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The Molecules of Immobility: Searching for the Skeleton Key

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Abstract: The transformation of soft connective tissue into heterotopic bone is a common feature of at least three distinct genetic disorders of osteogenesis in humans: fibrodysplasia ossificans progressiva, progressive osseous heteroplasia, and Albright's hereditary osteodystrophy. The molecular and cellular basis of transdifferentiation of a mature connective tissue phenotype is a remarkable biological phenomenon with enormous implications for the control of bone regeneration, fracture healing, and disorders of osteogenesis.

Introduction

A novel approach to the isolation, detection, and control of genes responsible for the induction and regulation of osteogenesis involves the identification and study of genetic diseases in which osteogenic induction is specifically dysregulated. Three such diseases of heterotopic ossification in humans are fibrodysplasia ossificans progressiva, progressive osseous heteroplasia, and Albright's hereditary osteodystrophy. In each of these genetic conditions, soft connective tissue is transformed into mature heterotopic bone. These disorders provide an unprecedented opportunity to identify and study osteogenic inductive genes and their relevant regulatory pathways. Detailed knowledge of the master genes and molecular pathways involved in the induction of ectopic osteogenesis would be

invaluable in designing better treatments to control fracture healing, bone regeneration, and osteogenesis in numerous pathologic conditions.

Fibrodysplasia Ossificans Progressiva

Fibrodysplasia ossificans progressiva is a rare genetic disorder of connective tissue characterized by congenital malformation of the great toes and by progressive, disabling heterotopic osteogenesis in predictable anatomic patterns (Table 1). Congenital malformation of the great toes is the earliest phenotypic feature of fibrodysplasia ossificans progressiva and is present in nearly all affected individuals [1--3]. Progressive heterotopic ossification begins early in life with the first involvement typically occurring along the neck and upper back [4,5]. Impending heterotopic ossification is heralded by the appearance of large painful tumors of highly vascular fibroproliferative tissue [6,7] involving tendons, ligaments, and skeletal muscle [8]. The anatomic progression of heterotopic ossification in fibrodysplasia ossificans progressiva occurs in specific patterns (or gradients) over time [4]. Involvement is typically seen earliest in dorsal, axial, cranial, and proximal regions of the body and later in ventral, appendicular, caudal, and distal regions [4]. These developmental patterns are similar to the patterns and progression of embryonic skeletal formation, although the exact cause of this precise pattern is unknown.

Table 1. Clinical features of fibrodysplasia ossificans progressiva, progressive osseous heteroplasia, and Albright's hereditary osteodystrophy

Feature	Fibrodysplasia ossificans progressiva	Progressive osseous heteroplasia	Albright's hereditary osteodystrophy
Sex distribution	Female = male	Female > male	Female > male
Genetic transmission	Autosomal dominant	Autosomal dominant	Autosomal dominant
Congenital malformation of great toes	+	–	–
Congenital papular rash	–	+	+/–
Cutaneous ossification	–	+	+
Muscle ossification	+	+	–
Superficial to deep progression	–	+	–
Severe limitation of mobility	+	+	–
Severe flare-ups of disease	+	–	–
Ectopic ossification after IM injections	+	–	–
Ectopic ossification after trauma	+	+/–	–

Regional patterns of progression	+	–	–
Superficial to deep progression	–	+	–
Definitive treatment available	–	–	–

