v. 7-5-01

FIVE-YEAR REPORT
OF THE PROGRESSIVE OSSEOUS HETEROPLASIA (POH)
COLLABORATIVE RESEARCH PROJECT
June 2001

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

POH was recognized as a clinically distinct disorder in 1994. The following year, in October 1995, Drs. Frederick S. Kaplan, Eileen M. Shore, and Michael A. Zasloff of the University of Pennsylvania School of Medicine established the POH Collaborative Research Project with the support of the Progressive Osseous Heteroplasia Association (POHA). This effort arose out of a mutual desire to identify the cause and to find a cure for POH.

The POH Collaborative Research Group is an international team of physicians and scientists who collaborate on clinical and basic research on POH. The international working group is dedicated to finding the cause and to establishing a cure for POH. In 1996, the group was awarded a research grant from the Progressive Osseous Heteroplasia Association (POHA) to study the molecular basis of POH. In this report, we describe the significant advances of our program over the past five years.

In addition to funding support from the POHA, a grant from the New Jersey Association of Student Councils (NJASC) was awarded in 1997 to expand the research on POH. The International Fibrodysplasia Ossificans Association (IFOPA) and the Center for Research in FOP and Related Disorders have also supported research on POH. During this past year, a grant proposal to study the genetics of POH was submitted and favorably reviewed by the National Institutes of Health (NIH). The three years of funding provided through this grant (Principle Investigator: Eileen Shore; co-PI: Frederick Kaplan) began in September 2000.

PROGRESSIVE OSSEOUS HETEROPLASIA (POH)

POH is a genetic disorder of heterotopic ossification (extra bone formation) that is characterized by bone formation within the skin during childhood followed by progressive heterotopic ossification of skin, subcutaneous fat, deep connective tissues, and skeletal muscle at sporadic locations throughout the body during life. POH is a very rare human condition, and as of June 2001 fewer than 50 people have been identified worldwide. However, our recent studies indicate that POH may be more closely related to several other genetic disorders than previously recognized, forming the extreme end of a spectrum of clinically distinct but genetically related conditions.

POH RESEARCH

Identification of the Altered Gene in POH

In 1998, the POH collaborative research group began the experimental analyses that led to the identification of the damaged gene responsible for POH. The gene that we have identified is called GNAS1 and is located on the long arm of human chromosome 20.

As far back as 1995, we had recognized the similarities between POH and a condition known as Albright hereditary osteodystrophy (AHO). Patients with AHO are generally recognized by characteristic skeletal morphology (such as the shape of the face and hands) and they frequently show a decreased response to various hormone signals. In addition, some patients with AHO have very mild ossification of the skin, although their bone formation does not progress to affect the deeper tissues such as muscle -- as is found in people who have POH. People who have POH do not show any characteristic skeletal features or have any altered response to hormones. Since bone formation in the skin is rare, however, we hypothesized that the GNAS1 gene, which was altered in many patients who had AHO (and had been determined to be the genetic cause of this disorder), might also be the cause of POH.

Our hypothesis that the GNAS1 gene is involved in both conditions was strengthened by the identification of two patients who had clinical features of both AHO and POH as well as reduced activity of the GNAS1 protein. Furthermore, a mutation in the GNAS1 gene was identified in one of these patients. These findings were not conclusive that alterations in the GNAS1 gene and/or activity of the GNAS1 protein (known as Gs-alpha) caused the extensive bone formation that occured in these patients, since it was possible that changes in the GNAS1 gene caused the AHO characteristics while a second independent gene alteration caused ectopic bone formation. However, concurrent with these investigations, we were also studying a child with unique POH-like heterotopic ossification (clinically described as plate-like osteoma cutis or POC). The discovery of a mutation in the GNAS1 gene of this child's DNA was the first example of a GNAS1 gene alteration that was associated with extensive heterotopic ossification independent of AHO features. (The scientific reports of these studies were published in the November 2000 issue of the Journal of Bone and Mineral Research.)

