NIH RELAIS Document Delivery

NIH-11341717

NIH w1 PE 167FG

NIH

5005/13

Michael T COLLINS
National Institute of Dental and Craniofacial Rese
NIH
Building 30 / Room 228
National Institute of Dental and Craniofacial Research
Bethesda, MD 20892

OSI

9/15/11

ATTN:
PHONE: 301-496-4913
FAX:
E-MAIL:

SUBMITTED: 2007-12-11 18:02:04
PRINTED: 2007-12-12 06:04:06
REQUEST NO.: NIH-11341717
SENT VIA: World Wide Web
PATRON TYPE: NED

NIH Regular Copy Journal

20

TITLE: Pediatric endocrinology reviews
VOLUME/ISSUE/PAGES: vol:4 Suppl 4 401 - 11
DATE: 2007
AUTHOR OF ARTICLE: Riminucci, Mara
TITLE OF ARTICLE: The pathology of fibrous dysplasia and the McCune-Albright syndrome.
ISSN: 1565-4753
OTHER NOS/LETTERS: 0792-6863
DELIVERY: E-mail: mcollins@dir.nidcr.nih.gov
REPLY: E-mail: mcollins@dir.nidcr.nih.gov

NOTICE: THIS MATERIAL MAY BE PROTECTED BY COPYRIGHT LAW (TITLE 17, U.S. CODE)

National Institutes of Health. Bethesda. MD 133/298

The Pathology of Fibrous Dysplasia and the McCune-Albright Syndrome

Mara Riminucci[1,2], MD, Pamela Gehron Robey[3], PhD, Paolo Bianco[2,4], MD

[1]Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy, [2]San Raffaele Biomedical Science Park, Rome, Italy, [3]Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD USA, [4]Department of Experimental Medicine, La Sapienza University, Rome, Italy

Corresponding author: Prof. Paolo Bianco, La Sapienza University, Viale Regina Elena 324, Rome 00161, Italy, Tel: 011 39 06 444 1049, Fax: 011 39 06 494 0896, E-mail: p.bianco@flashnet.it

Abstract

Fibrous dysplasia (FD) is the most serious and least understood clinical expression in patients with activating mutations of the GNAS gene. Since the discovery of the causative mutation, important progress has been made in the understanding of the pathology of FD and the pathogenesis of bone lesions. The histology of FD has been reinterpreted in light of the pathological effect of the genetic lesions on mutated skeletal stem cells. True histological hallmarks of the disease have emerged, along with genetic analysis, as additional tools to establish the correct diagnosis. Furthermore, the recognition of FD as a disease of excess, abnormal and imperfect bone formation has helped to explain relevant mechanisms of its clinical morbidity, based on which potential specific therapeutic approaches may be developed in the near future.

***Ref:** Ped. Endocrinol. Rev. 2007;4(Suppl 4):401-411*

***Key words:** Pathology; Fibrous Dysplasia; McCune-Albright Syndrome*

Introduction

Recognition of fibrous dysplasia as a distinct skeletal disease is commonly attributed to the description of an "osteitis fibrosa disseminata" occurring in conjunction with endocrinopathies and skin pigmentation, by Albright et al (1,2) and by McCune and Bruch (3), in 1937. The term "osteitis fibrosa disseminata" was meant to convey the resemblance of the newly recognized entity to von Recklinghausen's "osteitis fibrosa cystica" (4) (hyperparathyroid bone disease) and, at the same time, its distinction from it. Lichtenstein (5) and Lichtenstein and Jaffe (6) recognized that the same skeletal changes could occur as single or multiple lesions, with or without associated extraskeletal disorders, thus providing the first unifying concept of fibrous dysplasia. Lichtenstein (5) also coined the term Fibrous Dysplasia of bone (FD) and Lichtenstein and Jaffe (6) recommended its use in all cases and in all forms of the disease (monostotic, polyostotic, or associated with endocrinopathies and skin pigmentation as in the McCune-Albright syndrome). Following Lichtenstein and Jaffe's recommendation allows us to place emphasis on the bone lesions, which among all of the systemic forms of the disease, remain undoubtedly the most serious, the least understood and the least treatable.

Since the times of Albright, Lichtenstein and Jaffe, there had been little progress in the understanding of the pathology of FD, until the causative mutation was discovered. While pathology textbooks continued to describe FD essentially in the same way as it was originally described, pathologists continued to mistake, not infrequently, FD for other fibro-osseous disorders of bone and even to conceive FD as part of a "continuum" with such other disorders. The discovery of *GNAS* mutations as the genetic basis of FD (7,8) set a precise discontinuity between FD and genetically unrelated, but superficially similar, lesions like cemento-ossifying fibroma or osteofibrous dysplasia. At the same time, it prompted a reappraisal of the pathology of FD, in search of a connection between the newly recognized molecular etiology, the partial elucidation of its pathogenesis and the histological features themselves. The following pages are devoted to these links, to the link between the pathology and the clinical expression of the disease and to newly recognized facets of the histopathology of FD lesions that are either clinically or biologically significant.

Fibrous Dysplasia as a Genetic Disease of Bone Formation

Definitions of FD that are commonplace in textbooks of bone pathology, or in textbooks of metabolic bone disease, are often inaccurate and reflect specific biases. In pathology textbooks, FD is commonly described as an overgrowth of fibrous tissue in bone, reflecting a developmental disorder and associated with an arrested differentiation of bone cells. Deposition of bone is seen as occurring in the absence of osteoblasts, by "metaplasia" of an immature fibrous tissue. This definition is wrong, since bone can never be formed in the absence of bone forming cells (osteoblasts). Furthermore, FD does not represent an impairment either in prenatal bone development, or in postnatal bone formation. Although the causative mutations arise early in development, FD lesions are mostly non-congenital and actually develop after birth, during bone growth. In textbooks of metabolic bone diseases, FD is often defined as a "high turnover disease of bone remodeling". This adopts a point of view (rate of turnover) which is a fundamental tenet of the field, but does not conform to the specific biology of the disease. Bone turnover is indeed altered in FD, but accelerated turnover is a mere epiphenomenon, not a causative mechanism of the bone lesions.

Genetics of FD and MAS

The genetic defect in FD and MAS patients is an activating mutation of the *GNAS* gene, a complex locus on chromosome 20q13 encoding the α subunit of the stimulatory G protein Gs. Although missense activating mutations of *GNAS* may involve either the codon Q227 or the codon R201, only R201 mutations have been identified in MAS patients and in patients with isolated mono or polyostotic FD. Mutations at this site lead to the replacement of the arginine 201 in exon 8 with a histidine or a cystein (7,8) or, more rarely a different amino acid (9). However, the identity of the replacing amino acid does not relate to the cellular abnormality evoked by the specific mutation and does not correlate with clinical expressions thereof. Therefore, it is assumed that it is the absence of the unique lateral chain of arginine, rather than the biochemical properties of the replacing amino acid, that affects the function of the mutated Gs protein. *GNAS* mutations are never inherited, likely due to the lethality of the mutation in the germline (10). The post-zygotic occurrence of the mutation and the consequent somatic mosaic state, provides the most intuitive, although probably not the only, biological explanation for the clinical heterogeneity of FD patients. At the same time it indicates each FD patient as a new mutational event and the R201 codon (3'CGT5') as a mutational hot spot of the human genome (11). The Gs protein is a heterotrimeric protein involved in the cAMP dependent intracellular signaling pathway (12-17). The α subunit binds GTP and plays a critical role in Gs functions. Upon stimulation of Gs coupled surface receptors, the α subunit replaces GDP with GTP, dissociates from the beta-gamma complex and activates the adenylyl cyclase enzyme, thus increasing intracellular cAMP levels. Given its intrinsic GTPase activity, shortly after stimulation, the α subunit turns GTP into GDP thus turning off the system. Replacement of the arg201 in the mutated protein impairs its phosphatase activity and causes the constitutive activation of the pathway. Mutated cells are therefore exposed to the effects of excess endogenous cAMP production (18), which results from the inappropriate stimulation of adenylyl cyclase by the mutated $G_s\alpha$. In bone and marrow, the mutation impacts on cells of the osteogenic lineage at various stages of their maturation (19-21), causing different types of dysfunction.

