Introduction

Discovery of the RANKL/OPG/RANK signaling pathway as a major regulatory system for osteoclast formation and action, and thereby skeletal remodeling, began with identification of new receptors and ligands in the tumor necrosis factor (TNF) superfamily1. Subsequent studies of genetically altered mice uncovered physiologic roles for these molecules, especially concerning bone biology. Nevertheless, appreciation of the significance these proteins have in humans came from clinical characterization of several extremely rare, heritable diseases followed by revelation of their genetic bases2,3. These remarkable disorders that primarily involve the skeleton were found to reflect gene defects leading to constitutive activation of RANK or to deficiency of OPG. Specifically, in 2000, Hughes and colleagues investigated familial expansile osteolysis (FEO) and identified an activating 18-bp tandem duplication in the gene encoding RANK (TNFRSF11A) in three affected kindreds, and a similar 27-bp duplication in an unusual, familial form of early-onset Paget disease of bone (PDB) in Japan2. In 2002, Whyte and Hughes reported that a seemingly unique disorder designated familial expansile osteolysis (ESH) and identified an activating 18-bp tandem duplication in the gene encoding RANK (TNFRSF11A) in three affected kindreds, and a similar 27-bp duplication in an unusual, familial form of early-onset Paget disease of bone (PDB) in Japan2. In 2002, Whyte and Hughes reported that a seemingly unique disorder designated expansile skeletal hyperphosphatasia (ESH) was allelic to FEO and involved a 15-bp tandem duplication in RANK4. That same year, Whyte and co-workers documented homozygous complete deletion of the gene encoding OPG (TNFRSF11B) as the first molecular explanation for idiopathic hyperphosphatasia — more commonly called juvenile Paget disease (JPD)4.

Here, we review FEO, ESH and JPD to illustrate the crucial importance RANKL/OPG/RANK signaling has for skeletal homeostasis in humans.

Familial expansile osteolysis

FEO, also called hereditary expansile polyostotic osteolytic dysplasia, is a very rare, yet remarkably informative, autosomal dominant disorder (MIM #174810)*5. Affected individuals typically manifest deafness early in life followed by destruction of adult dentition and progressive focal lytic expansion within limb bones causing pain, fracture, and deformity6-8. The skeletal lesions initially have close clinical, radiographic and histopathologic resemblance to the active (osteolytic) phase of PDB (MIM #167250)*, but evolve into expanded, shell-like, fat-filled bones9,10. The pathogenesis is excessive RANK effect2.

History

FEO was first mentioned in 1976 with a brief communication by Osterberg from Northern Ireland11. However, initial characterization of the disorder came from two 1979 publications by Enderle and colleagues concerning affected brothers in Germany who suffered from an unusual seemingly "osteolytic-expansive" type of PDB12-14, but evolve into expanded, shell-like, fat-filled bones9,10. The pathogenesis is excessive RANK effect2.

* This 6-digit number is the entry number for the disorder in Mendelian Inheritance in Man (MIM; reference #5), a continuously updated catalog of human genes and genetic disorders. The online version (Online Mendelian Inheritance in Man; OMIM) is accessible from the National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD, through the World Wide Web (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM).
Subsequently, a series of nine reports beginning in 1987, commencing with the medical thesis of R.G.H. Wallace, delineated 46 affected family members in five generations of the Northern Ireland kindred7,8,13,19. A report by Whyte et al., recounting 30 years experience with a five-generation American FEO family, was published in 200220.

In 1994, FEO was mapped by Hughes and co-workers to chromosome 18q21.1-q2221. In 2000, these investigators discovered the molecular defect using a candidate gene approach19. At that time, RANKL (also called "osteoclast differentiation factor" or "OPG ligand") was recognized as a paracrine factor from mesenchyme-derived cells and activated T-cells in the bone marrow environment which could bind to RANK promoting osteoclastogenesis12,22. In both reported FEO families (from Germany and Northern Ireland) and in the as yet unpublished American kindred, Hughes et al. discovered identical, in-frame, 18-bp tandem duplications in exon 1 of the TNFRSF11A gene (MIM #603499) encoding RANK2. Transfection studies showed that nuclear factor-kappa B (NF-κB) (MIM #164011) activity was increased by the elongated RANK — extension of RANK’s signal peptide perhaps trapped it intracellularly, causing gain-of-function. Hence, FEO proved to be an "experiment-of-nature" featuring an activating mutation in TNFRSF11A which enhanced RANK and NF-κB effects in humans2.

The overview below, including a description of the American kindred [FEO(Am)] with relatively mild FEO, shows that epigenetic or additional genetic factors impact the clinical severity of FEO as the three affected families share the identical TNFRSF11A defect. Also, the efficacy of bisphosphonate therapy for FEO, shown in the American family, is discussed. Finally, we mention the insight FEO has provided for the etiology and pathogenesis of the far more common disorder, PDB.

