The Second Annual Report on the Fibrodysplasia Ossificans Progressiva (FOP) Collaborative Research Project August, 1992

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This year's extraordinary burst of activity in FOP research was catalyzed by The First International Symposium On FOP which convened in Philadelphia on September 25-26, 1991. Detailed accounts, and abstracts of this historic event have been published in *Calcified Tissue International*, *The University of Pennsylvania Orthopaedic Journal*, *The FOP Connection*, and *The I-Care Bulletin*. The symposium was documented on a television special which aired twice on prime-time TV in the Philadelphia metropolitan area, and in several news stories transmitted nationally and internationally by The Associated Press. The wide attention has served to invigorate and unite all aspects of the FOP community and it has catapulted awareness of this problem into a research perspective which encompasses osteoporosis, osteoarthritis, congenital limb defects, disorders of fracture healing, and other diseases involving abnormalities of bone formation.

Among the long-term consequences of the meeting was a heightened sense of urgency to discover the cause and find a cure for FOP. The most tangible expression of this accomplishment catalyzed an international collaboration of physicians, scientists, and FOP patients on the design of several two-to-five-year research proposals on the investigation of FOP.

The three major grants that have been written on FOP research include a two-year proposal to the Orthopaedic Research and Education Foundation, a four-year proposal to the National Institutes of Health, and a five-year proposal to the International FOP Association. These research proposals have been made possible by a series of accomplishments and discoveries relating to FOP during the past two years. These early studies have established that:

1. **FOP is a Genetic Disease.** Previous reports of the genetics of FOP were based upon the assumption of: spontaneous mutations, low reproductive fitness, a paternal age effect, several pairs of concordant twins, and anecdotal reports in the medical literature (prior to 1920) of several members of an affected family. We recently learned of a family from rural Georgia with an affected father and three affected children (two girls and a boy). A visit with the family at the Nemours Children's Clinic in Jacksonville, Florida stringently documented the presence of FOP in the father and in all three children. Blood samples were obtained for genetic studies which will permit for the first time the preliminary linkage analysis of potential candidate genes. A paper entitled "Genetic Transmission of FOP: Report of a Family" will be published in *The Journal of Bone & Joint Surgery* . Detailed molecular genetic studies on this family will begin this year.

2. **Bone Formation in FOP Occurs Predominantly Through a Precursor Cartilage Pathway.** In all vertebrates, normal bone formation occurs through one of two major pathways. Our studies on FOP have revealed that the extra bone formation characteristic of the disorder forms exclusively through a precursor cartilage pathway. Thus, a search for a candidate gene responsible for the disorder must take that fact into account. During this past year, we have been able to complete an extensive evaluation of twelve biopsy specimens on eleven patients with FOP. Analysis revealed bone formation through a cartilage pathway in all eleven patients. Specialized testing of the tissue revealed pre-osseous cartilage tissue in even the earliest of the lesions. These findings have been documented in a paper entitled "The Histopathology of Fibrodysplasia Ossificans Progressiva," and will be published in *The Journal of Bone & Joint Surgery*.

3. **Bone Formation in FOP is Not Random but Highly Predictable in its Patterns and Progression.** Preliminary hypotheses on the patterns of bone formation in FOP were described in a paper entitled "FOP: A Clue from the Fly," by Drs. Kaplan, Tabas, and Zasloff in 1989. During the past year, our group has completed a survey of forty-four patients of the international FOP community and has stringently confirmed highly predictable patterns of bone formation in the natural history of the disease. These patterns suggest that the involved gene (or genes) might also regulate pattern formation in bone development. The results of this extensive survey are documented in a paper entitled "The Natural History of FOP," which has been accepted for publication in *The Journal of Bone & Joint Surgery*.

4. **The Bone Morphogenetic Proteins (BMPs) are the Only Proteins Yet Discovered Which Are Capable of Forming Bone Outside of a Bony Environment.** This protein fraction was first described by Dr. Marshall Urist in the late 1960s, and the coding regions for these genes were cloned by Dr. John Wozney and colleagues at the Genetics Institute in 1988. To date, these remain the most plausible candidate genes for FOP.