Clinical and Radiographic Features of FD

As is well known, FD lesions can present as monostotic lesions, or as part of a polyostotic disease, with or without accompanying extraskeletal lesions, as in the McCune Albright syndrome. In polyostotic forms, bone lesions can be ipsilateral or amphilater, monomelic or polymelic and typically include craniofacial lesions. The proximal femur and the craniofacial bones are the two most common individual sites of involvement **(Figure 1)**. Bone lesions develop in infancy and adolescence and are not congenital, even though subtle radiographic anomalies can be detected at birth in bones that will become fibrous dysplastic over time. Bone lesions do not develop after puberty, but can grow after puberty. Radiographic changes can occur well into adulthood and even at mature age. However, these changes are brought about either by superimposed, secondary events

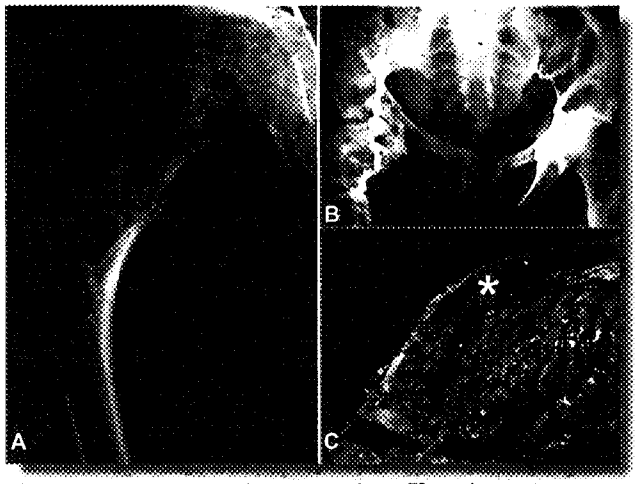

Figure 1. A-B. Radiographic images from FD patients showing a growing lesion of the femoral neck (A) and a case with extensive involvement of right femur and pelvic bones (B). C Post-mortem examination in a case of MAS with severe FD of craniofacial bones. The thickness of the calvaria is markedly increased due to the deposition of intramedullary FD bone (asterisk)

such as engraftment of an aneurysmal bone cyst, or by the continuing remodeling of preexisting lesional bone. This late remodeling may in some cases replace FD bone with normal-appearing bone [reviewed in(22)].

Craniofacial lesions are typically deforming and sclerotic. This reflects in part the higher degree of mineralization of the craniofacial skeleton compared to other bones. When extended to most of the cranium, FD lesions produce a striking and quite characteristic deformity, in which frontal bossing, elongation and flattening of the midface, depression of the nasal bridge and acromegalic traits combine to produce what has been called the "facies fibrodysplastica", a peculiar variety of leontiasis ossea. Limb lesions are typically metaphyseal or diaphyseal in site, central (medullary) in location. The well known "ground glass" appearance of limb FD lesions actually refers to medullary lesions seen in young children, before a host of secondary changes have occurred and express the two fundamental histological changes in FD bone - an excess of trabecular bone within the marrow space and its low degree of mineralization. This basic pattern can be altered by the appearance of areas of stippling, corresponding to foci of high mineral content, which may include remnants of mineralized cartilage or of unaffected bone, or by true lytic areas, indicating intralesional hemorrhage or cyst formation. In addition, peripheral "rinds" of sclerotic bone, or equivalent central regions of sclerosis, signify phases of arrest of the growth of the lesion. The FD lesions most commonly spare the epiphyses, but involvement of the epiphysis does not rule out FD in the individual case. The articular cartilage and joint space are never involved, except by secondary arthritic changes. When cortical bone is extensively involved, the lesion assumes an expansile profile. Focal involvement may result in scalloping of the internal aspect of the cortex. The cortex is never truly interrupted, except when malignancy has superimposed. Deformity is an inherent feature of FD bone, even when the severe measure characterizing the classical "shepherd crook" is not attained. In rare instances, FD lesions may present as bulging masses (so-called fibrous dysplasia protuberans) [reviewed in(22)].

Expression of $G_s\alpha$ in the Bone/Bone Marrow Environment

As revealed by immunolocalization and in situ hybridization studies, $G_s\alpha$ is expressed at comparatively low levels in preosteoblasts (such as cells in the cambial layer of the prenatal periosteum) and at much higher levels in mature osteoblasts, osteoclasts and cells of the microvascular walls (19) in normal bone. The same pattern of expression is observed in fibrous dysplasia (19). Here, osteogenic cells at the surface of bone trabeculae and pericytes of arterial capillaries exhibit the highest levels of $G_s\alpha$ mRNA and protein **(Figure 2)**. The "fibrous" tissue of FD expresses comparatively low levels of $G_s\alpha$, akin to the normal preosteoblasts. The increased levels of $G_s\alpha$ signal in mature osteoblasts compared to less mature osteogenic cells suggests that mutated $G_s\alpha$ is upregulated during osteoblast maturation. Thus, the differentiation of osteoblasts from their progenitors amplifies the effect of the disease genotype on bone tissue. Since cAMP mediated effects on normal osteoclasts are largely inhibitory in nature, one can expect that mutated osteoclasts would be less active in resorption than non-mutated ones. Hence, in spite of the high expression of $G_s\alpha$ in osteoclasts, the higher resorptive activity observed in FD bone in most cases, cannot easily be envisioned as a direct, cell-autonomous effect of $G_s\alpha$ mutation in osteoclasts and FD lesions themselves cannot be seen as an expression of osteoclast dysfunction. The sustained expression of $G_s\alpha$ in the marrow microvasculature on the other hand, is consistent with the notion that adrenergic receptors are in fact G protein coupled. The expression of high levels of $G_s\alpha$ in cells of the microvascular walls in bone does predict a participation of vascular cells in the genesis of FD lesions, in two different ways. On the one hand, recent observations point to pericytes in the marrow microvasculature as a prime source of osteoprogenitor cells in the marrow (reviewed in 23,24). On the other hand, as discussed below, vascular changes are an important component of FD lesions and are directly implicated in their evolution and complications.

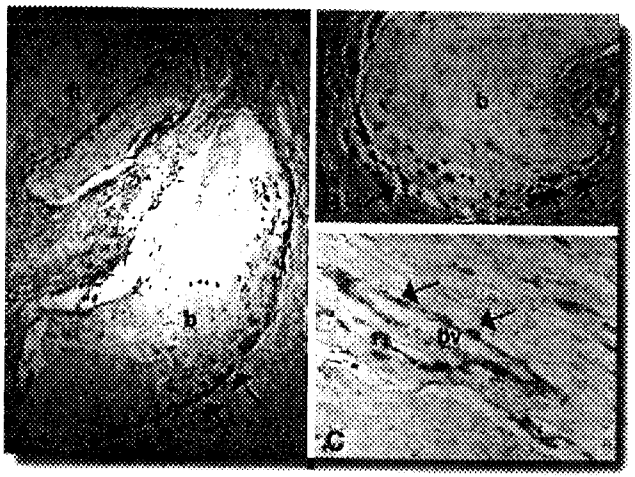

Figure 2. A-C. Immunolocalization of Gsα protein in FD. A strong labeling marks osteoblastic cells directly involved in the deposition of the bone matrix (A, B arrows) and a subset of perivascular cells (C, arrow) (ft fibrous tissue; b bone; bv blood vessel)