We immediately began collecting and analyzing DNA samples from as many people who have POH as possible, and discovered disease-causing alterations in the GNAS1 gene in a very high percentage of POH patients. Results of ongoing studies suggest that the inheritance patterns of mutations in the GNAS1 gene determine whether a GNAS1 mutation will result in POH or AHO in a given individual. In each case for which we can follow the inheritance of a GNAS1 mutation in a family with more than one member with POH, the inheritance of the condition is from a father to children. Families with AHO show the reciprocal pattern, with inheritance of the GNAS1 mutation from mothers to children. This genetic phenomenon, which has been recognized for several other genes, is known as genetic imprinting.

It should also be noted that there are approximately 20% of patients with clinically evident POH in whom we have not yet found a damaged copy (mutation) in the GNAS1 gene. This could mean that the genetic cause of POH in these affected individuals and families is in a regulatory portion of the GNAS1 gene that we have not yet examined. The regulatory regions of a gene are enormous in size and therefore more difficult to study and to pinpoint changes. It is also possible that the mutation exists in a completely different gene involved in the same bone formation pathway, and continued studies are needed to examine this possibility. Nevertheless, the tremendous knowledge we are gaining

about the genetic and molecular basis of POH is providing important clues that will eventually enable us to solve these puzzles.

At the present time, we are investigating the GNAS1 gene in all known patients with POH in order to develop a comprehensive understanding of the range of alterations in this gene that can cause POH. We will also study the GNAS1 gene in family members of POH patients in order to more fully understand the inheritance pattern of the GNAS1 gene -- necessary information for comprehending the expression and regulation of the GNAS1 gene. We have already initiated studies to examine the expression levels and functional activity of the mRNA and protein products of the GNAS1 gene in POH. Understanding the effects that reduced GNAS1 gene activity has on the functions of cells is critical to determining why mutations in this gene lead to the extensive bone that forms in POH patients and to determining how we can correct the effects of the misfunction of this gene.

The discovery of the POH gene is an extremely important development in bone biology and of paramount importance for understanding the earliest cellular and molecular pathways in bone formation. Identification of the gene that causes POH has profound implications for developing treatments for patients with POH and also for many more common diseases of bone formation. We have completed our initial set of mutational studies on POH patients, and are in the process of describing this important discovery in the peer-reviewed scientific and medical literature.

The GNAS1 Gene

The structure and regulation of the GNAS1 gene are extraordinarily complex. GNAS1 encodes a protein called Gs-alpha located on the inside of the cell membrane in nearly every cell in the body. The protein is extremely versatile and appears to have different functions in different cells. Generally, Gs-alpha functions as a relay switch in a multi-protein complex that monitors the environment of the cell and sends signals to the nucleus (the site of the chromosomes), providing instructions to direct cell "behavior".

An enormous amount of additional research is necessary to understand exactly how mutations in the GNAS1 gene and the corresponding abnormalities in the Gs-alpha protein trigger ectopic bone formation. Early clues suggest that the Gs-alpha protein may normally act as an inhibitor of bone

formation in soft connective tissue (skin, fat, and skeletal muscle) by suppressing 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 its sister disease, fibrodysplasia ossificans progressiva (FOP), if POH bone formation is as extensive in its distribution. We have some evidence that part of the bone inducing pathway that is mis-activated in POH is also involved in FOP, although the gene mutations that cause the two conditions are different. It is also interesting and important to note (as discussed above) that the GNAS1 gene that is damaged in POH is the same gene that causes several other severe bone diseases including fibrous dysplasia (or McCune-Albright syndrome and its variants), Albright Hereditary Osteodystrophy (AHO), pseudohypoparathyroidism (PHP), and plate-like osteoma cutis (POC). By understanding more about these disorders, a clearer understanding of POH will also be gained.

Families and the Inheritance of POH

With the identification of the gene alteration that causes POH, families of affected individuals will have many questions regarding the inheritance of the condition. Since we are still learning about the inheritance patterns of POH (and are very grateful to the families who have helped and who will help us understand these patterns), we do not yet have all of the answers.

However, we feel that it is very important for families to note that gene alterations are a very common occurrence in human biology -- in fact it is thought that all of us have a handful of genetic alterations. Some of these changes are readily detected (like POH), some may be expressed in later life (such as heart disease), and some will never have any substantial effect on us at all. These changes are thought to occur randomly at a low rate of frequency in our DNA. Most of the people who have POH likely have spontaneous mutations in the GNAS1 gene. This means that the altered GNAS1 gene first occurred in that individual and was not inherited from either parent.

