The POH Collaborative Research Group is an international team of physicians and scientists who have contributed to clinical and basic research on progressive osseous heteroplasia (POH). Research efforts dedicated to finding the cause and to establishing a cure for POH are continuing at the University of Pennsylvania School of Medicine with the support of the Progressive Osseous Heteroplasia Association (POHA). In 1996, the first POHA grant was awarded to study the molecular basis of POH. Since then, the POH research program has also been supported through grants from the National Institutes of Health (NIH) and a Focused Giving Award from Johnson & Johnson.

In this report of the POH Collaborative Research Project, we present an overview of POH and some of the key research findings by the collaborative research group and include the progress of our program over the past year.
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 followed by progressive heterotopic ossification of skin, subcutaneous fat, deep connective tissues, and skeletal muscle at sporadic locations throughout the body. The first signs of POH bone formation commonly occur during childhood. POH is a very rare human condition, with approximately 60 people identified worldwide. Our studies that identified mutations in the GNAS 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. However, it is likely that many patients who have POH are misdiagnosed. As information is disseminated about POH through scientific journals, meetings, the Progressive Osseous Heteroplasia Association (POHA), the National Organization for Rare Diseases (NORD), and the National Institutes of Health (NIH), more patients who have POH will be accurately diagnosed.

Defining the diagnostic criteria by which additional individuals who have POH can be identified 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.

Heterotopic ossification is the most obvious clinical characteristic of POH, however other more subtle features may also be associated. In order to better understand the clinical effects of POH, we evaluated clinical information in patients with POH and POH-like conditions and their families. We have identified clear clinical features that can serve as minimal diagnostic criteria to define and distinguish POH from related conditions. This study was published in the American Journal of Medical Genetics in 2008. During the last year, we have also initiated a study of a mouse model with some features of POH; this model will also help us learn about the effects of the POH mutation.

**Identification of the Altered Gene in POH**

In 1998, the POH collaborative research group began the experimental studies 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 change in gene nomenclature has replaced the previously used gene name “*GNAS1*” with “*GNAS*.”)

As far back as 1995, we 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 often 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 generally normal skeletal features and have normal response to hormones. Since bone formation in the skin is rare, however, we hypothesized that mutation of the *GNAS* gene, which had been determined to be the genetic cause in many patients who had AHO/PHPIa, 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 and who showed reduced
activity of the \textit{GNAS} protein. Furthermore, a mutation in the \textit{GNAS} gene was identified in one of these patients. These findings were not conclusive that alterations in the \textit{GNAS} gene and/or activity of the \textit{GNAS} protein (known as Gs-alpha; Gsα) caused the extensive bone formation that occurred in these patients, since it was possible that changes in the \textit{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 \textit{GNAS} gene of this child’s DNA was the first example of a \textit{GNAS} gene alteration that was associated with extensive heterotopic ossification independently of AHO/PHPIa features.

Our next studies examined DNA samples from all available people with POH, and we discovered disease-causing alterations in the \textit{GNAS} gene in a high percentage of POH patients. Ongoing studies support that the inheritance patterns of mutations in the \textit{GNAS} gene determine whether a \textit{GNAS} mutation results in POH or AHO/PHPIa in a given individual. In each case for which we can follow the inheritance of a \textit{GNAS} mutation in a family with more than one member having POH, we have consistently observed that the inheritance of the condition is from a father to his children.

\textbf{\textit{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. In a recent series of complex studies, we investigated whether identified \textit{GNAS} mutations in “spontaneous” cases (i.e. patients who are the only person with POH in their family) always occur on the chromosome inherited from their father. In these patients, the \textit{GNAS} mutation was not inherited; instead, the DNA change that caused the \textit{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” \textit{GNAS} mutation that caused POH occurs randomly on the \textit{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 \textit{GNAS} mutation
occurred on the chromosome inherited from the father in every case of spontaneous POH that was examined.