Nature of the FD Fibrous Tissue

The "fibrous" tissue filling the space between bone trabeculae in FD is certainly one of the most conspicuous features of FD lesions. For decades, this tissue has been interpreted as spent connective tissue, expressing a developmental arrest of osteogenic differentiation. Studies

using enzyme cytochemistry and immunohistochemistry have revealed that the "fibroblasts" of FD tissue express multiple phenotypic features of osteogenic progenitors (19) and resemble the normal stromal cells found in the marrow space in normal bone (24,25). The spaces between FD bone trabeculae are in fact abnormal marrow spaces, in which an excess of stromal cells accumulate, hematopoietic cells cannot localize and marrow adipocytes do not develop. The latter feature is fully consistent with the negative effects of $G_s\alpha$ overactivity on the differentiation of adipocytes (26,27). Since marrow adipocytes develop from marrow stromal cells (28), mutated stromal cells would be impaired in their ability to give rise to adipocytes within the FD tissue. The accumulation of osteogenic progenitors in FD tissue recalls the "endosteal fibrosis" of hyperparathyroid bone changes (29). Interestingly, in a mouse model in which a constitutively active PTH receptor is targeted to cells of osteogenic lineage, not only a marrow "fibrosis" similar to the one observed both in human hyperparathyroidism and in FD is observed, but over time, bone changes develop that mimic FD bone structure directly (30). Taken together, all these observations allow us to reinterpret the "fibrous" tissue of FD as an accumulation of osteogenic progenitor cells, a view that was confirmed by the observation that clonogenic progenitors of skeletal tissues can in fact be isolated in culture and assayed *in vitro* and *in vivo*. Most notably, clonogenic progenitors isolated from FD tissue can generate, upon in vivo transplantation, miniature replicas of FD bone (20). In other words, they behave as progenitors of FD tissue, in keeping with the demonstration of causative GNAS mutations in at least a fraction of all clonogenic progenitors that can be isolated from a given FD lesion (20). Overall, these observations portray FD as a disease of the osteoblastic lineage.

FD as a Disease of Bone Formation

Recognizing that FD is a disease of the osteoblastic lineage facilitates picturing FD as a disease of bone growth and modeling, rather than of bone development or remodeling. In a nutshell, FD is a disease of *excess* bone formation, of *abnormal* bone formation and of *imperfect* bone formation - all occurring during bone growth. It is a disease of *excess* bone formation, because it leads to a primary localized increase in bone mass. This is fully apparent in lesions of young patients, but may be obscured by subsequent, secondary changes in older lesions, arising from continuing, unabated remodeling and cyst formation. It is a disease of *abnormal* bone formation, because bone formation does not adhere to the architectural design of the affected bone segments. The spatial definition of cortical bone, trabecular bone and marrow space is lost in FD bone and mechanically unsound bone is formed with haphazard trabecular architecture and an irregular internal structure. Finally, FD is a disease of *imperfect* bone formation, because the matrix is abnormal in chemical composition, texture and mineral content. Whereas the excess bone formation and the architectural changes contribute to clinically apparent bone deformity, it is the imperfection of the bone matrix that leads to fracture. Secondary changes, such as cysts and hemorrhage, further contribute to generate clinically relevant events in the natural history of individual patients, as well as to diversify the histological picture observed in individual FD lesions.

Abnormal Bone Modeling in FD

Modeling of bone is the process that establishes and maintains through growth, the external shape and internal architecture of individual bone segments. It is through modeling that spatial specifications and architectural diversity of cortical bone, cancellous bone and marrow space are generated. A simple submacroscopic view of a bone segment affected by FD readily reveals that the distinction of cortical and trabecular bone is lost in it and marrow space is not sculpted. The cortical bone is too trabecular to be cortical and marrow space is filled by an excess of bone trabeculae. These changes emanate from an excess of bone resorption in the cortex and an excess of bone formation in the medullary space, which together tend to equalize the bone structure throughout, resulting in a plexiform architecture of short trabeculae. Both in the cortical and medullary regions, space between the abnormal bone trabeculae is filled with a "fibrous" tissue and does not accommodate hematopoiesis. These changes reflect the blurring of the architectural plan of the affected bone segment and denote FD as a disease of bone modeling.

Abnormalities in Bone Deposition

FD bone is deposited by osteoblasts, exactly like all kinds of bone. In tissue sections of FD, however, osteoblasts are not easily recognized due to their unusual shape. Whereas normal osteoblasts are cuboidal, FD osteoblasts are retracted and stellate in shape (**Figure 3A**). This makes them inconspicuous, leading to the common belief that FD trabeculae are typically not bordered by osteoblasts. The abnormal shape of FD osteoblasts is a direct in vivo correlate of classically known effects of cAMP on osteoblastic cells in culture (31). The bone trabeculae resulting from fibrous dysplastic bone formation are woven in structure. Within a given sample of FD tissue, scattered trabeculae that display a lamellar rather than woven structure usually represent remnants of pre-existing, partially resorbed normal bone (19). The edge of the fibrous dysplastic trabeculae are noted for arrays of collagen bundles running perpendicular to the trabecular surface, instead of parallel to it (32) (**Figure 3B**). These bundles are identical to Sharpey fibers, a normal feature of sites of tendon and ligament

insertion into bone as well as of sutural bone growth in cranial bones. Away from the bone edge, Sharpey fibers merge with the "fibrous" tissue next to bone. Towards the interior of the trabeculae, the Sharpey fibers merge with the woven texture of FD bone collagen. Together, the Sharpey fiber pattern and the retracted, abnormally shaped osteoblasts represent the most characteristic and consistent histological feature of FD bone. Although multiple overall patterns of FD bone architecture [so called "Chinese writing", sclerotic-pagetoid, hyperosteocytic (32)] can be encountered depending on the site and age of the individual lesion, the combination of Sharpey fibers and retracted osteoblasts is regularly observed and represents a true histological hallmark of the disease. Immunohistochemical studies of FD bone have revealed that FD osteoblasts produce a concoction of bone matrix protein that is conspicuously departed from the normal stoichiometry of normal postnatal bone matrix. Levels of non-collagenous proteins in particular (most notably bone sialoprotein and osteocalcin) are altered, both in terms of cellular immunoreactivity and in terms of deposition within the matrix.

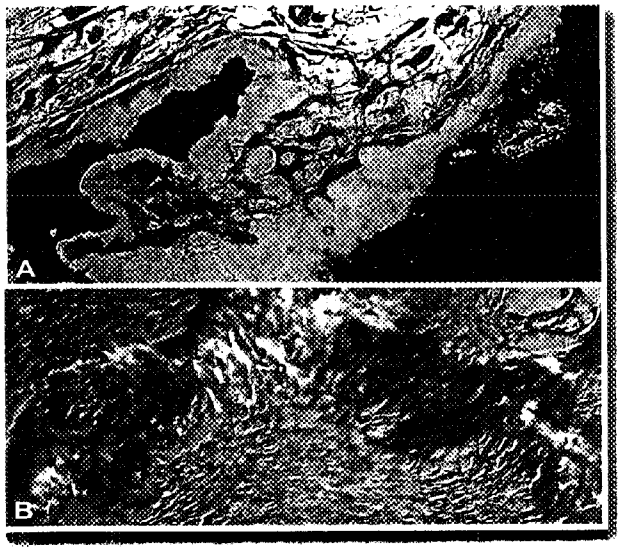

Figure 3. A. Mutated FD cells producing osteoid (o) along the border of bone trabeculae, are hardly recognized due to their abnormal stellate shape (arrows) B. Retracted cells are intimately associated with collagen fibers (Sharpey fibers, asterisk) perpendicularly oriented to bone surfaces (b) (A. undecalcified tissue stained with von Kossa stain; B H&E stain)

