

2004 ANNUAL REPORT
OF THE PROGRESSIVE OSSEOUS HETEROPLASIA (POH)
COLLABORATIVE RESEARCH PROJECT

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Only ten years have passed since progressive osseous heteroplasia (POH) was recognized as a clinically distinct disorder. Soon after this recognition, research was initiated, in October 1995, at the University of Pennsylvania School of Medicine with the support of the Progressive Osseous Heteroplasia Association (POHA) in order to identify the cause and to find a cure for POH. In this eighth report of the POH Collaborative Research Project, we present an overview of some of the key research findings by the collaborative research group and include the progress of our program over the past year.

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. During the past year, the POH research program has continued to be supported through the POHA. In addition, research funds to study the genetics of POH were provided by the National Institutes of Health (NIH) and through a Focused Giving Award from Johnson & Johnson.

PROGRESSIVE OSSEOUS HETEROPLASIA (POH)

POH is a genetic disorder of heterotopic ossification (extra-skeletal 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. POH is a very rare human condition, with fewer than 50 people identified worldwide. Our recent studies that identified a mutated gene in many POH patients have indicated that classic features of POH form the extreme end of a spectrum of genetically related conditions.

POH RESEARCH

Identification and Characterization of POH

POH was recognized and described as a unique developmental disorder in 1994. The distinguishing clinical characteristic of POH is the formation of bone in skin (dermis) and subcutaneous tissues followed by progressive and extensive bone formation in deeper soft tissues such as skeletal muscle, tendons, ligaments, and fascia.

The identification of additional individuals who have POH continues to be an important aspect of our research. Only by learning as much as possible about the condition, including the types and severity of associated symptoms, can we fully understand the effects that POH has on patients. The diagnosis of patients who have POH is important not only for advising and counseling those affected individuals and families, but also to help learn more about the condition so that the most productive research can be undertaken in order to develop the most effective treatments.

It is likely that many patients who have POH are misdiagnosed as having other conditions. As information is disseminated about POH through scientific journals, meetings, the Progressive Osseous Heteroplasia Association, the National Organization for Rare Diseases, the National Institutes of Health, and the internet, it is likely that more patients who have POH will be diagnosed.

During the past year, we compiled a list of clinical evaluations and questions that will help us to evaluate patients with POH and POH-like conditions. Rita Bhagat, R.N., the clinical coordinator of the Center for Research in FOP and Related Disorders, is working to obtain and compile as much of this information as possible from patients and their families.

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 identified is called *GNAS* and is located on the long arm of human chromosome 20. (Note: A recent change in gene nomenclature has replaced the previously used gene name “GNAS1” with “GNAS”.)

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 identified by characteristic skeletal morphology (such as the shape of the face and hands) and they frequently show a decreased response to various hormone signals. (When hormone resistance is noted, such patients are also described as having pseudohypoparathyroidism type Ia or PHPIa.) Some patients with AHO have mild ossification of the skin, although their bone formation typically does not progress to affect the deeper tissues such as muscle - as occurs in people who have POH. People with POH have normal skeletal features and have normal response to hormones. Since bone formation in the skin is rare, however, we hypothesized that the *GNAS* gene, which was altered in many patients who had AHO/PHPIa (and had been determined to be the genetic cause of these conditions), might also be the cause of POH.

Our hypothesis that the *GNAS* gene is involved in POH was strengthened by the identification of two patients who had clinical features of both AHO/PHPIa and POH as well as reduced activity of the *GNAS* protein. Furthermore, a mutation in the *GNAS* gene was identified in one of these patients. These findings were not conclusive that alterations in the *GNAS* gene and/or activity of the *GNAS* protein (known as Gs-alpha) caused the extensive bone formation that occurred in these patients, since it was possible that changes in the *GNAS* gene caused the AHO/PHPIa 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 *GNAS* gene of this child's DNA was the first example of a *GNAS* gene alteration that was associated with extensive heterotopic ossification independently of AHO/PHPIa features. (The scientific reports of these studies were published in the November 2000 issue of the Journal of Bone and Mineral Research.)

