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Fibrodysplasia Ossificans Progressiva: Searching For The Skeleton Key

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Preface

The format for the Fifth Annual Report of the FOP Collaborative Research Project is an editorial based upon the proceedings of the Second International Symposium on FOP (Philadelphia, PA; October 29-31, 1995). Selected portions of this report will be published in the scientific journal Calcified Tissue International along with the medical and scientific abstracts from the Symposium. The highly successful Second International Symposium provided a splendid opportunity to focus our attention on the progress of FOP research during the past five years, and to brainstorm on directions for future collaborative work.

During the next year, we will continue to pursue leads provided by the very important discovery of BMP4 over-expression in cells derived from the peripheral blood of patients who have FOP. This important genetic and molecular clue to the cause of FOP has propelled the development of a transgenic animal model in which the BMP4 gene is over-expressed. Detailed studies on these transgenic animals will determine their value for testing new drugs for treating FOP.

Work is proceeding on additional animal models that are critical to our understanding of the pathways that lead to the development of FOP lesions and heterotopic bone: (a) A transgenic animal model in which the fos gene (a tumor-associated gene involved in bone formation) is over-expressed in embryonic mice and leads to early FOP-like lesions. This work involves a productive ongoing collaboration with scientists in Vienna and London. (b) An animal model of focal heterotopic ossification based upon the implantation of recombinant human BMP in which FOP-like lesions predictably form. Such a model is being developed to test the potential efficacy of a novel anti-angiogenic steroid derived from the shark to inhibit the development of early FOP lesions. In addition to work on these animal models, robust activity will continue to better define the pathways that lead to FOP, involving BMP activity, BMP receptors, and cells that are likely responsible for interpreting the BMP signal to form bone.

We are very excited about these and many other related projects, and will continue work on FOP clinical studies in order to provide better advice on the evaluation, prevention, and treatment of complications. We will, of course, continue to report on important developments and findings in a timely manner. We are, as always, extremely grateful to our generous sponsors and collaborators.

INTRODUCTION

For three centuries, physicians and scientists have sought to understand the cause of the rare heritable disorder fibrodysplasia ossificans progressiva (FOP), but until recently to no avail. For the second time in four years, a small international symposium was convened in Philadelphia to focus on this devastating childhood disease, and to search for the key to this perplexing enigma. What is FOP, why has it proved to be such an elusive problem, and what is being done to find the key?

WHY HAS IT BEEN SO DIFFICULT TO FIND THE KEY TO FOP?

There are at least six factors that have impeded research progress:

1. The difficulty of obtaining tissue prospectively (except during emergency procedures for non-FOP-related problems) due to exacerbation of disease from surgical trauma.
2. The lack of relevant animal models.
3. The lack of large families (due to low reproductive fitness) for genetic linkage analysis.
4. The general lack of awareness in the medical community of the early features of the disease and thus, the subsequent high rate of misdiagnosis.
5. The absence, until recently, of an international patient support group and registry that could foster interest in the medical and scientific community (as well as communication among patient-members).
6. The absence, until recently, of broad insights from developmental molecular biology and molecular genetics that could serve as a framework for further understanding a disease with important implications for human development.

WHAT IS THE INTERNATIONAL FOP ASSOCIATION AND WHY HAS IT MADE A DIFFERENCE?

The modern era for FOP began in 1988 with the establishment of the International Fibrodysplasia Ossificans Progressiva Association (IFOPA) by Jeannie Peeper, a patient with FOP who wanted to end the social isolation imposed by this rare, debilitating disease. Conceived originally as a small pen-pal organization, the IFOPA grew within two years to a vibrant international organization that supports education, clinical care, research, and communication on FOP. The IFOPA rapidly became the nominal lifeline of the FOP universe.

The existence of the IFOPA has propelled the collaborative research that has taken place over the past four years and that was reported recently at The Second International Symposium on FOP (October 29-31, 1995, Philadelphia, PA).

THE SECOND INTERNATIONAL SYMPOSIUM ON FOP

The Second International Symposium on FOP was a milestone for the FOP community, and celebrated a steadfast commitment to the original missions: the discovery of the cause for FOP and the establishment of treatments that are genuinely useful to the patients. The major scientific goal of the symposium was to stimulate research in basic bone biology and clinical medicine that is applicable to the study and treatment of FOP and other disabling diseases of heterotopic osteogenesis in man.

