Paget's Disease of Bone and Genetic Disorders of RANKL/OPG/RANK/ NF-κB Signaling

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ABSTRACT: **Identification of the RANKL/OPG/RANK/NF-kB (receptor activator of nuclear factor κ-B ligand / osteoprotegerin) signaling pathway as the major regulatory system for osteoclastogenesis began with discovery of these ligands and receptors in the tumor necrosis factor (TNF) superfamily. Subsequently, genetically altered mice revealed physiologic roles for these proteins in bone biology. However, full appreciation of their significance for the human skeleton came from clinical characterization of several extremely rare, heritable disorders followed by discovery of their genetic bases. Familial expansile osteolysis (FEO) is an autosomal dominant disorder featuring constitutive activation of RANK due to an 18-bp tandem duplication in its gene (*TNFRSF11A*). A similar, 27-bp duplication causes what has been called a familial form of early-onset Paget's disease of bone (PDB2). Expansile skeletal hyperphosphatasia (ESH) is allelic to FEO and PDB2 and involves a 15-bp tandem duplication in *TNFRSF11A*. Autosomal recessive inheritance of deactivating mutations of the gene encoding OPG (*TNFRSF11B*) causes most cases of juvenile Paget disease. These disorders feature high bone turnover, deafness during early childhood, "idiopathic external lysis" of adult teeth, and sometimes focal lesions in appendicular bones that mimic active PDB. Biochemical markers indicate rapid skeletal remodeling. In FEO, osteolysis progresses to fat-filled bone rather than to osteosclerosis. Antiresorptive therapy with bisphosphonates can be effective for each disorder.**

KEYWORDS: **alkaline phosphatase; bisphosphonate; bone resorption; familial expansile osteolysis; fracture; hyperostosis; hyperphosphatasia; juvenile Paget's disease; NF-κB; osteoclast; osteolysis; osteoprotegerin; osteosarcoma; skeletal remodeling; tumor necrosis factor**

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INTRODUCTION

Discovery of the RANKL/OPG/RANK/NF-κB signaling pathway as the major regulatory system for osteoclast formation and action began with the identification of new ligands and receptors in the tumor necrosis factor (TNF) superfamily and culminated with studies of genetically altered mice.[1] Nevertheless, full appreciation of its importance for humans came from revelation of the genetic bases of several extremely rare, mendelian disorders shown to be caused by either constitutive enhancement of receptor activator of nuclear factor-kappa B (RANK) or deficiency of osteoprotegerin (OPG).[2] In 2000, Hughes and colleagues found a heterozygous, gain-of-function, 18-bp tandem duplication in the gene encoding RANK (*TNFRSF11A*) in three kindreds with *familial expansile osteolysis* (FEO), and a similar insertional 27-bp duplication in *early-onset Paget's disease of bone* in Japan (PDB2).[3] In 2002, Whyte and Hughes reported that *expansile skeletal hyperphosphatasia* (ESH) was caused by a 15-bp tandem duplication in *TNFRSF11A*.[4] Also that year, Whyte and co-workers uncovered homozygous deletion of the gene encoding OPG (*TNFRSF11B*) in Navajo patients with *juvenile Paget's disease* (JPD).[5] Mutation of *TNFSF11* encoding RANKL has not been encountered in humans.

In this article, FEO, ESH, PDB2, and JPD are reviewed to illustrate the significance RANKL/OPG/RANK/NF-κB signaling has for skeletal homeostasis in man. However, a brief synopsis of Paget's disease of bone (PDB) is presented beforehand to appreciate how these unusual disorders resemble this far more common condition that is increasingly understood to involve heritable predisposition and other genes.[7]

PAGET'S DISEASE OF BONE

PDB (MIM 167250)[6] is common in the United States (i.e., overall prevalence at least 1%, and perhaps as much as 2%).[8,9] The disorder features focally increased skeletal remodeling within axial or appendicular bones in the adult skeleton.[10,11] Any site can be affected. Initially, a "wave" of osteoclast-mediated osteolysis moves slowly but relentlessly through an individual bone, and is then followed by disorganized skeletal repair.[10,11] This leads to bony expansion as well as to osteosclerosis and hyperostosis (excessive woven and lamellar bone in trabeculae and cortices, respectively).[10,11] Pagetic bone is unsound and predisposes to pain, fracture, and deformity. Eventually, "mosaic bone" from repeated episodes of resorption and formation characterizes lesional trabeculae.[10,11] Deafness of multifactorial pathogenesis is common,[12] and dental problems include loosening and migration of teeth.[13] Rarely, there is malignant transformation to, for example, osteosarcoma and chondrosarcoma.[10,11].