Reports

Multigenerational FEO has been described thrice — a small two-generation family in Germany [FEO(Ger)], a large five-generation kindred in Northern Ireland [FEO(NI)], and a five-generation kindred in the United States [FEO(Am)]. In these three unrelated (determined from family history and haplotype analyses) patient groups, there were 3, 46 and 8 affected individuals, respectively6,8,11,12,20. In 2003, two additional, unrelated, American cases of FEO were reported33. A review of FEO by Hughes and Barr (pre-dating identification of the gene defect) was published in 1996, and a summary by Whyte and co-workers20 (after discovery of the genetic basis) appeared in 2002 when FEO(Am) was described in detail.

Too little is recorded about FEO(Ger) to know if all principal clinical features of FEO(NI) and FEO(Am) troubled these three affected German men. Notwithstanding, differences among FEO(Ger), FEO(NI), and FEO(Am) are apparent and merit discussion.

**FEO**

Two brothers with FEO(Ger) were reported in 197910,12. The proband had expansile disease in both fibulae, radii, and patellae noted at approximately age 25 years. His brother manifested similar clinical and radiographic findings18. Their father was probably affected because he died at age 47 years from metastatic osteosarcoma with focal skull lesions10 - a cancer that also occurs in FEO(NI) (see below).

In FEO(Ger), there was no mention of hearing loss or dental problems12. However, the light-microscopy findings were depicted in considerable detail. The skeletal changes included filigree-like trabeculae of woven bone with absence of a mosaic pattern, abundance of osteoclasts and osteoblasts lining the edges of trabeculae, giant osteoclasts with bizarre shapes and numerous nuclei, fibrous marrow, and significant subperiosteal bone formation10. Electron microscopy (EM) was not performed. Understandably, the authors believed FEO(Ger) to be an "osteolytic-expansive" form of familial PDB in the "initial"-"active" phase, but which fails to progress to the "inactive" phase19.

In retrospect, FEO(Ger) seemed more severe than FEO(NI), and especially FEO(Am), featuring unusually symmetrical, polyostotic disease in the extremities — particularly the radii and fibulae10.

**FEO**

The publications beginning in 19877, comprising eight reports by several groups of investigators9,13-19, documented almost complete penetrance of FEO(NI)6,16. Although the severity differed among affected family members, with few exceptions each suffered three major disease features16. First, progressive osteolytic lesions (90% prevalence) were especially common in the lower limbs and typically multifocal17. A tibia was invariably involved17,19. Most started (or near) the ends of long bones19 and then advanced relentlessly6,15,17. The maximum number of such lesions was 12 in one individual19. Second, conductive deafness (that became mixed-type) occurred in at least 95% of individuals with FEO(NI)17,19. Deafness presented as early as 4 years of age17, but more commonly was detected in the second decade14. The long process of the incus was typically absent or replaced by fibrous tissue6,17,19. Third, dental loosening and pain and/or fracture of secondary teeth troubled most FEO(NI) patients17,19. Deciduous teeth, however, were not involved13,15.

Radiographic abnormalities in FEO(NI) included altered modeling of some bones — particularly the humerus, radius, ulna and fibula13,17. Furthermore, there was always a tightly meshed ("fish-net") trabecular pattern variably present throughout the skeleton13, especially in metaphyses17 and sometimes in the mandible14. Bone scanning showed proportionately greater uptake of isotope in tibias compared to
femurs\textsuperscript{17}. Osteopenia, however, was not mentioned as a significant feature of FEO\textsubscript{(NI)} (or FEO\textsubscript{(Ger)})\textsuperscript{6}.

Histopathology of early osteolytic lesions in FEO\textsubscript{(NI)} resembled PDB\textsuperscript{9,17,19} and biochemical markers indicated increased rates of skeletal turnover\textsuperscript{17}. However, intermediate-stage lytic disease showed scanty skeletal matrix with increased fibrous tissue and extensive vascularity. Advanced disease featured almost total loss of cortex and trabecular bone. Instead, there was fatty replacement occupying medullary spaces\textsuperscript{6,17,19}. Osteoclasts in lytic lesions were particularly large with increased numbers of nuclei\textsuperscript{19}. Furthermore, all of these polykaryons studied by EM had microcylindrical nuclear inclusion bodies\textsuperscript{9,14,19} whose ultrastructure resembled measles, canine distemper, or respiratory syncytial virus\textsuperscript{9,17,19}. Nevertheless, iliac crest light microscopy appeared unremarkable\textsuperscript{19}. Several individuals with FEO\textsubscript{(NI)} suffered malignant transformation of bone\textsuperscript{6}.

Investigation of 27 affected individuals\textsuperscript{15,16} revealed that the principal histological disturbances of the dentition in FEO\textsubscript{(NI)} is extensive root resorption, reduction in the size of pulp chambers and root canals, and patchy narrowing of the periodontal ligament\textsuperscript{17,19}. However, the most remarkable dental complication is designated "idiopathic external resorption" leading to destruction of teeth (see below)\textsuperscript{15,16}.

Figure 1. The 71-year-old proband with FEO\textsubscript{(Am)} presented in 1971 with this expansile ("soap bubble") osteolytic lesion in his distal left humerus (see Fig 6). The radius and ulna were osteopenic with coarse trabeculae probably reflecting, disuse atrophy as well as accelerated bone turnover.

Figure 2. Dental radiographs of this 14-year-old boy with FEO\textsubscript{(Am)} showed characteristic external resorption of his teeth (black arrows). Note also that the tooth root is being destroyed.