A distinguished group was assembled from a wide spectrum of basic biological and applied medical sciences including: molecular biology, cell biology, developmental biology, pathology and laboratory medicine, hematology, immunology, genetics, oncology, pediatrics, general medicine, cardiology, rheumatology, orthopaedics, oral biology, physical medicine and rehabilitation, endocrinology, otorhinolaryngology, nursing, social work, statistics, and pharmacology.

Symposium participants discussed advances in FOP and FOP-related research during the past four years and strategies for continuing investigation. The convention of such an eclectic group of physicians and scientists stimulated a valuable interchange of new information and ideas that will aid in understanding the molecular pathophysiology of FOP, in developing relevant transgenic animal models, in formulating practical approaches to prevention of disabling complications, and in achieving the ultimate longterm goal of developing more effective treatment strategies.

The natural history and developmental patterns of heterotopic osteogenesis in FOP were described to all participants. The modelling and remodelling characteristics of the heterotopic skeleton were examined and support the hypothesis that the genetic defect in FOP involves normal skeletal morphogenesis at heterotopic sites. The histopathology of FOP was described in detail, and supports a predominant pathway of endochondral osteogenesis that leads to the formation of a heterotopic skeleton. Intense pre-osseous inflammatory changes were described in FOP and include acute perivascular lymphocytic infiltration into skeletal muscle.

The Mendelian genetics of FOP was examined in detail. Inheritance of this disease occurs by autosomal dominant transmission with complete penetrance and variable expressivity. The presence of gonadal mosaicism in one affected family is an important finding that will influence diagnosis, counseling, and further research.

Based upon molecular and phenotypic similarities between BMP gene expression in vertebrates and decapentaplegic gene expression in the fruit fly, an hypothesis was proposed that the molecular pathogenesis of FOP involves over-expression of one or more of the bone morphogenetic proteins (BMPs). BMPs have the ability to induce heterotopic endochondral ossification and to regulate pattern formation in the developing skeleton, two cardinal features of FOP. The importance of members of the BMP family was illustrated further by the discovery of naturally occurring genetic deletions of specific bone morphogenetic protein (BMP) genes in mice. These mutations led to specific abnormalities in the development of the axial or appendicular skeleton as well as abnormalities in post-natal fracture healing. Transgenic animals that over-express a specific BMP (growth/differentiation factor 5) were shown to develop heterotopic osteogenesis. These and other data help confirm the basis for considering BMPs as morphogens in vertebrate embryonic and postnatal bone induction.

Abundant BMP4 was discovered recently in pre-chondral FOP lesional tissue. This finding provided the first evidence of a relationship between the expression of a BMP and the occurence of FOP. In addition, the over-expression of BMP4 messenger RNA (mRNA) and protein in fibroproliferative cells from the earliest FOP lesions (but not in nonlesional muscle or tendon cells) was reported. Immortalized lymphoblastoid cell lines have been established from more than 50 FOP patients and have been used to assess the activity of a wide variety of genes which function in mesenchymal differentiation and osteochondrogenesis. Among all of the BMPs examined to date (BMP1-BMP8 and GDF5), BMP4 mRNA and protein alone are expressed in the lymphoblastoid cells derived from patients who have FOP, but not from unaffected individuals. Studies are being conducted to determine whether there is a mutation in the BMP4 gene in patients who have FOP.

An hypothesis on the pathophysiology of FOP was formulated based on the dysregulation of BMP4 in inflammatory cells and on the interaction of BMP4 with vascular and perivascular cells in involved connective tissue. The initiation of experiments to construct a BMP4 transgenic animal model was presented, based upon the finding of dysregulation of BMP4 mRNA and protein in lymphoblastoid cells of patients who have FOP.

Considering the involvement of one or more of the BMPs in FOP, the characterization of the genes which encode these proteins are important in understanding their cellular regulation and dysregulation. Eight BMPs (BMP1 thru BMP8) have been mapped to the human chromosomes, using somatic-cell hybrid panels and standard molecular techniques, and fine-mapping studies of human BMP4 are in progress based upon its consideration as a prime candidate gene for FOP. A human BMP4 genomic clone has been recovered and DNA sequence of its exons, introns, and putative adjacent regulatory sequences has been determined. The evaluation of the promoter of the human BMP4 gene has been initiated, and at least three alternately-spliced mRNAs of the human BMP4 gene have been discovered in an osteosarcoma cell line. An evaluation of the human BMP4 transcripts in FOP lymphoblastoid cells and in primary human bone cell lines is in progress.