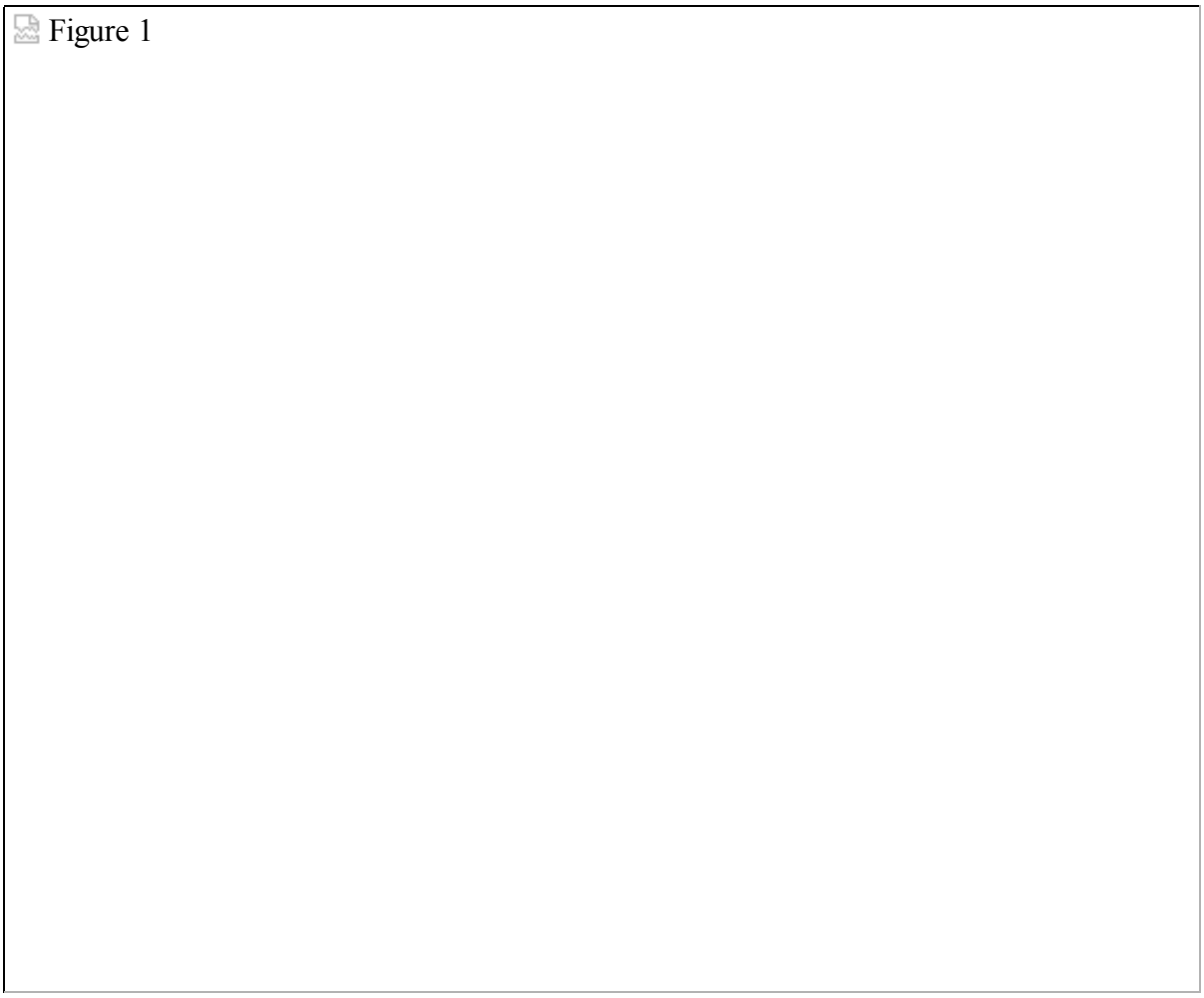

Fig. 1. Clinical Photograph and Skeleton of a Man with Fibrodysplasia Ossificans Progressiva. The rigid posture noted in this 25-year-old man with fibrodysplasia ossificans progressiva is attributable to ankylosis of the spine, shoulders, and elbows. Plates and ribbons of ectopic bone contour the skin over the back and arms **(A),** and can be visualized directly on the skeleton **(B)** (after death from pneumonia at age 40 years). Courtesy, Mütter Museum, College of Physicians of Philadelphia. Reprinted from the New England Journal of Medicine [16].

Progressive episodes of heterotopic ossification lead typically to ankylosis of all major joints of the axial and appendicular skeleton, rendering movement impossible. Although the rate of disease progression is variable [9], most patients are confined to a wheelchair by their early twenties and require lifelong assistance in performing activities of daily living [10]. People with fibrodysplasia ossificans progressiva have markedly reduced

reproductive fitness [1]. Surgical trauma associated with the resection of heterotopic bone, injections for dental work and routine immunizations lead to exacerbation of local ossification [11,12]. At present, there is no effective prevention or treatment.