Despite its obvious focal attributes, PDB has traditionally been called the second most common metabolic bone disease (after osteoporosis), where

generalized acceleration in skeletal remodeling has been attributed to increments in circulating parathyroid hormone levels.[14]

Although the precise etiology and pathogenesis of PDB remains unknown,[7,15,16] research advances are considerable. Accumulating evidence supports the name for the disorder suggested by Paget in 1877, that is, *osteitis deformans*.[17] Mindful that pathogens have not been cultured from pagetic bone, paramyxovirus infection of osteoclasts and their precursor cells at some remote time seems associated with the foci of excessive osteoclast action.[15,16,18–20] The marrow microenvironment is especially osteoclastogenic with viral proteins, transcripts, and inclusion bodies in osteoclasts from lesional tissue[15,16] and is reproduced in a measles virus-based mouse model for PDB.[21]

Additionally, increasing evidence indicates that PDB is often heritable and, therefore, develops on a systemic background. McKusick, in 1960,[22] recognized the familial predisposition and sometimes multigenerational occurrence of PDB and assigned to it autosomal dominant inheritance (MIM 167250).[6] Nevertheless, reports of large kindreds with PDB have, until recently, been rare.[10,23] Now, the prevalence of PDB in first-degree relatives of affected individuals is appreciated to be 12–40%, representing a sevenfold enhanced risk.[9,24]

In 1977, investigation of a few, small, PDB families suggested involvement of chromosome 6p21.3, near the HLA loci (PDB1) (MIM 167250).[6,25] Subsequent clinical characterization of FEO with its similarity to PDB (see below) and obvious autosomal dominant inheritance strengthened interest in a possible genetic basis for PDB.[26,27] In 1994, FEO was mapped to chromosome 18q21–22[28] and some studies of familial PDB indicated that this was also a chromosomal region predisposing to PDB (PDB2) (MIM 602080).[29,30] In fact, the as yet unpublished Japanese family with the 27-bp tandem duplication (75dup27) of *TNFRSF11A* in this region[3] (see below) was said to have an early-onset form of PDB (PDB2).[31] Linkage studies of PDB families were then consistent[29,32] or inconsistent[33,34] with a gene defect at 18q21.1–q22, but no further gene mutations were identified within this chromosomal region.[34] Additionally, PDB was shown not to involve *TNFRSF11A* mutations.[35,36]

Now, PDB is understood to be genetically heterogeneous,[6] and considerable effort is under way to identify all associated genes.[27] In 2001, PDB was mapped to chromosome 5q35-qter and 5q31 in French Canadian families (i.e., PDB3 and PDB4, respectively)[37,38] leading to discovery in 2002 of a mutation at 5q35-qter in the gene encoding sequestosome (*SQSTM1*).[39] *SQSTM1* defects were then identified worldwide in a large number of familial and some sporadic PDB cases.[39–43] Furthermore, the rare, autosomal dominant syndrome called inclusion body myopathy with early-onset Paget disease and frontotemporal dementia[44] (MIM 167320) was shown in 2004 to involve mutations in the gene encoding valosin containing protein (VCP).[45] SQSTM1 and VCP both seem to participate intracellularly in "ubiquination."[46] VCP may regulate nuclear factor-kappa B (NF-κB) signaling by binding to Inhibitor-κBα (IκBα)

affecting ubiquitin-dependent proteosome degradation of IκBα[46]; however, this syndrome too seems to be genetically heterogeneous.[45] Additional genetic loci for PDB include 2q36, 10p13, and 18q23 (PDB 5,6, and 7, respectively).[7,38,47] Perhaps a factor from 18q21 acts as a modifier for PDB in some families, reflecting digenic inheritance.[34]

RANK EXCESS

Familial Expansile Osteolysis

Constitutive activation of RANK from tandem duplications in exon 1 of *TNFRSF11A* has been found in three seemingly distinctive disorders: FEO, ESH, and PDB2.[2]

FEO (hereditary expansile polyostotic osteolytic dysplasia) is an extremely rare yet remarkably instructive, autosomal dominant disorder (MIM 174810).[6] Patients manifest deafness early in life that is followed by destruction of adult dentition and focal, lytic expansion of major appendicular bones causing pain, fracture, and deformity.[26,48,49] The skeletal lesions initially resemble the clinical, radiographic, and histopathologic findings of PDB in its osteolytic phase,[10,11] but without treatment affected bones eventually become expanded, shell-like, and fat-filled rather than coarse and dense.[50,51] Histology of FEO osteolysis reveals an extreme local excess of bone turnover. Furthermore, generalized osteopenia and a coarse trabecular pattern suggest that FEO is a systemic bone disease. Elevation in serum alkaline phosphatase activity and other biochemical markers of bone remodeling surely derives from focal lesions, but is also contributed to, in some patients, by diffuse acceleration in skeletal remodeling.[2]

History

FEO was briefly reported in 1976 by Osterberg from Northern Ireland.[52] However, detailed characterization commenced with two 1979 publications by Enderle and colleagues from Germany concerning two brothers with an unusual,[10,11,53] severe, "osteolytic-expansive" PDB.[51,54] Subsequently, nine papers beginning in 1987 delineated the Northern Ireland kindred.[49,50,55–61] In 2002, Whyte and co-workers recounted 30 years' experience with an American FEO family demonstrating relatively mild disease.[62] That same year, a Spanish kindred was described.[63]

After FEO was mapped by Hughes and colleagues to chromosome 18q21.1–q22[28] in 1994, they discovered the molecular defect in 2000 using a candidate gene approach.[3] RANKL, a paracrine factor from mesenchyme and activated T cells in the bone marrow, binds to RANK promoting osteoclastogenesis.[1,64–73]

In the Northern Ireland, German, and American kindreds, Hughes and coworkers uncovered an identical, in-frame, 18-bp tandem duplication of bases 84–101 (84dup18) in exon 1 of the *TNFRSF11A* gene (MIM 603499) encoding RANK.[3] Transfection studies showed increased NF-κB activity presumably due to extension of the RANK signal peptide trapping the receptor intracellularly.[3]