Our next studies examined DNA samples from all available people with POH, and we discovered disease-causing alterations in the *GNAS* gene in a high percentage of POH patients. Results of ongoing studies suggested that the inheritance patterns of mutations in the *GNAS* gene determined whether a *GNAS* mutation results in POH or AHO/PHPIa in a given individual. In each case for which we can follow the inheritance of a *GNAS* mutation in a family with more than one member with POH, the inheritance of the condition is from a father to his children.

***GNAS*: Mothers and Fathers Make a Difference**

Since we know only a small number of families that show inheritance of POH from parent to child, the possibility that the observed paternal inheritance pattern is just an “interesting coincidence” could not be excluded. During the past year, we conducted a series of complex studies to investigate whether identified *GNAS* mutations in “spontaneous” cases (i.e. patients who are the only person with POH in their family) always occurs on the chromosome inherited from their father. In these patients, the *GNAS* mutation was not inherited from a parent; the DNA change that caused the *GNAS* mutation occurred in that person, likely at a very early stage of embryonic development. Since half of the chromosome set of each cell is inherited from a person's father and half from a person's mother, we investigated whether the “spontaneous” *GNAS* mutation that caused POH occurs randomly on the *GNAS*-containing chromosome from either parent or specifically on a chromosome of a particular parental origin. Consistent with our observations in inherited POH, we determined that the *GNAS* mutation occurred on the chromosome inherited from the father in every case of spontaneous POH that was examined.

Families with AHO/PHPIa often show the reciprocal pattern, with inheritance of the *GNAS* mutation from a mother to her children. This genetic phenomenon, in which the parental origin of a

gene affects its expression in cells, has been recognized for several other genes and is known as genetic imprinting.

Our conclusive demonstration that POH is dependent on *GNAS* mutations that occur on the paternally-inherited chromosome, provide us with important information that we will use to continue our investigation of the expression and regulation of the *GNAS* gene and how this expression and function leads to bone formation in POH.

***GNAS* and POH: What Else...**

It should also be noted that we have not yet found a damaged copy (mutation) of the *GNAS* gene in about one third of examined patients with clinically evident POH. This could mean that the genetic cause of POH in these affected individuals and families is in a regulatory portion of the *GNAS* 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.

We are continuing to investigate the *GNAS* 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 are also examining the *GNAS* gene in family members of POH patients in order to more fully understand the inheritance pattern of the *GNAS* gene - necessary information for comprehending the expression and regulation of the *GNAS* gene. Understanding the effects that reduced *GNAS* 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 altered functioning 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. This discovery was published in the New England Journal of Medicine in January 2002.

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.

The *GNAS* Gene

The structure and regulation of the *GNAS* gene are extraordinarily complex. *GNAS* 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 *GNAS* gene and the corresponding abnormalities in the Gs-alpha protein trigger ectopic bone formation. One likely possibility is 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.

In cells from POH patients, the mRNA that encodes the Gs-alpha protein occurs at 20-60% of the average levels for non-POH individuals. This is consistent with our findings that in most POH patients we only detect the mRNA that is synthesized from the non-mutated *GNAS* gene copy. The mRNA from the mutated *GNAS* gene copy is either not synthesized or is rapidly degraded. Likewise, we found that the Gs-alpha protein is synthesized at reduced levels. During the past year, we have extended these studies to investigate the activity of the Gs-alpha protein in POH cells and have confirmed that the function of the Gs-alpha protein is also reduced. Most of these experiments have been conducted by laboratory research specialist Meiqi Xu.

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 and 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 harbor a handful of genetic alterations. The effects of 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 impact on us. These genetic changes are thought to occur randomly and at a low frequency in our DNA. Most of the people who have POH likely have spontaneous mutations in the *GNAS* gene. This means that the altered *GNAS* gene first occurred in that individual and was not inherited from either parent.

However, once an individual has a mutation that causes POH (or AHO/PHPIa), 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/PHPIa. 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/PHPIa. 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/PHPIa symptoms.

Our studies on the variable expression and the inheritance patterns of *GNAS* mutations are still continuing and more remains to be learned. 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.

The Role of *GNAS* in Bone Cell Differentiation

With the identification of *GNAS* as the mutated gene that causes POH, we now have the opportunity to investigate the role of this gene in directing the fate of cells to become bone. Understanding the cellular and molecular pathways in bone formation that are controlled by *GNAS* gene products, will help us develop treatments for patients with POH and also for many more common diseases of bone formation.