Fibrodysplasia ossificans progressiva is an autosomal dominant disorder, and most cases are attributable to new gene mutations. The genetic defect and pathophysiology of the disorder are not known, although the bone morphogenetic protein (BMP) genes and other genes in the BMP pathway have been implicated as plausible candidate genes [13--16]. Studies to identify the cause of fibrodysplasia ossificans progressiva are currently focused on the candidate-gene approach since karyotypic abnormalities have not been detected in patients with the disorder and lesional tissue is not readily available for study [17]. Definitive linkage analysis and positional cloning is not possible, since only two small families with inheritance of fibrodysplasia ossificans progressiva have been identified worldwide [9,18--21].

Pathology of Fibrodysplasia Ossificans Progressiva

Histologic examination of early fibrodysplasia ossificans progressiva lesions reveals an intense peri-vascular lymphocytic infiltration followed by lymphocytic invasion into muscle, and robust development of fibroproliferative tissue with extensive neovascularity [22]. Tissue from fibrodysplasia ossificans progressiva lesions at a later stage of maturation shows characteristic features of endochondral ossification including chondrocyte hypertrophy, calcification of cartilage, and formation of woven bone with marrow elements. Fractures through heterotopic bone appear to heal normally [23].

A role for hematopoietic cells in the induction of heterotopic osteogenesis has been suggested [24]. Immunohistochemical evaluation of lymphocyte markers in the early lesions of fibrodysplasia ossificans progressiva revealed a mixed population of perivascular B-lymphocytes and T-lymphocytes and a population of predominantly T-lymphocytes weakly positive for bone morphogenetic protein-4 invading the skeletal muscle [22]. Whether the early lymphocytic infiltrate is part of an inductive cascade, a reaction to it, or both, cannot be determined from the observations in this small sample of patients. The intermediate lesions of fibrodysplasia ossificans progressiva are histologically indistinguishable from those of aggressive juvenile fibromatosis, a condition which does not progress to form bone [6]. However, recent studies document the expression of BMP-4 in cultured fibroproliferative cells and in intact tissue specimens from pre-osseous lesions in patients with fibrodysplasia ossificans progressiva [16], but not from tissue specimens from patients with aggressive juvenile fibromatosis [6].

Levels of basic fibroblast growth factor (bFGF), an extremely potent angiogenic peptide, are markedly elevated in the urine of patients with fibrodysplasia ossificans progressiva during times of disease flare-up [25]. Elevation of urinary bFGF correlates with the appearance of a vascular fibroproliferative lesion.

Molecular Genetics of Fibrodysplasia Ossificans Progressiva

The usual approach of identifying the genetic basis of a disease by performing genetic linkage analysis and positional cloning is presently impossible for fibrodysplasia ossificans progressiva due to the small number of affected individuals and the lack of multi-generational families showing inheritance of the disease. The candidate gene approach has been pursued as an alternative indirect method of attempting to identify the damaged gene. In selecting candidate genes for fibrodysplasia ossificans progressiva, the main diagnostic criteria (congenital malformations of the great toes, heterotopic endochondral ossification, and distinct spatial patterns of ectopic bone formation) must be considered. A

candidate gene for fibrodysplasia ossificans progressiva would need to be one that is functional during normal embryonic development (to account for the malformations of the great toes), and one that could also be activated postnatally to induce severe generalized heterotopic ossification in tendon, ligament, fascia, and skeletal muscle. In addition, the protein product of the responsible gene should be able to induce the entire program of endochondral bone formation.

Among known genes, those that seem to best fit these criteria are the bone morphogenetic protein (BMP) genes [14, 26--32]. Mutations in the genes of two members of the BMP family that result in skeletal abnormalities during embryogenesis have been identified in the mouse. Homozygous deletions of the BMP-5 gene cause malformations of the axial skeleton and abnormal fracture repair [33]. Homozygous mutations of Gdf-5 (growth-differentiation factor-5) result in malformations of the appendicular skeleton [34]. A mutation in the human homologue of the Gdf-5 gene, CDMP-1 (cartilage-derived morphogenetic protein 1), is associated with a recessive human chondrodysplasia, acromesomelic chondrodysplasia, Hunter-Thompson type [35]. Thus, naturally occurring mutations in BMP genes provide evidence for a direct role of at least some of the BMPs in embryonic and postnatal bone formation [29,31].