An early report of FEO\textsubscript{(NI)} described symptomatic relief (without radiographic change) after salmon calcitonin injection therapy\textsuperscript{11}. Dichloromethylene diphosphonate treatment was not effective\textsuperscript{19}. Intravenous infusions of pamidronate seemed most helpful\textsuperscript{15,16}. However, responsiveness of FEO\textsubscript{(NI)} to antiresorptive therapy was considered unsatisfactory overall\textsuperscript{6}.

**FEO**\textsubscript{(Am)}

Our 30-year experience involving FEO\textsubscript{(Am)} confirmed that the excessive RANK/NF-\textgreek{b} activity discovered by Hughes, et al.\textsuperscript{2} can cause early-onset deafness, loss of adult dentition, and expansile osteolysis of major appendicular bones in humans (Figure 1)\textsuperscript{20}. However, FEO\textsubscript{(Am)} also clearly illustrates that the molecular defect involving RANK has systemic consequences. Osteopenia is a common and sometimes important fourth feature of FEO\textsubscript{(Am)} in adults – reflecting generalized, accelerated bone remodeling and a form of high-turnover osteoporosis that occasionally results in fractures. Familiarity with FEO\textsubscript{(Am)} suggests that trauma could be an inciting factor for the osteolytic lesions\textsuperscript{20}. Furthermore, as discussed below, FEO\textsubscript{(Am)} shows that the clinical severity of FEO can be significantly diminished without pharmacologic intervention. Finally, alendronate therapy can heal early osteolytic lesions and fully suppress the systemic bone disease in adults\textsuperscript{20}.

In FEO\textsubscript{(Am)}, early-onset hearing loss from degeneration of middle ear bones is characteristic\textsuperscript{14}. Deafness is the first symptom – generally presenting anytime from early childhood to young adult life, but perhaps not until age 60 years. Premature tooth loss (Figure 2), however, is exceptional in FEO\textsubscript{(Am)}, although the characteristic external resorption of secondary dentition can occur suddenly\textsuperscript{16}. Additionally, FEO\textsubscript{(Am)} in contrast to FEO\textsubscript{(NI)}, is not always fully penetrant. The four major disease features of FEO manifested in only some subjects. The proband did not describe deafness, although hearing loss troubled all others. At age 29 years, one affected man lacked skeletal symptoms yet he alone suffered
characteristic tooth loss. An obligate carrier woman, who lived 89 years, reportedly had deafness and tooth loss (but only late in life) with no symptoms of osteolytic disease.

In FEO(Am), elevated serum alkaline phosphatase (ALP) activity early in life reflects the fundamental pathogenetic disturbance, i.e., rapid turnover of the skeleton. It is not known, however, if hyperphosphatasemia can be present at birth. This biochemical abnormality manifested prior to generalized radiographic changes. In fact, the skeletal disease of FEO(Am) typically seems to first disturb middle ear ossicles causing deafness by early childhood. It is unclear if some hearing loss is congenital. Accelerated bone remodeling then becomes associated with generalized osteopenia and probably accounts also for the characteristic, coarse trabecular pattern. Such coarsening could be seen radiographically as early as age 20 years. Osteolysis could begin any time during young adult life or middle age, and might present without laboratory evidence (hyperphosphatasemia) of accelerated bone turnover. The lytic lesions had radiographic and histopathologic features of the active phase of PDB. Nevertheless, subnormal bone mass or a coarse trabecular pattern were not always present when lytic lesions were discovered. Osteopenia distal to the expansile lesions was common and likely reflected partially disuse atrophy of bone. Generally, little radiographic evidence of the osteosclerosis characteristic of advanced PDB occurred in FEO(Am) unless anti-resorptive pharmaceuticals were administered.

In FEO(Am), four patients (ranging from 27 to 67 years of age) underwent biopsy of an expanding bone lesion (Figure 3). The findings on light microscopy were always typical of active PDB. Nevertheless, subnormal bone mass or a coarse trabecular pattern were not always present when lytic lesions were discovered. Osteopenia distal to the expansile lesions was common and likely reflected partially disuse atrophy of bone. Generally, little radiographic evidence of the osteosclerosis characteristic of advanced PDB occurred in FEO(Am) unless anti-resorptive pharmaceuticals were administered.

Advanced (and seemingly quiescent) disease in FEO(Am) has not been examined histologically. Iliac crest specimens showed no features of PDB. Hence, in FEO(Am) only the lytic appendicular lesions resembled PDB.

Figure 3. An undecalcified specimen from an osteolytic lesion of the distal humerus in FEO(Am) shows woven bone, fibrous tissue accumulation in the marrow spaces, and numerous osteoclasts (Masson trichrome stain x 80).

