The Eighth Annual Report of the FOP Collaborative Research Project

by Frederick S. Kaplan, M.D. and Eileen M. Shore, Ph.D.

Drs. Frederick Kaplan and Eileen Shore with FOP patient Carol Orzel at a reception to commemorate the establishment of the Isaac and Rose Nassau Professorship of Molecular Orthopaedic Medicine.

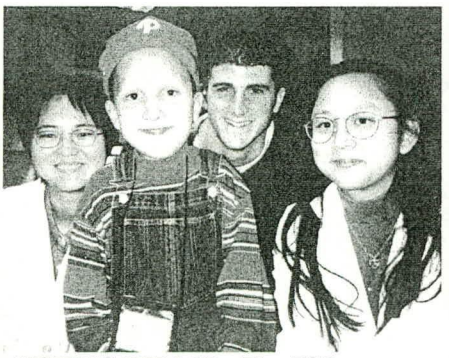
Nineteen ninety-eight was a year of major advances in FOP research. Here we describe some of the most significant developments:

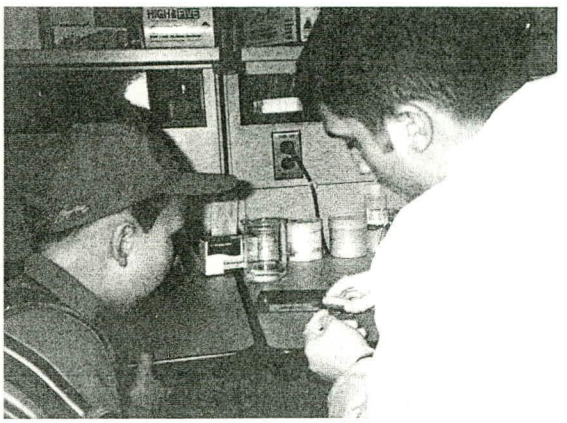
1. The FOP gene was localized to a small region on the long arm of human chromosome 4, a truly remarkable accomplishment considering that it was achieved using DNA samples from only five small multi-generational families that show inheritance of FOP. This scientific approach to localizing the gene for FOP, called a **genome-wide linkage analysis**, was inconceivable just two years ago because of the paucity of multi-generational families, and emphasizes the extraordinary power of cooperation in medical research. This vitally important milestone in FOP research was accomplished with the collaboration of physicians and scientists from the United States, the United Kingdom, and France, and provides an important landmark for all future investigations of the FOP gene.

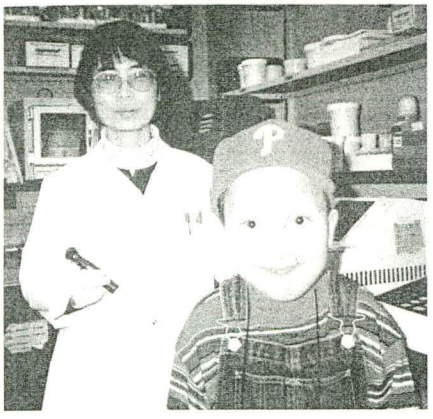
Additional multi-generational families with FOP are required to substantially narrow the identified interval on human chromosome 4. Only then can positional cloning techniques be employed to pinpoint which gene within the interval is the damaged gene responsible for FOP. Consider the following analogy: imagine that all of the approximately one hundred thousand genes in a human cell were stretched out between New York City and Philadelphia—a one hundred mile distance. We have presently narrowed the location of the FOP gene to an interval between two genetic "milestones" that are one mile apart. The FOP gene is somewhere in that one mile stretch, but, in order to find the gene, we must know the street, the house, the room, and even the piece of furniture where the gene is located before we can start opening-up drawers to find it. It is literally like looking for a tiny little key in a crowded drawer. Knowing the one mile stretch where the gene is located is a great start, but we need to narrow the interval substantially before we can find the damaged gene. More multi-generational families will be essential in that search. There is, however, one possible shortcut to that requirement. And that is the **candidate gene approach** or "the best guess approach." It's a very logical place to start, and we'll explain it in more detail in the next section.