The BMP genes have been highly conserved throughout evolution [14]. The genes with the highest degree of homology to members of the mammalian BMP family have been found in the fruit fly, Drosophila melanogaster [14,29,31]. The BMP-2 and BMP-4 genes, which produce proteins that are about 90% similar to each other, are homologous to the Drosophilia decapentaplegic (dpp) gene. The DPP protein shows 75% amino acid identity to BMP-2 and BMP-4 in the mature carboxyl-terminal region of these proteins.

These BMPs play critical roles in early embryogenesis and in skeletal formation, important criteria in considering them as candidate genes for fibrodysplasia ossificans progressiva. BMP-4 and DPP are secreted peptides and seem to function by directing cell fate in a gradient-dependent manner [14,36]. The absence of BMP-4 in a transgenic knockout mouse is lethal early in embryogenesis, showing little or no mesodermal differentiation, and no hematopoiesis [30,37]. BMP-4 has also been implicated in patterning of the developing mouse limb. Over-expression of BMP-4 in the chick embryonic limb bud is associated with polarizing defects in limb formation [38].

Whereas the gene structures of Drosophila dpp and human BMP-2 and BMP-4 are very similar, the functional similarities of their protein products are even more striking. Experiments have demonstrated that the BMP-4 gene can rescue embryonic dorsal-ventral lethal pattern mutations of dpp-deficient flies [39]. Furthermore, when implanted into an animal assay system used to monitor bone induction by BMPs, DPP protein can induce bone formation [40].

Drosophila genetics and developmental biology have provided us with several clues to understand BMP function and to select the BMPs as plausible candidate genes for fibrodysplasia ossificans progressiva [14]. Recent studies have examined the expression of many of the BMPs in cells from fibrodysplasia ossificans progressiva patients [6,16].

Early fibrodysplasia ossificans progressiva lesions are histologically indistinguishable from those of aggressive juvenile fibromatosis. However, these two disorders can be distinguished by immunohistochemistry with BMP-2/4 antibodies [6]. Whereas tissue from aggressive juvenile fibromatosis lesions (which does not progress to form bone) shows no binding of the BMP2/4 antibody, fibrodysplasia ossificans progressiva lesional tissue binds the antibody, indicating the presence of the BMP proteins within early stage lesions that will progress to endochondral ossification. Although the antibody used for these experiments cannot distinguish between BMP-2 and BMP-4, the activity of these two BMP genes can be distinguished by examining specific mRNA expression [6].

Northern analysis and ribonuclease protection assays were used to specifically examine the expression of BMP-2 and BMP-4 mRNAs in cells from fibrodysplasia ossificans progressiva patients. Cells derived from a pre-osseous fibrodysplasia ossificans progressiva lesion and from immortalized lymphoblastoid cell lines established from fibrodysplasia ossificans progressiva patients showed increased expression of BMP-4 but not BMP-2 compared with controls. Correlation of BMP-4 expression with fibrodysplasia ossificans progressiva was also observed in a family showing inheritance of fibrodysplasia ossificans progressiva: the affected father and three affected children over-expressed BMP-4, whereas the unaffected mother did not [16]. Further studies have verified that BMP-4 protein is over-expressed in cells from patients who have fibrodysplasia ossificans progressiva [41,42].

In a recent study, semi-quantitative competitive reverse transcription polymerase chain reaction was used to quantify steady-state levels of mRNA expression for BMP-4 and the BMP receptors. These data confirmed the previous finding of elevated steady state levels of BMP-4 mRNA in lymphoblastoid cell lines of affected individuals in a family that exhibited autosomal dominant inheritance of fibrodysplasia ossificans progressiva [42]. However, there were no differences in the steady state levels of mRNA for either the type I or type II BMP-4 receptors between affected and unaffected individuals in the same family. These data support the hypothesis that the molecular basis of BMP-4 signaling is abnormal in fibrodysplasia ossificans progressiva [41].