However, once an individual has a mutation that causes POH, this person has a 50% chance of passing that mutation to his or her child. If no mutation is inherited by the child, he/she will have

neither POH nor AHO. If a mutation is inherited by the child, the gender of the parent who transmits the mutated gene may determine whether the child develops POH or AHO. However, we have also uncovered two cases in which a mutation appears to be completely "silent" and these individuals are free of either POH or AHO symptoms.

Our studies on the inheritance patterns of GNAS1 mutations are still in their early stages, and we currently cannot make any general statements or final conclusions until further investigations are completed. As we learn more about the altered gene in POH and its inheritance patterns, we will be better able to trace the inheritance within a family. While this information may be uncomfortable for some families to know (and we will not reveal details to any family who does not wish to know this information), these family inheritance studies are critical to providing a foundation for development of the best possible treatments for POH.

SUMMARY:

WHAT WE HAVE LEARNED ABOUT POH SINCE THIS WORK BEGAN

During the past five years, the working group on POH has:

1. Discovered, named, and identified POH as a distinct developmental disorder of heterotopic ossification in humans, and provided a detailed clinical description of the disease phenotype.
2. Defined the histopathology (microscopic tissue characteristics) of heterotopic ossification in POH.
3. Established risk profiles for heterotopic ossification in patients who have POH.
4. Distinguished POH from fibrodysplasia ossificans progressiva (FOP), another autosomal dominant disorder of heterotopic ossification in children.
5. Noted the similarities and differences between POH and Albright Hereditary Osteodystrophy (AHO), an autosomal dominant disorder that can exhibit cutaneous and subcutaneous heterotopic ossification.
6. Identified a child with unilateral hemimelic POH and reported a multigenerational family with POH.
7. Identified and/or examined 40 patients with POH (19 males and 21 females).
8. Identified two children with features of both AHO/PHP and POH.

9. Postulated a putative connection between the molecular genetics of AHO/PHP and POH.
10. Established GNAS1 as the leading candidate gene for POH.
11. Discovered a heterozygous 4-bp deletion in GNAS1 in a patient with severe plate-like osteoma cutis (POC), a variant of POH.
12. Discovered heterozygous frameshift mutations in approximately 20 GNAS1 families with classic expression of POH.
13. Wrote and published "*What is POH? A Guidebook for Families.*"
14. Organized and hosted the **First International Workshop on POH**, as part of the Second International Symposium on FOP (October 1995). This meeting was attended by sixty physicians and scientists and by three POH families. The Workshop provided the scientific basis for establishing an international POH collaborative working group.
15. We have recently organized the **Second International Workshop on POH** which was held as part of the Third International Symposium on FOP (November 2-5, 2000). This meeting was attended by approximately two hundred physicians and scientists and by nine POH families.

THE NEXT GOALS OF POH RESEARCH

Three hypotheses provide the focus for our ongoing research:

Hypothesis 1: Heterozygous inactivating mutations of the GNAS1 gene are the cause of progressive osseous heteroplasia (POH), and result in reduced levels and/or activity of GNAS1 messenger RNA and/or Gs-alpha protein.

Hypothesis 2: The osteogenic phenotype of inactivating GNAS1 mutations in POH is influenced by maternal/paternal inheritance (imprinting) of the GNAS1 locus and may be caused by the absence of Gs-alpha protein expression in POH lesional cells.

Hypothesis 3: Heterozygous inactivating mutations of the GNAS1 gene, through a reduction in functional GNAS1 mRNA and/or $G_s\alpha$ protein, alter cellular signaling pathways that direct osteoblast differentiation.