2. We know the "one mile stretch" on human chromosome 4 where the FOP gene is located. Within this "genetic neighborhood", we have recently identified about 8 genes that could plausibly cause FOP. We are now methodically studying these plausible candidate genes and examining them for possible mutations. Many of these candidate genes are involved either directly or indirectly in BMP4 signaling pathways. BMP4 is a powerful bone-forming hormone that is overproduced by lesional cells and by certain types of blood cells from patients who have FOP. For many years, we relied on the candidate gene approach as we searched among all the genes in the entire human genome for the FOP gene. The identification of a specific region on chromosome 4 in which the FOP gene resides now allows us to focus our search to that region alone. We also know that many genes that have not yet been identified also exist in that "genetic neighborhood." These genes are currently nameless, and their functions completely unknown. It is entirely possible that the FOP gene is one of those as yet undiscovered genes.

It is technically quite simple, although extremely labor-intensive, to check out all of the plausible candidate genes (about 8) in the "genetic neighborhood," but if none of







VISITING THE LAB. The FOP Molecular Biology Laboratory at the University of Pennsylvania loves getting visitors. These pictures show FOP patient Cody Hickmott, age 10, of Ellsworth, South Dakota getting a personal tour of the research lab from our wonderful research staff. Young Cody even gets an opportunity to participate in ongoing research—and look, he even gets to sit at Dr. Kaplan's desk!

the suspicious culprits turns out to be the FOP gene, then it will be even more critical to substantially narrow the "genetic neighborhood" where the FOP gene is located by finding more multi-generational families. While we meticulously focus on the promising candidate genes, we will continue to search assiduously for more multi-generational families, a dual approach that will allow us to narrow the "genetic neighborhood" while at the same time focusing on promising leads.

3. We have identified the regulatory switch of the human BMP4 gene and are investigating the complex signaling network in which BMP4 acts during skeletal formation and regeneration. This "big picture" approach allows us to map out a "wiring diagram" to better understand how BMP4 is inappropriately "turned on" in FOP and triggered to cause the explosive bone formation that characterizes the condition. In many ways, FOP is like an "atom bomb" that is set to go off. Knowledge of the proteins that control the BMP4 switch will be critical in effectively preventing the bomb from exploding. BMP4 is not simply a powerful protein that triggers bone formation, but part of a highly controlled and exceedingly complex molecular circuitry that regulates the formation and regeneration of the skeleton. During the past several years, at least 25 different genes have been shown to influence BMP4 activity. Many more such genes are likely to be discovered, and our laboratory is dedicated to understanding the complete and relevant "wiring diagram" for BMP4 action as it relates to FOP. Much of our work is devoted to this effort. The more we know about it, the more effectively we will be able to deactivate the explosive bone formation.

4. Considerable progress was made in 1998 toward identifying the earliest cell involved in the formation of an FOP lesion. In essence, we must identify not only the genetic signal that triggers the explosive bone formation, but the cells that reside within the normal muscle, tendon, and ligament that receive that signal and begin the "chain reaction" that leads to the formation of an FOP lesion and eventually bone. Preliminary data indicate that a cell within the muscle tissue (not a muscle cell, but possibly a non-muscle cell residing near or around small blood vessels within the muscle) is involved in the formation of the early lesion. Our current studies are focused on identifying the molecular signature of the responding cell. This will provide us with important knowledge for treating FOP. For example, one approach to therapy is to prevent the renegade signal that triggers "the chain reaction," while another approach is to interrupt the signal by "jamming the receptors" on the responding cell. That cannot be accomplished unless the primary responding cell has been clearly identified.

5. We have made considerable progress in the last year in understanding the range and scope of BMP4 expression in blood cells from people with FOP and

from normal volunteers. This work has fundamental implications for understanding the interaction between the blood-immune system and the skeletal system, and eventually for effectively treating FOP. It has been known for over a century that bone makes blood. What we are learning from FOP is that the blood plays an important role in making bone. The "cause and effect" relationship between the blood-immune system and the skeletal system is exceedingly complex but of central importance to the study of FOP. We now have a much better understanding of the types of cells in normal blood that make BMP4, as well as a clearer perspective on the overproduction of BMP4 by very specific types of blood cells in FOP patients. Transgenic animals (in which BMP4 is overexpressed in various cells of the immune system) have been extremely helpful in trying to dissect the complex pathways by which the blood-immune system interacts with cells responsible for bone formation.