Given the evidence of BMP-4 over-expression associated with heterotopic ossification in fibrodysplasia ossificans progressiva, several directions are being pursued to understand the exact involvement of BMP-4 in the pathophysiology of the disease. Recent results have indicated that the increased levels of BMP-4 mRNA in fibrodysplasia ossificans progressiva cells are attributable to an increased rate of transcription of the BMP-4 gene [41]. The increased activation of BMP-4 in fibrodysplasia ossificans progressiva cells may be attributable to a mutation within the BMP-4 gene itself or to a mutation in another genetic locus that causes over-expression of BMP-4 in the cells of fibrodysplasia ossificans progressiva patients. The structure and function of the human BMP-4 gene is being examined to understand how the BMP-4 gene is regulated, and the BMP-4 genes of patients with fibrodysplasia ossificans progressiva are being screened for mutations, although none have yet been found. Genetic linkage exclusion analyses is also being conducted using informative polymorphic miscrosatellite markers near the BMP-4 gene.

The appearance of large aggregates of B-cell and T-cell lymphocytes in the intramuscular perivascular space of the earliest detectable lesions of fibrodysplasia ossificans progressiva provides support that lymphocytes and perivascular cells are involved in the induction of osteogenesis [22]. These findings suggest a mechanism to explain the pathophysiology of heterotopic bone formation in this disorder. We hypothesize that lymphocytes capable of expressing BMP-4 circulate in the peripheral blood of patients with fibrodysplasia ossificans progressiva, and are recruited to connective tissue sites after soft-tissue injury [16]. Alternatively, an event at a soft-tissue site may cause an immune-like response and recruitment of lymphocytes, with cells within the soft tissue induced to produce BMP-4. Type IV collagen, a primary constituent of the basement membrane of endothelial cells, muscle cells, and myoblast-like satellite cells, avidly binds BMP-4, and could result in increased local concentrations of BMP-4 [27]. At high concentrations, BMP-4 acts as a morphogen capable of upregulating its own expression [28]. Such an autoregulatory cascade could lead to the development of pre-osseous lesions around muscle satellite cells or pericytes [43] capable of transducing the BMP signal. To test the hypothesis that lymphocyte-mediated BMP expression can result in fibrodysplasia ossificans progressiva lesions, transgenic animal models are being developed to over-express BMP-4 in B-lymphocytes and T-lymphocytes. The expression of BMPs and BMP receptors in hematopoietic stem cells is also being investigated

The stringent temporal and spatial patterns of postnatal heterotopic ossification in patients with fibrodysplasia ossificans progressiva are reminiscent of the patterns of mesenchymal cell condensation during skeletal embryogenesis and suggest a common molecular basis for prenatal and postnatal osteogenesis. Postnatal osteogenesis in humans most commonly occurs during fracture healing. Fracture callus and heterotopic bone formation in fibrodysplasia ossificans progressiva form by endochondral pathways and both involve increased BMP-4 expression [44,45]. BMP-4 over-expression at connective tissue sites leads to focal osteogenesis at those sites [46,47].

Presently, a direct link between fibrodysplasia ossificans progressiva and the BMP-4 gene has not been proven and remains circumstantial. The genetic mutation(s) in fibrodysplasia ossificans progressiva could plausibly reside anywhere in the BMP-4 signaling pathway, or in other molecular pathways that have effects on the level of BMP-4 expression [48].

Progressive Osseous Heteroplasia

Progressive osseous heteroplasia is a distinct genetic disorder of osteogenesis characterized by dermal ossification during infancy and by progressive heterotopic ossification of subcutaneous and deep connective tissue during childhood (Table 1) [49]. The disorder was first described in 1994 [49], and can be distinguished from fibrodysplasia ossificans progressiva, another developmental disorder of heterotopic ossification, by the absence of congenital skeletal malformations, by the absence of predictable regional patterns of heterotopic ossification, and by the predominance of intramembranous rather than endochondral ossification [2]. There have been 13 classic case reports of progressive osseous heteroplasia, 11 in females, and 2 in males [49--53].

The first signs of the disease are the appearance of cutaneous plaques of intramembranous ossification during infancy. The plaques eventually coalesce and progress to invade the deeper connective tissues. Extensive ossification of the deep tissues results in ankylosis of affected joints and focal growth retardation of involved limbs. Patients with progressive osseous heteroplasia have normal intelligence, normal developmental milestones, and lack sustained biochemical or endocrine abnormalities.

The long-term prognosis for patients who have progressive osseous heteroplasia is uncertain, as only several cases have been followed beyond adolescence. At present, there is no definitive prevention or treatment available for children with progressive osseous heteroplasia. The extensive coalescence of ossified skin plaques and the progressive ossification of deep tissues pose perplexing therapeutic dilemmas.

