be monitored and manipulated in the limb while the embryo is still in the egg, and the outcome of the manipulation and the underlying mechanisms of regulation can be studied conveniently. Squalamine, a steroid isolated from shark (and shown to be a potent inhibitor of angiogenesis in other systems) was used to block angiogenesis.

Using microsurgical procedures, control beads or beads soaked in various doses of squalamine were placed near the early chick limb bud. In the control animals, immature cartilage appeared as expected, and slowly transformed into bone through an endochondral process. In sharp contrast, the limbs of the squalamine-implanted embryos were much smaller and made entirely of cartilage. Close inspection revealed that in the squalamine-treated specimens, the cartilage cells had failed to reach a predicted developmental stage, and consequently, invasion by bone marrow and vascular precursor cells was severely deficient. However, the most striking finding was the lack of blood vessel invasion in the early skeletal element and the resultant dramatic inhibition of bone formation.

The second study focused on an earlier stage of skeletogenesis which is called mesenchymal condensation (the stage analogous to the early fibroproliferative FOP lesions before transformation into cartilage and bone). These high cell density structures form at prescribed times and sites in the chicken embryo and represent future skeletal elements, such as an arm bone, a leg bone, or a rib, for example. It has long been known that formation of mesenchymal condensations is preceded by the disappearance of blood vessels.

To test whether blood vessel removal is needed for condensation and skeletogenesis, the experiments used a gain-of-function approach. Beads containing the potent angiogenic (blood vessel promoting) factor vascular endothelial growth factor (VEGF) were placed in the vicinity of future skeletal condensations in early chick embryos. Over time, angiographic analysis showed that

VEGF, as expected, was able to induce a large increase in local vasculature. This increased vasculature was accompanied by the absence of mesenchymal condensations or cartilage formation. Thus, in the earliest stages of skeletal formation, vascular invasion paradoxically inhibits the growth of the early skeletal element.

In conclusion, the results of this project provide extremely important insights into the relationship between the developing skeleton and the developing vasculature and identifies factors that may mediate these interactions. Taken together with the findings from the Goldhamer and Shanahan laboratories, the data from the Pacifici laboratory add to the understanding and importance of the vasculature (and the control of vascular development) in the formation of FOP-like lesions. If we keep in mind the occurrence of lymphocytic infiltration around the blood vessels of skeletal muscle in the early FOP lesions, a powerful picture begins to emerge of the interaction of the immune system and the vascular system in the formation of heterotopic bone.

Powerful insights from the FOP Core Laboratory, the Goldhamer Laboratory, the Shanahan Laboratory and the Pacifici Laboratory, strongly implicate the importance of the immune system and the vasculature in general and the lymphocyte and endothelial cell specifically in the evolution of BMP4-induced heterotopic bone formation. Intense investigation will continue in this area in the future, and we will look forward to reporting results to you as they evolve.

We are also proud to announce that two students have received their Ph.D. degrees for work on these FOP-related projects. They are Raga Ramachandran (Goldhamer laboratory) and Melinda Yin (Pacifici laboratory). Raga and Melinda

are, to our knowledge, the first students ever to receive Ph.D. degrees for basic science research on FOP. Our congratulations go to both of these young scientists and their mentors for their important contributions to FOP research.

### **Continued Need For FOP Lesional Tissue**

Finally, it is essential to reiterate that none of the important experiments, findings, or discoveries noted above would have been possible without the all-important FOP biopsy samples that you have so graciously provided to us. While the experiments described above used 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, the FOP lesional biopsies are invaluable for determining the features of FOP at a cellular and tissue level.

If you contact us, 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**

### **The Importance of Animal Models for FOP**

The development of relevant animal models for FOP is a major stepping-stone in the development of effective treatments. While FOP-like conditions have been described sporadically in domestic house cats, in several pigs, and last year in a dog, no known living animals are currently available for study. It is even doubtful whether the FOP-like condition in the cat, pig, or dog is truly FOP. The achievement of a truly reliable animal model for FOP in humans will likely have to await the discovery of the gene responsible for FOP. After that discovery occurs and is verified, immediate attempts will be made to develop a truly relevant animal model based upon manipulation of the identical gene in the mouse.



Ms. Joan Gilmore is in charge of FOP animal colonies.

### **The BMP4-Matrigel System: A Useful Animal Model**

In the meanwhile, the most reliable model system for the induction of isolated FOP-like lesions is recombinant (genetically-engineered) human BMP4 protein mixed with a heterogeneous carrier substance called matrigel that is injected into a muscle of a mouse. This continues to be the most useful system for reproducing all of the known stages of FOP-like heterotopic ossification. These stages include lymphocytic and mast cell infiltration, the death of skeletal muscle cells, the formation of a highly angiogenic fibroproliferative lesion, the transformation of the fibroproliferative lesion into cartilage, the calcification of cartilage, and the eventual replacement of the calcified cartilage with mature heterotopic bone containing bone marrow elements. We have used this model to study the early inflammatory events associated with BMP-induced heterotopic ossifications including lymphocytic and mast cell infiltration as well as to test the effects of anti-angiogenic compounds such as squalamine and BMP antagonists such as noggin. The recombinant BMP4-matrigel mouse muscle implant model continues to be a useful model system to assess various treatments for FOP and will likely continue to be so until a better animal model can be developed based on the precise gene mutations(s) causing FOP. We will refer to this animal model later in the treatment section when we review the recent groundbreaking “proof-of-concept” discovery using noggin gene therapy to inhibit BMP4-induced heterotopic ossification in the mouse (first described last year). A major scientific paper containing the description of the BMP4 - matrigel system was recently submitted for peer-review and publication.

## **Lymphocytes from FOP Patients Induce Early FOP-like Lesions in Nude Mice**

During the past year, we have continued the development and validation of a lymphocyte-derived model cell culture system relevant to the early molecular pathology and histopathology of FOP. The validity of this lymphocyte-cell system is based on a series of observations and experimental findings in FOP lymphocytes as well as in BMP4 signal transduction pathways in relevant cells:

1. Perivascular accumulation of B-lymphocytes and T-lymphocytes (with subsequent infiltration and death of skeletal muscle) are the earliest histopathological findings in FOP.
2. BMP4 signaling regulates early lymphocyte differentiation.
3. BMP4 is overexpressed in lesional lymphocytes in FOP patients.
4. Routine immunizations (iatrogenic activation of the immune system) lead to heterotopic ossification of skeletal muscle at the injection site in FOP patients but not in normal controls.
5. Circulating lymphocytes in FOP patients exhibit dysregulation of BMP signaling.

These data suggest that the lymphocytes are an informative model cell relevant to the early molecular pathology and histopathology of FOP. The readily available and safely obtainable lymphocytes from peripheral blood (through routine venipuncture) can be immortalized in the laboratory and used for studies in an animal-based system.

In order to determine the ability of FOP lymphoblastoid cells to induce FOP lesions, we subcutaneously implanted lymphoblastoid cells obtained from FOP patients and from unaffected family members into athymic nude mice (immune compromised mice that will not reject cells from a different species such as human). Cells from unaffected individuals either did not grow or formed small masses with little evidence of a fibrotic or angiogenic response. In dramatic contrast, cells from FOP patients gave rise to solid tumor-like masses in the animals.