Dr. Robert Pignolo M.D., Ph.D. is working with us to develop cellular systems that will help us address the role of *GNAS* in bone cell differentiation (osteogenesis). Because of the close association of POH bone formation with the dermal subcutaneous fat layer, and with the fat tissue that occurs within skeletal muscle, we have hypothesized that the cells that differentiate into heterotopic osteoblasts (bone cells) may normally be directed toward an adipocyte (fat cell) fate. Using the mouse (which has a *GNAS* gene that is 99% identical to the human gene) as a model system, Dr. Pignolo has successfully developed the methodology to isolate “stem cells” from peripheral adipose tissue. This tissue is analogous to the dermal layer in which bone forms at early stages of POH. We are using assays to induce and evaluate adipocyte and osteoblast differentiation and find that the peripheral fat “stem cells” (but not stem cells from at least some other tissues) have an enhanced osteogenic potential when the expression of the *GNAS* gene is reduced. This fascinating finding is very consistent with the development of heterotopic bone in the dermal fat of POH patients but not, for example, in the bone marrow cavity which is a rich source of osteogenic precursor cells.

Dr. Pignolo, was a Hartford Foundation Geriatrics Fellow in the Department of Medicine at the Hospital of the University of Pennsylvania when he began working with us on 2002. He became a member of the faculty at the University of Pennsylvania School of Medicine in July 2003, and is supported, in part, through a grant from the Center for Research in FOP and Related Disorders. His laboratory and office are near our laboratories in the Department of Orthopaedics and he continues to work together with us on POH.

A post-doctoral fellow, Reem Kanaan, Ph.D., joined our lab group in November 2003 in order to work use the cellular systems that have been developed by Dr. Pignolo and to extend these studies on the effects of *GNAS* gene inactivation in bone cell development. Dr. Kanaan is supported by the J&J Focused Giving Grant.

POH and FOP

POH can be as disabling as its sister disease, fibrodysplasia ossificans progressiva (FOP), when POH bone formation is as extensive in its distribution. Although the gene mutations that cause the two conditions are different, we suspect that part of the bone inducing pathway that is mis-activated in POH is also involved in FOP bone formation. It is also interesting and important to note that the *GNAS* 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.

SUMMARY:

WHAT WE HAVE LEARNED ABOUT POH SINCE THIS WORK BEGAN

Since the initiation of the POH research program, 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 ~60 patients with POH and POH-like conditions.
8. Identified four children with features of both AHO/PHP and POH.
9. Postulated a putative connection between the molecular genetics of AHO/PHPIa and POH.
10. Established *GNAS* as the leading candidate gene for POH.
11. Discovered a heterozygous 4-bp deletion in *GNAS* in a patient with severe plate-like osteoma cutis (POC), a variant of POH.
12. Discovered heterozygous *GNAS* mutations in more than 20 families with classic expression of POH.
14. Determined that, at least in some cells from POH patients, there is haploinsufficiency of expression of *GNAS* mRNA transcription and of the Gs-alpha protein.
15. Determined that, at least in some cells from POH patients, there is reduced functional activity of the Gs-alpha protein as determined by cAMP activity.
16. Determined that POH is dependent on mutations in the paternal *GNAS* allele in both familial and spontaneous cases of POH, further supporting an important role of imprinting in POH.
17. Initiated studies on the role of *GNAS* expression in the regulation of bone cell formation and differentiation.
18. Developed cellular systems to investigate the role of *GNAS* in regulating bone and fat cell differentiation in stem cells derived from various tissues.
19. Wrote and published "*What is POH? A Guidebook for Families.*" The first edition was published in 1997. The guide was updated and revised, and the second edition was published in 2003.
20. 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.
21. Organized and hosted the **Second International Workshop on POH** 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

In our Five-Year Summary Report in 2001, we presented the goals and specific aims for our studies on POH. These goals are incorporated within our NIH and Johnson & Johnson Research Grants and continue to provide the immediate focus for our research.

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

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

Hypothesis 3: POH is molecularly related to Albright Hereditary Osteodystrophy (AHO), and broadens the spectrum of human disorders of osteogenesis associated with inactivating mutations of *GNAS*.