Mineralization of FD Bone

Although classical descriptions of FD bone included mention of the fact that the affected vertebra of a child could be shaved and cut with a nail (33), the fact that FD bone is usually markedly undermineralized has escaped perception for decades. Indeed, the fibrous dysplastic calvarium of a child can be cut with a scalpel even if ten-fold thicker than normal. The reason for such prolonged lack of recognition of a mineralization defect in FD bone is somewhat trivial. Sent to routine histology laboratories serving surgical pathology units, FD samples are usually demineralized prior to processing, which makes it impossible to detect mineralization defects in paraffin sections. Observation of plastic embedded sections stained for mineral readily reveals the severe mineralization defect that occurs in FD (21) (34). Contrary to a common belief, FD bone is not reduced in mass, compared to normal cancellous bone. It is reduced in mineral content. Fatigue fracture and deformity, including the classical "shepherd's crook" deformity of the proximal femur, find in the undermineralization of bone tissue the most simple and direct explanation. Undermineralization is expressed in tissue sections by a large amount of unmineralized osteoid **(Figure 4A)**. When analyzed by polarized light microscopy, this has a woven collagen texture and includes Sharpey fibers **(Figure 4B)**. Woven bone is a

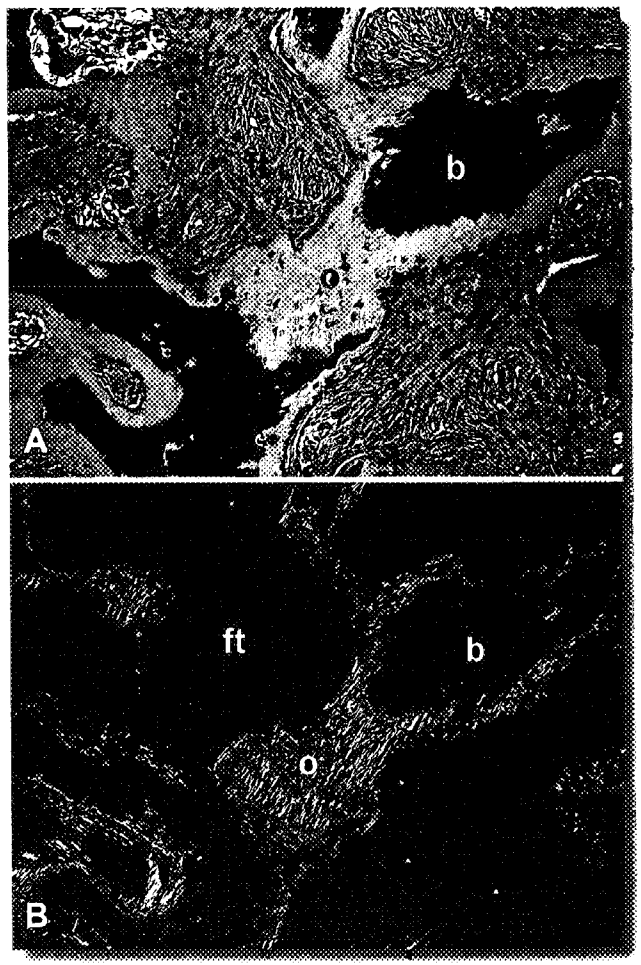

Figure 4. A-B. Undecalcified, plastic embedded, von Kossa stained FD tissue. Bone trabeculae are bordered by a thick layer of unmineralized osteoid (A. standard light microscopy) that is woven in structure (B. polarized light microscopy) (ft. fibrous tissue; o.osteoid; b. mineralized bone matrix)

normal feature of normal fetal bone. The woven texture expresses a high rate of deposition and a peculiar matrix stoichiometry noted for a relatively high content of highly hydrated non-collagenous proteins and a relatively low content in collagen, compared to adult lamellar bone (35) (36). For the very same reasons, (normal) woven bone is always rapidly mineralized, at a faster rate and, to an extent, greater than adult lamellar bone. Therefore, the undermineralized woven bone seen in FD is, in terms of bone structure, a conspicuous anomaly in nature and represents per se a severe form of mineralization deficit.

When analyzed using histomorphometry parameters, FD bone reveals values of osteoid thickness (OTh) and of osteoid surfaces (OsBs%) that fall well within the range conventionally considered diagnostic for a true osteomalacic change in lamellar bone (34). Tetracycline labeling in FD bone reveals the absence of dual labels and a smeared pattern of single labeling, again consistent with a genuine osteomalacic change (34). To analyze the degree of mineralization of mineralized FD bone, rather than osteoid, quantitative back-scattered electron imaging has been used (37). This technique reliably measures mineral density in bone. Its application to the study of FD bone revealed that the degree of mineralization attained in mineralized portions of the FD bone is itself lower than normal (34). This is not the case in other genetic diseases of the skeleton noted for high levels of bone turnover and deposition of "immature" and fragile bone. In Osteogenesis imperfecta, for example, the "immature" bone is even more mineralized than normal bone (38). Hence, the mineralization defect seen in FD is a unique feature of the disease. Some determinants of this histological phenomenon of major clinical relevance have been elucidated at this time. Renal phosphate wasting, hyphosphatemia and low levels of $1,25(OH)_2D_3$ are major determinants of the mineralization defect observed within FD lesions (39). It is important to note that the same determinants underlie the picture of generalized hypophosphatemic rickets that can superimpose on FD (40-42). Whereas these cases represent instances of generalized osteomalacia, the localized mineralization defect can occur and does occur in most cases, independent of the presence of a true generalized osteomalacia. FD lesions can be seen as localized forms of osteomalacia. At the same time, the dissociation of local osteomalacia from general osteomalacia suggests that additional, intrinsic local mechanisms of impaired mineralization may operate in FD bone and remain to be clarified.

The similarities between the phosphate wasting syndrome complicating FD and tumor-induced osteomalacia (TIO) prompted a search for potential common mechanisms operating in the two conditions. This led to the recognition that FGF-23, the humoral factor mediating renal phosphate wasting in TIO, is implicated in the phosphate syndrome of FD (43). More important, it was recognized that FD bone itself represents the source of the excess circulating FGF-23 in FD patients and that a correlation can be found between disease burden and FGF-23 levels. Cells of osteogenic lineage appear to be the major source of FGF-23 in FD bone, as revealed by in situ hybridization studies (43). FD osteoblasts, osteocytes, fibrous cells and a population of perivascular adventitial cells all express significant levels of FGF-23 mRNA in situ, indicating that the whole osteogenic lineage participates in its production **(Figure 5)**. However, at the single cell level, a mutation dependent excess production of FGF-23 could not be documented in *GNAS*-mutated osteogenic cells compared to normal cells. Hence, the excess production of FGF-23 in FD should rather be ascribed to the production of the factor at normal rates by an excess of cells competent to produce it (osteogenic cells). While setting a major distinction with true TIO (in which a vast excess of circulating FGF-23 is sustained by minute and often occult tumors), these observations also revealed that bone itself, as a source of FGF-23, directly contributes to the hormonal regulation of renal phosphate handling, as a true diffuse, mesodermal endocrine gland.

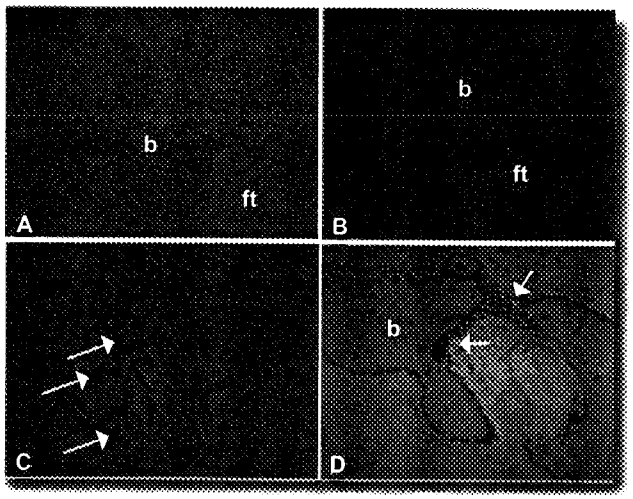

Figure 5. *A-C. Expression of FGF-23 mRNA in FD tissue. By in situ hybridization, FGF-23 mRNA is detected in osteogenic cells in the fibrous marrow (ft) and in bone trabeculae (b). High levels of FGF-23 expression characterize mature bone forming cells along and within the bone matrix (C, arrows). D. Expression of FGF-23 mRNA in normal bone. ISH labeling of osteoblastic cells in a healing fracture (arrows) reveals that FGF-23 is normally produced by mature osteogenic cells at the site of bone growth.*