Histopathologic evaluation of these lesions indicated that FOP cells induced angiogenesis and a fibrotic response in the host mouse, similar in appearance to early FOP lesions. FOP-like cell-induced lesions were probed for human-specific genetic sequences, confirming that the cellular masses contained human cells as well as host cells. These results suggest that cells of FOP patient origin induce changes in cell growth and/or differentiation and mimic events in early FOP lesions. Hence, implantation of FOP-derived cells in nude mice is beginning to provide an extremely useful cell model system for examining the early stages of FOP lesion formation, and ultimately in providing an intermediary model system for testing potential medications.

## **The Zebrafish as an Animal Model for FOP**

The zebrafish, a fresh water tropical fish that can be raised in the laboratory, has become an increasingly popular vertebrate model organism. The advantageous features of the zebrafish include the manageable size and optical transparency of its embryos, factors which allows visual inspection of internal organs and tissues in live embryos. Fertilization and

embryonic development occurs external to the mother's body, allowing easy access to embryos. Furthermore, the zebrafish is the most amenable of all vertebrates to genetic analysis.

In November 2000, Dr. Mary Mullins, an associate professor of cell and developmental biology at The University of Pennsylvania, and one of the world's experts in zebrafish development, attended the Third International FOP Symposium in Philadelphia. She said, "I attended the FOP symposium here in Philadelphia and was struck by the debilitating effects of this disease and the lack of effective treatments for the children. The strong implication of ectopic BMP signaling in the development of FOP led me to consider how our BMP studies in the zebrafish and my expertise in the zebrafish might be applied to understanding and possibly developing treatments for FOP."

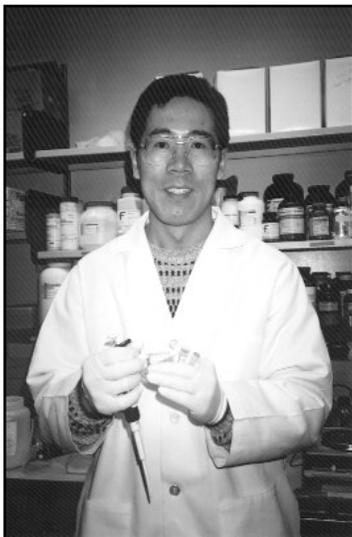
In her laboratory, Dr. Mullins has isolated mutations in several BMP signaling pathway genes including the zebrafish homologues of BMP2 and BMP4 as well as several of the BMP receptors. Mutations in all of these genes cause defects at very early stages of embryonic development in the zebrafish and Dr. Mullins has studied these genes extensively.

In 2002, Dr. Mullins was awarded a Developmental Grant from The Center for Research in FOP & Related Disorders to develop a BMP-inducible overexpression model of FOP in the zebrafish. Dr. Mullins will also test the function of several BMP antagonists and mixed agonist-antagonists in the regulation of skeletogenesis.

As a member of the FOP Center, Dr. Mullins is aware of the discovery of abnormally high levels of an activated BMP receptor on the surface of FOP cells. Using new molecular inducible expression systems that she has

developed, Dr. Mullins can test whether elevated levels of various BMP receptors are sufficient to cause an FOP-like syndrome in the zebrafish. Already, Dr. Mullins has generated heat shock inducible transgenes of a critical BMP receptor by fusing the heat shock protein promoter to the coding sequence of a BMP receptor gene. She has shown in multiple transgenic lines that this construct can rescue its respective mutant phenotype, thus demonstrating the utility of this transgene as an inducible expression tool. In the past, such studies have relied on messenger RNA injection to overexpress genes, but this approach limits the overexpression study to early embryonic stages of development. The heat shock transgene developed by Dr. Mullins allows overexpression of BMP pathway components throughout later stages of development and into adulthood in the zebrafish.

These "stealth-guided" molecular tools will be used in ongoing studies to overexpress various components of the BMP4 signaling pathway in the zebrafish and will be developed as a potential animal model system for FOP. This is a powerful and important new tool for FOP research and you will be hearing much more about it in future reports.



Dr. Chuanzhao Li, Ph.D.

## V. TRIGGERS

### The Immune System and FOP

Mounting evidence from all levels of investigation suggests involvement of the immune system in FOP. The presence of lymphocytes and mast cells in early FOP lesions, lymphocyte-associated death of skeletal muscle, flare-ups following viral infections, the intermittent timing of flare-ups and the beneficial response of early flare-ups to corticosteroids are all important pieces of evidence to support involvement of the immune system in the pathogenesis of FOP flare-ups. Some have also indicated that the clinical and pathological features of FOP suggest an autoimmune component to the condition, perhaps an autoimmune trigger.

Autoimmune diseases, with the exception of rheumatoid arthritis and autoimmune thyroid inflammation, are individually rare, but together affect approximately 5 percent of the population in western countries. Autoimmune disorders are a fascinating but poorly understood group of diseases. An autoimmune disease is defined as a clinical condition caused by the activation of T-lymphocytes or B-lymphocytes or both in the absence of an ongoing infection or other discernible cause. Genetic susceptibility, environmental and internal triggers of autoreactivity, changes in pathologic processes as the disease progresses, and multiple mechanisms of tissue injury have been considered as causes of autoimmune disorders. A useful division of autoimmune diseases distinguishes between those in which there is a general alter-

ation in the selection, regulation, or death of T-lymphocytes or B-lymphocytes and those in which an aberrant response to a particular antigen causes autoimmunity.

### Lymphocyte-Endothelial Cell Interaction: Early Markers of Inflammation

The migration of lymphocytes from an intravascular location to a location just outside of the endothelial cell membrane is the earliest microscopically-observed event in an FOP flare-up. How does the lymphocyte leave the blood vessel and gain access to the skeletal muscle where subsequent death of skeletal muscle cells occur? Integrins, cellular sensors that act as signaling molecules, are expressed by most lymphocytes. Integrins interact with integrin receptors such as vascular-cell adhesion molecules on the surface of endothelial cells to regulate the infiltration of lymphocytes into solid organs such as muscle. Alpha-4 integrin, a glycoprotein, is expressed on the surface of activated lymphocytes and monocytes and plays a critical role in their adhesion to the vascular endothelium and in their subsequent migration into various organs.

We are currently investigating the identity of these integrin markers on lymphocytes and endothelial cells in the limited FOP tissue that we have available. Identification of specific integrins on activated lymphocytes in FOP lesions could provide important therapeutic targets for pharmacologically available humanized monoclonal antibodies at the earliest stages of an FOP lesion. This will continue to be an important topic of investigation in 2003, and you will be hearing more about it in future reports.

## VI. TREATMENTS

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

### **BMP4 Antagonists: Paving the Way**

*Last year, we described an important discovery in FOP research in the field of gene therapy. The discovery, more appropriately labeled “a proof of concept,” provides the foundation for ongoing work in this area. In order to present a comprehensive account of this important work, we plan each year, to review the relevant background so that those new to this story will be able to more coherently follow its development and progress.*

Several years ago, after the discovery of the overexpression of BMP4 in FOP cells, Brigid Hogan, a distinguished developmental biologist from Vanderbilt University in Nashville, Tennessee wrote in the journal **Science**, “With so much being discovered about how the BMPs act, it might be possible to develop drugs that would block some part of the BMP4 pathway — and therefore prevent the progression of what is a horrible, nightmare disease.”