Pathology of Progressive Osseous Heteroplasia

The heterotopic ossification of progressive osseous heteroplasia occurs predominantly by an intramembranous pathway (Table 2) [49]. Recent reports of progressive osseous heteroplasia describe islands of endochondral ossification in the deeper connective tissue with the sporadic appearance of marrow elements [54]. Although the osteogenesis seen in progressive osseous heteroplasia is similar to that observed in Albright's hereditary osteodystrophy, the lesions in Albright's hereditary osteodystrophy are limited to the skin, whereas those in progressive osseous heteroplasia may also involve the deep mesenchymal tissues of the limbs [2].

Table 2. Pathologic features of fibrodysplasia ossificans progressiva, progressive osseous heteroplasia, and Albright's hereditary osteodystrophy

Feature	Fibrodysplasia	Progressive	Albright's hereditary

	ossificans progressiva	osseous heteroplasia	osteodystrophy
Predominant mechanism of ossification	Endochondral	Intramembranous	Intramembranous
Inflammatory perivascular and muscle infiltrate	+	–	–
Hematopoietic marrow in ectopic bone	+	+/–	–
Parathyroid hormone resistance	–	–	+
Generalized hormone resistance	–	–	+
Hypocalcemia, hyperphosphatemia	–	–	+
Pathogenesis	Involves increased expression of BMP4 in most patients	Unknown	Unknown
Genetic mutations	Unknown	Unknown	Inactivating mutation of alpha subunit of G-stimulatory protein of adenylyl cyclase

Molecular Genetics of Progressive Osseous Heteroplasia

Some cases of progressive osseous heteroplasia are sporadic, whereas some are familial [49]. Once the disease appears, it is inherited in an autosomal dominant Mendelian manner with mosaic distribution in affected individuals and variable expressivity between individuals [49]. The etiology and pathogenesis of the disease are unknown.

The anatomic distribution of lesions in progressive osseous heteroplasia suggests that the pathogenesis may involve variable expression of the mutant gene in mesenchymal stem cells destined for widespread mosaic distribution [55]. Although dermal fibroblasts and internal limb structures arise embryonically from limb bud mesenchyme, the fate map of the blastoderm mammalian embryo suggests that specific cell types, such as muscle or bone, are polyclonal in origin. Conversely, in the mature organism, a single cell such as a hematopoietic stem cell or connective tissue stem cell can, under various conditions, generate a wide variety of cell types. At present, little is known about the molecular mechanisms of the signal and response system of mesodermal induction.

A recently discovered promising candidate gene for progressive osseous heteroplasia is Osf2/Cbfa1. Osf2/Cbfa1 is an obligate transcriptional activator of osteoblast differentiation. Osf2/Cbfa1 binds to the OSE-element in the promoter of numerous bone-associated genes to regulate the expression of the osteogenic phenotype. The

spurious expression of Osf2/Cbfa1 in pluripotent mesenchymal cells and in mouse skin fibroblasts induces a mature osteoblast-specific phenotype. Osf2/Cbfa1 is positively regulated by at least several of the BMPs and inhibited by 1,25-dihydroxyvitamin D in the mouse [56,57]. Homozygous knockout of the Osf2/Cbfa1 gene in the mouse leads to complete lack of bone formation by both the endochondral and intramembranous pathways because of a failure of osteoblastic differentiation [57]. Mice heterozygous for the Osf2/Cbfa1 deletion exhibited phenotype abnormalities characteristic of the human skeletal disorder cleidocranial dysplasia [58]. Mutations in Osf2/Cbfa1 cause cleidocranial dysplasia in humans, and heterozygous loss of Osf2/Cbfa1 function is sufficient to produce the phenotype [59]. These and other findings raise the critically important questions: "What is the relationship of Osf2/Cbfa1 to osteoblast commitment and to sustained phenotype expression; and how is the expression of Osf2/Cbfa1 regulated" [60]? Could the mis-expression of Osf2/Cbfa1 in pluripotent mesenchymal cells derived from embryonic somites plausibly lead to the progressive osseous heteroplasia phenotype? Linkage exclusion analysis using polymorphic microsatellite markers closely linked to the Osf2/Cbfa1 gene on human chromosome 6q21 in multi-generational families with progressive osseous heteroplasia may be revealing.