Other genes and cellular pathways that are associated with osteogenesis were also discussed. A human BMP2 genomic clone has been isolated and its characterization is in progress. The expression pattern of BMP2 and BMP4 receptors during mammalian embryogenesis and the potential implication of these findings for FOP were discussed. Embryonic over-expression of the c-fos-proto-oncogene in the mouse leads to an FOP-like condition, and sustained c-fos embryonic over-expression leads to the activation of BMP2 and/or BMP4. The roles of homeotic genes and paired box genes were considered in the genetic control of osteogenesis. A prostaglandin-like substance in the serum of patients who have FOP has been identified, a finding of potential importance for understanding the role of prostaglandins in pathways of endochondral osteogenesis.

The involvement of mesenchymal stem cells in the pathophysiology of heterotopic osteogenesis in FOP was addressed in seminar. Microvessel cells and mesenchymal stem cells may be bone or cartilage precursor cells, and possible target cells in the induction of post-natal FOP lesions. The concept of BMP-induced transcription factors was introduced.

The identification of an FOP-like condition in domestic short-hair and domestic long-hair cats was described, a finding of potential importance for the development of an animal model for the study of this condition.

Extensive clinical studies on FOP have been conducted during the past four years and have further elucidated the phenotypic features of the condition. The high rate of initial misdiagnosis, and the importance of early diagnosis of FOP (in order to avoid unnecessary and harmful medical and surgical treatments) was emphasized. Age and joint-specific risk profiles have been established for heterotopic ossification in patients who have FOP; this information provides a guide for planning individual patient needs and anticipating auxiliary social services. Several mild variants in the expression of FOP have been described, findings that will influence diagnosis, counselling, and research. The patterns of fracture healing in the heterotopic skeleton were described. The natural history and pathogenesis of spinal deformity in patients who have FOP was elucidated, providing guidelines for the management of spinal complications. The devasting problem of site-induced heterotopic ossification following childhood intramuscular immunizations was described, an important finding that helped

establish immunization guidelines for FOP children. Similar complications of dental injections in patients who have FOP were examined and provided guidelines for safer and more effective dental care. The clinical features and treatment of submandibular swelling were described and provided guidelines for the management of this life-threatening complication. The natural history and pathogenesis of limb swelling in patients who have FOP was described and the implications of this problem at a molecular level were discussed. The myriad patterns of hearing loss in patients who have FOP were presented for the first time and suggestions for further studies were raised. The cardiopulmonary complications of FOP were studied in detail. A prospective study involving a questionnaire, physical examination, electrocardiogram, echocardiogram, and pulmonary function tests was conducted during the Symposium. The utility and limitations of 13-cis-retinoic acid for the prevention of new regional heterotopic ossification were discussed, based upon the results of a recently-completed 10 year prospective study.

Every disorder of heterotopic ossification has potential implications for the study of FOP, just as the information uncovered in the investigation of FOP may help elucidate other disorders of osteogenesis. A new developmental disorder of heterotopic ossification in humans (progressive osseous heteroplasia; POH) was described and the condition was compared to FOP. Associations of POH with Albright's Hereditary Osteodystrophy were discussed and possible connections between the G-stimulatory proteins and pathways of osteogenesis were explored.

For the benefit of people who have FOP (along with their families, caretakers, physicians, and communities) two books have recently been published by the IFOPA:

WHAT IS FOP? A GUIDEBOOK FOR FAMILIES
and
WHAT IS FOP? QUESTIONS AND ANSWERS FOR THE CHILDREN.

The Keynote Address at the Symposium was delivered by Victor McKusick (Johns Hopkins) who spoke on "MAP-BASED GENE DISCOVERY: IMPLICATIONS FOR FOP." He outlined the map-based approach of positional cloning that has led to numerous disease-based gene discoveries, an approach which is unlikely to be applicable for a disease like FOP due to the paucity of families available for linkage analysis. Dr. McKusick outlined the candidate-gene approach, a more feasible strategy for a disease like FOP. The candidate-gene approach has been the paradigm for research in FOP over the past four years, and has led to many of the discoveries outlined above.