### **Noggin: Blocking the BMP4 Pathway**

The protein noggin, discovered by our collaborator Dr. Richard Harland from The University of California–Berkeley, was known to have powerful effects on antagonizing BMP4 activity. The day after our discov-

ery of the overexpression of BMP4 was published in **The New England Journal of Medicine**,

Dr. Harland and his colleagues published a seminal paper in the journal **Cell** showing that the noggin protein directly binds to the native BMP4 molecule and prevents it from interacting with its own receptor. The importance of noggin to the FOP story became apparent immediately, and noggin was brought to the forefront of development for FOP treatment.

Noggin is involved in controlling the amount of skeleton that is formed by regulating the concentrations of BMP4 available to the body’s tissues. For this reason, noggin offers promise for controlling the rampant bone growth of FOP. Soon after the discovery of BMP4 overexpression in FOP, we were contacted by scientists from Regeneron Pharmaceuticals suggesting that a collaboration be formed between the FOP laboratory, Dr Harland’s laboratory, the scientists involved in the development of gene therapy vectors, and the scientists at Regeneron Pharmaceuticals, to explore the possibility of developing noggin as a treatment for FOP. We have been working together since then.

### **A Proof-of-Concept Experiment In Noggin Gene Therapy**

In 2001, we began a critical “proof-of-concept” experiment. The results of this experiment demonstrated dramatically that a genetically-engineered form of the protein noggin can be produced by the mammalian liver after targeted delivery of the modified noggin gene and circulate systemically to completely block BMP4-induced heterotopic ossification in the mouse.

The purpose of the study was to develop an effective gene therapy approach for the prevention of BMP4-mediated heterotopic ossification that would be applicable in principle to patients with FOP. To achieve this goal, we used an adenovirus –mediated transfer of a genetically-modified noggin gene.

Naturally occurring noggin protein does not circulate in the bloodstream, and has a very short half-life in the body. The reason that noggin does not circulate through the blood is that the noggin protein sticks to complex sugars (called heparan sulfate proteoglycans or HSPGs) on blood vessel walls. Such “stickiness” of noggin would not be amenable to treating a disease like FOP. Therefore, if noggin is to be applicable for systemic (body-wide) delivery, the gene encoding the noggin protein first must be modified to allow the noggin protein to circulate through the blood and to have a long half-life in the circulation.

The native human noggin gene was modified by removing the DNA sequences that encode the portion of the protein that allows it to stick to blood vessel walls. This modification of the noggin gene was accomplished, and the protein produced by the modified gene was able to circulate through the blood vessels without sticking to the vessel walls while still retaining its ability to bind and inactivate the BMP4 molecule. Furthermore, this modification in the noggin gene and its associated protein increased the half-life of noggin within the circulation, making it even more desirable for systemic delivery.

The mouse model of BMP4-induced heterotopic bone formation was used in the definitive experiment. This model system, described earlier in this annual report, allowed us to identify the various stages of BMP4-induced bone formation that closely

mimic the stages of bone formation of an FOP lesion. Following approval by The Investigational Animal Care and Use Committee, a subcutaneous muscle in 32 mice was injected on one side of the midline with matrigel alone and on the contralateral side with matrigel combined with recombinant human BMP4. A dose of BMP4 was selected to consistently produce heterotopic ossification. Half of the animals were pretreated for four days with an intravenous injection of recombinant adenovirus carrying the modified human noggin gene. Half of the animals were used as controls and were pretreated for four days with recombinant adenovirus that contained no insert. The matrigel implants were recovered at seven days and 14 days after injection. Standard histological techniques were used to evaluate the various stages of bone formation and to identify specific cell types present in the tissue.

Experimental and control implants were recovered from all animals. The implants containing recombinant human BMP4 induced an aggressive fibroproliferative lesion with early cartilage formation at seven days and heterotopic ossification at 14 days in the control animals. However, in animals pre-treated with the modified noggin gene, the BMP4 implants caused no lesion formation at either seven days or 14 days.

This remarkable study demonstrates that the delivery of a modified noggin protein through a systemic gene therapy approach successfully prevents BMP4-induced heterotopic ossification in a mouse model. It provides "proof-of-concept" that a genetically-modified morphogen antagonist (noggin) can be produced *in vivo* and act systemically to prevent BMP4-mediated heterotopic ossification that is clinically relevant to the treatment of FOP.

*This major breakthrough in advanced therapeutics proves 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 liver can act as a factory for producing an active form of the modified protein, that*

*the modified 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.*



## Noggin Gene Therapy: Major Developments in 2002

*Our ground-breaking proof-of-concept experiment was first reported in last year's annual report (and reproduced above). There have been four major developments in noggin gene therapy in 2002:*

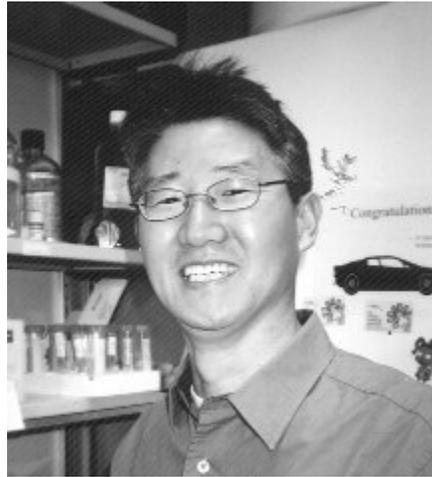
1. The development of a highly sensitive and specific radioimmunoassay for measuring the concentration of the modified noggin protein in the blood.
2. The exploration, development and use of safer viral vectors (adeno-associated viruses) for systemic noggin gene delivery.
3. Studies to determine if the genetically-engineered form of noggin causes organ-specific toxicity in the mouse.
4. Exploration of specific cell-based delivery systems for genetically-engineered noggin gene therapy.



Dr. Kaplan's Secretary  
Kay Rai

The development of a highly sensitive and specific radioimmunoassay for measuring the concentration of the genetically engineered noggin protein in the blood is necessary for all future experimental work in animals and eventually in humans. This work was performed in the core FOP laboratories this past year. The noggin radioimmunoassay is critical not only for determining the level of modified noggin protein in the circulation of the experimental animals, but also in determining whether or not the mice are developing antibodies to the modified noggin protein. It is possible that the mouse may, over time, develop antibodies to the human noggin protein. Although the human and mouse noggin proteins are extremely similar in sequence, they are perhaps different enough to elicit an antibody response.

Of greater concern, however, for human studies, will be whether or not the modification of the native human noggin gene elicits an antibody response in patients. The act of truncating the noggin gene to encode a modified but circulating noggin protein that is missing its heparin-binding domain leads to a molecule that has a slightly different shape and confirmation



Dr. Yun-Sik Lee

in three-dimensional space than the native noggin protein. Such a modified protein might be recognized as a foreign protein by the human immune system. As a result, detailed and intensive immunological studies will eventually be necessary for further development of noggin gene therapy in humans. We are well aware of those considerations, and they are already figuring largely into our thinking about the design of a more definitive FOP therapy. For example, it might be possible to target the native noggin gene (rather than the modified noggin gene) to the exact cells in skeletal muscle where the FOP lesions begin. Emerging knowledge of those cell types as described earlier in this report might enable such an approach in the future.

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. Although adenovirus is an excellent delivery vehicle for demonstrating proof-of-concept of noggin gene therapy, the adenovirus vector elicits an intense systemic immune response and is unacceptable for long-term human use.