Kindred Reports

Multigenerational FEO comprises the two-generation family in Germany [$FEO_{(Ger)}$], the five-generation kindred in Northern Ireland [$FEO_{(NI)}$], the five-generation kindred in the United States [$FEO_{(Am)}$], and the four-generation kindred in Spain [$FEO_{(Sp)}$] with 3, 46, 8, and 20 affected individuals, respectively.[26,50–52,54–62] Two additional, unrelated, American cases were reported in 2003.[74] Description separately of $FEO_{(Ger)}$, $FEO_{(NI)}$, $FEO_{(Am)}$, and $FEO_{(Sp)}$ is worthwhile because their *TNFRSF11A* defects are identical,[3] yet the families are unrelated and their disease expressivities differ.

$FEO_{(Ger)}$: For $FEO_{(Ger)}$, it is not know if all of the principal clinical features of FEO were manifest. The proband had expansile disease in his fibulae, radii, and patellae at ~age 25 years.[51,54] His brother had similar clinical and radiographic findings.[51] Their father was probably affected because he died at 47 years of age from metastatic osteosarcoma[51]—a cancer with increased prevalence in PDB and in $FEO_{(NI)}$[26] (see below). However, hearing loss or dental problems were not mentioned.[54] Detailed light microscopy of the bone lesions showed filigree-like trabeculae of woven bone (yet no mosaic pattern), abundant osteoclasts and osteoblasts lining trabeculae, giant osteoclasts with bizarre shapes and numerous nuclei, fibrous marrow, and subperiosteal bone formation.[51] Electron microscopy was not performed. The authors believed $FEO_{(Ger)}$ to be an "osteolytic-expansive" form of familial PDB in the "initial" "active" phase that failed to progress to the "inactive" phase.[51]

$FEO_{(NI)}$: Beginning in 1987,[49] eight reports by several groups of investigators[50,55–61] documented nearly complete penetrance of $FEO_{(NI)}$.[26,58] Severity differed among family members yet, with few exceptions, each suffered three major manifestations: osteolytic lesions, deafness, and tooth loss.[58] Osteolytic defects (90% prevalence) were especially common in the lower extremities and typically multifocal,[26,59] and a tibia was always involved.[59,61] Most of these lesions started at or near the end of a long bone[61] and then advanced[55] 6.5–22 mm yearly[56,59,61] like in PDB.[10,11] Pain in $FEO_{(NI)}$ began between the ages of 18 and 44 years, sometimes leading to limb amputation.[55–57] The maximum number of such defects in one patient was 12.[61] Conductive deafness (ultimately mixed-type) troubled at least 95% of affected individuals.[59,61] Deafness presented as young as the age of 4 years,[59] but more commonly in the

second decade.[56] The long process of the incus was usually absent or fibrous tissue.[26,59,61] Loosening and pain and/or fracture of adult teeth distressed most patients.[59,61] Primary teeth, however, were not involved.[57,58]

Radiographic findings in $FEO_{(NI)}$ included altered long bone modeling, particularly involving the humerus, radius, ulna, and tibia.[55,59] Furthermore, a tightly meshed ("fish net") trabecular pattern was variably present throughout the skeleton,[55] especially in metaphyses,[59] and sometimes in the mandible.[56] Bone scanning showed greater isotope uptake in tibias compared to femurs.[59] Osteopenia, however, was not mentioned as a significant feature of $FEO_{(NI)}$ [or $FEO_{(Ger)}$].[26]

Biochemical markers indicated accelerated skeletal turnover.[59]

Histopathology of the osteolytic lesions in $FEO_{(NI)}$ resembled PDB.[50,59,61] Osteoclasts were particularly large with increased numbers of nuclei.[61] On electron microscopy, all had microcylindrical nuclear inclusion bodies with ultrastructure resembling measles, canine distemper, or respiratory syncytial virus[50,59,61] similar to PDB.[15,50,56] Intermediate-stage disease, however, showed scanty skeletal matrix with abundant fibrous tissue and vascularity. Advanced disease featured almost total loss of cortical and trabecular bone, and fat occupied medullary spaces.[26,59,61] Nevertheless, light microscopy of the iliac crest was unremarkable.[61]

In $FEO_{(NI)}$ dentition, major histological disturbances included extensive root resorption, reduction in the size of pulp chambers and root canals, and patchy narrowing of the periodontal ligament.[57–59,61] However, the most remarkable finding was "idiopathic external resorption" that destroyed teeth (see below).[57,58]

$FEO_{(Am)}$: Experience with $FEO_{(Am)}$ supported heritable, excessive RANK/NF-κB activity[3] as a cause of early-onset deafness, loss of adult dentition, and expansile osteolysis of major appendicular bones (FIG. 1),[62] but it also underscored its systemic consequences on the skeleton. Osteopenia in adults was a common and sometimes clinically important feature of $FEO_{(Am)}$. Pathologic fractures occurred at sites other than those with osteolysis. Iliac crest histology did not show PDB. Notably, trauma seemed to incite osteolytic lesions in several patients (see below).[62] Additionally, $FEO_{(Am)}$ showed that the severity of FEO differed significantly among affected kindreds despite the identical 84dup18 *TNFRSF11A* duplication.[62]

