The First Annual Report On The Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project August, 1991

Frederick S. Kaplan, M.D.* and Michael A. Zasloff, M.D., Ph.D.

The University of Pennsylvania collaborative research efforts in FOP were established in September 1989, by Dr. Michael Zasloff, Chief of the Division of Molecular Biology and Human Genetics at Children's Hospital of Philadelphia, and by Dr. Frederick S. Kaplan, Chief of the Division of Metabolic Bone Disease at the Hospital of the University of Pennsylvania. These efforts arose out of a mutual desire to establish the cause and find a cure for this disabling disease. For the past 15 years, we have been involved in the evaluation and treatment of patients with FOP and have been frustrated by the lack of a comprehensive molecular basis for understanding developmental patterns and progressive deformity in this disorder.

FOP is an extremely rare development disorder of connective tissue characterized by congenital malformations of the hands and feet and by progressive bone formation in the tendons, ligaments and muscles of the spine, torso, and limbs. Progressive bone formation usually begins during the first decade of life and is often heralded by painful swellings of the spine and limbs. These swellings often mature into normal bone that bridges and rigidly immobilizes the joints, making movement virtually impossible. Bone formation can be triggered by slight trauma but can occur spontaneously. Once new bone has formed, it remains for life. FOP is different from other forms of abnormal bone formation in that any attempt at surgical removal stimulates an even more robust response of new bone formation. To date, no standard medication or therapy purported to be helpful in slowing other forms of abnormal bone formation have been helpful in FOP.

Patients with FOP may experience a normal life span, or may be imperiled by complications of this disease, most commonly respiratory compromise — a result of abnormal bone formation bridging the chest and restricting the mechanisms of breathing.

In order to intelligently search for a cause of the disease, we decided first to reexamine the mechanisms by which bone forms in FOP, and then examine those genes thought to be involved in the earliest induction of such bone development.

We realized that even our earliest search would be hindered by these obstacles:

1. Sources of biopsy specimens would be very rare and sporadic due to both the rarity of the disease and the knowledge that surgical biopsy would stimulate new bone formation.
2. Lack of a central repository for such specimens.
3. Mistaken diagnosis of FOP for other more limited disorders of abnormal bone formation.

We overcame these obstacles by:

a) Contacting our patients and other known patients with FOP who had known previous biopsies.

b) Contacting hospitals around the nation and obtaining release of surgical specimens with patient's approval.

c) Contacting the Armed Forces Institute of Pathology (AFIP) which is known to have a collection of human tissue pathology specimens especially in the musculoskeletal field.

d) The formulation of stringent inclusion and exclusion criteria for the diagnosis of FOP, so that it would not be confused with other disorders with features similar to FOP.

Through these efforts, we were able to identify 13 potential cases of FOP nationwide in which a surgical or biopsy-specimen was available for examination. After applying very strict diagnostic criteria for the presence of FOP, we were able to certify that six of the patients, in fact, had the disease. We reviewed all of the cases with radiologists and bone pathologists and were able to document a very specific mechanism of bone formation in all cases.

Bone forms normally by one of two mechanisms. In one type of bone formation, bone cells manufacture new bone directly on top of old bone. Such bone formation occurs in the skull, ribs, and on the outer surface of the long bones of the limbs. It is also the mechanism by which the human body repairs and maintains the skeleton throughout life.

In the other major type of bone formation, bone forms around a preformed sponge-like cartilage scaffold. It is through this mechanism that our bones grow in length, and repair and regenerate following a fracture. This latter type of bone which arises from a cartilage precursor (endochondral) is the type of bone formation we identified in all documented cases of FOP. That startling finding provoked us to seek the genes that controlled such bone formation in humans.

*Chief of the Division of Metabolic Bone Disease, Hospital of the University of Pennsylvania, Philadelphia, PA, 19104

We were aware of the seminal work of Dr. Marshall Urist at UCLA School of Medicine (who isolated and attempted to purify a factor called bone morphogenetic protein (BMP). BMP is a protein factor which is responsible for the formation of bone by the mechanism of mesenchymal tissue transformation. This work was later undertaken by Dr. John Wozney of the Genetics Institute in Boston. Dr. Wozney isolated and cloned the cDNAs for the bone morphogenetic proteins. He showed that several of the recombinant BMPs expressed from the cDNA clones were uniquely responsible for the formation of normal bone through a preformed cartilage model, and that these proteins were uniquely capable of forming bone outside of the normal skeleton. This major breakthrough occurred in late 1988 and was very timely for our work on FOP.