Our colleagues at the Institute for Human Gene Therapy at Penn have isolated new adeno-associated viruses and are developing them as vectors for human gene therapy. In general, adeno-associated viruses are much less toxic systemically (but still have some toxicity) and elicit a much 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. That, in fact, was the case. These new delivery vectors have been used in our modified noggin gene therapy experiments this past year and are extremely effective in delivering adequate doses of the modified noggin protein systemically to inhibit heterotopic ossification in an animal model. These novel adeno-associated viruses are much less toxic to the mice than the original adenoviruses we used, and they elicit a much weaker inflammatory response, especially in the liver where the modified noggin protein is manufactured. Also, this safer viral gene delivery vehicle is necessary in animal studies in order to examine and isolate any potential toxic effects of the modified noggin protein at high-sustained dosage levels. In other words, it is first necessary to minimize the toxic effects of the delivery vehicle (virus) which carries the modified noggin gene into the body before we can effectively study any possible toxic effects of the modified noggin protein itself. The studies to determine whether these novel adeno-associated viruses could be used to deliver the modified noggin protein at high enough levels to inhibit heterotopic ossification were extremely successful.

Preliminary 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 adeno-virus, 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. Thus, if noggin is to be considered for human use, it must be precisely regulated and be kept at the lowest effective levels possible for the briefest periods of time to inhibit heterotopic ossification. As a result, it is even more compelling now that we explore regulated and inducible systems for the delivery of BMP antagonists (such as noggin) and then target the noggin gene therapy to cells that are either direct targets of BMP action or to cells in the vicinity of the BMP targets.

Earlier in this Annual Report (Section III. Cells), we discussed important breakthroughs in determining cellular targets of BMP action within skeletal muscle. These new and exciting discoveries in the cell biology of heterotopic ossification will be exceedingly important in designing the most appropriate cell-based therapies to target gene therapy. Ultimately, an inducible promoter for the native noggin gene might best be targeted to those exact cells that would respond to the BMP4 signal within skeletal muscle and begin the transformation into bone. While the laboratory findings and treatment investigations are often presented in separate sections of the annual report, the overall integrated nature of the FOP research should not escape anyone's attention.

Presently, we are exploring these new cell-based gene delivery options while at the same

time working to develop an inducible promoter for the novel adeno-associated virus modified noggin gene delivery. We are currently working with attorneys and scientists at two pharmaceutical companies to facilitate the development of an inducible promoter (a drug-regulated molecular switch) that can be used in the next phase of the noggin gene therapy studies in 2003.

*Hope is the thing with  
feathers that perches  
in the soul,  
And sings the tunes without  
words . . .*

Emily Dickinson



## **Noggin Gene Therapy: The Future**

*Numerous major technical obstacles need to be overcome before noggin gene therapy could be considered for the treatment of patients who have FOP. These include the refinement of safe and effective viral vectors for use in humans, the successful development of inducible and regulated delivery of the noggin gene, complete toxicity studies on systemically-administered noggin and modified noggin, and ultimately the development of improved animal models based upon knowledge of the molecular genetics of FOP. Nevertheless, for patients suffering from FOP, stable gene transfer of an inducible BMP4 antagonist may eventually offer hope where all other modalities have failed.*

**S**uccessful gene therapy in FOP as with any genetic disease, will require the coordinated and collaborative work of geneticists, virologists, immunologists, biochemists, cell biologists and clinicians. The optimal viral vector for noggin gene therapy in FOP will be administered by non-invasive delivery routes, targeted to a specific tissue or cell, and induced to express a therapeutic amount of noggin protein for a regulated length of time. All of these aspects of noggin gene therapy are currently being pursued with intense activity. As already described, virologists have generated safe and efficient viral vectors such as AAV for introducing the extra copies of the noggin gene into the human body. Molecular biologists will help to design vectors capable of cell-specific and tissue-specific expression of the noggin gene. Immunologists will continue to develop ways to prevent unwanted immunological consequences of the viral delivery vehicles and their modified noggin cargo. Cell biologists will devise ways to facilitate gene transfer to various tissues and will take the lead in identifying muscle or blood stem cells to which the vector can be introduced. Clinicians will carry out clinical trials on patients with FOP with the best vectors that the scientists can supply. To achieve successful gene therapy in patients, many branches of biology and medicine must contribute to this endeavor.

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. Whether or not noggin gene therapy will eventually be realized in patients with FOP depends on many major technical hurdles mentioned above and many scientific hurdles, which have been outlined earlier in this and last year's Annual Report. What is most important is that the FOP gene be determined, that all of the target cells be identified, and that the molecular pathways be elucidated. Clearly then, the best treatment for FOP will emerge. For the meanwhile, we will move ahead swiftly with our experimentation on noggin gene therapy, as it is the best hope we now have.

### **Noggin: Crystal Clear**

In related work, our colleague Dr. Jay Groppe, an x-ray crystallographer working at The Salk Institute in La Jolla, California recently published a major article in the journal **Nature** on the x-ray crystallographic (molecular 3D) structure of the noggin protein as it is bound to a BMP molecule. The work is an extraordinary technical tour-de-force. In the article entitled, "**Structural Basis of BMP Signaling Inhibition by Noggin, a Novel Cystine Knot Protein**", Dr. Groppe and colleagues discuss how the interplay between bone morphogenetic proteins and their antagonists govern numerous developmental and cellular processes in the human body and throughout the animal kingdom.

In the article, they show exactly and elegantly how the

three-dimensional structure of noggin enables it to inhibit BMP signaling by blocking the interaction of BMP with both of its transmembrane receptors. They further demonstrate that the binding of BMP to noggin sequesters the BMP molecule in an inactive complex.

In a special visit to the Center for Research in FOP and Related Disorders in late November 2002, Dr. Groppe explained how noggin and various BMPs appeared to have evolved from a common ancestral gene. The proteins encoded by the BMP genes and the noggin gene are highly related not only in structure but in shape. In fact, when noggin binds to BMP, it binds in a back-to-back fashion with both molecules in a planar configuration. "It is as if two butterflies are linked back-to-back with the larger noggin molecule holding the smaller BMP molecule in a full Nelson,"

Dr. Groppe explained.

Dr. Groppe went on to show how both BMP and noggin have heparin-binding domains – amino acid residues in their respective

proteins that allow the molecules to bind to cell-surface heparan sulfate proteoglycans (HSPGs) that concentrate the molecules near the site of cell signaling at the membrane surface. Most importantly, he showed how the heparin-binding sites of both BMP and noggin are at opposite ends of the molecules from which the two molecules bind to each other, thus allowing the heparin-binding sites of each molecule to be attached to respective cell surfaces. This has enormous implications for the regulation of BMP signaling.

Even more importantly, Dr. Groppe showed graphically and



dramatically how the molecular modification of the noggin gene to remove the heparin-binding site would not at all affect the ability of the molecule to bind and sequester BMPs, an important confirmation for our modified noggin gene therapy experiments described in the previous section. Experimental evidence at the biochemical level has shown that that in fact is the case, but the beauty and power of the x-ray crystallography studies showed why in fact that was true.

Dr. Groppe discussed how the extracellular regulation of BMP activity is unique among signaling pathways in that duplications of both BMP genes and BMP receptor genes may have led to the evolution of structurally-related antagonists (such as noggin) to downregulate the activity of the BMPs. Dr. Groppe writes in the last sentence of the article, "The surprising structural homology between agonists (BMPs) and antagonists (such as noggin) provide evidence of the ancient origin and evolution of BMP-related regulated signaling pathways."