6. We discovered unexpectedly that many non-FOP patients who undergo heart valve replacement surgery form bone in their diseased heart valves, and that BMP is involved in the process. This is an example of an important discovery based on our work on FOP that could help hundreds of thousands of people around the world. Although people with FOP do not form bone in their heart valves, much of what we have learned about FOP has prepared us to make this discovery. Similarly, what we are learning about the process of bone formation in diseased heart valves is already providing important clues to the understanding and treatment of FOP. Reproduced below are excerpts from a news release that accompanied the presentation of the paper on the discovery of bone formation in heart valves.

Laboroatory Co-director Eileen Shore, Ph.D. at her desk. That's a painting from IFOPA member Jack Sholund in the background.

Hard Hearts: New Discovery of Bone in Heart Tissue May Explain Valve Disease

For the first time, researchers at the University of Pennsylvania Medical Center have confirmed that bone — similar to that found in the human skeleton — is present in a substantial portion of diseased heart valves. This finding, which sets the stage for more in-depth research on the biochemical process by which heart valves transform into bone, could lead to the development of therapies to prevent or treat heart-valve disease. The broader problem of valve calcification is the leading reason for heart-valve replacement surgery. According to the American Heart Association, more than 71,000 Americans received the lifesaving procedure in 1995.

This line of inquiry should also help scientists better understand how calcium deposits form in the arteries of patients with atherosclerosis and other vascular disease. And, the information gleaned will be useful to researchers who are studying rare disorders - such as **fibrodysplasia ossificans progressiva** where bone forms outside the skeleton.

"While the problem of valve calcification has been recognized for over 100, years, this is the first study to look at a large series of diseased heart valves and find bone," explains Emile R. Mohler, III, M.D., Director of Vascular Medicine at Penn. Mohler and his colleagues, Frederick Kaplan, Eileen Shore, Frank Gannon and Carol Reynolds presented their findings at the annual meeting of the American College of Cardiology in Atlanta.

The team studied 228 valves removed from patients who underwent valve-replacement surgery from 1994 to 1997 at the Hospital of the University of Pennsylvania. Organized bone tissue, identical to that found in a living human skeleton, was found in 30 of the valves.

The findings of lymphocytes and endochondral bone formation inside the thickened leaflets of the damaged heart valves is nearly identical to those seen in an FOP lesion. But that still leaves the question: How did bone cells get into the heart? "One theory is that, under the right conditions, either valve cells or inflammatory cells at the area of heart valve damage undergo a genetic change and start making bone-cell proteins," suggests Mohler. "But the most important question is: What's the trigger?" The team has identified the overproduction of BMP in the diseased heart valves forming bone, similar to their findings in FOP.

The team's ultimate goal is to devise a treatment to prevent the bone in the first place. Knowing how and why bone forms in the soft tissue of the heart valve may lead to

a preventative or corrective therapy. This is a great example of how research in a rare disease like FOP can help solve a common problem that threatens the life of thousands of people every year, and how the two together can bring better answers for both.

7. In a remarkable and unexpected discovery, the Progressive Osseous Heteroplasia (POH) collaborative research group discovered the damaged gene responsible for POH (a condition related to FOP, and first described by us in 1994). The gene was discovered serendipitously after seeing several patients whose symptoms provided a clue to a plausible candidate gene. When that candidate gene was examined, changes in the DNA sequence of the gene were found. Several of these changes could be immediately recognized as causing the production of a non-functional protein. At the present time, we are investigating all patients with POH (about 30 that we have identified from around the world) to determine if they all harbor a mutation in the same gene. The gene that we have identified for POH encodes a protein located on the inside of the cell membrane in nearly every cell in the body. The protein is versatile and appears to have different functions in different cells. Generally, the protein functions as a relay switch in a multi-protein complex that monitors the environment of the cell and sends messages to the nucleus instructing the cell not what to do, but rather what to become. An enormous amount of additional work is necessary to understand exactly how this abnormal protein triggers ectopic bone formation, but early clues indicate that it may normally act to block the activity of other genes involved in bone formation. When the switch is broken, the inhibition ceases, and the cell becomes a bone cell by default. In children who have POH, bone formation occurs in the skin and fat tissue underneath the skin and then progresses into deeper tissue such as muscle, tendon, and ligament. POH can be as disabling as FOP if it is extensive in its distribution. We also discovered that part of the molecular pathway identified in POH is also involved in FOP even though the genes that cause the two conditions are different (the FOP gene is located on chromosome 4, while the POH gene is located on chromosome 20).