Remodeling of FD Bone

The best known architectural abnormalities of FD bone [the bizarre trabecular shapes described as C-shaped, S-shaped, Chinese writing or alphabet soup (33,44)] arise from remodeling of FD bone, not from its primary deposition **(Figure 6)**. In fact, these patterns are seen in long-standing FD lesions, in which

multiple cycles of remodeling have occurred, but are not seen, or are not prominent, in young lesions (young children). Likewise, independent of patient's age, these patterns are less expressed at skeletal sites in which remodeling is inherently slow, independent of FD, such as the craniofacial bones. Lesions of gnathic bones for example often exhibit a unique pattern, sharply departed from the "Chinese writing" pattern, which has been termed hyperosteocytic (32). In this type of lesion, parallel plates of FD bone are laid down, featuring regular rows of osteoblasts at homologous sides. Whereas the Chinese writing pattern arises from remodeling, the hyperosteocytic pattern arises from modeling. Location of osteoblasts at single and homologous sides of the individual rods in the "hyperosteocytic" pattern indicates a modeling drift as a contributor to the origin of the peculiar histological pattern. In contrast, the "Chinese writing" pattern arises mostly from tunneling resorption (resorption from within) of FD bone. Tunneling resorption is the normal pattern of resorption in Haversian remodeling of cortical bone. In normal remodeling of cancellous bone, resorption is predominantly a surface phenomenon. Tunneling resorption of trabecular bone, however, is a known common finding in hyperparathyroidism (45). Tunneling resorption reflects the recruitment and activation of osteoclasts along the internal bone surface bordering intratrabecular vascular spaces. This compartment is usually not activated with steady state bone remodeling, but it is recruited to remodeling in hyperparathyroidism and FD. Mechanisms leading to increased osteoclast differentiation and activity have been elucidated only partially. High expression of the IL-6 gene has been shown in FD tissue in situ at the sites of osteoclastic differentiation (46) and increased production of IL-6 in FD cells in culture has also been reported. Enhanced IL-6 production may arise as a consequence of the upregulation of c-fos in mutated cells (47).

Histomorphometric indices of bone resorption (osteoclast numbers and surfaces) are usually higher in FD bone compared to age-matched reference values and correlate strictly with urinary pyridinium cross-links (34). However, excess resorption within FD is neither uniform, nor a necessarily autonomous expression of the disease. Significant regional variations in osteoclastic activity are observed in FD and edges of lesions are often noted for a relatively more prominent osteoclastic activity. Significant variations also occur in individual patients. In a series in which histomorphometric parameters could be correlated with serum and clinical features, it was observed that only a subset of FD lesions were noted for high values of histomorphometric parameters of bone resorption. These correlated tightly with levels of circulating PTH. Indeed, hyperparathyroidism secondary to low $25(OH)D_3$ can occur in FD and it is in these cases that the most dramatic increases in osteoclast activity is observed histologically. In these cases, tunneling resorption is more obvious and extensive than usual and large clusters of osteoclasts and mononuclear TRAP-positive cells (mini-brown tumors) are observed. Based on these findings, indeed, secondary hyperparathyroidism superimposed on FD can even be blindly predicted by a bone pathologist with specific experience.

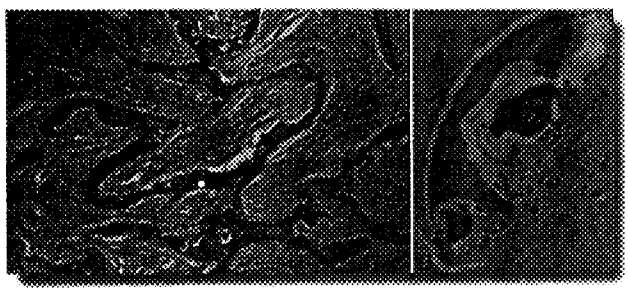

Figure 6. *A. Chinese writing pattern of bone formation in FD. Thin bone trabeculae (b) are haphazardly distributed in the fibrous background (ft) and show bizarre shapes. B. FD bone trabecula (bt) with the typical C-shape resulting from the increased resorption by osteoclasts (ocs) (H&E stain)*

The occurrence of hormonally-directed tissue changes within FD bone demonstrates that the FD lesion remains hormonally responsive and that in fact the histological picture observed can represent a dual input, including changes arising from the inherent dysfunction of local cells and changes reflecting a specific hormonal climate. Whereas the blend of FD-autonomous and PTH-induced changes has been somewhat precisely recognized, further study will be necessary to determine whether other common hormonal imbalances or changes in MAS/FD patients (hyperthyroid states, GH excess, puberty or precocious puberty, pregnancy) are in turn reflected in recognizable changes in the lesion histology.

Vascularity and Intralesional Bleeding

Rare recorded instances of high output cardiac failure in FD (48) are attributed to extensive arterovenous shunts generated by remodeling of the local, rich vascularity of FD tissue. Surgeons often experience unusually high bleeding of FD bone (49). Engrafted aneurysmal bone cysts often complicate FD lesions (50) (51) (52) generating an acute event reflected in a marked expansion of the lesion and of the bone contour. When occurring in craniofacial FD, acute bleeding can cause catastrophes [sudden blindness from encroachment on the optic nerves (53)], or less ominous consequences such as the formation of pseudocysts with air-fluid or fluid-fluid levels [often improperly referred to, when occurring in sinusal space, as "mucocoeles" or aneurysmal cysts (53,54)]. The native structure of craniofacial bones, namely their rich porosity and wealth of macroscopic and microscopic foramina giving passage to vascular and nervous structures, makes them particularly prone to bleeding complications, also through herniation and constriction of the richly vascular FD tissue. This wealth of bleeding-related complications of FD all emanate from a common root that is the unusually rich and

architecturally abnormal vascularity of FD tissue. Both arterial capillaries and venous sinusoids are increased in number per unit volume of tissue in FD and ectatic capillaries engorged with blood are commonly seen along and even inside the trabecular surfaces (49). This excess vascularity reflects the coupling of osteogenesis and angiogenesis, which leads to excess vascularity in the presence of excess osteogenic tissue, as in FD. FD vessels are extremely prone to bleeding and microhemorrhages are common. At times, even microscopic but otherwise histologically typical aneurysmal bone cysts can be detected at biopsy.

Diagnostic Significance of FD Histology

In general, the clinical picture of the full blown McCune Albright syndrome is sufficiently expressive to dispel the need for histological confirmation of the nature of the bone lesions. In endocrinopathy- free forms of polyostotic involvement, skeletal angiomatoses and enchondromatoses, more rarely multiple non-ossifying fibromas, do enter a differential diagnosis, which is readily addressed by the typical histological features of FD. In monostotic forms, particularly those occurring in the craniofacial region or in the tibia, diagnostic confusion may arise clinically, particularly versus ossifying fibromas and osteofibrous dysplasia. In these cases, awareness of the true histological hallmarks of FD is of great help in establishing the correct diagnosis on histological grounds. Based on the frequency of misdiagnosis of FD in such circumstances (17% in a large retrospective series collected in Europe and 5% in a larger series in North America) the task is apparently not trivial in many cases. Both in polyostotic and monostotic forms in which a diagnostic dilemma may be encountered, analysis of *GNAS* mutation can effectively complement histology, as none of the disorders entering a differential diagnosis has ever been found to be associated with *GNAS* mutations. Mutation analysis may be of specific help in those cases of craniofacial disease in which a significant resemblance of FD tissue to non-ossifying fibroma (NOF) is encountered (55). In mutation-positive FD, formation of cementum or psammoma bodies (characteristic of ossifying fibroma) may be found (in fact, psammoma bodies and cementum like structures can even be found in long bone FD, arising from dystrophic, single cell mineralization of FD cells). In these cases, the presence or absence of *GNAS* mutation can clearly assign an individual lesion to FD versus ossifying fibroma. However, it must be emphasized that mutation analysis must always be performed on the pathological tissue and that, given the mosaic condition of FD patients, negative results obtained on peripheral blood lymphocytes may not be reliable.