5. **The BMP Genes in Man are Strikingly Similar to a Gene (decapentaplegic: dpp) in the Fruit Fly Which Regulates Pattern Formation and Limb Development.** These genes are highly conserved throughout evolution, and we believe that they provide valuable clues to the pathogenesis of FOP in man.

6. **One of the BMP Genes in Man Can Rescue a Lethal Mutation in the Fly.** This study was recently completed by our colleagues Drs. Padgett, Wozney, and Gelbart, and was submitted to a journal for peer review. This experiment establishes the extraordinary conservation of the dpp/BMP genes over half-a-billion years of evolution. This experiment provides even more tangible evidence that the overexpression of the BMPs may be associated directly or indirectly with FOP in man.

7. **All of the Known Human BMP Genes Have Been Assigned to Chromosomes in Man.** This chromosomal assignment study has provided extremely valuable information for chromosomal localization of disorders of cartilage and bone formation possibly associated with these genes. This work has provided valuable information to scientists and physicians working in numerous fields related directly or indirectly to the problems of FOP. A report documenting the human chromosomal localization of BMP1, BMP2, and BMP3 has been published in the journal *Genomics*, by Tabas, et al. in 1990. A report on the human chromosomal localization of BMP4 has been submitted for peer review. A report on the human chromosomal localization of BMP5, BMP6, and BMP7 has been accepted for publication in *Genomics*. A recently completed report on the sublocalization of BMP1 on human chromosome 8 has also been submitted to *Genomics*. These data will become part of the human genome data bank and will provide landmark information on the location of these genes and possible disease associations.

8. **There Are Elevated Levels of a Substance That Cross Reacts with an Antibody to Prostaglandin E_2 (PGE_2) in the Blood of Patients With FOP.** This molecule is not a bone stimulating prostaglandin, but a small polar molecule that cross reacts with the antibody to PGE_2. Studies during the past year have confirmed the presence of this molecule in the serum of patients with FOP. Although the molecule is enriched in the blood of patients with other bone forming diseases, the highest levels were seen in FOP patients. The quantity of the molecule is very small, and further isolation and characterization will be needed.

9. **A Distinct New Bone Disease Related to FOP Has Been Discovered.** This disease called Progressive Osseous Heteroplasia (POH) is similar to FOP in its ability to form bone in abnormal locations but distinctly different from FOP in the method, pattern, and progression of bone formation. This disease is even more rare than FOP; and to date, only seven patients in the world have been documented with it. In addition, patients with this disorder form bone in the skin. The recent discovery of two children with this disorder, (one from New Orleans, Louisiana, and the other from Portland, Oregon) has substantially increased our knowledge of this rare but important problem. Studies on the DNA from the children in New Orleans, and Portland as well as available DNA on a patient from New Zealand will enable a much more profound understanding of the molecular nature of this disease. This disorder will be documented in a research paper in *The Journal of Bone & Joint Surgery*. The discovery of this disease provides a startling contrast to FOP and an important focus for comparative molecular research. To date, the severe manifestations of Progressive Osseous Heteroplasia have been seen only in girls.

Other major accomplishments in FOP research during the past year have included:

10. **The Establishment of a Laboratory Devoted Primarily to the Investigation of the Molecular Genetics of FOP and its Candidate Genes — The Bone Morphogenetic Proteins.** This laboratory has been established in the Department of Orthopaedic Surgery at the University of Pennsylvania School of Medicine and is co-directed by Dr. Frederick Kaplan, Dr. Michael Zasloff, and Dr. Eileen Shore (research scientist). Dr. Shore received her Ph.D. from the University of Pennsylvania and completed post-doctorate work in molecular genetics at the Fox Chase Cancer Center in Philadelphia. The establishment of a molecular genetics laboratory whose efforts are focused on FOP research will enable a more rapid advancement of research in FOP. As funding for various projects becomes available, we look forward to approximately six full-time positions in FOP and related research.