FEO phenotypic variation

Delineation, thus far, of only three kindreds worldwide with FEO limits conclusions about phenotypic or interfamilial variation despite the identical defect (84dup18) in TNFRSF11A. Because the mutations were identical in FEO, it was important that Hughes et al. showed by genealogy and haplotyping that the families were not related. Early on, significant phenotypic differences due to subtle mutation heterogeneity in TNFRSF11A was suggested by unpublished information about the Japanese family with the 27-bp TNFRSF11A tandem duplication (75dup27) causing PDB. In 2003, detailed clinical, radiographic, and histopathologic findings were reported by Nakatsuka et al. which appeared to support this variation. Furthermore, the disorder we called expansile skeletal hyperphosphatasia (see below) also seemed different from FEO. In the affected mother and daughter, hyperostosis in major limb bones, rather than large osteolytic lesions, as well as episodic hypercalcemia appeared considerably different from FEO. In 2002, ESH was shown to be caused by a unique, yet remarkably similar 15-bp tandem duplication (84dup15) in exon 1 of TNFRSF11A (see below). Nevertheless, some potential modifying factors, e.g., responsiveness to pharmacologic therapy, etc., may have obscured differences or similarities among these disorders.

We do not fully understand why FEO(Am) is less virulent than FEO(NI) and especially FEO(Ger), which is most severe, despite identical molecular defects in TNFRSF11A. In FEO(Am), only one long bone (humerus, ulna, femur, tibia, or fibula) developed lytic disease per patient, and only one patient suffered the characteristic tooth loss. Although malignant transformation of the skeleton occurs in PDB and osteosarcoma has not developed in FEO(Am). There does not seem to be a significant gender influence in FEO(Am). Furthermore, observations in FEO(Am) exclude a
physical effect from any left-right dominance pattern condition where osteolysis appears. However, we have reviewed evidence for trauma, especially that caused by orthodonture, inciting the osteolytic expansile disease (see below)20. Perhaps greater dietary calcium intake, higher vitamin D levels, different dental care, earlier therapeutic intervention or compliance with medical therapy (see below), or genetic background diminishes the severity of FEO20.

Treatment

The medical history of one FEO(Am) patient suggested that pregnancy (a time when skeletal turnover accelerates) can exacerbate the osteolytic disease15. Conversely, the mother with expansile skeletal hyperphosphatasia (see below) experienced worsening of her disease during lactation5,38. Therefore, the effects of birth control pills or hormone replacement therapy on the FEO skeleton will require especially close observation and understanding. If overt or microscopic fracture incites osteolysis in FEO, avoiding trauma and orthodonture seems prudent.

In 1988, medical treatment was considered unsatisfactory for FEO(NI), but pamidronate infusions were reportedly the best option6. FEO(Am) patients received anti-resorptive pharmaceuticals as they became available in the United States at various times during the past three decades20. Overall, calcitonins and bisphosphonates slowed accelerated skeletal turnover with different efficacies demonstrated by reductions in biochemical markers of bone remodeling. In some patients, radiographic improvement followed, including partial healing of osteolytic fronts with remineralization and cortical thickening of lesions20. Such responses also occur in PDB35,40. Additionally, some FEO(Am) patients showed increases in low bone mass at skeletal sites not involved by lytic disease. Nevertheless, therapeutic outcomes reviewed below seemed fair at best until our recent excellent experience using alendronate treatment20.

Parenteral salmon calcitonin therapy for one year proved minimally helpful for one FEO(Am) patient, with transient halving of serum ALP activity but no beneficial clinical response. Nasal salmon calcitonin treatment seemed ineffective35. In a few FEO(Am) patients, injections of synthetic human calcitonin were better than salmon calcitonin in lowering serum ALP activity and correcting urinary hydroxyproline (OHP) levels and were followed by beneficial radiographic changes featuring cortical and trabecular thickening of lytic lesions20. However, improvements were transient with eventual resistance to human calcitonin.

Etidronate lowered serum ALP and urinary OHP levels and diminished radioisotope uptake on bone scanning in one subject with FEO(Am)20. In two individuals with FEO(Am) and active osteolysis, elevated biochemical markers of skeletal turnover corrected following a six-month course of 40 mg alendronate orally each day and small osteolytic defects healed completely20. In another patient who was older, alendronate corrected serum ALP as well as urinary OHP and deoxypyridinoline levels, but had no effect on the radiographic appearance of her advanced tibial disease that had markedly expanded the entire bone20. We speculated that such refractoriness occurs when bones become fat-filled and the lytic disease is "burnt-out"9,10. Nevertheless, improvements in lumbar spine BMD could occur. No FEO(Am) patient, however, reported any change in deafness although external tooth resorption could be halted (unpublished). Off alendronate therapy for two years, sustained clinical, biochemical and radiographic improvement with increased BMD was documented in one individual. A small lytic area in a cystic distal humerus remineralized during a second, lower-dose, prolonged course of alendronate, and BMD improved. It seemed that alendronate therapy during the early stages of FEO(Am) when lesions most closely resemble active PDB9,10, produces excellent and long-term clinical, biochemical and radiographic improvement that can persist for some time after treatment is withdrawn. We have no experience using other bisphosphonates for FEO.