In the long bones, non-ossifying fibromas may both mimic and be mimicked by FD. A storiform (from Latin *storea*, mat) arrangement of fibrohistiocytic cells with interspersed giant cells, foam cells and hemosiderin deposits may be seen in "aged" FD lesions and contribute to mimic a non-ossifying fibroma, particularly when radiographic studies provide evidence of cortical involvement. NOF-like changes reflect post-hemorrhagic reactions in FD. Multiple NOFs can occur along with skin pigmentation and various extraskeletal disorders in the Jaffe-Campanacci syndrome (JCS) (56) and the multiple bone lesions may be unilateral both in the JCS and in polyostotic FD. Thus, a differential diagnosis between FD and NOF is in order when dealing either with individual lesions, or with multifocal, polyostotic disease. There has been no attempt to demonstrate *GNAS* mutations in the JCS as a potential cause to date. Osteofibrous dysplasia [ossifying fibroma of long bones, Campanacci's lesion (44,57)] affects the tibia and fibula of young children in a highly restricted fashion. It is readily distinguished histologically from FD, also based on the abundance of typical cuboidal osteoblasts. Osteofibrous dysplasia is not associated with *GNAS* mutations (58). The so-called liposclerosing myxofibrous tumor, an FD-like lesion affecting the proximal femur of young adult, represents true mutation positive FD in many cases and probably in all (59).

Tumors in FD

Occasionally, malignant bone tumors develop from pre-existing FD lesions of bone. Neither the extent of the disease, nor the concurrence of endocrine dysfunction necessarily predicts the risk for malignant change in FD. In a seminal study in which 28 cases of malignant bone tumors were found to complicate FD in a series of 1122 cases (60), the vast majority of these tumors in fact complicated monostotic FD, even though criteria for classification of FD in that series might have been less accurate than they would be in a prospective study. A history of radiation therapy was available for about half of the tumors, indicating that part of the risk of malignancy is linked to improper radiation therapy (no longer in use nowadays), but also that transformation can indeed occur independent of irradiation. In a series of studies, different types of clonal chromosomal aberrations in FD [**t(6;11)**, +2, rearrangements involving chromosome band 12p13 and others (61-63)] have been reported. A "true" neoplastic nature of FD per se has been contended on this basis. Rather than supporting the contention, these observations may however simply relate to a phase of secondary clonal evolution of FD. Depending on the nature of the associated secondary chromosomal changes, this kind of event may ultimately, but not necessarily, result in the development of a malignant tumor upon a "second hit" (as per the classical Knudson's two-hit hypothesis). These events may thus signify the emergence, within FD, of true pre-neoplastic changes. Considering the low but definite risk of malignancy in FD, this is not inconceivable.

Tumors that complicate FD most often develop in the craniofacial skeleton. They are usually aggressive and have a poor prognosis (64). Osteogenic sarcoma is the most common type of tumor, but chondrosarcoma, fibrosarcoma, malignant fibrous histiocytoma and rarely, angiosarcoma have all been reported (65) (66) (67). This variety of tumor types may reflect the involvement of skeletal progenitor cells (osteo, chondro and fibrogenic progenitors) as the target of the transforming events. The occurrence of tumors of angiogenic lineage, in turn, may be taken as further evidence for the impact of *GNAS* mutations on the bone/bone marrow vascularity as a contributor to FD-related tissue changes.

FD and certain malignant bone tumors may mimic each other clinically and histologically. Low-grade central osteogenic sarcoma (LGCOS) may on occasion be disguised as FD clinically and radiographically and be misdiagnosed as such (68). A subset of these tumors is even referred to as "FD-like" low grade osteogenic sarcoma. Recently, a *GNAS* mutation has been demonstrated in one case of so-called FD-like osteogenic sarcoma (68). This in fact complicates the use of mutation analysis for differential diagnosis between FD and LGCOS, but suggests that FD may evolve into a tumor retaining resemblance to the original FD lesion.

Pathology of Extraskeletal Lesions

Unlike fibrous dysplasia, which represents a highly characteristic pathological pattern in bone, lesions caused by activating *GNAS* mutations in extraskeletal organs are not characteristic, for the most part. To mitigate this statement, it should be noted that formal studies aiming at identifying mutation-linked patterns of pathology have seldom if ever been conducted in extraskeletal organs. Nonetheless, in all organs that are the target or the source of significant pathology in MAS, a hyperplastic change in parenchymal tissues is the common denominator. In keeping with the proliferative effects of excess cAMP in parenchymal tissues (an effect not observed in mesenchymal tissues such as bone), changes in endocrine organs such as thyroid, pituitary, ovary and testis are readily accounted for as either true adenomatous proliferation, or simple hyperplasia (**Figure 7**). In the ovary, nondescript follicular cysts underlie or associate with precocious puberty. In the testis, Leydig cell hyperplasia or submacroscopic adenoma can be observed. Primary adrenocortical hyperplasia underlies Cushing's syndrome in most cases, although one report has documented an activating *GNAS* mutation in the pituitary of a child with true Cushing's disease (69). Thyroid hyperfunction is most commonly associated with either hyperfunctioning adenoma or goitrous changes. Whereas a large proportion of isolated somatotroph adenomas are in fact caused by *GNAS* mutations, GH excess in the context of FD seems to be more commonly associated with diffuse somatotroph hyperplasia, which can however evolve into a sizable adenoma over time. Muscular myxomas associate with FD in Mazabraud's syndrome and the *GNAS* mutation can be detected in the muscle. However, the histology of mutation associated myxomas is not different from usual myxomas. In the rare instances in which skin lesions become available for histological study, a nonspecific diffuse pigmentation of the basal and suprabasal layers, with or without a lentiginous hyperplasia of melanocytes is observed.

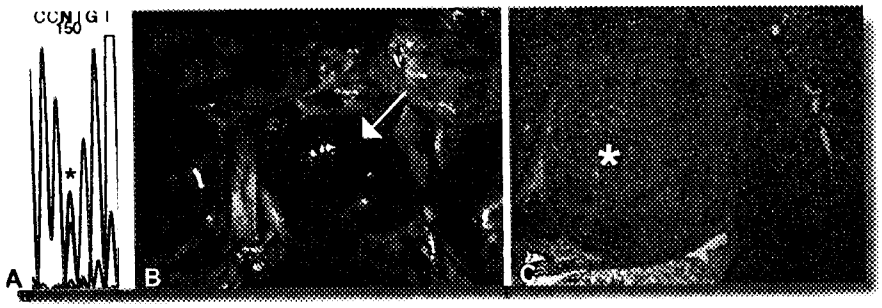

Figure 7. A. Sequence of the relevant fragment of the GNAS gene in a MAS patient. A heterozygous missense mutation in the CGT codon specifying the Arg 201, is revealed by the presence of a double peak. In this case, a G > A transition is observed that replaces the Arg 201 with a Cys in the Gsα protein. GNAS R201 mutations cause FD and a wide range of extraskeletal lesions. MAS patients are typically affected by endocrine lesions such as pituitary adenomas (B, arrow) and primary hyperplasia of the adrenal cortex (C, asterisk)

Lesions in other organs may in contrast reflect unique patterns of expression of the consequences of *GNAS* mutations. Instances of cholestatic neonatal liver disease have been reported, as associated to detection of *GNAS* mutations in the liver. In some of these cases, unusual patterns of liver progenitor cell proliferation are observed (our unpublished observations), deserving closer scrutiny. It is interesting to note that liver changes seem unique of newborns and transient in nature. Full recovery of normal liver function has been documented in at least some cases and no clinically significant liver disease or dysfunction has been reported in adults with MAS. Finally, the complex effects of individual organ dysfunction brought about by *GNAS* mutation at specific sites may generate equally complex patterns of secondary changes at a distance. The range of these almost coincides with human pathology and need only to be mentioned to remind involved physicians of the extraordinarily pleomorphic patterns of pathology that can be observed in individual patients with disseminated *GNAS* disease.