Albright's Hereditary Osteodystrophy and G Proteins

Albright's hereditary osteodystrophy is an autosomal dominant disorder that involves the dermatologic, skeletal, and endocrine systems, with variable features including cutaneous and subcutaneous ossification, pseudohypoparathyroidism, hypoparathyroidism, gonadotropin resistance, obesity, brachydactyly, short metacarpals, and plethoric round facies (Table 1) [61--66]. Interestingly, the most common form of Albright's hereditary osteodystrophy (pseudohypoparathyroidism type 1A) is caused by inactivating mutations of the same *GNAS-1* gene, the activating somatic mutations of which lead to McCune-Albright Syndrome and sporadic fibrous dysplasia lesions (Table 2) [67--79].

Patients with Albright's hereditary osteodystrophy have unusual physical features involving the skeleton and the skin and have resistance to multiple hormones that activate adenylate cyclase [61--79]. Patients with Albright's hereditary osteodystrophy (pseudohypoparathyroidism type 1A) have a 50% reduction in the activity of the stimulatory G protein of adenylate cyclase in plasma membranes of multiple cell types [70]. In contrast to the activating mutations of the *GNAS-1* gene in McCune-Albright Syndrome that lead to increased activity of the stimulatory G protein, a functional inactivation of the stimulatory G protein leads to the multiple organ resistance of patients who have Albright's hereditary osteodystrophy [77--79].

In Albright's hereditary osteodystrophy, the steady-state content of both the long and short forms of the -subunit of the stimulatory G protein are equally reduced [67]. In most patients with Albright's hereditary osteodystrophy (pseudohypoparathyroidism type 1A), the disease is caused by an inherited single-base mutation in the *GNAS-1* gene [67--79].

Recent studies have shown that parathyroid hormone (PTH) and PTH-related protein use a common cell membrane receptor linked to the alpha-subunit of the stimulatory G protein [80]. The normal physiologic roles of PTH-related protein include not only calcium homeostasis but also embryonic bone and cartilage development [80--82]. Germline homozygous mutations in the gene for PTH-related protein are fatal and lead to gross malformations of the skeleton [81]. Heterozygous mutations in the GNAS-1 gene disrupt embryonic signal transduction of PTH-related protein and may contribute to the short stature, brachydactyly, and cutaneous ossification seen in patients who have Albright's hereditary osteodystrophy.

Albright's Hereditary Osteodystrophy and Progressive Osseous Heteroplasia

Whereas cutaneous and subcutaneous ossification occur commonly in patients who have Albright's hereditary osteodystrophy and progressive osseous heteroplasia, progressive ossification of deep connective tissues is not known to occur in patients who have Albright's hereditary osteodystrophy. Similarly, patients with progressive osseous heteroplasia have not been noted to have primary endocrine dysfunction. Recent clinical observations have identified two children from different families who have prominent features of both Albright's hereditary osteodystrophy and progressive osseous heteroplasia [83]. The occurrence in the same patient of two distinct disorders of heterotopic ossification is intriguing and suggests that a common molecular mechanism may be responsible for the unique phenotypic features of Albright's hereditary osteodystrophy and progressive osseous heteroplasia in these two patients.

The exact mechanism by which an inactivating mutation in the alpha-subunit of the stimulatory G protein of adenylate cyclase may lead to progressive osseous heteroplasia remains elusive, as it does for the cutaneous and subcutaneous ossification seen classically in Albright's hereditary osteodystrophy. It is plausible that the molecular basis of at least one form of progressive osseous heteroplasia consists of an as yet undiscovered mutation in the alpha-subunit of the stimulatory G protein of adenylyl cyclase or in a related signaling pathway plausibly involving the common G-linked protein receptor for PTH- and PTH-related protein.

Summary

Three rare genetic and developmental disorders of heterotopic ossification in humans (fibrodysplasia ossificans progressiva, progressive osseous heteroplasia, and Albright's hereditary osteodystrophy) have the potential to illuminate molecular and genetic pathways of osteogenic induction. Insights gained from the study of rare disorders of heterotopic ossification will enhance our understanding of the normal pathways of bone induction, fracture healing, and regeneration. Such knowledge will be useful in designing more effective therapies for disorders of osteogenesis.

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