FEO and PDB

PDB is common in the United States (i.e., overall prevalence at least 1%, and perhaps as much as 2%)41 affecting at least one skeletal site in ≈ 15% of Americans older than age 65 years35,39,42. PDB features focal accelerated skeletal remodeling (monostotic or polyostotic) in axial and/or appendicular bone35,39,43. Progressive, osteoclast-mediated osteolysis occurs first, and is followed by disorganized skeletal repair leading to areas of hyperostosis and osteosclerosis (excessive newly-formed woven and lamellar bone in cortices and trabeculae, respectively)35,39. Nevertheless, PDB has been regarded as the second most common metabolic bone disease (after osteoporosis) and generalized disturbances in bone remodeling have been described35,39.

Notably, many of the clinical, biochemical, radiographic and histopathologic features of PDB seem to manifest in FEO. The most remarkable is the focal, expansile, osteolytic lesions which involve major appendicular bones in FEO35,39. Additionally, however, PDB2 in the Japanese family is associated with axial bone lesions like in PDB37. Histology of FEO osteolysis confirms the extreme local excesses of bone turnover, etc., as in PDB. However, the generalized osteopenia and coarse trabeculation identified in FEO(Am)1 indicates that FEO is a systemic metabolic bone disease. Elevation in serum ALP activity and other biochemical markers of bone remodeling in FEO(Am) is probably not entirely from focal lesions. This assumption was supported by identification of the molecular basis of FEO2.
tance44 although the gene defect(s) was unknown (MIM #167250, #602080). Nevertheless, until recently, reports of large families or kindreds with PDB had been rare35,36. Now the prevalence of PDB in first-degree relatives of affected individuals is appreciated to be 12–40%, representing a 7-fold enhanced risk42,44,45. In 1977, investigation of small PDB families suggested genetic predisposition involving chromosome 6, near the HLA loci (PDB1, MIM #167250).44,46 Subsequently, the similarity of FEO to the osteolytic phase of PDB and its autosomal dominant inheritance understandingly re-activated interest in a genetic basis for PDB43–47. Then, in 1994, linkage studies mapped FEO(NI) to chromosome 18q21 and subsequent reports suggested a second genetic region (also on chromosome 18q21) for PDB (PDB2, MIM #602080)23,48,49. Initially, linkage studies of further PDB families were consistent48,50 or inconsistent51,52 with a gene defect at chromosome 18q21.1-q22. The Japanese family with the 27-bp tandem duplication (75dup27) of TNFRSF11A was considered to have a form of early-onset PDB rather than FEO (PDB2, MIM #602080)53. However, one of these families with linkage to 18q21, was later shown to have a sequestosome mutation (chromosome 5q35, see below)52. No PDB mutations have yet been identified in the other families with linkage to 18q2149. Nevertheless, FEO is not a variant of "garden variety" PDB (MIM #167250). PDB has been shown not to involve TNFRSF11A mutations53,54. Linkage to chromosome 18q21 suggests that an as-yet unknown genetic defect at 18q21 may act as a modifier for PDB in some families with a sequestosome mutation, invoking digenic inheritance52.

Now, PDB is understood to be genetically heterogeneous5, and considerable efforts are underway to identify additional causal genes7,21. In 2001, PDB was also mapped to chromosome 5q35-qter and 5q31 in French Canadian families (i.e., PDB3 and PDB4, respectively)55,56. This led to the discovery of mutations in the gene encoding sequestosome (SQSTM1) in at least 29 families and 33 sporadic cases7,58. Subsequently, several additional mutations were identified in SQSTM1 for a large number of familial and sporadic PDB cases26–29 – supporting McKusick’s designation and showing at least a heritable predisposition to PDB. In fact, our review of the literature suggests mutations in SQSTM1 account for 39% of familial PDB cases but fewer sporadic instances of PDB7,59,61. Additional genetic loci for PDB include 2q36, 10p13, and 18q23 (distinct from the 18q21.1-q22 locus)56,62. A rare, autosomal dominant syndrome of limb-girdle muscular dystrophy, frontotemporal dementia, and PDB55 was mapped to chromosome 9p13.3-p12 in 200146. In 2004, this disorder was shown to involve mutations in the gene encoding valosin-containing protein (VCP) in 13 families45. SQSTM1 and VCP are both involved in the intracellular process of "ubiquination"46. Further, VCP may regulate NF-κB signaling by binding to Inhibitor-κBα (IκBα) and affecting ubiquitin-dependent proteosomal degradation of IκBα46. However, this syndrome – caused by VCP mutation in muscle, brain, and bone – also seems to be genetically heterogeneous45,55. Despite considerable recent progress, the precise pathogenesis of PDB is still unknown43,67. Increasing evidence supports the name given to PDB by Sir James Paget in 1876, i.e., osteitis deformans48. Viral infection involving the cellular lineage of osteoclasts seems important for developing the skeletal manifestations of PDB43,53,57,69,70. In PDB, the marrow microenvironment is especially osteoclastogenic43. Paramyxovirus infection has been investigated as a necessary pathogenic insult43,67,70,71. In fact, osteoclasts in FEO(NI) contain nuclear inclusions similar to PDB9,14,43. However, we identified PDB-like nuclear inclusions in only one of the twelve osteoclasts examined within the lytic lesion of one individual with FEO(Am)20. Two other FEO(Am) patients studied by EM had no inclusion bodies. The differences in severity between relatively mild FEO(Am) versus FEO(NI) or FEO(Ger) suggest that viral infection may not be required for osteolytic disease in FEO, but such infection could be an exacerbating factor43. Nevertheless, FEO illustrates how focal bone lesions can be a major finding in an autosomal dominant skeletal disease20.