Acknowledgments

Personal work mentioned in this article was supported by Telethon Fondazione Onlus (grant GGP04263 to PB).

Disclosure

The authors do not have any conflicts of interest.

References

1. Albright F, Scoville B, Sulkowitch HW. Syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation and a gonadal dysfunction: further observations including the report of two more cases. Endocrinology 1938;22:411-421
2. Albright F, Butler AM. Hampton AO Smith P. Syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation and endocrine dysfunction with precocious puberty in females. N Engl J Med, 1937;216:727-746
3. McCune DJ, Bruch H. Progress in Pediatrics: Osteodystrophia fibrosa. Am J Dis Child, 1937;54:806-848
4. von Recklinghausen F. Die fibrose order deformierende Ostitis, die Osteomalazie und die osteoplastische Karzinose in ihren gegenseitigen Beziehungen, in Festschrift fur Rudolf Virchow. 1891 Reiner: Berlin
5. Lichtenstein L. Polyostotic fibrous dysplasia. Arch Surg 1938;36:874-879
6. Lichtenstein L, Jaffe HL. Fibrous dysplasia of bone: a condition affecting one, several or many bones, the graver cases of which may present abnormal pigmentation of skin, premature sexual development, hyperthyroidism and still other extraskeletal abnormalities. Arch Pathol 1942;33:777-797
7. Weinstein LS. The stimulatory G protein alpha-subunit gene: mutations and imprinting lead to complex phenotypes. J Clin Endocrinol Metab, 2001;86:4662-4666
8. Schwindinger WF, Francomano CA, Levine MA. Identification of a mutation in the gene encoding the alpha subunit of the stimulatory G protein of adenylyl cyclase in McCune-Albright syndrome. Proc Natl Acad Sci USA 1992;89(11):5152-5156
9. Riminucci M, Fisher LW, Majolagbe A, Corsi A, Lala R, De Sanctis C, Robey PG Bianco P. A novel GNAS1 mutation, R201G, in McCune-Albright syndrome. J Bone Miner Res 1999;14(11):1987-1989
10. Happle R. The McCune-Albright syndrome: a lethal gene surviving by mosaicism. Clin Genetics 1986;29:321-324
11. Cooper DN, Krawczak M. The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. Hum Genet 1990;85:55-74
12. Spiegel AM. Mutations in G proteins and G protein-coupled receptors in endocrine disease. J Clin Endocrinol Metab 1996;81(7):2434-2442
13. Spiegel AM. Defects in G protein-coupled signal transduction in human disease. Annu Rev Physiol 1996;58:143-170
14. Spiegel AM. Inborn errors of signal transduction: mutations in G proteins and G protein-coupled receptors as a cause of disease. J Inherit Metab Dis 1997;20(2):113-121
15. Spiegel AM. The molecular basis of disorders caused by defects in G proteins. Horm Res 1997;47(3):89-96
16. Ourne HR, Landis CA, Masters SB. Hydrolysis of GTP by the alpha-chain of Gs and other GTP binding proteins. Proteins 1989;6(3):222-230
17. Landis CA, Masters SB, Spada A, Pace AM, Bourne HR Vallar L. GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. Nature 1989;340(6236):692-696
18. Yamamoto T, Ozono K, Kasayama S, Yoh K, Hiroshima K, Takagi M, Matsumoto S, Michigami T, Yamaoka K, Kishimoto T Okada S, Increased IL-6-production by cells isolated from the fibrous bone dysplasia tissues in patients with McCune-Albright syndrome. J Clin Invest 1996;98(1):30-35
19. Riminucci, M, Fisher LW, Shenker A, Spiegel AM, Bianco P, Gehron Robey P. Fibrous dysplasia of bone in the McCune-Albright syndrome: abnormalities in bone formation. Am J Pathol 1997;151:1587-1600
20. Bianco P, Kuznetsov S, Riminucci M, Fisher LW, Spiegel AM Gehron Robey P. Reproduction of human fibrous dysplasia of bone in immunocompromised mice by transplanted mosaics of normal and Gs-alpha mutated skeletal progenitor cells. J Clin Invest 1998;101:1737-1744
21. Bianco, P, Riminucci M, Majolagbe A, Kuznetsov SA, Collins MT, Mankani MH, Corsi A, Bone HG, Wientroub S, Spiegel AM, Fisher LW Robey PG. Mutations of the GNAS1 gene, stromal cell dysfunction and osteomalacic changes in non-McCune-Albright fibrous dysplasia of bone. J Bone Miner Res 2000;15(1):120-128
22. Bianco P, Gehron Robey P, Wientroub S. Fibrous dysplasia, in Pediatric Bone - Biology and Disease, e. Glorieux F. Editor, Academic Press New York 2003;509-540
23. Bianco P, Gehron Robey P. Marrow stromal stem cells. J Clin Invest 2000;105(12):1663-1668
24. Bianco P, Riminucci M, Gronthos S, Robey PG, Bone Marrow Stromal Stem Cells: Nature, Biology and Potential Applications. Stem Cells, 2001;19(3):180-192
25. Bianco, P, Riminucci M, Kuznetsov S, Robey PG. Multipotential cells in the bone marrow stroma: regulation in the context of organ physiology. Crit Rev Eukaryot Gene Expr, 1999;9(2):159-173
26. Wang H, Watkins DC, Malbon CC. Antisense oligodeoxynucleotides to Gs protein alpha subunit sequence accelerate differentiation of fibroblasts to adipocytes. Nature 1992;358:334-337
27. Watkins DC, Rapiejko PJ, Ros M, Wang H, Malbon CC. G protein mRNA levels during adipocyte differentiation. Biochem Biophys Res Commun 1989;165:929-933
28. Bianco P, Costantini M, Dearden LC Bonucci E. Alkaline phosphatase positive precursors of adipocytes in the human bone marrow. Br J Haematol 1988;68(4):401-403
29. Bianco P, Bonucci E. Endosteal surfaces in hyperparathyroidism: an enzyme cytochemical study on low-temperature-processed, glycol-methacrylate-embedded bone biopsies. Virchows Arch A Pathol Anat Histopathol 1991;419(5):425-431
30. Kuznetsov SA, Riminucci M, Ziran N, Tsutsui TW, Corsi A, Calvi L, Kronenberg HM, Schipani E, Gehron Robey P Bianco P, The interplay of osteogenesis and hematopoiesis: Expression of a constitutively active PTH/PTHrP receptor in osteogenic cells perturbs the establishment of hematopoiesis in bone and of skeletal stem cells in the bone marrow. Journal of Cell Biology 2004;167:1113-1122
31. Miller SS, Wolf AM, Arnaud CD. Bone cells in culture: morphologic transformation by hormones. Science 1976;192:1340-1343
32. Riminucci M, Liu B, Corsi A, Shenker A, Spiegel AM, Gehron Robey P, Bianco P. The histopathology of fibrous dysplasia of bone in patients with activating mutations of the Gs alpha gene: site-specific patterns and recurrent histological hallmarks. J Pathol 1999;187:249-258
33. MacMahon HE. Albright's syndrome - thirty years later. Pathol Annu 1971;6:81-146
34. Corsi A, Collins MT, Boyde A, Riminucci M, Howell PGT, Gehron Robey P, Bianco P. Osteomalacic and hyperparathyroid changes in Fibrous dysplasia of bone: Core biopsy studies and clinical correlations. J Bone Min Res 2003;18:1235-1246
35. Bianco P. Structure and mineralization of bone. in Mineralization in biological systems, B E, Editor CRC Press: Boca Raton 1992;243-268
36. Gehron Robey P, Bianco P. The cellular biology and molecular biochemistry of bone formation, in Disorders of Bone and Mineral Metabolism, FCaM Favus, Editor. in press, Raven Press: New York
37. Boyde A, Maconnachie E, Reid SA, Delling G Mundy GR. Scanning electron microscopy in bone pathology: review of methods, potential and applications. Scan. Electron Microsc 1986;Pt 4:1537-1554
38. Boyde A, Travers R, Glorieux FH Jones SJ. The mineralization density of iliac crest bone from children with osteogenesis imperfecta. Calcif Tissue Int 1999;64:185-190