Taken together, Dr. Groppe's work has profound implications for BMP4 signaling in FOP and for noggin gene therapy as well as for biologists in numerous fields of molecular, developmental, and structural biology. Finally, in a related and recent article in the journal **Nature**, other investigators showed how the relationship between BMP4 and noggin plays an important role in the development of feathers and likely played a critical role in the evolution of dinosaurs to birds.

### **The Natural Regulation of Noggin**

In addition to the ability of noggin to bind and sequester multiple BMPs and thus prevent them from activating the BMP

pathway, several of our colleagues have been pursuing work to better understand how noggin is regulated *in vivo*. During a visit to the Center for Research in FOP & Related Disorders in early November 2002, our colleague Dr. Aris Economides from Regeneron Pharmaceuticals, explained how in the fusion of cranial sutures (the soft parts in a baby's skull that allow the expansion of the bones that make-up the skull) during development, the fibroblast growth factor (FGF) family of molecules and their cognate receptors dampen the ability of BMPs to upregulate the expression of their antagonists (such as noggin). Thus, overactivity of the fibroblast growth factor receptors (as seen in the craniosynostoses, conditions where the skull bones fuse prematurely), prevents BMPs from properly upregulating the expression of noggin. As a result, there is a dangerous imbalance between BMP and noggin leading to excessive bone formation and premature fusion of the bones of the skull. Most importantly, the molecular pathology of the craniosynostoses and FOP demonstrates that it is not simply too much BMP, but too little of the antagonists, that may cause the problems.

In related work, preliminary data from Dr. David Diefenderfer at the University of Pennsylvania School of Dental Medicine (who was recently awarded a Developmental Grant from the FOP Center) indicates that glucocorticoids (medications like prednisone) may have the ability to elevate the expression of noggin *in vitro*. Such findings, if confirmed *in vivo*, would have valuable and important implications for the therapy of conditions like FOP, and other disorders of heterotopic ossification.

## Antiangiogenic Agents and the Phase I Clinical Trial of Squalamine

Development and growth of the human embryo as well as growth and regression of tumors are dependent on the control of new blood vessel formation (angiogenesis). Angiogenesis is also an absolute requirement for the formation and development of the skeleton, for the successful healing of fractures, and for the formation of heterotopic bone. The early stages of skeletal embryogenesis correspond to the highly vascularized pre-osseous fibroproliferative lesions seen in FOP. Angiogenesis, a prominent histopathologic feature of pre-osseous FOP lesions, thus becomes a potential target for therapy.

The goal of anti-angiogenic therapy in FOP is to inhibit new blood vessel formation in order to retard new bone formation once a new lesion has appeared. Angiogenesis may potentially be minimized with anti-angiogenic agents such as thalidomide, squalamine, cyclooxygenase-2 (cox-2) inhibitors, aminobisphosphonates (to be discussed in the next section), and vascular endothelial growth factor traps.

Squalamine, a new anti-angiogenic agent, with potential interest for FOP, was discovered in 1992 by Dr. Michael Zasloff. Dr. Zasloff isolated squalamine from the liver of the dogfish shark, and discovered its anti-angiogenic properties. Squalamine is a naturally occurring cholesterol-like molecule that inhibits the proliferation of endothelial cells (blood vessel cells) and exhibits potent anti-angiogenic activity in laboratory animals and humans. Several years ago, the cellular mechanism of action of squalamine was

elucidated. Squalamine modifies the response of endothelial cells to proteins that organize their shape and structure.

Squalamine is currently produced synthetically under sterile conditions and does not have to be obtained from sharks. In pre-clinical studies, squalamine has been shown to inhibit angiogenesis and the subsequent growth of some solid tumors. By directly blocking the angiogenic process, squalamine has the potential to slow the progression of the FOP lesions in muscle. As described in a previous section of this report, squalamine was shown to be a potent inhibitor of cartilage maturation and bone formation in a developmental assay system relevant to the study of FOP.

A Phase I clinical trial of squalamine in FOP has been approved by the U.S. FDA and is targeted to a small group of adult FOP patients who are having severe pre-osseous flare-ups. The initial study is designed to evaluate the safety and efficacy of intravenous squalamine on the inhibition of angiogenesis in early FOP flare-ups, and permits the enrollment of no more than 10 adult patients with FOP. The study has been fully approved by the FDA, the Institutional Review Board of The University of Pennsylvania, The General Clinical Research Center of the Hospital of the University of Pennsylvania, The Radiation Safety Board, The Nursing Committee, The Pharmacy Committee, and The Data Safety Monitoring Board of The University of Pennsylvania School of Medicine. Further information on this Phase I clinical trial is now available online at [www.ifopa.org](http://www.ifopa.org).



**Dr. Nader Hebela**

### **Prostaglandins & the Cox-2 Inhibitors: Inflammation & FOP**

In previous reports, we noted an important new category of drugs with previously unexpected and important implications for the treatment of FOP. These are the cyclo-oxygenase-2 (cox-2) inhibitors, medications that specifically target pro-inflammatory prostaglandins. Data from the FOP laboratory has shown a dramatic and robust expression of the cox-2 enzyme in the fibro-proliferative cells of the early FOP lesions.

The body essentially produces two types of prostaglandins: “physiological” prostaglandins and “inflammatory” prostaglandins. Physiological prostaglandins are normally produced in many of the body’s tissues and protect organs such as the stomach from metabolic injury. Inflammatory prostaglandins are produced in response to injury as an active byproduct of the breakdown of cell membrane components and play a major role in the inflammatory response to injury. Traditional non-steroidal anti-

inflammatory drugs such as aspirin, ibuprofen and indomethacin inhibit the formation of both the physiological and inflammatory prostaglandins. The new cyclo-oxygenase 2 (cox-2) inhibitors primarily block the formation of the inflammatory prostaglandins and leave the physiological prostaglandins relatively intact.

Inflammatory prostaglandins are potent co-stimulatory molecules along with BMPs in the induction of heterotopic bone. Studies published in the orthopaedic literature have shown that lowering prostaglandin levels in experimental animals dramatically raises the threshold for heterotopic ossification, thus, making it more difficult (but not impossible) for bone to form. Animals pretreated with large doses of prostaglandin inhibitors failed to form heterotopic bone following intramuscular injections of BMP-containing demineralized bone matrix. In contrast, animals treated with prostaglandin inhibitors at the same time as or following a demineralized bone matrix injection still formed heterotopic bone. These data suggest that in order for prostaglandin inhibitors to be truly effective in preventing heterotopic ossification, the medication must be “in the system” (in other words circulating in the blood at the therapeutic levels) before a bone-induction signal is delivered to a target cell. In addition to their potent anti-inflammatory properties, a series of recent studies have demonstrated that the new cox-2 inhibitors have potent anti-angiogenic properties as well as anti-inflammatory properties, a feature that makes them even more desirable for consideration in FOP.

An important paper published in 2002 by colleagues from The University of Rochester showed convincingly that ani-

mals genetically engineered to lack both copies of the gene encoding the cox-2 enzyme (cox-2 knockouts) failed to generate new bone formation at a fracture site, thus demonstrating the importance of the cox-2 enzyme in inflammatory bone formation. While pharmacologic doses of cox-2 inhibitors (medications that block the activity of the cox-2 enzyme) given to normal animals had a similar effect, the inhibition of bone formation in both sets of animals (cox-2 knockouts and animals treated with cox-2 inhibitors) could be overcome with massive amounts of recombinant BMP, indicating that cox-2 activity occurs upstream of BMP signaling and that intense overactivity of the BMP pathway (as can be seen in FOP) could plausibly overcome a cox-2 blockade. Similar results were reported in a separate study published in 2002 by a research group led by a former FOP Laboratory fellow who now works at The University of Medicine and Dentistry of New Jersey.