The poorer skeletal repair of FEO compared to PDB (osteolytic expansion of the entire long bone without osteosclerosis or hyperostosis) is not understood35,39. Although bones are not equally impacted in FEO(Am), extensive woven bone suggests that the RANK abnormality disproportionately compromises osteoblasts compared to osteoclasts in FEO(Am) in contrast to PDB. Once the osteolysis runs its course, expanded bone in FEO(Am) becomes fat-filled, perhaps because precursor mesenchymal stem cells have differentiated excessively to adipocytes9,10. Understandably, advanced expansile lesions are then unresponsive to bone anti-resorptive therapy20. We speculate that skeletal injury sometimes explains the focal and lytic nature of the appendicular bone defects in FEO. Indeed, trauma has been posulated to initiate PDB lesions35,39. Several FEO(Am) patients gave histories consistent with this possibility20. Perhaps microscopic or macroscopic fracture in FEO (or PDB) initiates skeletal repair that becomes deranged because excessive RANK effect, etc., enhances osteoclast numbers and activity. Osteolysis could then progress until the whole bone is involved35,39. In fact, the best evidence for trauma in the pathogenesis of the osteolysis of FEO could be the remarkable tooth loss in one boy with FEO(Am) who was fitted with orthodontic braces at about 11 years of age20. Only he among his kindred had undergone this procedure that moves teeth by activating osteoclasts to cause osteolysis within alveolar bone10. However, destruction of the dentition is common in FEO(NI) and therefore orthodontic trauma can not always be the trigger for the idiopathic external resorption of the teeth15,16. Against the notion of pathogenetic trauma in FEO(Am), some patients suffered fractures or had iliac crest biopsies, yet did not develop osteolytic lesions. Pathogenetic trauma seems more plausible for FEO than PDB, because PDB often affects the skull, pelvis, scapula, and other axial bones that are not often fractured35,39. Additionally, axial lesions occurred in the Japanese family with PDB27.
Expansile skeletal hyperphosphatasia

Expansile skeletal hyperphosphatasia (ESH) is a singular disorder characterized clinically by Whyte and co-workers in 2000 in an Australian mother and daughter and featuring early-onset deafness, premature loss of teeth, progressive hyperostotic widening of long bones causing painful phalanges in the hands (Figure 4), accelerated bone remodeling, and episodic hypercalcemia inherited as an autosomal dominant trait. Initially, absence of large osteolytic lesions with cortical thinning in major long bones together with hypercalcemia indicated that ESH is not a variant of FEO. However, in 2002, Whyte and Hughes found that ESH is caused by a 15-bp tandem duplication (84dup15) in TNFRSF11A that is remarkably similar to the mutations in the FEO and PDB2 families (see before). Hence ESH, FEO and PDB2 are allelic diseases involving RANK, and ESH probably reflects increased activity of the RANK targets, NF-κB, etc., in the skeleton.

Clinical features

ESH was characterized after investigation of the affected individuals at 36 and 11 years of age, respectively. Like in FEO, deafness is the earliest clinical problem. Both the mother and daughter developed a hearing deficit in infancy that was complete by early childhood. Compression in the VIIIth cranial nerve did not seem to be the cause, because the daughter’s skull radiographs were unremarkable at age 11 years. In fact, the mother reportedly had absence of middle ear bones, perhaps like the necrotic degeneration of these structures with other abnormalities causing deafness in FEO. Alternatively, hearing loss was also frequent in JPD (see below), where conductive deficits had been reported. Finally, deafness from a variety of mechanisms was common when PDB involves the temporal bone.

Premature tooth loss during childhood or early adult life also seems to characterize ESH. The pathogenesis initially seemed unclear. We did not observe radiographic abnormalities in the mandible of the daughter. Her mother’s X-rays showed edentia. However, the daughter subsequently lost adult teeth. Notably, early tooth loss is also a feature of FEO and JPD, and sometimes occurs in PDB.

Skeletal symptoms in ESH began before puberty, at about 10 years of age. Bony expansion at the proximal interphalangeal joints of the daughter was understandably worrisome for “arthritis”, yet these joints did not feel hot, although they could be tender. The hands in ESH suffer the greatest discomfort and deformity. Possibly, pain is due to periosteal stretching or joint distortion. Whether the generalized skeletal disturbance of ESH manifests long before bone symptoms is not known. Of interest, the daughter’s bone pain and finger swelling started soon after a forearm fracture, perhaps provoking a sustained, generalized skeletal reaction leading to symptoms.

Radiographic survey of the mother showed that ESH eventually disturbs the entire skeleton. Her calvarium (excluding the basal occiput), thoracic vertebral bodies, and long bones were affected most. The principal abnormalities were expansion (undertubulation) as well as hyperostosis (cortical thickening) of large and small long bones. Additionally, bowing of long bones, particularly the femurs, was noted. The daughter’s radiographic changes were similar, although less severe.