Hypothesis 4: Heterozygous inactivating mutations in the *GNAS* gene alter cellular signaling pathways that direct osteoblast differentiation.

Our current research is intended to accomplish seven specific short-term aims. Many of these aims have been successfully accomplished, but will be strengthened by the addition of information obtained from samples of additional patients.

1. Continue to screen genomic DNA for mutations in the *GNAS* gene in POH patients by PCR amplification and DNA sequencing.
2. Compare the identified *GNAS* mutations in POH patients to those found in patients with AHO/PHPIa, in order to further understand the differences and similarities between these two conditions.
3. Examine expression of the *GNAS* gene in POH patients by quantitation of (a) *GNAS* mRNA (by RT-PCR) and (b) Gs-alpha, the protein product of the *GNAS* gene (by immunoblot analysis).

4. Perform functional analysis of Gs-alpha protein in POH patients' cells (by G-protein-mediated cAMP activity assays).
5. Investigate Gs-alpha protein expression in POH lesional tissue by immunohistochemical analysis.
6. Evaluate the effects of maternal/paternal inheritance of the defective *GNAS* gene on phenotypic expression in all available pedigrees showing inheritance of POH.
7. Examine the effects of *GNAS* inactivation on the cell signaling pathways that result in bone formation.
8. In order to facilitate our ability to test treatments to impede heterotopic ossification, develop transgenic animal models based on reproducing the identical POH-disease mutations in *GNAS* in mice.

Aim 1 will be continuously ongoing. Aim 2 is in progress, and we expect to compile a clinical comparison of POH and AHO/PHPIa over the next year. Aims 3 and 4 have been completed in LCLs (cell lines that have been established from blood) and will be investigated in soft tissue stem cells. Initially, these studies will be conducted in cells from mouse model systems. The immunohistochemical approach in Aim 5 has not been greatly informative, and Gs-alpha protein expression will be further examined more directly in stem cells (Aims 3 and 4).

The genetic studies in Aim 6 have been very successful and will be expanded to further investigate *GNAS* imprinting in POH. These studies provide the basis to investigate how mutations in *GNAS* lead to ectopic bone formation in the skin (osteoma cutis) in AHO or to severe ectopic bone formation in POH. Both mouse and cell culture systems (Aims 7 and 8) are proving to be very useful and successful in our lab for investigating *GNAS* in bone cell formation. These studies are currently working to identify the cells in the skin and other target tissues that are specifically effected by the *GNAS* mutations.

As noted before, *GNAS* encodes a protein (Gs-alpha) that functions as one component of a tripartite relay switch on the inside of the cell membrane. With the addition of a new postdoctoral researcher (to be supported through the POHA grant), we will begin to investigate the cell receptors and effectors that activate the Gs-alpha relay switch and the signaling pathway components that

regulate genes to control bone formation. The components of this signal transduction pathway will be future drug targets for the treatment of POH.

THE IMPORTANCE OF POH RESEARCH

At present, there are no effective treatments or prevention for POH. Analysis of the molecular genetics of POH will increase the 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 treating POH.

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 than its rarity might indicate. 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. Research in POH, therefore, has the possibility of elucidating the pathophysiology of 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.

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 Italian Progressive Osseous Heteroplasia Association (IPOHA)
- 3) The International Fibrodysplasia Ossificans Progressiva Association (IFOPA)
- 4) The Center for Research in FOP and Related Disorders
- 5) The New Jersey Association of Student Councils (NJASC)
- 6) The Four Schools (University of Pennsylvania, Johns Hopkins University, Duke University, Washington University) Medical Student Fellowship Program.
- 7) National Institutes of Health (NIH); National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS).
- 8) Johnson & Johnson Focused Giving Program
- 9) The Hartford/American Federation for Aging Research Academic Fellowship Program.

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REPORTS ON POH RESEARCH

1. The results of some of our research findings on POH have been presented at scientific meetings by members of our research group. Published abstracts are listed:

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.)

Xu, M-q., G. Feldman, M. LeMerrer, Y.Y. Shugart, D.L. Glaser, J.A. Urtizberea, M. Fardeau, J.M. Connor, J. Triffitt, R. Smith, E.M. Shore, F.S. Kaplan (2000). Linkage exclusion and mutational analysis of the noggin gene in patients with fibrodysplasia ossificans progressiva (FOP). *J. Bone Min. Res.* 15 (Suppl 1), S213. (Presented at the American Society for Bone and Mineral Research Annual Meeting; Toronto, Canada, September 2000.)