$FEO_{(Am)}$ was not always fully penetrant. The four major features of FEO manifested in only some individuals. Deafness from degeneration of middle ear bones[75] was the first symptom—generally presenting from early childhood to young adult life, but perhaps not until the seventh decade. Premature tooth loss was exceptional, although idiopathic external resorption of secondary dentition could develop suddenly.[58]

In $FEO_{(Am)}$, it was not known if elevated serum alkaline phosphatase activity could occur at birth. The skeletal disease seemed to first disturb middle

ear ossicles causing deafness. Accelerated bone remodeling then accompanied generalized osteopenia and probably accounted also for the characteristic, coarse trabecular pattern.[62] Osteolysis had the radiographic features of active PDB[10,11] and could begin any time during young adult life or middle age, and might present without hyperphosphatasemia. Osteopenia or a coarse trabecular pattern was not always evident when lytic lesions were discovered.[62]

Light microscopy of osteolytic lesions was always typical of active PDB.[10,23,50,51] Dr. Barbara Mills concluded that nothing distinguished the findings from "garden variety" PDB.[62] There was osteoclast-mediated bone resorption, membranous bone, and marrow fibrosis. However, "mosaic bone," the hallmark of advanced PDB,[10] was rare—perhaps reflecting the extreme rates of bone remodeling. Instead, woven bone seemed to be rapidly deposited and did not mature or remodel into cortical bone. This helped explain the

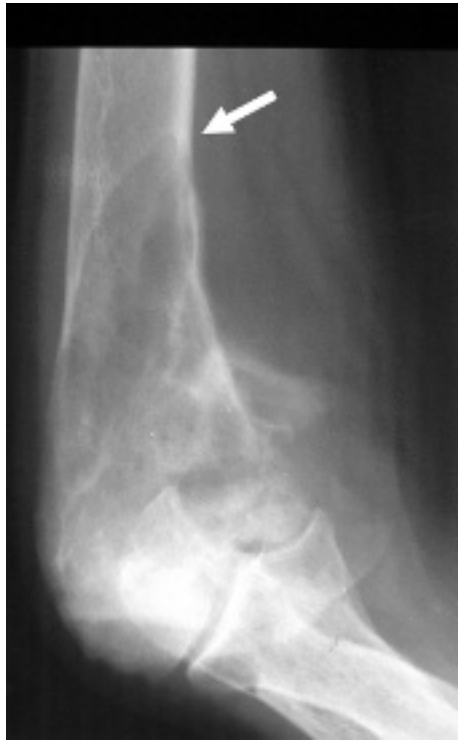

FIGURE 1. Lateral radiograph of the left elbow of the proband with $FEO_{(Am)}$ at the age of 71 years, a few years before elective amputation of the limb because of severe pain, shows an expansile ("soap bubble") osteolytic lesion in his distal left humerus, which is moving proximally (arrow) and predisposing to fracture. The radius and ulna are osteopenic with coarse trabeculae, probably reflecting disuse atrophy of muscle and bone distally as well as generalized acceleration of bone turnover.

absence of osteosclerosis.[62] Generally, little radiographic osteosclerosis occurred unless antiresorptive pharmaceuticals were administered.[62] Osteolytic lesions contained fibrous structures often involving most of the bone. Eroded areas of fibrous trabeculae had foci of numerous, enlarged, giant cells with many nuclei, some nestled in Howship's lacunae and appearing like PDB osteoclasts. Unlike in PDB, the lesions in untreated $FEO_{(Am)}$ had no "cutting cones" containing extensive concentrations of osteoclasts. On electron microscopy, paramyxovirus inclusions in osteoclasts were rare.[62] Iliac crest specimens showed no features of PDB. Advanced, appendicular, lesional disease has not been examined histologically in $FEO_{(Am)}$.

$FEO_{(Sp)}$. The fourth kindred with FEO, reported in Spain in 2002, had 20 affected individuals in four generations troubled by deafness, tooth loss, and osteoporosis with variable penetrance, but osteolytic lesions were a distinctly uncommon finding.[63] $FEO_{(Sp)}$ is caused by the same 18-bp *TNFRSF11A* insertional duplication (84dup18) found in the other three FEO kindreds.[3]

Additional Cases

The two unrelated American patients with FEO[74] manifested early-onset deafness and dental disease and especially tibial expansion. They had an 18-bp duplication in exon 1 of *TNFRSF11A* that is shifted proximally by one base-pair compared to the FEO kindreds yet resulted in substitution of the same six amino acids in the signal peptide of RANK.[74]

Treatment

The earliest report concerning FEO described, in 1976, symptomatic relief for Northern Ireland patients after injections of synthetic salmon calcitonin.[52] Later, dichloromethylene diphosphonate treatment was ineffective.[61] Intravenous infusions of pamidronate seemed most helpful.[26,61] Nevertheless, antiresorptive therapy was generally unsatisfactory.[26]

$FEO_{(Am)}$ patients received antiresorptive pharmaceuticals as they became available.[62] Calcitonins and bisphosphonates diminished biochemical markers of skeletal turnover with different efficacies. Radiographic changes were observed for some patients, including partial healing of osteolytic fronts with remineralization and cortical thickening of lesions,[62] as occurs in PDB.[11,76] Additionally, some $FEO_{(Am)}$ patients showed improvements in their generalized osteoporosis. Nevertheless, outcomes still seemed fair at best until recent excellent successes using oral alendronate treatment. Alendronate could mineralize early osteolytic lesions when they most closely resembled active PDB,[50,51] halt external tooth resorption (unpublished), increase bone density

from the associated osteoporosis (unpublished), and fully correct biochemical markers of bone turnover using at first a 6-month course of 40 mg orally each day as for PDB.[62] Sustained clinical, biochemical, and radiographic improvement with increased bone mineral density could persist 2 years after stopping this therapy. However, refractoriness to antiresorptive treatment seemed to be present when bones were fat filled and lytic disease had "burnt-out."[50,51] No $FEO_{(Am)}$ patient, however, reported any change in deafness.