Serum ALP activity and additional markers of bone remodeling were considerably elevated in both affected individuals before anti-resorptive therapy. This was in keeping with the histopathological findings indicating rapid skeletal turnover. Reportedly, the mother’s skeletal disturbance was influenced by intercurrent illnesses and pregnancies. Her episodic hypercalcemia occurred spontaneously during childhood as well as later during intercurrent illnesses and lactation. Chosich and colleagues postulated that the hypercalcemia reflected such perturbations acting on rapid skeletal turnover in JPD—uncoupling of bone remodeling was due to factors that enhanced osteoclastic activity, including lowered estrogen levels during lactation.

Figure 4. The hand of this 36-year-old woman with ESH shows a remarkably expanded proximal phalanx of the middle finger with areas of hyperostosis and an irregular and dense trabecular pattern.
in the mother, later appeared in her daughter\(^4\). Hence, ESH seems to be a progressive disorder, with periods of exacerbation, at least until middle-age.

Histologic studies of the ESH skeleton\(^76\) showed increased numbers of osteoblasts and osteoclasts\(^38\). In the daughter, accelerated bone turnover was suggested by the enhanced cellular resorative activity\(^38\). Dynamic histomorphometry after tetracycline labeling\(^76\) revealed, however, that her bone accretion rate was normal\(^38\). Her total bone volume in the iliac crest was normal (no morphologic signs of osteopenia were present), but was markedly increased in the mother\(^38\). Paratrabecular fibrosis was not seen. There were reversal lines, but not those of PDB\(^38\). The findings were consistent with the radiographic studies. The coarse trabecular lines evident on X-ray were possibly explained by cortical bone loss providing a "window" on thicker trabecular struts\(^38\). In the mother’s bone, resorption was noted primarily in the cortex. The mother showed early erosion of Haversian canals in the cortex without corresponding resorption in the trabecular compartment, but she had received bisphosphonate treatment years earlier\(^38\). EM showed disorganized collagen bundles as well as necrotic and apoptotic bone cells, but no osteocytic osteolysis\(^38\). Measles virus gene transcripts were not detected in her peripheral blood monocytes\(^38\).

Although the mother’s skeletal symptoms had not diminished during treatments elsewhere with anti-resorptive therapy (salmon calcitonin, etidronate, or pamidronate), this approach seemed helpful for her hypercalcemia\(^72\). Alendronate treatment, however, eventually proved quite beneficial\(^4\).

In our original report\(^38\), ESH did not seem to be a variant of FEO in part because expansile osteolysis is a hallmark of FEO\(^76\). In ESH, we did not find osteolytic lesions with cortical bone thinning in widened, major long bones\(^38\). Instead, these bones were broad with hyperostosis (cortical thickening) (Fig. 5). Furthermore, episodic hypercalcemia was a remarkable feature of ESH\(^38\).

Nevertheless, ESH and FEO manifested with sufficient likeness to explore a shared etiology and pathogenesis\(^20,38\). Both affected individuals had lost hearing in early childhood and suffered premature shedding of teeth\(^38,73\). Skeletal pains began just before puberty\(^38\). Accordingly, after Hughes and co-workers discovered in 2000 that the molecular basis for FEO is an 18-bp tandem duplication (84dup18) in \(TNFRSF11A\)\(^4\), this gene was studied in ESH. In 2002, Whyte and Hughes showed that ESH is caused by a 15-bp tandem duplication (84dup15) in \(TNFRSF11A\)\(^4\). Despite the phenotypic differences, the mutations causing FEO, PDB2, and ESH proved remarkably similar, i.e., overlapping 18-bp, 27-bp, and 15-bp tandem, in frame, duplications in exon 1 of \(TNFRSF11A\) (84dup18, 75dup27, and 84dup15), respectively\(^4\). The duplications were predicted to lengthen the signal peptide of RANK by 6, 9, and 5 amino acids, respectively\(^4\).

Hence, like FEO\(^2\) and PDB2, ESH probably reflects excessive RANK activity and NF-\(\kappa\)B, etc., effects within the skeleton although transfection studies of the 84dup15 mutation have not been performed\(^4\). Of course, with few patients described worldwide with \(TNFRSF11A\) disorders, it is uncertain if future reports will show greater phenotypic overlap to no longer warrant distinct labels for these entities\(^4\).

As discussed, additional genetic or epigenetic factors could (once again) be explaining differences observed among the FEO kindreds, ESH, and the Japanese family with PDB2. In fact, after we studied these ESH patients and they returned to Australia their dental problems were subsequently reported to be remarkably similar to FEO\(^73\). Additionally, our suggestion for a trial of alendronate therapy\(^4,38\), based in part on the favorable observations in FEO\(^20\), led to symptomatic relief and normalization of the biochemical disturbances\(^38,73\).

**Phenotype/genotype correlation**

Until additional kindreds or individual patients with various types of activating \(TNFRSF11A\) defects are reported, ESH might best be considered allelic to FEO\(^7\). In any case, ESH broadened the phenotype for activating \(TNFRSF11A\) disorders that lengthen RANK’s signal peptide\(^2\) and the radiographic hyperostosis in ESH added another similarity of \(TNFRSF11A\) activation to "garden variety" PDB\(^35,39\).