Xu, M., S.M. Jan de Beur, C. Li, N. Hebela, S. Fitzgerald, M.A. Levine, F.S. Kaplan, E.M. Shore. Expression of mRNA and protein for the stimulatory G protein alpha subunit (Gsa) of adenylyl cyclase in progressive osseous heteroplasia (POH) patients with inactivating GNAS1 mutations. *J. Bone Min. Res.* 17 (Suppl 1), S210. (Presented at the American Society for Bone and Mineral Research Annual Meeting; San Antonio, TX, September 2002.)

Pignolo, R.J., F. S. Kaplan, and E. M. Shore. The GNAS1 gene as a regulator of osteoblast differentiation and molecular target for the stimulation of bone formation. (Presented at the American Geriatric Society Annual Meeting, Baltimore, MD; May 2003.)

Pignolo, R. J., S. Blythe, J. Gilmore, F. S. Kaplan, and E. M. Shore. Differential osteogenic potential in bone marrow and soft tissue stromal cells mediated by the Gnas/GNAS1 gene. *J. Bone Min. Res.* 18 (Suppl 2), S143. (Presented at the American Society for Bone and Mineral Research Annual Meeting; Minneapolis, MN; September 2003.)

2. During the past several years, Dr. Shore and Dr. Kaplan have presented several scientific talks on POH. Presentations for 2003-2004 are listed:

“Two inherited disorders of ectopic bone formation.” Indiana University School of Medicine, Division of Endocrinology and Metabolism, Indianapolis, IN; January 6, 2003.

“POH and FOP: Two inherited disorders of progressive ectopic ossification.” Harvard Institutes of Medicine, New England Baptist Bone and Joint Institute, Beth Israel Deaconess Medical Center, Boston, MA; January 23, 2003.

“FOP and POH - Two genetic disorders of ectopic ossification.” Johns Hopkins University School of Medicine, Division of Pediatric Endocrinology, Baltimore, MD; January 30, 2003.

“GNAS1 inactivating mutations in patients with progressive osseous heteroplasia (POH).” Osteoblast Signaling Pathway Discussion Group (Video-conference, Merck facilitated); January 31, 2003.

- “Inherited disorders of heterotopic ossification - FOP and POH.” Department of Orthopaedic Surgery Grand Rounds, HUP; March 13, 2003.
- “Two inherited disorders of ectopic ossification.” University of Western Ontario, Department of Physiology and Pharmacology and the Arthritis and Skeletal Biology Program, London, Ontario, Canada; April 14, 2003
- “Progressive osseous heteroplasia: The discovery of a new disease and its causative gene.” Kinderklinik and Rheumakinderklinik; Garmisch-Partenkirchen, Germany; August 14, 2003.
- “G-protein mutations in an inherited disorder of ectopic ossification.” 2003 Gordon Conference on Bones and Teeth; Meriden, NH; August 20, 2003.
- “G-protein mutations in progressive osseous heteroplasia (POH), an inherited disorder of ectopic ossification.” Rutgers University, Department of Genetics; New Brunswick, NJ; November 10, 2003.
- “Differential osteogenic potential in bone marrow and soft tissue stromal cells.” 17th Annual Johnson & Johnson Focused Giving Scientific Symposium; New Brunswick, NJ. Invited poster presentation. December 2, 2003.
- “Progressive Osseous Heteroplasia – What we have learned.” Orthopaedic Surgery Bone Discussion Group. January 23, 2004.
- “Rare Diseases of Bone Development.” Penn-CHOP Bone Interest Group. February 2, 2004.
- “Progressive Osseous Heteroplasia.” Penn Epigenetics Interest Group; April 12, 2004.
- “Two inherited disorders of ectopic bone formation.” University of Pittsburgh, Division of Endocrinology and Metabolism, Pittsburgh, PA; April 15, 2004.
- “G-protein mutations in progressive osseous heteroplasia (POH), an inherited human disorder of ectopic ossification.” University of Pennsylvania, Department of Genetics Faculty Retreat; Morris Arboretum, Philadelphia, PA; April 29, 2004.