39. Collins MT, Chebli C, Jones J, Kushner H, Consugar M, Rinaldo P, Wientroub S, Bianco P, Robey PG. Renal phosphate wasting in fibrous dysplasia of bone is part of a generalized renal tubular dysfunction similar to that seen in tumor- induced osteomalacia. J Bone Miner Res 2001;16(5): 806-813
40. Dent CE, Gertner JM. Hypophosphataemic osteomalacia in fibrous dysplasia. Q J Med 1976; 45(179):411-420
41. Yamamoto T, Miyamoto K, Ozono K, Taketani Y, Katai K, Miyauchi A, Shima M, Yoshikawa H, Yoh K, Takeda E, Okada S, Hypophosphatemic rickets accompanying McCune-Albright syndrome: evidence that a humoral factor causes hypophosphatemia. J Bone Miner Metab 2001;19(5):287-295
42. Ryan WG, Nibbe AF, Schwartz TB, Ray RD. Fibrous dysplasia of bone with vitamin D resistant rickets: a case study. Metabolism 1968;17(11):988-998
43. Riminucci M, Collins MT, Fedarko S, Cherman N, Corsi A, White KE, Waguespack S, Gupta A, H T, Econs MJ, Bianco P, Gehron Robey P. Fibroblast Growth Factor-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. J Clin Invest, 2003;112:683-692
44. Dorfman HD, Czerniak B. Bone Tumors. 1997, St. Louis, MO: Mosby
45. Delling G. Bone morphology in primary hyperparathyroidism - a qualitative and quantitative study of 391 cases. Appl Pathol 1987;5:147-159
46. Riminucci M, Kuznetsov SA, Corsi A, Bianco P, Gehron Robey P. Osteoclastogenesis in fibrous dysplasia of bone: In situ and in vitro analysis of IL-6 expression. Bone 2003;33:434-442
47. Candeliere GA, Glorieux FH, Prud'Homme J, St-Arnaud R. Increased expression of the c-fos proto-oncogene in bone from patients with fibrous dysplasia. New Eng J Med, 1995;332:1546-1551
48. Fischer JA, Bollinger A, Lichtlen P, Wellauer J. Fibrous dysplasia of the bone and high cardiac output. Am J Med 1970;49(1):140-146
49. Ippolito E, Bray EW, Corsi A, De Maio F, Exner GU, Gehron Robey P, Grill F, Lala R, Massobrio M, Pinggera O, Riminucci M, Snela S, Zambakidis C, Bianco P. Natural history and treatment of Fibrous dysplasia of bone: a multicenter clinico-pathologic study promoted by the European Pediatric Orthopaedic Society. J Ped Ortho B 2003;12:155-177
50. Arden RL, Bahu SJ, Lucas DR. Mandibular aneurysmal bone cyst associated with fibrous dysplasia. Otolaryngol Head Neck Surg, 1997;117(6):S153-S156
51. Bandiera S, Bacchini P, Bertoni F. Secondary aneurysmal bone cyst simulating malignant transformation in fibrous dysplasia. Orthopedics 2000;23(11):1205-1207
52. Burd TA, Lowry KJ, Stokesbary SJ, Allen IC. Aneurysmal bone cyst associated with fibrous dysplasia. Orthopedics 2001;24(11):1087-1089
53. Dowler JG, Sanders MD, Brown PM. Bilateral sudden visual loss due to sphenoid mucocele in Albright's syndrome. Br J Ophthalmol 1995;79(5):503-504
54. Hirabayashi S, Kagawa K, Ohkubo E, Sugawara Y, Sakurai A. Craniofacial fibrous dysplasia with rapidly increasing proptosis due to a mucocele behind the globe. Ann Plast Surg 1998;40(2):182-185
55. Riminucci M, Collins M, Corsi A, Boyde A, Murphey MD, Wientroub S, Kuznetsov SA, Cherman N, Robey PG, Bianco P. Gnatho-diaphyseal dysplasia: A syndrome of fibro-osseous lesions of jawbones, bone fragility and long bone bowing. J Bone Miner Res 2001;16:1710-1718
56. Campanacci M, Laus M, Boriani S. Multiple non-ossifying fibromata with extraskeletal anomalies: a new syndrome? J Bone Joint Surg 1983;65B:627-632
57. Park YK, Unni KK, McLeod RA Pritchard DJ. Osteofibrous dysplasia: clinicopathologic study of 80 cases. Hum Pathol 1993;24(12):1339-1347
58. Sakamoto A, Oda Y, Iwamoto Y, Tsuneyoshi M. A comparative study of fibrous dysplasia and osteofibrous dysplasia with regard to Gsalpha mutation at the Arg201 codon: polymerase chain reaction-restriction fragment length polymorphism analysis of paraffin- embedded tissues. J Mol Diagn 2000;2(2):67-72
59. Corsi A, De Maio F, Ippolito E, Cherman N, Gehron Robey P, Riminucci M, Bianco P. Monostotic fibrous dysplasia of the proximal femur and liposclerosing myxofibrous tumor: which one is which? J Bone Miner Res 2006;21(12):1955-1958
60. Ruggieri P, Sim FH, Bond JR, Unni KK. Malignancies in fibrous dysplasia. Cancer 1994;73(5):1411-1424
61. Dal Cin P, Bertoni F, Bacchini P, Hagemeijer A, Van den Berghe H. Fibrous dysplasia and the short arm of chromosome 12. Histopathology 1999;34(3):279-280
62. Dal Cin P, Sciot R, Brys P, De Wever I, Dorfman H, Fletcher CD, Jonsson K, Mandahl N, Mertens F, Mitelman F, Rosai J, Rydholm A, Samson I, Tallini G, Van den Berghe H, Vanni R, Willen H. Recurrent chromosome aberrations in fibrous dysplasia of the bone: a report of the CHAMP study group. CHromosomes And MorPhology. Cancer Genet Cytogenet 2000;122(1):30-32
63. Dal Cin P, Sciot R, Speleman F, Samson I, Laureys G, de Potter C, Meire F, van Damme B, van den Berghe H. Chromosome aberrations in fibrous dysplasia. Cancer Genet Cytogenet 1994;77(2):114-117
64. Beuerlein ME, Schuller DE, DeYoung BR. Maxillary malignant mesenchymoma and massive fibrous dysplasia. Arch Otolaryngol Head Neck Surg 1997;123(1):106-109
65. Blanco P, Schaeverbeke T, Baillet L, Lequen L, Bannwarth B, Dehais J. Chondrosarcoma in a patient with McCune-Albright syndrome. Report of a case. Rev Rhum Engl Ed 1999;66(3):177-179
66. Cheng MH, Chen YR. Malignant fibrous histiocytoma degeneration in a patient with facial fibrous dysplasia. Ann Plast Surg 1997;39(6):638-642
67. Fukuroku J, Kusuzaki K, Murata H, Nakamura S, Takeshita H, Hirata M, Hashiguchi S, Hirasawa Y. Two cases of secondary angiosarcoma arising from fibrous dysplasia. Anticancer Res 1999;19(5C):4451-4457
68. Pollandt K, Engels C, Kaiser E, Werner M, Delling G. Gsalpha gene mutations in monostotic fibrous dysplasia of bone and fibrous dysplasia-like low-grade central osteosarcoma. Virchows Arch 2001;439(2):170-175
69. Riminucci M, Collins M, Lala R, Corsi A, Matarazzo P, Robey PG, Bianco P. An R201H activating mutation of the GNAS1 (Gs alpha) gene in a corticotroph pituitary adenoma. Mol Pathol 2002;55:58-60