The unexpected and serendipitous discovery of the POH gene is an important development in bone biology and of paramount importance in understanding the earliest cellular and molecular pathways in bone formation. This discovery has profound implications in developing treatments for POH, but is also highly relevant in understanding critical cellular, and molecular pathways in FOP and in many more common diseases of bone formation. It is also a wonderful example of how the candidate gene approach ("best guess approach") can sometimes pinpoint the damaged gene without having to resort to detailed family studies. POH is even rarer than FOP, with fewer than three dozen known patients throughout the world. We are completing mutational studies on all known POH patients, and will write-up this important discovery for the peer-reviewed scientific and medical literature.

8. The Center for Research in FOP and Related Disorders was established in December 1998 at the University of Pennsylvania in order to expand research efforts on FOP beyond the core laboratories into related disciplines. A multi-disciplinary approach to understanding FOP is critical to developing a cure for this devastating disorder. The proposal for the Center grew out of lengthy discussions between Mr. John Cali (patron of FOP research at The University Pennsylvania), Dr. Kaplan, Dr. Shore, Dr. William N. Kelley (Dean of the School of Medicine), and Dr. Robert H. Fitzgerald, Jr. (Chairman of the Department of Orthopaedics). There is substantial interest at The University to broaden our research efforts into disciplines such as immunology and gene therapy that will be germaine to the cure for FOP.

A major function of the Center will be the funding of a developmental grants program that will allow other investigators at the University (in disciplines related to our primary mission) to become involved in FOP research in order to find a cure for FOP. The Center Director is Dr. Frederick Kaplan, and the Center Co-Director is Dr. Eileen Shore.

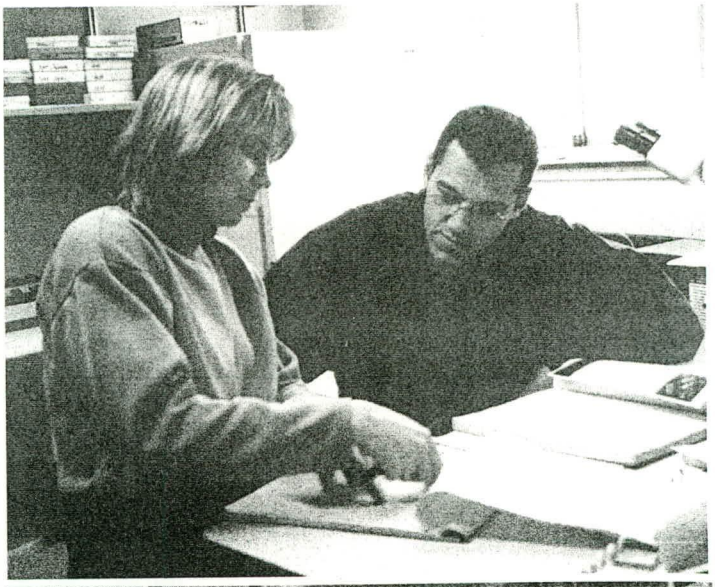
9. Phase I human safety trials of squalamine, a potent anti-angiogenic molecule that inhibits the formation of small blood vessels continues in two cancer therapy studies in humans. Comprehensive safety data should be forthcoming by mid 1999, and are required by the FDA before clinical trials with squalamine can begin in patients who have FOP. Our collaborators at a pharmaceutical company continue to work at a robust pace to identify the cellular protein receptors on which squalamine acts to inhibit the formation of blood vessels. Such information will be essential in identifying the squalamine receptors in early FOP lesions, and will allow us to determine the most effective "time window" for squalamine therapy when treating an FOP flare-up. Also, considerable progress has been made (using several animal models) in deciphering the role and timing of blood vessel formation in the earliest stages of BMP-induced bone formation. Without blood vessels, bone does not form. In summary, comprehensive data on the safety of squalamine in humans, and comprehensive biochemical data on the identification, location, and distribution of squalamine receptors in FOP tissues should be forthcoming in 1999, and will allow us to proceed to the next step in designing clinical protocols for testing squalamine in FOP. We anticipate that the squalamine clinical trials in FOP will be targeted to the treatment of early pre-osseous flare-ups.