Inflammatory prostaglandins directly stimulate the induction of angiogenic proteins which can further promote bone formation. Inflammatory prostaglandin levels are dramatically elevated in the urine of patients who have FOP, especially during times of a disease flare-up. These observations suggest the following hypothesis: lowering baseline prostaglandin levels in patients with FOP may raise the threshold for heterotopic ossification even in the presence of substantial endogenous levels of BMP4. Thus, while cox-2 inhibition may not effectively block all BMP-induced FOP lesions, they may potentially raise the threshold (make it more difficult) for bone to form. This hypothesis is amenable to clinical testing and will be the focus of a placebo-controlled study to assess the safety and efficacy of cox-2

inhibitors in the prevention and treatment of FOP flare-ups. This proposed study will permit enrollment of children with FOP and has been given tentative approval by both a major pharmaceutical company who has agreed to supply both drug and placebo as well as by the U.S. FDA. The study is being prepared for submission to a long list of required regulatory committees at The University of Pennsylvania and, of course, will require final approval by both the pharmaceutical company and by the U.S. FDA.

While the potential benefit of the new cox-2 inhibitors in preventing heterotopic ossification is no greater than the parent class of non-steroidal anti-inflammatory medications, the new cox-2 inhibitors offer the possibility of a slightly lower gastrointestinal risk profile than the parent compounds. In addition, the half-life of some of the new cox-2 inhibitors is conducive to a once-daily dosage regimen, a factor which helps promote patient compliance. Cox-2 inhibitors are available by prescription. While not officially approved for use in children, they are currently being tested in children with rheumatoid arthritis, and are being used sporadically by pediatric specialists for the treatment of severe inflammatory conditions such as FOP where few other treatment options exist.

While the cox-2 inhibitors are generally safe, their action must be carefully monitored, especially in those who are taking the medications for long periods of time, since rare but life-threatening side-effects and kidney-damaging effects can occur.

As with any condition, the relative risks and benefits of potential therapies must be weighed against the potential risks of the underlying condition being studied or treated.

## Aminobisphosphonates: A Gold Mine or Fool's Gold?

**B**isphosphonates are a potent class of medications that have profound effects on bone remodeling and exert their primary effect by decreasing the life span of osteoclasts, the bone-eating cells of the body. Bisphosphonates are thus widely used in the treatment of numerous bone diseases where bone resorption exceeds bone formation — disorders such as osteoporosis, Paget's disease, fibrous dysplasia, and bone cancer.

The original bisphosphonate, Etidronate, when administered at high doses also has a potent effect on inhibiting mineralization of newly formed cartilage and bone protein and had been proposed as a possible treatment for FOP and other disorders of heterotopic ossification as far back as 30 years ago. While its effectiveness in FOP is questionable, Etidronate has enjoyed limited use in the treatment of more focal disorders of heterotopic ossification such as those that arise following soft tissue trauma or injuries to the central nervous system. Unlike Etidronate, the newer bisphosphonates including the aminobisphosphonates have no appreciable effect on inhibiting mineralization, but are hundreds to thousands of times more potent than Etidronate in inhibiting bone resorption, a property that dictates their current utility in a wide range of bone diseases characterized by excessive bone resorption such as osteoporosis.

So, why would such drugs, which act primarily to inhibit bone resorption, even be discussed or considered in the context of FOP, a condition where decreased bone resorption (at least in the heterotopic skeleton) would not be desirable?

At first glance, there would

appear to be little rational use for compounds such as the newer aminobisphosphonates in the treatment of FOP. However, the story is not that simple.

We are all aware that medications have side effects, but it is an interesting sidelight of medical practice that, on occasion, medications have been used either mistakenly or coincidentally with unanticipated beneficial effects. Very often, a new use for a medication is discovered serendipitously or accidentally only after a medication has been released for a specific use. Such was the case, for example, with the discovery (in the early 1940s) that antihistamines were effective not only in treating allergies, but also in preventing motion sickness. An internist at The Johns Hopkins Hospital School of Medicine, treating a patient for allergies with a newly approved antihistamine, asked the woman how the medication was working. The patient told her doctor that her allergy symptoms were much improved with the new antihistamine, but that amazingly, she no longer got motion sickness while riding the trolley car, a problem that she had experienced her entire life. She attributed the absence of motion sickness to the antihistamine as she noticed that she got motion sickness when she didn't take the antihistamine, but didn't feel sick when she took the medication. That single observation led to a series of studies that stringently validated the patient's observations. Thus, the encounter of one physician with one patient led to the fortuitous discovery of the use of antihistamines for the treatment of motion sickness — a discovery that had an immediate impact on the overseas transport of troops from the United States to

theaters of operation in the South Pacific and Europe during World War II.

A similar scenario occurred recently with the use of the aminobisphosphonates in the treatment of FOP. In the past year, we have learned of several extremely credible and anecdotal reports from physicians and FOP patients worldwide regarding the response of FOP flare-ups to Pamidronate, one of the newer aminobisphosphonates. But, why would Pamidronate even be considered for the treatment of FOP flare-ups? Ironically, in all three cases we heard about, the medication had been used with the mistaken belief that Pamidronate was more potent than Etidronate in inhibiting mineralization. It is not. None of the newer bisphosphonates including Pamidronate have any effect whatsoever on inhibiting mineralization. Nevertheless, all three patients reported substantially decreased swelling, redness, and pain following high dose intravenous Pamidronate administration during a new flare-up. In one patient, the Pamidronate was administered alone, while in the other two patients, it was administered along with an oral steroid (such as Prednisone) for several days during the early phases of a new FOP flare-up.

All of us in the FOP community know that such anecdotal observations could be purely coincidental - that is, that the flare-ups might have receded spontaneously without treatment and that the Pamidronate might have had nothing to do whatsoever with the reported improvement. Also, one cannot discount a potent placebo effect in any uncontrolled observation. Nevertheless, we also know that such observations of potential improvement in an FOP flare-up cannot be ignored. It is entirely possible to stumble on something worthwhile even for the wrong reason!

As word of this Pamidronate-associated response (with or without steroids) spread throughout the FOP community in the past several months (generally by internet communications among patients and families), nearly a dozen patients (in consultation with us and their local physicians), have used Pamidronate empirically (either alone or with steroids) in the treatment of acute flare-ups especially those involving major joints. In 10 of the 13 patients (77%), there was reported improvement in the symptoms of an FOP flare-up. In three of the 13 patients (23%), there was no reported improvement in the appearance of the lesion to either the physician or the patient. Interestingly, there seemed to be no protective effect whatsoever on the occurrence of subsequent flare-ups in any of the patients treated with either a single dose or brief course of intravenous Pamidronate. Therefore, whatever anecdotal improvement there may have been was transient and affected only the lesion present at the time of the therapy.

While these patient reports are not scientifically valid, they constitute an important set of anecdotal observations that compel further stringent scientific inquiry in controlled laboratory and clinical studies. The

treatment protocols varied slightly between the patients (depending on age, body weight, and site of involvement) but in general were similar. Details on the treatment protocols used to date will be included in the updated **FOP Treatment Guidelines**, presently in preparation. These will be available online at [www.ifopa.org](http://www.ifopa.org) when they are completed in the next several months.