Families with AHO/PHPType typically show the reciprocal pattern, with inheritance of the \textit{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 genomic imprinting.

Our conclusive demonstration that POH is dependent on \textit{GNAS} mutations that occur on the paternally-inherited chromosome is a very exciting finding. It tells us that there is a special function for the \textit{GNAS} gene copy when it is inherited from fathers. We are using this important information to continue our investigation of the expression and regulation of the \textit{GNAS} gene and how this expression and function leads to bone formation in POH.

\textbf{The \textit{GNAS} Gene}

Our studies continue to examine the \textit{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 also examine the \textit{GNAS} gene in family members of POH patients in order to more fully understand the inheritance pattern of the \textit{GNAS} gene - necessary information for comprehending the expression and regulation of the \textit{GNAS} gene. Understanding the effects that reduced \textit{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. We are very interested in studying the \textit{GNAS} gene in as many people with POH as possible in order to continue to learn about the genetic changes that can cause POH.

The structure and regulation of the \textit{GNAS} gene are extraordinarily complex. \textit{GNAS} encodes a protein called Gs-alpha (G_{\alpha}) located on the inside of the cell membrane. 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."
Much more additional research is necessary to understand exactly how mutations in the \textit{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. It is possible that 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, assays to study the expression and activity of the \textit{GNAS} gene have shown that the messenger RNA (mRNA; a molecule that is transcribed from DNA sequence of a gene and acts as a “blueprint” for the protein product of the gene) 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 \textit{GNAS} gene copy. The mRNA from the mutated \textit{GNAS} gene copy is either not synthesized or is rapidly degraded. Likewise, we found that the Gs-alpha protein is synthesized at reduced levels and we have confirmed that the function of the Gs-alpha protein is also reduced.

Although Gs-alpha is the main gene product of the \textit{GNAS} gene, \textit{GNAS} also produces other mRNAs and proteins. Synthesis of these additional gene products is regulated by a special type of gene regulation known as “genomic imprinting.” Genes that are “imprinted” synthesize mRNAs only from one of the two gene copies in the cell. A \textit{GNAS} mRNA known as Nesp55 is synthesized only from the gene copy that is inherited from the mother, while the XL-alpha-s and 1A mRNAs are synthesized only from the gene copy that is inherited from the father. Our experiments have shown that POH cells have reduced amounts of XL-alpha-s and 1A mRNAs, suggesting that the clinical expression of \textit{GNAS} inactivating mutation in POH patients may not be solely dependent on the level of Gs-alpha expression, but may also be influenced by the amounts of other \textit{GNAS} gene products.

One well-known molecular mechanism through which genomic imprinting regulates gene expression is through specific patterns of DNA modifications known as DNA methylation. Methylation is a modification of DNA that is associated with gene expression. To determine whether
altered DNA methylation could be the mechanism regulating changed GNAS expression in POH, we examined the GNAS gene from POH cells to look for changes in the DNA methylation pattern. Although the data suggest some variability, no significant correlation of altered DNA methylation and POH appears to occur, indicating that altered DNA methylation and dysregulation of imprinting do not appear to contribute to the pathophysiology of POH. These studies therefore exclude one potential explanation for the cause of POH and direct our focus to other possibilities.

The Role of GNAS in Bone Cell Differentiation

Having identified GNAS as the mutated gene that causes POH, we 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.

Because of the close association of POH bone formation with the subcutaneous fat layer and with the fat tissue that occurs within skeletal muscle, we are investigating the possibility that the cells that differentiate into heterotopic (extra-skeletal) 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, we can isolate “stem cells” (progenitor cells) from peripheral adipose tissue. This tissue is analogous to the subcutaneous layer in which bone forms at early stages of POH. We are using assays to induce and evaluate adipocyte and osteoblast differentiation. Over the past year, we have completed an extensive series of experiments and found that the peripheral fat “stem cells” (but not stem cells from at least some other tissues) have an enhanced osteogenic (bone cell forming) potential when the expression of the GNAS gene is reduced. Our results are 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 also a rich source of osteogenic precursor cells. Using supplemental funding for our NIH grant that was awarded through the federal stimulus American Reinvestment and Recovery Act (ARRA), we are extending this set of
studies to examine *GNAS/Gnas* gene (upper case used for the human gene, and lower case for mouse) expression during adipogenesis.