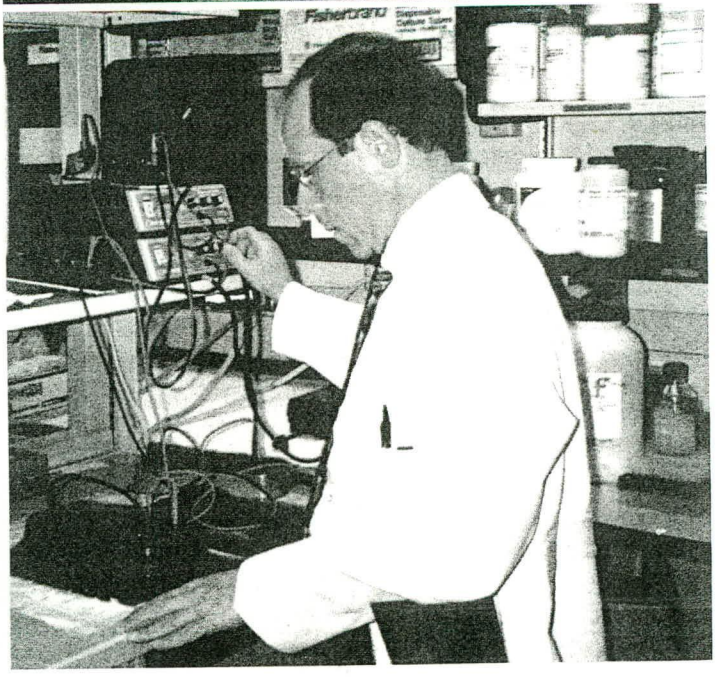
10. A Phase I safety-efficacy trial of thalidomide has been approved by the FDA for 15 patients with FOP, and patients with active flare-ups are currently being enrolled. Basic fibroblast growth factor is overproduced during FOP flare-ups. Thalidomide is an inhibitor of fibroblast growth factor-induced blood vessel formation. In addition, thalidomide is an immune-modulator (affects cells of the immune system) and may play a role in the very early FOP lesions where lymphocytes (circulating blood cells from the immune system) attack the body's skeletal muscles, and replace them with bone cells. Therefore, thalidomide may be a useful drug in limiting the development of early FOP lesions. Preliminary data from this limited Phase I study will be forthcoming in 1999.

11. An anti-BMP4 protein has been shown to block BMP induced bone formation and is being developed for gene therapy treatment for FOP. In 1996, studies in our laboratory showed that BMP4, a powerful bone-inducing hormone, was overproduced in specific blood cells and lesional cells of patients who have FOP. At exactly the same time, in another laboratory, the protein **noggin** was discovered. Noggin avidly binds to BMP4 and antagonizes its activity. Animals genetically engineered to develop without any noggin protein have an overabundance of active BMP4 and form excessive amounts of bone during development. These and other findings suggested that noggin may be useful in treating FOP. Studies were conducted last year to examine the effects of noggin on inhibiting BMP-induced bone formation in an animal model. **The results of these studies dramatically proved the principle that noggin can effectively inhibit BMP-induced heterotopic bone formation!**

Naturally produced noggin protein has a very short half-life in the body and would have to be administered frequently for any sustained therapeutic effect in FOP. Therefore, our collaborators at a pharmaceutical company developed several modified forms of noggin that have longer half-lives in the body. During the past year, we performed experiments to determine whether these modified noggin molecules were also effective in inhibiting BMP-induced bone formation. **The results of the experiments showed conclusively that modified noggin molecules were as effective as naturally produced noggin in inhibiting BMP-induced bone formation.**

A highly attractive alternate strategy to industrially-manufactured noggin is a gene-therapy approach where the body would manufacture its own modified-noggin protein, and would do it much more economically and at a vastly higher capacity than could be accomplished industrially. Gene therapy is a powerful new technology that enables the production and delivery of a therapeutic protein under pharmacologic control by transferring extra copies of the desired gene to the body for production.