Pamidronate therapy administered intravenously was very helpful for another patient.[74]

In case overt or microscopic fracture incites osteolysis in FEO, avoiding trauma and orthodonture seemed prudent (see below).[62]

Phenotypic Variation

Delineation, thus far, of only four kindreds worldwide with FEO limits conclusions about the range of interfamilial variation despite sharing the identical 84dup18 in *TNFRSF11A*. In retrospect, $FEO_{(Ger)}$ seems to be the most severe FEO—featuring unusually symmetrical, polyostotic disease in the extremities; particularly the radii and fibulae.[51] $FEO_{(Am)}$ and $FEO_{(Sp)}$ appear more mild than $FEO_{(NI)}$[26] and especially $FEO_{(Ger)}$.[51,54] In $FEO_{(Am)}$, only one long bone per patient (humerus, ulna, femur, tibia, or fibula) developed lytic disease, and only one patient suffered tooth loss.[62] Additionally, despite elective amputation for one patient, no one with $FEO_{(Am)}$ developed an osteosarcoma[10,11] which affected one patient with $FEO_{(Ger)}$ and several with $FEO_{(NI)}$. Perhaps genetic background or greater dietary calcium and higher vitamin D levels suppressing circulating parathyroid hormone concentrations diminished the severity of $FEO_{(Am)}$ and $FEO_{(Sp)}$ compared to $FEO_{(Ger)}$ and $FEO_{(NI)}$.[62] Furthermore, we identified nuclear inclusions in only 1 of the 12 osteoclasts examined within the lytic lesion of one individual with $FEO_{(Am)}$.[62] Two other $FEO_{(Am)}$ patients studied had no inclusion bodies. The difference in clinical severity between relatively mild $FEO_{(Am)}$ versus $FEO_{(NI)}$ suggests that paramyxovirus infection could be an inciting or exacerbating factor.[62] Finally, different dental care (see below) and earlier therapeutic intervention or compliance with medical therapy could also influence the FEO phenotype among affected families. The two additional American patients, with 83dup18, also seemed to have FEO. Remarkably, the consequences of RANK activation appeared to differ according to the subtle mutation heterogeneity in exon 1 in *TNFRSF11A* demonstrated by the Japanese family with PDB2 caused by a 27-bp *TNFRSF11A* tandem duplication (75dup27)[3,31] and ESH caused by a 15-bp tandem duplication (84dup15)[4] (see below).

For one FEO patient, pregnancy (when skeletal turnover accelerates) seemed to exacerbate her osteolytic disease.[55] Additionally, the mother with ESH (see below) experienced worsening of her disorder during lactation.[4,77]

Nevertheless, any effect of birth control pills or hormone replacement therapy on FEO remains unknown.

In FEO, there seems to be no significant gender influence or physical effect from left-right dominance conditioning where osteolysis appears. However, it may be that skeletal injury partly explains the focal nature of FEO. Indeed, trauma has been considered as an initiating factor for some PDB lesions.[10,11] Several $FEO_{(Am)}$ patients gave histories consistent with this hypothesis.[62] Microscopic or macroscopic fracture in FEO might initiate skeletal repair that becomes deranged because excessive RANK effect enhances osteoclast numbers and activity. Osteolysis could then progress until an entire bone is involved.[10,11] In fact, the best evidence for this hypothesis could be the remarkable tooth loss of one young man with $FEO_{(Am)}$ who was fitted with orthodontic braces at about 11 years of age.[62] Only he among his kindred lost adult teeth by idiopathic external osteolysis and had undergone orthodonture, which moves teeth by activating osteoclasts to cause osteolysis within alveolar bone.[58] However, destruction of the dentition has been common over generations in $FEO_{(NI)}$ and other American patients[74] and, therefore, orthodontic trauma is not always the trigger.[57,58] Against the notion of pathogenetic trauma in FEO, some $FEO_{(Am)}$ patients suffered fractures or had iliac crest biopsies, yet did not develop osteolytic lesions at those sites. Furthermore, osteolytic disease was not a major disease feature in ESH or the Japanese family with PDB2[31] (see below). Pathogenetic trauma seems more plausible in FEO than PDB, because PDB often affects the skull, pelvis, scapula, and other axial bones that are not often fractured.[10,11]

FEO illustrates how focal bone lesions can be an important manifestation of an autosomal dominant skeletal disease.[62] Similar changes can also appear in ESH, PDB2, and JPD (see below) showing that such seemingly isolated skeletal defects are characteristic of disorders of RANKL/OPG/RANK/NF-κB signaling. The paucity of skeletal repair in untreated FEO (osteolytic expansion of the entire long bone without osteosclerosis or hyperostosis) compared to PDB is not understood.[10,11] The RANK abnormality seems to disproportionately compromise osteoblasts compared to osteoclasts. Once osteolysis runs its course, expanded bone becomes fat-filled, perhaps because the mesenchymal stem cell pool has differentiated excessively to adipocytes.[50,51] Understandably, advanced expansile lesions are then unresponsive to antiresorptive therapy.[62]