Our immediate research is intended to accomplish the following specific aims:

1. Continue to screen genomic DNA for mutations in the GNAS1 gene in POH patients by PCR amplification and DNA sequencing.
2. Compare the identified GNAS1 mutations in POH patients to those found in patients with AHO, in order to further understand the differences and similarities between these two conditions.
3. Examine expression of the GNAS1 gene in POH patients by quantitation of (a) GNAS1 mRNA (by RT-PCR) and (b) Gs-alpha, the protein product of the GNAS1 gene (by immunoblot analysis).
4. Perform functional analysis of Gs-alpha protein in POH patients' cells (by G-protein-mediated cAMP activity assays).
5. Evaluate the effects of maternal/paternal inheritance of the defective GNAS1 gene on phenotypic expression in all available pedigrees showing inheritance of POH.
6. Investigate Gs-alpha protein expression in POH lesional tissue by immunohistochemical analysis.
7. Examine the effects of heterozygous GNAS1 gene inactivation on osteoblast and adipocyte differentiation.

8. Examine the differential expression of GNAS1 mRNAs during osteoblast and adipocyte differentiation in GNAS1 mutant and control cells.

At present, there are no effective treatments or prevention for POH. Analysis of the molecular genetics of POH will greatly foster an understanding of the cellular and molecular pathways that initiate skeletogenesis and osteogenesis in POH and will lead to development of a more rational diagnostic and therapeutic approach to a treating POH.

THE IMPORTANCE OF POH RESEARCH

The importance and implication of POH research for affected children and their families is unquestionable. However, the importance of POH research for the general medical community is far greater that its rarity might indicate. Research on POH is relevant to all disorders of renegade bone formation. By unraveling the complex pathogenesis of POH, there is great hope that more common disorders of bone formation will become understandable and treatable.

Knowledge gained from this work has the likelihood of elucidating not only the basic molecular mechanisms of POH, but also the basic molecular mechanisms involved in disorders as diverse as congenital limb anomalies, bone cancer, osteoarthritic bone spurs, osteoporosis, and abnormal fracture repair. Recently, our laboratory (propelled by discoveries in FOP and POH) has identified bone formation in heart valves as a common finding in patients who have endstage valvular heart disease. Research in POH, therefore, has the possibility of elucidating mechanisms of phenotype stability in disorders as fundamental as cancer, aging, and valvular heart disease**.**

During the past several years, great progress has been made in understanding not only the cellular and molecular mechanisms involved in normal bone formation, but also in understanding the complex mysteries of POH. The work undertaken by the collaborative research group is focused on elucidating the underlying molecular causes of POH, and using that knowledge to design medications and treatments that will be genuinely useful to the children and adults who have POH.

SYMPOSIUM 2000

An international scientific symposium on Progressive Osseous Heteroplasia (POH) and Fibrodysplasia Ossificans Progressiva (FOP) was hosted by the Center for Research in FOP and Related Disorders at the University of Pennsylvania School of Medicine and was held at the Wyndham-Franklin Plaza Hotel on November 2-5, 2000. The symposium featured medical, scientific and family-related sessions on POH and FOP. This symposium greatly increased interest in POH within the scientific community and will stimulate new research and therapeutic directions.

SPONSORS

The members of the POH collaborative research project greatly acknowledge the generous support of our sponsors in helping us to achieve our long-term goals.

1) The Progressive Osseous Heteroplasia Association (POHA)
2) The International Fibrodysplasia Ossificans Progressiva Association (IFOPA)
3) The New Jersey Association of Student Councils (NJASC)
4) The Four Schools (University of Pennsylvania, Johns Hopkins University, Duke University, Washington University) Medical Student Fellowship Program.
5) National Institutes of Health (NIH); National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).

POH COLLABORATIVE RESEARCH PROJECT MEMBERS (in alphabetical order):