Side-effects of the intravenous infusions included flu-like symptoms of fever, chills, and muscle aches. These symptoms can often be lessened by pre-treatment with acetaminophen. One patient developed tetany (uncontrolled muscle contractions due to a low vitamin D level in the blood), and one



**Krystyna Knight bringing tissue culture supplies to the lab.**

patient developed peripheral phlebitis (inflammation of the vein) at the intravenous infusion site which required inpatient intravenous antibiotic treatment.

An important question that these observations from routine clinical care of FOP patients raises is: "What might be the physiological basis for any potential beneficial effect of aminobisphosphonates in the treatment of FOP flare-ups? As a consequence of its potent inhibition of bone resorption, the aminobisphosphonates effectively inhibit the release of growth factors and morphogens (such as BMPs) which are stored in the extracellular bone matrix of the skeleton. The action of the bisphosphonates on the suppression of bone resorption is exceedingly long, longer than for any other class of medications, and is on the order of months to years. Therefore, if aminobisphosphonates inhibited FOP lesions by decreasing the release of BMPs sequestered in the skeleton, one would expect a more pronounced effect on the prevention of subsequent flare-ups which was not seen in the patients treated. Clearly, if the aminobisphosphonates are truly beneficial in the treatment of FOP flare-ups, there must be a mechanism of action that is very brief and substantially different from that of osteoclast inhibition from which the medication derives its beneficial effects in the normotopic skeleton.

While the potential mechanism of action of the aminobisphosphonates on early FOP lesions or BMP-induced FOP-like lesions remains to be determined, two papers published in 2002 provide some tantalizing clues. These two papers which were published in the peer-reviewed cancer literature document the extremely potent antiangiogenic effects (decreased new blood vessel formation) of Pamidronate and several other aminobisphosphonates *in vitro*

and *in vivo*. Also, Pamidronate administered intravenously was shown to dramatically decrease vascular endothelial growth factor (VEGF) levels and basic fibroblast growth factor (bFGF) levels in cancer patients with bone metastasis. Both VEGF and basic FGF are potent tumor-associated angiogenesis factors.

Angiogenesis is one of the most prominent histopathologic features of pre-osseous FOP lesions, and thus a potential target for therapy (as discussed previously). Also, bFGF is an extremely potent *in vivo* stimulator of angiogenesis and has been implicated in the growth of solid tumors as well as FOP lesions. Urinary bFGF levels are markedly elevated in FOP patients especially during acute flare-ups. Furthermore, bFGF is highly expressed in lesional cells of FOP biopsy specimens. These data strongly suggest that bFGF may be a biochemical marker for disease activity and provide a biochemical basis for considering anti-angiogenic therapy and anti-bFGF therapy at early stages of the disease process. The goal of anti-angiogenic therapy in FOP (regardless of the medication used) is to inhibit new blood vessel formation in order to slow down or inhibit the subsequent production of new bone formation once a new lesion has appeared.

The effect of Pamidronate and other aminobisphosphonates on angiogenesis in mice was totally unanticipated but not surprising in light of the extraordinarily potent effects of these medications as adjuvant therapies in the treatment of various cancers. Potential anti-angiogenic effects of Pamidronate in FOP are also compatible with the known brief half-life of the medication in the circulation prior to its long-term stable deposition in the skeleton and could explain why the medication may have an

effect on active lesions but not on the prevention of new lesions.

Intravenous Pamidronate has also been shown in several studies to reduce various lymphocyte subpopulations in the circulation and may be responsible for its dose-related side-effects of causing flu-like symptoms. We cannot yet rule-out the possibility that Pamidronate may affect the early lymphocytic infiltration into skeletal muscle seen in both BMP4-induced FOP-like lesions and in FOP lesions themselves.

Other possible mechanisms by which the Pamidronate might affect FOP lesions include a direct inhibition on the proliferation of a rapidly dividing population of cells. Such an effect was noted recently in a study investigating the effects of aminobisphosphonates on cancer cells *in vitro*. It is certainly possible that Pamidronate may affect one or more cell types in an early FOP lesion.

Finally, one must consider the stark possibility there may be no positive effects whatsoever from the Pamidronate on FOP lesions and that the reports to date are the results of observational bias and/or coincidence. Only rigorous controlled investigations *in vitro* and *in vivo* in the laboratory, as well as placebo-controlled clinical trials will be able to definitively decipher these possibilities and provide a solid rational basis for determining whether or not one or more of the aminobisphosphonates will have a beneficial role in the treatment of FOP.

Laboratory studies to assess both the potential therapeutic benefit and potential mechanism of aminobisphosphonate action in the model of BMP-induced heterotopic ossification as well as in the model of lymphoblastoid cell implantation (discussed earlier in this report) will be underway rapidly. Simultaneously, we are beginning to design a controlled clinical study to assess



**Dr. Xiangyang Jiao and Dr. Glaser in the lab.**

the potential benefit of intravenous Pamidronate in the treatment of acute FOP flare-ups. The study will then be subject to rigorous approval by numerous regulatory Investigational Review Boards at The University of Pennsylvania, The Children's Hospital of Philadelphia, the pharmaceutical company co-sponsoring the study, and the FDA, as well as numerous local review boards associated with the private practices where a child or an adult may be seen and treated.

We are presently contemplating a phase I/II study in which a course of intravenous Pamidronate (or placebo) would be given as an outpatient and the patient carefully monitored with daily surveys filled out by the parents and physician, as well as daily lesional photographs and measurements made along with occasional blood tests. We would like to design the study so that it could be carried-out at multiple sites and so that transportation to Philadelphia will not be an issue. While the logistics of this may be difficult, we will work on it.

The results of such a carefully controlled clinical study would almost immediately give us better insight into the potential efficacy of Pamidronate in the treatment of FOP flare-ups. However, as mentioned above, it is extremely important to have rigorous controlled observations in order to understand the potential use of this medication (and other aminobisphosphonates) for the long-term.

Will Pamidronate and the newer generation of the aminobisphosphonates be a goldmine for FOP therapy or will it simply be fool's gold? Only time and rigorous experimental approaches will provide clear answers to that question. While noggin gene therapy and related approaches such as the development of BMP receptor antagonists and BMP pathway antagonists may eventually prove to be more definitive in the ultimate treatment and prevention of FOP, we hope that the use of more immediately available medications such as glucocorticoids, leukotriene inhibitors, mast cell inhibitors, cox-2 inhibitors, and perhaps the aminobisphosphonates will allow us to buy time for FOP patients. As Jeri Licht, the mother of Daniel Licht stated so eloquently and passionately in the **BBC** documentary, ***The 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."