Although gene therapy has had some difficult technical problems, the successful delivery of a therapeutic protein following gene transfer to live animals was achieved recently and reported in the January 1, 1999 edition of the journal, **Science.** This work was conducted by The Institute for Human Gene Therapy at the University of Pennsylvania.

We embarked on an exciting collaboration in late December with the pharmaceutical company and the Institute for Gene Therapy to develop regulated production and delivery of noggin by gene therapy. The goal of gene therapy with noggin will be to deliver extra copies of the modified noggin gene to the body to enable the body's cells to make the protein and deliver it in high concentration to the site of an early FOP flare-up, or to an operative site where heterotopic bone has been surgically removed. This will eventually be accomplished by injecting the modified noggin gene (packaged in an inactivated carrier virus) into the bloodstream where it will be taken up by the liver and used to manufacture and secrete noggin.

In addition to the genetic production of the modified-noggin protein, the technology now exists for regulating when the modified noggin is produced by the body. This will eventually be accomplished by swallowing a pill or liquid that will activate the body's production of noggin only when it is needed. Our immediate goal during the next year is to establish the safety and efficacy of noggin gene therapy (using the pharmaceutically-modified noggin gene) in an animal model.

These exciting new developments in the field of gene therapy, pioneered by our colleagues at Penn, provide an extremely practical approach to the use of noggin in inhibiting heterotopic ossification in FOP. In addition, noggin gene therapy will provide the safest and most controlled method of applying the potential benefits of noggin to the treatment of FOP.

MEETINGS, REPORTS AND PUBLICATIONS

In July 1998, The European Neuromuscular Center (ENMC) sponsored a follow-up international workshop on FOP in Naarden, The Netherlands. The small meeting was attended by eight members of the international FOP consortium from England, France, The Netherlands, and the United States. Professor Roger Smith (Oxford), Frederick Kaplan (University of Pennsylvania), and Eileen Shore (University of Pennsylvania) were co-chairpersons of the workshop.

The purpose of the workshop was to review progress in FOP research over the past year; specifically the international collaborative efforts to localize the FOP gene through a genome-wide linkage analysis. The workshop was extremely helpful in allowing the small group of international collaborators to exchange information rapidly, and to engage in intense discussion on the molecular, cellular, and physiologic aspects of FOP. Tremendous progress was reported in the collaborative FOP research project during the past year. This progress was highlighted by the localization of the FOP gene to the long arm of human chromosome 4. As a result of the meeting, two additional FOP families were identified. At the present time, there are a total of five multigenerational families with FOP identified worldwide that are included in the genome-wide linkage analysis. We anticipate the addition of a sixth family within the next few months.

In 1998, major presentations of FOP research were made by members of the FOP laboratory at:
The ENMC International Workshop on FOP, Naarden, The Netherlands; Cornell University School of Medicine, New York City, New York; Jefferson Institute of Molecular Medicine, Philadelphia, Pennsylvania; National Institutes of Health, Bethesda, Maryland; Ohio State University Medical Center, Columbus, Ohio; Oxford University; Oxford, United Kingdom; University of Zagreb School of Medicine, Zagreb, Croatia; American College of Cardiology, Atlanta, Georgia; American Society for Bone and Mineral Research, San Francisco, California; International Bone & Mineral Society, San Francisco, California; Boston University School of Medicine, Boston, Massachusetts.

In 1998, the Isaac and Rose Nassau Professorship of Orthopaedic Molecular Medicine was "officially" established at the University of Pennsylvania School of Medicine. The Isaac and Rose Nassau Professor of Orthopaedic Molecular Medicine is Frederick Kaplan. The professorship was established by Mrs. Diane Nassau Weiss to honor the memory of her father and mother, Isaac and Rose Nassau, and to promote research to comprehend and to conquer two devastating genetic disorders of renegade bone formation in children: fibrodysplasia ossificans progressiva (FOP) and progressive osseous heteroplasia (POH).

This unique professorship endows a mission of discovery that will bring the bounty of molecular biology to the bedside of patients who suffer from these and other crippling disorders that demand our most creative attention. It is the hope of all of us in the FOP Laboratory that our journey to find answers for FOP and POH will allow us to find cures for these disorders, and as a result of those discoveries to develop therapies for more common disorders of the skeleton that affect so many millions of people throughout the world. The gift that Mrs. Weiss has so generously provided is the gift of time to achieve these goals and to rekindle hope. The discovery this past year of the genetic location of the FOP gene, the proof of noggin's effectiveness, the technology necessary for

effective gene therapy, the identification of the gene responsible for POH, and the discovery of the formation of bone in diseased heart valves, are only a few of the many exciting discoveries that put us on course to achieving our mission.