As an alternate strategy to investigate a regulatory role for *GNAS/Gnas* during osteoblast and adipocyte differentiation, we are quantifying the expression of the GNAS/Gnas transcripts during cell differentiation in the pluripotent mouse embryonic stem (ES) cell model. We have determined that GNAS mRNAs are expressed at low levels during the early stages of osteogenesis, consistent with the reduced *GNAS* expression in POH. Furthermore, if we treat cells to over-stimulate the cell signaling pathways that are mediated by GNAS/Gs-alpha (the reverse of what happens in POH cells), we find an inhibition of osteogenic differentiation (similar to findings in patients with fibrous dysplasia who have activating mutations in *GNAS*). We further found that this effect is specific for the very earliest stages of osteoblast differentiation and is consistent with our hypothesis that this signaling pathway re-directs the fates of cells.

*GNAS/Gs-alpha* signaling plays many important roles in cells and it appears likely that targeting this pathway for therapeutic intervention would likely have many unwanted side-effects. However, cell signaling pathways do not function in isolation within cells, but instead form a network of interacting pathways. An important area of investigation that we have begun to explore is to identify what pathways are changed in response to the *GNAS* mutations in POH. We hope to identify the pathways regulated by *GNAS* that directly influence bone formation and then target these pathways as a strategy to block POH bone formation. One of our most interesting recent projects is examining whether the GNAS/Gs-alpha signaling pathway interacts with the BMP signaling pathway that is altered in patients who have fibrodysplasia ossificans progressiva (FOP; see below); our findings suggest a convergence of cellular events in the two diseases that lead to heterotopic ossification and raise the possibility that similar therapeutic strategies could be effective for both conditions.

**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 \textit{GNAS} gene. This means that the altered \textit{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 \textit{GNAS} 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 \textit{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.

\textbf{POH and FOP}
When POH bone formation is extensive in its distribution, POH can be as disabling as its sister disease, fibrodysplasia ossificans progressiva (FOP). Although the gene mutations that cause the two conditions are different, we suspect that part of the bone-inducing pathway that is dysregulated in FOP is also involved in POH bone formation.

In May 2006, we reported the identification of the gene that is mutated in FOP. This gene, known as \textit{ACVR1}, encodes a bone morphogenetic protein (BMP) type I receptor. BMP and GNAS are parts of distinct cell signaling pathways, however experimental evidence indicates that these two pathways interact to regulate bone and cartilage cell differentiation. Such possibilities will continue to be investigated as part of our future cell signaling studies.

It is also of interest and importance to note that the \textit{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 the initiation of the POH research program, the working group on POH has made numerous advancements toward understanding POH both on clinical and cellular/molecular levels.

• Clinical observations and studies led to the discovery, naming, and identification of POH as a distinct developmental disorder of heterotopic ossification in humans, and provided a detailed clinical description of the disease phenotype, including the histopathology (microscopic tissue characteristics) of heterotopic ossification in POH. POH has been clearly distinguished from fibrodysplasia ossificans progressiva (FOP), another autosomal dominant disorder of heterotopic ossification in children. We have also now clearly defined similarities and differences between POH and other related disorders with cutaneous and subcutaneous ossification.

• Approximately 110 patients with POH and other GNAS-related conditions have been identified and/or examined, and risk profiles for progressive heterotopic ossification have been established. Some patients with atypical presentations (a child with unilateral hemimelic POH; four children with features of both AHO/PHP and POH) have also been examined and may provide additional insight into POH. A small number of multigenerational families with POH are known.