Laura Audi, M.D. - Hospitals de Sant Celoni and Materno-Infantil Vall d'Hebron, Barcelona, Spain
J. Michael Connor, M.D. - University of Glasgow, Glasgow, UK
Betsy Olmsted Davis, Ph.D. - University of Pennsylvania, Philadelphia, PA
Mark Eddy, M.D. - Washington University, St. Louis, MO
John Emery, Ph.D. - SmithKline Beecham, King of Prussia, PA
Franklin Epstein, M.D. - Harvard University, Cambridge, MA.
Gerald Finkel, M.D. - Jefferson University, Philadelphia, PA
Steven Fitzgerald, B.A. - University of Pennsylvania, Philadelphia, PA
Francis H. Gannon, M.D. - Armed Forces Institutes of Pathology, Bethesda, MD
R.J. McKinlay Gardner, M.D. - Royal Children's Hospital, Melbourne, Australia
Nader Hebela, B.S. - University of Pennsylvania, Philadelphia, PA
Suzanne Jan De Beur, M.D. - Johns Hopkins University, Baltimore, MD
Frederick S. Kaplan, M.D. - University of Pennsylvania, Philadelphia, PA
Martine Le Merrer, M.D. - Association Francaise Contre les Myopathies, Paris, France
Michael A. Levine, M.D. - Johns Hopkins University, Baltimore, MD
Ming Li, B.S. - University of Pennsylvania, Philadelphia, PA
Sameer Mathur, M.D. - University of Pennsylvania, Philadelphia, PA
Pamela Gehron Robey, Ph.D. - National Institutes of Health, Bethesda, MD
Eileen M. Shore, Ph.D. - University of Pennsylvania, Philadelphia, PA
Roger Smith, M.D. - Oxford University, Oxford, UK
Larry Suva, Ph.D. - University of Arkansas, Little Rock, AK
Angels Ulied, M.D. - Hospitals de Sant Celoni and Materno-Infantil Vall d'Hebron, Barcelona, Spain
Jon Andoni Urtizberea, M.D. - Association Francaise Centre les Myopathies, Paris, France
Michael Whyte, M.D. - Washington University, St. Louis, MO
Ashley Wivel, M.D. - University of Pennsylvania, Philadelphia, PA
Mei-qi Xu, B.S. - University of Pennsylvania, Philadelphia, PA
Suzanne Yandow, M.D. - Shriner's Hospital, Salt Lake City, UT
George Yeh, M.D. - University of Pennsylvania, Philadelphia, PA
Michael A. Zasloff, M.D., Ph.D. - University of Pennsylvania, Philadelphia, PA

Of special note in this Annual Report are the contributions by Drs. Michael A. Levine and Suzanne Jan de Beur (Johns Hopkins University) and Drs. Michael P. Whyte and Mark Eddy (Washington University, St. Louis) toward the identification of the GNAS1 gene as the genetic alteration in POH patients.

REPORTS ON POH RESEARCH

1. Research publications:

Kaplan FS, Craver R, MacEwen GD, Gannon FH, Finkel G, Hahn G, Tabas J, Gardner RJM, Zasloff MA (1994). Progressive osseous heteroplasia: A distinct developmental disorder of heterotopic ossification. J Bone Joint Surg 76-A, 425-436.

Rosenfeld SR, Kaplan FS (1995). Progressive osseous heteroplasia in male patients. Clin Ortho Rel Res 317:243-245.

Urtizberea JA, Testart H, Cartault F, Boccon-Gibod L, LeMerrer M, Kaplan FS (1998). Progressive osseous heteroplasia J. Bone Joint Surg. 80B: 768-771.

Kaplan, F.S. and E.M. Shore (1999). Illustrative disorders of ectopic skeletal morphogenesis: A childhood parallax for studies in gravitational and space biology. Gravitational and Space Biology Bulletin 12(2) pp. 27-38.

Shore , E.M., D.M. Glaser, F.H. Gannon (2000). Osteogenic induction in hereditary disorders of heterotopic ossification. Clin. Ortho. Rel. Res. 374, 303-316.

Yeh, G., S. Mathur, A. Wivel, M. Li, F.H. Gannon, A. Ulied, L. Audi, E.A. Olmsted, F.S. Kaplan and E.M. Shore (2000). GNAS1 mutation and Cbfa1 misexpression in a child with severe congenital plate-like osteoma cutis. J. Bone Min. Res. 15, 2063-2073.

Eddy, M.C., S.M. Jan de Beur, S.M. Yandow, W.H. McAlister, E.M. Shore, C. d'Amato, C.H. Meyers-Seifert, F.S. Kaplan, M.P. Whyte, and M.A. Levine (2000). Deficiency of the α-subunit of the stimulatory G protein and severe extraskeletal ossification. J. Bone Min. Res. 15, 2074-2083.

Kaplan, F.S. and E.M. Shore (2000). Progressive osseous heteroplasia: a perspective. J. Bone Min. Res. 15, 2084-2094.