Expansile Skeletal Hyperphosphatasia

ESH was characterized clinically in 2000 in a mother and daughter at 36 and 11 years of age, respectively, and, like FEO and PDB2, is inherited as an autosomal dominant trait.[77] ESH features early-onset deafness, accelerated bone remodeling, premature loss of teeth, progressive hyperostotic widening of long bones causing painful phalanges in the hands, and episodic hypercalcemia. At first, hypercalcemia together with absence of large osteolytic lesions or cortical

thinning—rather hyperostosis in major long bones—suggested that ESH was not a variant of FEO.[77] However, in 2002, Whyte and Hughes identified a 15-bp tandem duplication (84dup15) in *TNFRSF11A* in both ESH patients that is remarkably similar to the mutations causing FEO and PDB2 (see before).[4] Hence FEO, ESH, and PDB2 are allelic conditions involving RANK, and ESH likely reflects systemic activation of NF-κB, etc., in the skeleton.[4]

As in FEO,[62] deafness was the earliest problem in ESH.[77,78] Both affected individuals developed hearing loss in infancy that was complete by early childhood. The mother had absence of middle ear bones[77] perhaps resembling the necrotic degeneration with other abnormalities causing deafness in FEO.[26,75]

Premature tooth loss during childhood or early adult life also seemed to characterize ESH.[79] In the daughter, adult teeth were lost early, and the dental pathology was that of FEO.[79]

When the generalized skeletal disturbance of ESH first manifests is not known. Skeletal symptoms began at about 10 years of age.[77] The hands suffered the greatest discomfort and deformity.[77] Bony expansion at proximal interphalangeal joints of the daughter was understandably worrisome for "arthritis," and the joints could be tender, but they did not feel hot. Possibly, pain was due to periosteal stretching or joint distortion.[77]

Radiographs of the mother revealed that ESH eventually disturbs the entire skeleton.[77] Her calvarium, thoracic vertebral bodies, and long bones were affected most. The major abnormalities were expansion (undertubulation) as well as hyperostosis (cortical thickening) of large and small long bones.[77] However, the most remarkable changes involved her fingers where phalangeal widening could be extraordinary. The daughter's radiographic changes were similar, but less severe.[77] Of interest, the daughter's bone pain and finger swelling started soon after a forearm fracture.[77]

Reportedly,[78] the mother's skeletal disease was influenced by intercurrent illnesses and pregnancies.[4] Episodic hypercalcemia occurred spontaneously during childhood and later during illnesses and lactation. Hypercalcemia eventually manifested in her daughter.[4] Chosich and colleagues[78] postulated that the mother's hypercalcemia could reflect hormonal changes, including lowered estrogen levels during lactation, acting on rapid skeletal turnover in JPD.[78]

Serum alkaline phosphatase activity and additional markers of bone remodeling in ESH were considerably elevated before antiresorptive therapy.[77] Coarse trabeculae on X ray were possibly explained by thin cortices providing a "window" on thicker trabecular struts.[77]

Histological studies of the iliac crest showed increased numbers of osteoblasts and osteoclasts.[77] In the daughter, accelerated bone turnover was suggested by the appearance of enhanced cellular resorptive activity.[77] Total bone volume was substantially increased in the mother.[77] There were reversal lines, but not in the mosaic pattern of PDB.[77] Peritrabecular fibrosis was not seen. Electron microscopy of the mother's bone showed disorganized collagen bundles as well as necrotic and apoptotic bone cells, but no osteocytic

osteolysis.[77] Measles virus gene transcripts were not detected in her peripheral blood monocytes.[77]

Because ESH and FEO had sufficient likeness for a shared etiology and pathogenesis,[20,38] Whyte and Hughes tested *TNFRSF11A* as a candidate gene and reported in 2002 that ESH was caused by a 15-bp, tandem duplication (84dup15) in exon 1.[4] Despite the phenotypic differences, the mutations causing FEO, PDB2, and ESH proved remarkably similar, i.e., overlapping, in-frame, duplications in exon 1 of *TNFRSF11A* (84dup18, 75dup27, and 84dup15, respectively)[4] predicted to lengthen the signal peptide of RANK by six, nine, and five amino acids, respectively.[4] Hence, FEO,[3] PDB2, and ESH probably reflect excessive RANK activity, although transfection studies of the 84dup15 mutation have not been performed.[4] Additional genetic, epigenetic, or environmental factors could explain differences between FEO and ESH. Finally, after we studied these ESH patients, our suggestion for a trial of alendronate therapy,[4,77] based on the favorable observations in $FEO_{(Am),}$ [62] led to symptomatic relief and normalization of the biochemical disturbances.[77,79]

It is of interest that in 2005 we briefly described an American teenager who seems to be a phenocopy for ESH.[80] She manifested sporadic, early-onset, conductive deafness, tooth loss after orthodonture, expansion of a metacarpal, lytic lesion in a humerus, and hyperphosphatasemia, but had no defect in exon 1 of *TNFRSF11A*.