• A connection between the molecular genetics of AHO/PHP1a and POH was recognized and we established GNAS as the leading candidate gene for POH. Heterozygous GNAS mutations have now been discovered in many families with classic expression of POH, as well as a heterozygous 4-bp deletion in GNAS in a patient with severe plate-like osteoma cutis (POC), a variant of POH.

• Our molecular studies have demonstrated that, at least in some cells from POH patients, there is reduced expression of GNAS mRNA and Gs-alpha protein, and that the functional activity of the Gs-alpha protein, as determined by cAMP activity, is reduced as well.

• We determined that POH is dependent on mutations in the paternally-inherited GNAS allele in both familial and spontaneous cases of POH, supporting effects of imprinting in POH. The GNAS gene products that are specifically expressed from the paternally-inherited GNAS allele (XL-alpha-s and 1A) show little expression in cells from POH patients, suggesting that their function may be important in POH.
• We are conducting studies on the role of GNAS expression in the regulation of bone cell formation and differentiation using newly-developed cellular systems to investigate the role of GNAS in regulating bone and fat cell differentiation in stem cells derived from various tissues. Our studies support that low expression levels of GNAS are correlated with osteoblast differentiation.

• We have determined that activity of the BMP signaling pathway that is dysregulated in FOP is influenced by alterations in the cellular pathways that are changed in response to GNAS activity, as occurs in POH patients. This suggests that the heterotopic bone formation that occurs in each of these diseases may share a common cellular mechanism.

• With the support and contributions of the POHA, we wrote and published “What is POH? A Guidebook for Families.” The first edition, published in 1997, was updated and revised in 2003. The First International Workshop on POH was held as part of the Second International Symposium on FOP (October 1995) and was attended by sixty physicians and scientists and by three POH families. This Workshop provided the scientific basis for establishing our international POH collaborative working group. The Second International Workshop on POH 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 GOALS OF POH RESEARCH

We will continue to screen genomic DNA for mutations in the \textit{GNAS} gene in POH patients. Our recent evaluation (Adegbite et al., 2008) has provided us with a much better picture of the clinical changes that are associated with these DNA sequence changes. Additional mutation data from patients will continue to contribute to a more complete understanding of POH and its related conditions.

The mRNA and protein products of the \textit{GNAS} gene determine the function of the gene. Investigations of the relative expression levels of the multiple \textit{GNAS} products will proceed along with studies to investigate the gene regulatory mechanisms that may be altered in POH. The specific regulation of the \textit{GNAS} gene copy that is inherited from fathers is of particular interest.

Having established that heterozygous inactivating mutations of the \textit{GNAS} gene (one affected gene copy) are the cause of progressive osseous heteroplasia (POH), and that these mutations occur on the \textit{GNAS} gene copy that is inherited from fathers, an important goal is to define how these \textit{GNAS} gene changes alter cellular signaling pathways that direct the formation of bone cells.

As we better understand the effects of \textit{GNAS} inactivation on cell signaling pathways that influence bone formation and the role of \textit{GNAS} during the bone cell differentiation, we will identify cellular targets for developing and testing treatments to impede heterotopic ossification in 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 of POH research for affected children and their families is unquestionable. However, the significance 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 uncovering 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 pathophysiologic aspects of common disorders such 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 understanding 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.
REPORTS ON POH RESEARCH

Presentation of our research on POH - through national and international conferences, university seminars and journal publications – provides opportunities to educate the medical and scientific community about POH. Dissemination of such information stimulates new ideas and approaches for understanding POH and encourages others to investigate relevant research questions.

1. The results of some of our research findings on POH have been presented at scientific meetings and reported in scientific/medical journals (2009-2010):


2. During the past year, Dr. Shore and Dr. Kaplan have presented work on POH at scientific conferences and universities. Presentations in 2009-2010 are listed:


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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, University of Pennsylvania
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