**Research Specialist Mei-qi Xu**

## VII. Presentations, Meetings, Reports, and Publications

During 2002, major lectures on FOP were presented by members of the FOP laboratory at the:

- Advances in Mineral Metabolism Conference of the American Society for Bone & Mineral Research; Snowmass, Colorado
- American Association of Allied Health Professionals; Myrtle Beach, South Carolina
- American Society of Gene Therapy; Boston, Massachusetts
- Annual Meeting of FOPev; Valbert, Germany
- Baylor College of Medicine; Houston, Texas
- Bristol Myers-Squibb Research Foundation; Wallingford, Connecticut
- Cornell University School of Medicine; New York City, New York
- European Calcified Tissue Society; Zagreb, Croatia
- First International FOP Symposium of the United Kingdom; Manchester, United Kingdom
- Institute for Musculoskeletal Sciences, Nuffield Orthopaedic Center – Oxford University; Oxford, United Kingdom
- Institute of Genetic Medicine – Keck School of Medicine, University of Southern California; Los Angeles, California
- Institute of Myologie - Association Française Contre Les Myopathies - Hôpital De La Salpêtrière; Paris, France
- International Conference on Bone Morphogenetic Proteins; Sacramento, California
- Johnson & Johnson Research Foundation; New Brunswick, New Jersey
- Musculoskeletal Center of The Children's Hospital of Philadelphia; Philadelphia, PA
- Orthopaedic Research Society; Dallas, Texas
- Royal College of Surgeons, England; London, England
- University of Arizona Health Sciences Center; Tucson, Arizona
- University of Paris, Faculty of Medicine; Paris, France
- University of Texas Health Science Center at San Antonio; San Antonio, Texas
- University of Zagreb School of Medicine; Zagreb, Croatia
- Wright State University, Dayton, Ohio

Five major presentations on FOP were made by members of the FOP Laboratory at The American Society for Bone and Mineral Research (ASBMR) in San Antonio, Texas in September 2002. Three of these papers were presented at the Plenary Session of the meeting which was attended by over 6,000 physicians and scientists worldwide. As always, it was a wonderful opportunity to present major highlights and discoveries from the FOP and POH laboratory to an extraordinarily large and distinguished group of scientists and physicians worldwide.

### Center for Research in FOP & Related Disorders

Writing in the **Scientist**, John Trojanowski, M.D., Ph.D., Director of The Institute on Aging at The University of Pennsylvania, stated: "Most of today's biological problems require multidisciplinary research strategies that entail the use of multiple, diverse methodologies that no single investigator can master. The most tangible benefit of Center and program grants may be the rapid and economical acquisition of insights into complex biological questions that result from the research conducted by dedicated investigators working synergistically." The Center for Research in FOP & Related Disorders was established several years ago by the Cali Family Endowment with the same spirit in mind.

During the past year, the Developmental Grants Program of the Center for Research In FOP and Related Disorders has sponsored eight major projects in affiliated laboratories at The University of Pennsylvania. Several of these projects have been highlighted in previous sections of this report, and have already produced important results and insights for FOP

## The Medical Management of FOP - Online

research. They include work on the definitive identification of target cells in BMP-induced heterotopic ossification, characterization of the role of angiogenesis at two critical phases of skeletogenesis and the potential role of anti-angiogenic agents in modulating those effects, work on developing new adeno-associated viral vectors for use in the noggin therapy experiments, the role of the fibroblast growth factor pathway in BMP antagonist signaling, the role of neural crest stem cells in osteogenesis, the role of glucocorticoids in modulating the BMP-antagonist response to ambient BMP4 levels, the use of microarray gene expression studies in determining the response of chondrocytes to fluoroquinolone-stimulated apoptotic signals in chondrocytes, and the development of the zebrafish as a viable vertebrate animal model for FOP. We are fortunate to have an extraordinarily talented group of research collaborators working with us at The University of Pennsylvania, and we are delighted with the progress and promise that this unique program supports.



Daniel Licht visits with Dr. Kaplan and Dr. Glaser.

In July 2001, we published the comprehensive first edition of **The Medical Management of Fibrodysplasia Ossificans Progressiva (FOP): Current Treatment Considerations.**

The document has been distributed to all members of the IFOPA, and to thousands of physicians and family members around the world. The document is available on the IFOPA website ([www.ifopa.org](http://www.ifopa.org)), can be easily downloaded, and is available to all interested individuals. Due to the gracious help of friends and translators, the document is now available in several languages including English, Spanish, French, Polish, and Portuguese. This important document will once again be updated later this year

### Papers in Press

In 2002, there were six major peer-reviewed publications on FOP and POH. Already for 2003, there are five major peer-reviewed research papers in press, as well as three recently submitted for peer review. Several others are in preparation, and there are more to come!

## VIII. YOUR FOP LABORATORY



Administrative Secretary  
Mrs. Susan Lippo.

*While the core FOP laboratory occupies approximately 2000 square feet of space, our walls have expanded during the past three years with the establishment of the Developmental Grants Project and the wonderful collaborations with colleagues in many departments and at several schools throughout The University of Pennsylvania. The pictures of our FOP and POH 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 laboratory, "This is really **your** laboratory." We love when you come and visit.*

**D**uring 2002, the staff of the FOP Core Research Laboratory included 17 researchers: three principal investigators, five research specialists, four post-doctoral fellows, two graduate students, one medical student, and two pre-medical students. In addition, five undergraduate college students worked on FOP projects in the laboratory during the summer of 2002.



The busy staff takes time out for a photo op when Mr. Richard Simcox from Aberdeen, Scotland, visits the lab. Mr. Simcox, of Roemex, Inc., is second from the left. In 2002, the FOP Core Research Laboratory staff included 17 researchers.

## IX. ACKNOWLEDGEMENTS

In the war against FOP, all of us find ourselves involved in a great battle. The battlefield itself is small, but the consequences for our community are large, and the consequences for the larger world of those who suffer more common afflictions of the skeleton should not be underestimated.

These are hopeful times in our war against FOP. We are thankful that dramatic progress continues to be made. We are proud that important discoveries that are being made in the FOP core and collaborative laborato-

ries are now beginning to be translated into clinical protocols and into pilot studies that can be tested in a stringent scientific manner. When a cure is found, the war will be won. Until then, we will fight the war on all fronts until it is won. We will not tire; we will not yield; we will not relent.

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 a cure. Our work continues to be highly focused and we believe that a great deal of our success to date has been an unrelenting focus on our

goals.

In summary, 2002 was a year of major developments for FOP research and was highlighted by important discoveries on the dysregulation of BMP receptor signaling in FOP, by the initiation of the powerful FOP microarray experiments, by the definitive identification of stem cells from both the muscle and vascular lineage of skeletal muscle that lead to BMP-induced heterotopic ossification, by the development of new FOP animal model systems, by the identification of potential new drugs and drug targets and by the development of safer and potentially more effective viral delivery systems for noggin gene therapy in FOP. As before, we have much work yet to do. We are hopeful that 2003 will be a year of even greater milestones in FOP

research and that exciting discoveries will highlight the year ahead.

The FOP community has traveled a long and difficult road in the past 12 years but it is amazing how far we have come. We continue to be, in fact, a real 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. 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.

The FOP Collaborative Research project arose out of a mutual desire to establish the cause and to find a cure for this disabling condition. We will accomplish that goal and we will prevail. As always, finding a 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 Fellowships in Orthopaedic Molecular Medicine
- The Stephen Roach Memorial Fellowship
- The Roemex Fellowship
- The Grampian Fellowship
- 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
- Genaera Pharmaceuticals
- Johnson & Johnson, Inc.
- Regeneron Pharmaceuticals
- The People of Santa Maria (10 years of extraordinary service)
- And the many individuals, families, and friends throughout the world who contribute generously and tirelessly to the FOP effort.

FOP continues to be one of the most obstacle-ridden and perplexing quandries of the human condition. We have often said that if it were as common as muscular dystrophy or cancer, thousands of scientists and thousands of laboratories around the world would be working to solve the mystery of FOP. 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 skeleton. It is fundamentally the skeleton key.

Thank you, as always, for

your continued generous and heartfelt support of this vital effort.