During the past year, more than 30 research articles on FOP were published by members of the FOP research laboratory. In addition, seven papers on FOP and POH were presented by members of the FOP Laboratory at the combined international meeting of The American Society for Bone and Mineral Research and the International Bone and Mineral Society in San Francisco, California on December 1-6, 1998.

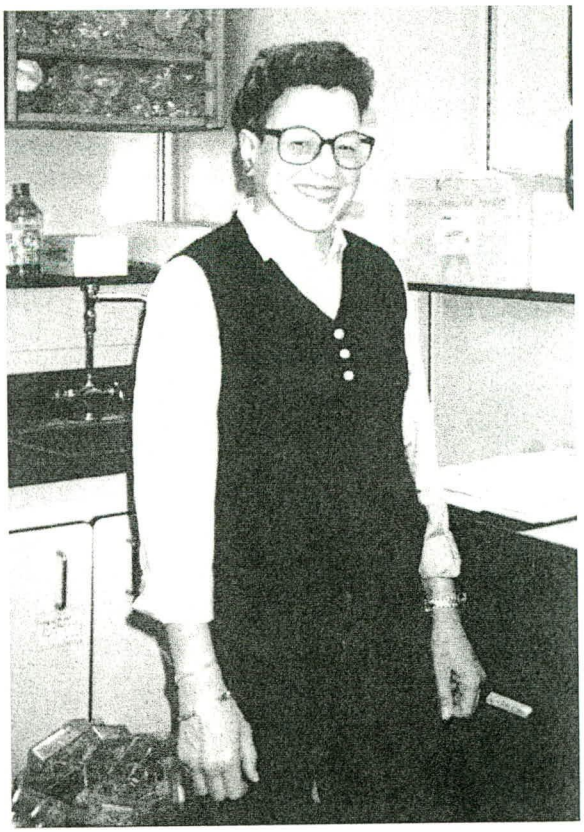
In addition to these peer reviewed scientific articles, work from our Laboratory was featured in an article entitled, "A Few Hundred People Turned To Bone" by Thomas Mader in the February 1998 edition of ***The Atlantic Monthly.*** An article by Andy Coughlin entitled, "Facing Down Medusa: An Illness That Turns Muscle to Bone Is Yielding Its Secrets" was published in the October 17, 1998, edition of ***New Scientist*** (London, England), and featured just a few of the new findings highlighted in this report. Also in 1998, the article "Bound By Bone," written by Corey Ullman and published in the Winter 1997 edition of ***PENN Medicine***, was awarded the top prize for excellence in feature writing by the American Association of Medical Colleges. The article on FOP was selected as the best story published by any medical center in the United States in the past year.

THE FOP LABORATORY

The current staff of the FOP Research Laboratory includes 16 researchers: 2 principal investigators, 7 research specialists (one part-time), 3 post doctoral fellows (one part-time), 1 graduate student, and 3 medical students. The medical students are supported by extramural grants from The Howard Hughes Medical Institute, The Four Schools Program (University of Pennsylvania, Johns Hopkins, Washington University, Duke), and the Gund-Raiffe Student Fellowship. During 1998, the FOP Laboratory doubled its size to nearly two thousand square feet of space. The pictures of our FOP and POH children adorn the hallways and are a constant reminder of our goals and our mission. This is really their laboratory. Please come and visit us!

CAUSE AND CURE

We are thankful that dramatic progress continues to be made in understanding the genetic, molecular, cellular and physiologic basis of FOP. *Cause* and *cure* are the two words that continue to propel the international collaborative research effort and provide the guiding principle for all we do: to discover the exact molecular cause of FOP and to use that knowledge to develop therapies that will be truly effective in preventing, treating, and curing FOP. Nineteen-ninety-eight was a year of tremendous discovery for FOP. We are hopeful that 1999 will be a year of additional great milestones in FOP research. We have all come a long way in eight years, but still have a long way to go.



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