Dr. Brigid Hogan from Vanderbilt University showed, through a series of elegant studies in mice, that BMP mRNA was expressed at the earliest stages of limb formation. Thus, evidence was available to substantiate that these gene products could stimulate new bone formation by the same mechanism that had been identified in normal bone growth, fracture healing, bone development, and FOP. The discovery of these gene products by Wozney and colleagues permitted a search of the genetic data base for any similar proteins in humans or other species. The BMPs were found to be members of a large family of regulatory molecules called transforming growth factors that determine tissue differentiation pathways.

It was a great surprise, however, to learn that within this family of molecules, the one most closely related to BMP (in fact nearly 75% similar) was a specific gene product in the fruitfly called *Dpp*. The amazing similarity of the BMP gene product in man to the *Dpp* gene product in the fruitfly across so large an evolutionary distance seemed too striking to be coincidental and suggested that the BMP genes in man and the nearly identical gene in the fruitfly might have been derived from a similar ancestral gene. Since flies lacked bones, what could the connection be? What did this gene called *Dpp* regulate in the fly, and by inference what might we learn about its BMP homologue in man? We met with Dr. William Gelbart, the Chief of Developmental Biology at Harvard and the discoverer of the *Dpp* gene. We learned that mutations in the *Drosophila Dpp* gene in the fly led to striking abnormalities in pattern formation almost identical to the patterns of progressive abnormal bone formation seen in patients with FOP.

To summarize:

1. We knew that bone formed in FOP from induction of mesenchyme through a preformed cartilage model.

2. Bone formed in FOP in highly specific patterns that mimicked the embryological patterns of normal trunk and limb development in man.

3. Several newly discovered BMP gene products were uniquely responsible for the formation of bone by those endochondral mechanisms outside of the normal skeleton.

4. Several BMP gene products were strikingly similar to gene products that regulated body plan in the fruitfly. Abnormalities in these fruitfly genes mimicked abnormalities in patterns of bone formation seen in patients with FOP. This startling revelation prompted us to formulate a new theory in which we proposed that inappropriate expression of BMP genes in non-skeletal connective tissue might be responsible for FOP in man. At the very least, learning about the expression of BMP in man, would likely help us learn more about renegade bone formation in FOP. We developed a theory in a paper entitled, "FOP: A CLUE FROM THE FLY." The paper was published in *Calcified Tissue International* in August 1991, and the response of the international community was robust and extremely gratifying.

Our next step was to obtain blood samples on a large panel of normal individuals not affected with FOP and methodically identify the normal variability in the BMP genes within the population. Only then would we be able to begin to discern whether any variations that may be seen in FOP patients were unique to the disease.

To date, we have identified the natural variability in two BMP genes and are beginning to examine the DNA of patients with FOP to see whether it harbors any evidence of large mutations or rearrangements that could implicate it as a prime factor in the causation of FOP. So far, seven BMP genes have been identified by Wozney and colleagues and much work remains to be done even on this very isolated and tantalizing avenue of investigation.

To help advance our understanding of the role of BMP genes in humans, our laboratory has undertaken the chromosomal localization (or mapping) of these important bone formation and regulating genes. The localization of a gene to a specific region in the human chromosomes establishes a genetic landmark that can be used as a valuable molecular tool to further identify diseases either already associated with that chromosome or that will later be mapped to that or nearby locations. Earlier this year, our laboratory localized three of the BMP genes — each to a separate human chromosome. The results of our first three localizations were published earlier this year in the journal, *Genomics*, and drew international attention.

We have recently completed the localization of a fourth gene to yet another chromosome, and are pursuing the localization of the three remaining bone morphogenetic protein genes thus far identified.

Our ongoing work on the search for a molecular cause for FOP has necessitated the use of precious DNA extracted from the blood of patients with the disease. In order to assure a robust supply of DNA for future work, and to alleviate the necessity of repeated blood tests, we have enlisted the collaboration of a colleague, Dr. Max Muenke, of the Division of Human Genetics at Children's

Hospital, to participate in the culturing and immortalization of blood cells from FOP patients. This will virtually ensure a continuous source of precious DNA from FOP patients for ongoing and future research. During the next year, we will further intensify our research efforts to identify the normal variability in all seven BMP genes and meticulously screen panels of DNA from FOP patients to see whether or not any gross rearrangements, additions, or deletions might have occurred within those BMP genes.