Early-Onset PDB2

"PDB2" was found to reflect a 27-bp tandem duplication (75dup27) in *TNFRSF11A* in 2000,[3] but the disease features of the six affected individuals spanning three generations in this Japanese family were first reported in 2003.[31] Subjects with PDB2 suffered hearing impairment and tooth loss beginning in the second or third decade of life, and later pain and deformity in the lower limbs.[31] One individual developed transient hypercalcemia during immobilization. Radiographs of affected bones revealed lytic and sclerotic lesions as well as bony enlargement and deformity. The majority of PDB2 patients also had pelvic and skull involvement, and all had particularly striking disease of the maxilla and mandible.[31] Most had enlargement of the small joints of the hands. Serum alkaline phosphatase activity was elevated 2–17 times above the normal range. An iliac crest specimen from an affected 51-year-old man showed evidence of increased bone turnover and woven bone considered typical of PDB.[31] The 75dup27 was believed to cause a PDB-like phenotype that is distinct from, but overlaps, FEO and ESH.[3,4]

Phenotype/Genotype Correlations

Unlike FEO, both ESH and PDB2 share episodic hypercalcemia, involvement of the skull and pelvis, bony enlargement in the hands, and absence of

osteolytic lesions of major long bones.[31,77] The RANK transfection studies in 2000 had demonstrated that 75dup27 causing PDB2 increased NF-κB signaling, but perhaps not as much as the 84dup18 causing FEO.[3] How this difference in NF-κB stimulation could account for these differences is not clear. Studies (*in silico*) of the secondary structure of exon 1 of *TNFSF11A* revealed a possible explanation for its inclination to duplicate.[74]

Until additional kindreds or individuals with various activating *TNFRSF11A* defects are reported, ESH and PDB2 might best be considered allelic to FEO[4]—broadening the phenotype for *TNFRSF11A* enhancement due to lengthening of RANK's signal peptide[3] and adding similarity to PDB.[10,11] However, with so few patients described worldwide with RANK activation, future reports may show greater phenotypic overlap leading to their classification as a single disorder of "RANK excess."[2]

OPG DEFICIENCY

JPD [MIM 23900], also called idiopathic or hereditary hyperphosphatasia,[6] is typically diagnosed in infants or young children.[81,82] Exceptionally, the bone disease does not manifest until later in childhood.[5,82] There also seems to be an especially unusual and relatively mild type of JPD associated with mental retardation.[82] Approximately 40 case reports have been published.[6,82] Unlike autosomal dominant FEO, ESH, and PDB2, all forms of JPD are considered autosomal recessive disorders.[6]

JPD affects the entire skeleton.[83] This, in part, has prompted objection in the past to the disorder being called a type of PDB. However, JPD as well as FEO, ESH, and PDB2 seem increasingly to share a number of features with PDB (see below).

Severe JPD causes bone pain, fracture, and deformity.[6,81,84–86] Premature loss of teeth and deafness[87] are also typical manifestations. Radiographs show marked undertubulation of long bones with osteopenic cortices.[87,88] Rapid rates of skeletal remodeling are inferred from substantially elevated biochemical markers of bone turnover,[81] including extreme hyperphosphatasemia,[5,81,82] but confirmed by histopathologic findings.[82] However, a mosaic pattern in bone characteristic of PDB is not found in JPD (or FEO, ESH, or PDB2). Measles virus transcripts, commonly identified in PDB,[20] were not detected in peripheral blood in mild[89] or severe cases of JPD (M.P. Whyte, R.V. Reddy, G.D. Roodman; unpublished).

Mild JPD is characterized by fewer fractures and less bony deformity.[5,82] Radiographs show diffuse, acquired hyperostosis and osteosclerosis associated with biochemical and histological evidence of accelerated skeletal turnover.[82] Understandably, Chosich and colleagues suggested that the mother with ESH had a mild form of JPD (see before).[78]

We have encountered a further similarity between JPD, PDB, FEO, and ESH—a focal, expansile, osteolytic ("soap bubble") defect. This occurred in

the distal humerus of our Navajo proband with JPD.[2] Furthermore, even severe JPD will respond to antiresorptive therapy, including human calcitonin[5] and bisphosphonates.[84,90]

In 2002, we speculated that OPG deficiency could explain JPD, because OPG suppresses bone turnover by functioning as a decoy receptor for RANKL,[1] and reported complete, selective deletion of the gene encoding OPG (*TNFRSF11B*) in two apparently unrelated Navajos with this disorder.[5] Both affected individuals had identical break points on chromosome 8q24.2.[5] The deletion spanned ~ 100 kb, but neighboring genes seemed to be intact. Serum levels of OPG and RANKL were undetectable and markedly increased, respectively. However, we detected no splice-site or exon mutations in the OPG gene in two unrelated women with relatively mild JPD.[5] Soon after, Cundy and colleagues reported an Iraqi sibship with JPD and a homozygous 3-bp *TNFRSF11B* deletion.[91] In 2003, they described a variety of deactivating *TNFRSF11B* mutations in additional JPD patients. The nature of the gene defect seemed to these investigators to predict the severity of the skeletal disease.[92] In 2003, in a preliminary communication,[93] we reported that homozygous defects in *TNFRSF11B* are found in nearly all JPD patients having OPG deficiency—illustrating the importance of a founder as a cause for this inborn error of metabolism.[93] Homozygous deletion of *TNFRSF11B* in our two Navajo patients likely reflected a unique founder defect emerging in this "bottleneck" population that had decreased to approximately 6,000 people in 1868[94] and then re-expanded to approximately 225,000 by 1990.[95] Because three JPD patients from apparently separate Navajo families had been identified since the 1960s, we calculated that ~1 in 100 Navajo is a carrier of this *TNFRSF11B* deletion.[96]