2. Conference abstracts:

Khan, F.S., E.A. Olmsted, F.S. Kaplan, and E.M. Shore (1998). A molecular characterization of progressive osseous heteroplasia. (Presented at the American Society for Bone and Mineral Research 1998 Annual Meeting).

Yeh, G., E. Olmsted, S. Mathur, A. Ulied, L. Audi, F. Kaplan and E. Shore (1998). Characterization of the use of alternate first exons of the human Cbfa-1 gene. (Presented at the American Society for Bone and Mineral Research 1998 Annual Meeting).

Yeh, G., E. Olmsted, S. Mathur, E. Shore, F. Gannon, A. Ulied, L. Audi, and F. Kaplan (1998). Mis-expression of the Cbfa-1 gene in dermal fibroblasts from a patient with an atypical form of progressive osseous heteroplasia. (Presented at the American Society for Bone and Mineral Research 1998 Annual Meeting).

Eddy, M.C., S.M. Jan de Beur, S.M. Yandow, W.H. McAlister, F.S. Kaplan, E.M. Shore, M.P. Whyte, M.A. Levine (1998). Mutation in exon 1 of the guanine stimulatory a-subunit (Gsa) gene [GNAS1] manifesting as severe ectopic bone formation. Bone, 23(5), S544. (Presented at the American Society for Bone and Mineral Research Annual Meeting; San Francisco, CA, December 1998.)

Shore, E.M., M. Li, N. Hebela, S.M. Jan de Beur, M.C. Eddy, M.P. Whyte, M.A. Levine, F.S. Kaplan (1999). Mutations in the gene encoding the stimulatory G protein of adenylyl cyclase in progressive osseous heteroplasia (POH). J. Bone Min. Res. 14 (Suppl 1), S342. (Presented at the American Society for Bone and Mineral Research Annual Meeting; St. Louis, MO, September 1999.)

3. Books/Chapters

Kaplan, F.S., Wagman, R.B., S.R. Wheeler, and F. Garner (1997). What is POH? Progressive Osseous Heteroplasia: A Guidebook for Families, First Edition. Medical Editors: F.S. Kaplan and F.H. Gannon; Scientific Editors: E.M. Shore and M.S. Zasloff. Progressive Osseous Heteroplasia Association, Indian Head Park, IL.

Shore, E.M., F.S. Kaplan, and J.S. Khurana (2000). Inherited and dysplastic conditions of the skeleton. In: A Compendium of Skeletal Pathology. J.S. Khurana, L. Fitzparick, B. Alman, S.V. Kattapuram, S.S. Gill, G.A. Raj, Editors. United Pathologists Press, USA. pp. 135-140.

4. Scientific talks on POH by Drs. Shore and Kaplan:

"Progressive Osseous Heteroplasia" - St. Luke's Medical Center, Milwaukee, WI, and the Marshfield Clinic, Marshfield, WI; May 1999.

"Progressive Osseous Heteroplasia: The Discovery and Molecular Basis of a Distinct Disorder of Heterotopic Ossification in Children" - FOPeV, Garmisch-Partenkirchen, Germany; November 1999.

"Skin and Bones: the Discovery and Molecular Genetics of Progressive Osseous Heteroplasia - a Unique Disorder of Heterotopic Skeletogenesis in Man" - Prague International Pediatric Rheumatology Symposium, Prague, Czech Republic; November 1999.

"Skin and Bones: Progressive Osseous Heteroplasia (POH), a Genetic Disorder of Severe Heterotopic Ossification" - Craniofacial and Skeletal Diseases, NIDR, National Institutes of Health, Bethesda, MD; December 1999.

"FOP and POH: Two Inherited Disorders of Heterotopic Ossification" - SmithKline Beecham, King of Prussia, PA; January 2000.

"Mutations in the GNAS1 Gene in Progressive Osseous Heteroplasia" - Advances in Mineral Metabolism; Snowmass, CO, March 2000.

"What is Progressive Osseous Heteroplasia?: A Perspective" - Third International Symposium on FOP, Philadelphia, PA; November 2000.

"The Genetic Cause of POH" The Alfred Gilman and Martin Rodbell Lecture in Molecular Genetics - Third International Symposium on FOP, Philadelphia, PA; November 2000.