In addition to our search for a molecular and genetic basis of the disease, we have recently begun a complex series of biochemical experiments based upon a discovery of one of us (M.Z.) that indicated an elevation of a hormone like molecule in the serum of patients with FOP. These prostaglandin-like molecules have profound effects on the functioning of many organs, and have been shown to play an important role in the regulation and formation of bone.

Other efforts of our FOP working group have been the identification of a wide spectrum of bone-forming tumors seen in patients with FOP. These benign tumors all involve abnormalities in the transformation of connective tissue to cartilage and bone, and had not previously been recognized as part of the spectrum of FOP. These findings provided additional support for the pathological basis of the condition. The results of this work have been submitted for publication. The results of the primary histopathological findings, and yet another study that explores the relationship between bone formation in FOP, and primary fracture repair callus are being prepared for publication.

A recently completed project involved the identification of a unique family with FOP with two afflicted family members in two successive generations. Despite the working hypothesis that FOP is a genetic condition, reproductive fitness is low among adults afflicted with the disorder. There has been a striking paucity of tangible data on the genetic transmission of the disease. Our study provides evidence for the hypothesis on the genetic basis of FOP and will soon be submitted for publication in a peer-review journal.

While the search continues for the molecular basis of FOP, patients with FOP continue to suffer the intemperate ravages of the disease and its subsequent devastating disability. One of us, (M.Z.) had observed years ago, that certain Vitamin-A analogues caused birth defects along the same connective tissue to cartilage pathways that appeared to be inappropriately stimulated in FOP. However, these compounds do not affect cartilage once formed, and, would not likely adversely affect the growth of normal bones. However, they could possibly be used to impair the transformation of primitive tissue into cartilage, and thus its transformation into bone. A compassionate-use study was begun to treat children with FOP with these Vitamin-A analogues in an attempt to retard the transformation of primitive tissue to cartilage, a known precursor of bone formation in FOP.

Preliminary phase-I results are encouraging but await more detailed analysis and a larger recruitment of patients.

We have recently completed the largest prospective survey on the natural history of FOP. These results will provide valuable information to clinicians and families and extend previous detailed observations by Connor and others on the natural history of the disease. Those findings will be presented at an international meeting on bone metabolism in California in August 1991, and will be submitted for publication.

In order to accommodate a greater number of research scientists in the FOP research project, we will during this upcoming year be increasing our available laboratory space by approximately 1500 square feet, along with a substantial expansion of equipment and facilities.

Finally, the First International Symposium on FOP convened at Children's Hospital of Philadelphia and at The University of Pennsylvania School of Medicine on September 25-26, 1991.

This marked an historic milestone for the FOP community, as world leaders in the basic and clinical sciences of bone metabolism, genetics, and developmental biology gathered to exchange data and ideas on the complex pathophysiology of FOP.

Although FOP is thought to afflict fewer than 2500 people worldwide, a complete understanding of this devastating disorder will likely lead to advances in our understanding of more well-known and common disorders of bone formation such as osteoporosis and certain forms of bone cancer.

In summary, these past two years have been productive ones in the collaborative FOP research project. But it is a mere beginning of what must be accomplished. The members of the FOP collaborative research project greatly acknowledge the generous support of our sponsors in helping all of us to achieve our ultimate goals.

Sponsors:

1. International Fibrodysplasia Ossificans Progressiva Association (IFOPA).
2. Jud Bogard Invitational Golf Tournament Sponsors.
3. American Heart Association Student Fellowship Program.
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5. The Hartford Foundation.

FOP Collaborative Research Project Members:

Michael A. Zasloff, M.D., PH.D., Co-Director,
Frederick S. Kaplan, M.D., Co-Director.

FOP Research Fellows and Scientists:

1. John Campbell, M.D., 2. Randolph Cohen, B.A., 3. Frank Gannon, M.D., 4. Gregory Hahn, B.A., 5. Craig Levitz, B.A., 6. Max Muenke, M.D., 7. Jeffrey Tabas, M.D.