Discovery of OPG deficiency in JPD provided both a cause and a mechanism for this osteopathy.[5] OPG is normally secreted into the marrow space by cells derived from mesenchyme, such as preosteoblasts and osteoblasts.[1] Absence of OPG biosynthesis would in turn cause high circulating (and presumably marrow space) unbound RANKL, which would bind to RANK and markedly accelerate osteoclastogenesis and bone turnover. In fact, mice lacking OPG from homozygous *TNFRSF11B* knockout had been reported to have osteoporosis, yet numerous osteoclasts and rapidly remodeling woven bone were noted.[97] Accordingly, these animals can be said to manifest JPD. Mice heterozygous for OPG deficiency could be osteopenic,[97] but the carrier parents of our JPD proband had unremarkable skeletal radiographs and bone densitometry (DXA) (unpublished), although their serum OPG levels were ~ 50% of control values.[5]

Furthermore, observations in JPD complement *TNFRSF11B* knockout mouse model studies that suggest a potential role for OPG in vascular calcification.[97–99] In these mice, aorta and renal artery mineralization was detected by histopathologic methods.[97] Nevertheless, no calcification was observed in these same arteries of our second Navajo JPD patient at age 23 years using computed tomography and "bone windows" sensitive for mineral.[5] Initially,

our proband with marked hypercalciuria had nephrocalcinosis and perhaps several small calculi[5] on renal sonography; however, computed tomography of his kidneys, including renal arteries, was recently negative for calcifications (unpublished). Hence, we found no evidence for permanent, macroscopic, ectopic mineralization in severe JPD, at least during childhood or early adulthood. Nevertheless, the literature concerning JPD includes "calcifying arteriopathy," on histopathologic analysis of the internal elastic membrane of a temporal artery, in a 6-year-old boy.[100] Striking changes consistent with *pseudoxanthoma elasticum* (MIM 177850, 264800),[6] including granular and coarse deposits of calcium in the membranes and intima of the muscular arteries and arterioles, were reported in all autopsy tissues from a 26-year-old man with JPD.[101] It is of interest that the prevalence of vascular calcification also seems increased in PDB.[11,102]

JPD belongs among the disorders characterized by excessive signaling in the RANKL/OPG/RANK/NF-κB pathway leading to increased osteoclast action and accelerated rates of osseous tissue turnover. Nevertheless, JPD has some clinical and radiographic features (see above) that seem different compared to autosomal dominant FEO, ESH, and PDB2, suggesting that additional factors modify the effects of this pathway when activated by mutation in the genes encoding OPG verses RANK. Chromosome 8q24.2 contains *TNFRSF11B*, but has not been reported as one of the susceptibility loci for PDB. The coding sequence of *TNFRSF11B* is not mutated in PDB.[35]

Antiresorptive treatment using calcitonin or bisphosphonates has been beneficial for JPD.[5,84,89] Furthermore, if recombinant OPG will not be rejected as a foreign protein when there is deletion or mutated *TNFRSF11B*, OPG replacement might be helpful.[5] Recently, recombinant OPG has been effective for adult siblings with JPD.[103] Anti-RANKL antibody could be another therapeutic approach.

Finally, although similarities continue to emerge for PDB and disorders of the RANKL/OPG/RANK/NF-κB signaling pathway, henceforth it might be best to call JPD, caused by *TNFRSF11B* deactivation, "OPG deficiency."

CONCLUSIONS

FEO, ESH, and PDB2 (caused by constitutive activation of RANK) and JPD (due to OPG deficiency leading to increased access of RANKL to RANK) are the heritable disorders identified to date, which directly involve the RANKL/OPG/RANK/NF-κB signaling pathway.[2] Activating mutations in *TNFRSF11A* encoding RANK and deactivating mutations in *TNFRSF11B* encoding OPG cause systemic bone disease featuring accelerated bone turnover, low bone mass, deafness early in life, and loss of dentition by enhancing signaling by the RANK pathway. Osteopetrosis, featuring defective osteoclast action and dense, "marble bones," would be expected for deactivation of RANK or

activation of OPG, but has not yet been reported in humans. Similarly, defects in the *TNFSF11* gene encoding RANKL have not been identified in humans.[6]

Despite the heritable (systemic) basis for these three autosomal dominant (FEO, ESH, PDB2) and one autosomal recessive (JPD) conditions, focal expansile osteolytic lesions may variably manifest in the phenotype. Perhaps such defects can begin from further local activation of osteoclast-mediated bone resorption due to skeletal trauma and are influenced by past exposure of bone to paramyxovirus. In fact, these four disorders resemble PDB, which can be inherited as an autosomal dominant trait featuring focal osteolytic disease, sometimes with deafness and tooth loss, and increasingly associated with mutations in other genes involved in intracellular processes. Although rare, further investigation of similar entities will likely reveal additional heritable disorders involving the RANKL/OPG/RANK/ NF-κB signaling pathway.

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