11. **Immortalized Cell Lines Have Been Established in 41 FOP Patients and Related Family Members.** This collaborative work undertaken with Dr. Max Muenke at Children's Hospital of Philadelphia will ensure a perpetual source of extremely valuable FOP DNA for ongoing research efforts.

12. **The Identification of Normal Variations in the BMP Gene Pattern in Patients With FOP.** These preliminary studies have identified variations in BMP gene patterns in the normal population as well as in patients with FOP. A recent finding of potentially great significance is the discovery of a unique BMP gene pattern in a child with FOP. Blood samples were obtained on other family members, none of whom have the pattern seen in the child with FOP. Extensive research during the next year will attempt to determine the exact nature of the rearrangement of the BMP gene in this child. Complete clones of the normal gene will be obtained from our collaborators and compared with changes seen in this child. If confirmed, this finding could provide valuable insight into the genetic nature of FOP.

13. **Work Has Begun on Cloning the Regulatory Regions of All of the BMP Genes.** Although the protein coding regions of the genes have been identified, the regulatory control regions have not yet been identified. Such knowledge will be critical to a more complete

understanding of bone formation in FOP and related disorders.

14. **A Dental Survey Was Completed and Is Being Prepared for Publication**. This information, obtained from more than forty patients with FOP, will help provide guidelines for dental care.

15. **Immunization Guidelines Have Been Developed**. A major concern of parents of children with FOP has been the policy of pre-school innoculations. As intramuscular injections can stimulate new bouts of bone formation, a modification of immunization policy is necessary for children with FOP. Preliminary guidelines have been established in cooperation with the immunization section of the Centers for Disease Control and have been published in *The FOP Connection*.

16. **Data on the Eight-Year Accutane Therapy Project For Treatment of FOP Have Been Collated From Records At The National Institutes of Health, and are being statistically Evaluated For Significant Findings**. The conclusions of this drug-evaluation program will be made available to the FOP community immediately upon completion, in late 1992. A full scientific report will be prepared for subsequent publication.

In summary, this has been an extraordinary year for FOP research. The establishment of a new laboratory devoted and dedicated to FOP research will advance the goal of discovering the cause and eventually establishing a cure for FOP.

Once again, the members of the FOP collaborative research project greatly acknowledge the generous support of our sponsors.

1) International Fibrodysplasia Ossificans Progressiva Association (IFOPA).
2) Jud Bogard Invitational Golf Tournament Sponsors.
3) American Heart Association Student Fellowship Program.
4) Merck Pharmaceutical Foundation Student Fellowship Program.
5) The Hartford Foundation for Research in Aging.

FOP Collaborative Research Project Members:

1) Michael A. Zasloff, M.D., Ph.D., Co-Director
2) Frederick S. Kaplan, M.D., Co-Director
3) Eileen Shore, Ph.D., FOP Research Scientist
4) William Gelbart, Ph.D., (Harvard Univ.), Research Collaborator
5) John Wozney, Ph.D., (Genetics Institute), Research Collaborator
6) J. Michael Connor, M.D., (Univ. of Glasgow), Research Collaborator

FOP Research Fellows & Scientists:

1) John Campbell, M.D.
2) Randolph Cohen, M.D.
3) Gerald Finkel, M.D.
4) Francis Gannon, M.D.
5) Gregory Hahn, B.A.
6) Craig Levitz, M.D.
7) Maximillian Muenke, M.D.
8) Christopher Strear, B.A.
9) Jeffrey Tabas, M.D.

Invited National and International Lectures on FOP:

1) University of California - Dr. Zasloff.
2) Harvard University (Department of Pathology) - Dr. Kaplan.
3) University of Colorado - Dr. Kaplan.
4) Academic Orthopaedic Association, Chicago - Dr. Kaplan.
5) American Society for Bone and Mineral Research - Drs. Kaplan, Cohen and Hahn.