In addition to participation by physicians and scientists, there were 43 FOP families in attendance from 14 nations. Such a robust turnout by patient-members is testimony to the extraordinary interest, organization and motivation of the IFOPA and the international FOP community. Patients and family members conducted seminars and support groups while physicians and scientists discussed their clinical and laboratory findings. Patients and family members attended many of the scientific sessions, and some of the scientists and physicians joined the patients in their group discussions. Everyone met for meals and social events. Such interactions fostered understanding and compassion for each other's perspectives, needs and directions; and ultimately invigorated the pursuit for answers.

Through the generosity of The Mutter Museum of The College of Physicians of Philadelphia, the skeleton of a patient who had FOP was placed on exhibit at the meeting for those who wished to study the detailed patterns of heterotopic ossification that are such a defining feature of the condition.

Finally, three days of clinics were conducted and attended by nearly all of the patients and a wide sampling of doctors from around the world. Such interactions provided a unique opportunity to learn first-hand about the devastating problems faced by patients who have FOP, and also, provided an opportunity to collect data for further prospective studies, and to clarify concerns and misconceptions about the condition.

Jeannie Peeper, the President of the IFOPA, noted in her introductory comments: "Over the past seven years, I have witnessed our organization take shape, expand, and form battle lines against FOP. We have accomplished our original goals of finding each other and of encouraging and supporting each other. Thanks to the IFOPA, the isolation many of us felt in dealing with FOP is diminished. Hope is critical to all of us, perhaps most critical to new patients and their families. For those of you who are new to the symposium and to the effects of FOP, we offer our friendship and the hope that our experiences will benefit you. No-one is more aware of what you are dealing with than your fellow FOP families, and no-one is more eager to help you than we are. We are all in this fight together! Finally, we are small in number, but great in heart, and hope. We can stand tall in our fight against a devastating disease, and we know in our comradeship lies our best hope for a brighter future. We must continue to encourage one another, and support the work of our researchers. Over the past seven years, we have travelled a long way down the path that will ultimately lead to the conquering of FOP. I look forward to continuing the journey with all of you."

CONCLUSION

After three hundred years, the gap between ignorance and knowledge is beginning to narrow in the study of FOP. FOP is among the rarest of human afflictions but the key to FOP may be the key to many more diseases of the heterotopic skeleton - as well as of the normotopic skeleton. We hope that at the end of the search there will be such a skeleton key. But, if it unlocks only this one door, it will be enough.

Symposium participants discussed advances in FOP, and FOP-related research, during the past four years. Members of the international working group on FOP have:

1. Defined the Mendelian genetics of FOP, and the unequivocal autosomal dominant transmission of the disease.
2. Described a form of gonadal mosaicism in the transmission of the disease that will influence diagnosis, counseling, and research.
3. Defined the natural history and developmental patterns of heterotopic osteogenesis in FOP, and provided a basis for considering bone morphogenetic protein (BMP) candidate genes.
4. Defined the modelling and remodelling characteristics of the heterotopic skeleton in patients who have FOP, and provided support for the hypothesis that the genetic defect in FOP leads to the formation of a heterotopic skeleton that involves normal skeletal morphogenesis at heterotopic sites.
5. Defined the spectrum of pre-osseous inflammatory changes in FOP including acute lymphocytic infiltration into skeletal muscle.
6. Defined the pathways of endochondral osteogenesis leading to the formation of a heterotopic skeleton in FOP.
7. Identified a prostaglandin-like substance in the serum of patients who have FOP, and discussed the role of prostaglandin-like molecules in the pathways of endochondral osteogenesis.
8. Formulated a hypothesis on the molecular pathogenesis of FOP, based upon molecular and phenotypic similarities between BMP gene expression in verte-

brates and decapentaplegic gene expression in the fruit fly.

9. Discussed the implications for BMPs as morphogens in vertebrate embryonic postnatal bone induction.

10. Demonstrated the presence of BMP 4 protein in pre-chondral FOP lesional tissue, thereby providing the first evidence of a relationship between a BMP and FOP.

11. Demonstrated the dysregulation of BMP4 messenger RNA (mRNA) and protein in fibroproliferative cells from the earliest FOP lesions, but not in nonlesional muscle or tendon cells.

12. Produced immortalized lymphoblastoid cell-lines from more than 50 FOP patients and used the cell-lines to assess a wide variety of genes related to mesenchymal differentiation and osteochondrogenesis.

13. Demonstrated dysregulation of the BMP4 gene in lymphocytes derived from the peripheral blood of patients who have FOP.

14. Formulated a hypothesis on the pathophysiology of FOP based on the dysregulation of a morphogen in inflammatory cells.

15. Mapped eight BMPs (BMP1 thru BMP8) to the human chromosomes, using somatic-cell hybrid panels and standard molecular techniques, and began fine-mapping studies of human BMP4 based upon its consideration as a prime candidate gene for FOP.

16. Recovered a human BMP4 genomic clone, and determined its DNA sequence.

17. Began evaluation of the promoter of the human BMP4 gene.

18. Discovered at least three alternately-spliced mRNAs of the human BMP4 gene in an osteosarcoma cell line.

19. Began evaluation of the mRNA start-site of the human BMP4 gene in FOP lymphoblastoid cells and in primary human bone cell lines.

20. Recovered a human BMP2 genomic clone, and began determination of its DNA sequence, and promoter activity.

21. Discovered that embryonic overexpression of the c-fos-proto-oncogene in the mouse leads to an FOP-like condition, and that sustained c-fos embryonic over-expression may lead to the activation of BMP2 and/or BMP4.

22. Considered the role of homeotic genes, and paired box genes, in the genetic control of osteogenesis.

23. Discussed the concept of mesenchymal stem cells in the pathophysiology of heterotopic osteogenesis in FOP.

24. Discovered that microvessel cells and mesenchymal stem cells may be bone or cartilage precursor cells, and possible target cells in FOP.

25. Investigated BMP-induction of transcription factors in mesenchymal stem cells.

26. Discussed the expression pattern of BMP2 and BMP4 receptors during mammalian embryogenesis and discussed the potential implication of these findings for FOP.

27. Discovered an FOP-like condition in domestic short-hair, and domestic long-hair cats.

28. Discovered naturally occurring genetic deletions of specific mutations in bone morphogenetic proteins (BMPs) in mice that enhance understanding of the developmental roles of the BMPs.

29. Identified transgenic animal models of growth differentiation factor and BMP over-expression that lead to heterotopic osteogenesis.

30. Began work on the construction of a BMP4 transgenic animal model relevant to the study of FOP, based upon the finding of dysregulation of BMP4 mRNA and protein in lymphoblastoid cells of patients who have FOP.

31. Discovered that the transgenic expression of the SV-40-T antigen in mice leads to heterotopic endochondral osteogenesis in epithelial sweat glands.

32. Documented the importance of early diagnosis of FOP, in order to avoid unnecessary and harmful medical and surgical treatments.

33. Established age and joint-specific risk profiles for heterotopic ossification in patients who have FOP that provide a guide for planning individual patient needs and anticipating auxiliary social services.

34. Described several mild variants in the expression of FOP that will influence diagnosis, counselling, and research.

35. Defined patterns of fracture healing in the heterotopic skeleton.

36. Defined the natural history and pathogenesis of spinal deformity in patients who have FOP, and provided guidelines for the management of complications.

37. Defined the complications of childhood, intramuscular immunizations in patients who have FOP, and established immunization guidelines for FOP children.

38. Defined the complications of dental injections in patients who have FOP, and provided guidelines for safer and more effective dental care.

39. Described the manifestations and treatment of submandibular swelling in patients who have FOP, and provided guidelines for the management of this life-threatening complication.

40. Defined patterns of hearing loss in patients who have FOP.

41. Defined the natural history and pathogenesis of limb swelling in patients who have FOP, and discussed implications of limb swelling at a molecular level.

42. Studied cardiopulmonary complications of FOP. This study involving a questionnaire, physical examination, electrocardiogram, echocardiogram, and pulmonary function tests was conducted during the Symposium.

43. Defined the utility and limitations of 13-cis-retinoic acid in the prevention of new regional heterotopic ossification in patients who have FOP, based upon the results of a prospective study.

44. Described a new developmental disorder of heterotopic ossification in humans (progressive osseous heteroplasia; POH) and compared this condition to FOP.

45. Discussed associations of POH with Albright's Hereditary Osteodystrophy, and began to explore possible connections between the G-stimulatory proteins and pathways of osteogenesis.

46. Published: WHAT IS FOP? A GUIDEBOOK FOR FAMILIES.

47. Published: WHAT IS FOP? QUESTIONS AND ANSWERS FOR THE CHILDREN.