**Juvenile Paget Disease**

Hyperphosphatasia refers generically to rare disorders featuring marked elevation in serum alkaline phosphatase (ALP) activity (i.e., hyperphosphatasemia)\(^77,78\). Included among these conditions are several entities characterized by focal or generalized acceleration of skeletal remodeling explaining this biochemical disturbance by selective increases in the circulation of the bone isoform of the tissue-non-
specific isoenceyme of ALP. Examples are severe polyostotic fibrous dysplasia, including the McCune-Albright syndrome, and JPD. FEO and ESH could now be considered among these disorders.

JPD [MIM # 23900], also called idiopathic or hereditary hyperphosphatasia, is typically diagnosed in infants or young children. Approximately 40 case reports have been published. Skeletal remodeling is greatly accelerated, often causing extreme hyperphosphatasemia. Exceptionally, JPD is more mild and the skeletal disease does not manifest until later in childhood. Accordingly, severe and especially rare mild forms of JPD have been described. There also appears to be a particularly unusual type associated with significant mental retardation. All forms of JPD are considered autosomal recessive conditions.

JPD affects the entire skeleton. It is not regarded as a focal bone disorder and this, in part, has engendered objections to its being called a type of Paget disease of bone (PDB). However, this chapter recounts how JPD as well as FEO, PDB2, and ESH seem increasingly to share pathogenetic themes with PDB (see below).

Severe JPD causes bone pain, fracture and deformity. Radiographs show marked expansion (underrubulation) of long bones with osteopenic cortices. These changes reflect extremely rapid rates of skeletal remodeling inferred by markedly elevated biochemical markers of bone turnover and supported by histopathologic findings in any area of the skeleton. In fact, severe JPD will respond to anti-resorptive therapy, including human calcitonin and bisphosphonates. Premature loss of teeth and deafness are also typical manifestations.

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

A mosaic pattern in bone, characteristic of PDB, is not found in JPD (or FEO). Measles virus transcripts, commonly identified in PDB, were not detected in peripheral blood leukocytes in mild or severe cases of JPD (M.P. Whyte, R.V. Reddy, G.D. Roodman; unpublished observations).

In 2002, we speculated that OPG deficiency could explain JPD because OPG suppresses bone turnover by functioning as a decoy receptor for RANKL. We reported complete deletion of the gene encoding OPG (TNFRSF11B) in two apparently unrelated Navajos with JPD. Both patients had identical break points on chromosome 8q24.2. The deletion spanned ~100 kb, but neighbouring genes seemed to be intact. Serum levels of OPG and soluble RANKL were undetectable and markedly increased, respectively, and consistent with the DNA-based findings.

Discovery of homozygous deletion of TNFRSF11B in two native Americans with JPD provided both a cause and a mechanism for this osteopathy. OPG is normally secreted into marrow spaces by cells derived from mesenchyme.

Homozygous deletion of TNFRSF11B precluded the biosynthesis of OPG, in turn causing high circulating (and presumably marrow space) levels of biologically active RANKL, which in turn markedly accelerated bone turnover. Mice lacking OPG from tnfrsf11b knockout were initially reported to have "osteoporosis," but numerous osteoclasts and rapidly remodeling woven bone were noted rather than a paucity of lamellar bone. Accordingly, OPG knockout animals manifest JPD. Although mice heterozygous for OPG deficiency could be osteopenic, our patient’s heterozygous (carrier) parents, whose serum OPG levels were approximately 50 percent of control values, had skeletal radiographs and bone densitometry (DXA) that were unremarkable (unpublished).

Additionally, our observations in JPD complemented studies of a potential role for OPG in atherosclerosis. The tnfrsf11b knockout mouse model implicated OPG deficiency in this process because of aortic and renal artery calcification detected by histopathological studies. Nevertheless, no mineralization was observed in the aorta or renal arteries of our second Navajo JPD patient who underwent computed tomography (with bone windows sensitive for calcification) of her abdomen at age 23 years. Our proband initially had microscopic hematuria and renal ultrasound revealed nephrocalcinosis and several tiny echogenic foci in his kidneys, perhaps representing small calculi. These calculi could have formed from his marked hypercalciuria due to impaired skeletal growth and perhaps the calciuric effects of salmon calcitonin.
therapy. However, computed tomography of his kidneys was recently negative for calcifications (unpublished). Hence, we found no evidence that OPG deficiency causes macroscopic ectopic mineralization, at least in childhood or early adulthood. Nevertheless, the literature concerning JPD includes "calcifying arteriopathy," detected by renal sonography and confirmed by histopathological analysis of the internal elastic membrane of a temporal artery, in an affected 6-year-old boy96. Striking changes consistent with pseudoxanthoma elasticum (MIM #s 177850 and 264800)7, 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 JPD90.

We detected no splice-site or exon mutations in TNFRSF11B in two unrelated women with relatively mild JPD3. However, also in 2002, Cundy and colleagues reported an Iraqi sibship with a homozygous 3-bp TNFRSF11B deletion92, and in 2003 a variety of deactivateing TNFRSF11B mutations in additional JPD patients in whom the nature of the gene defect seemed to correlate with the severity of the skeletal disease92. Subsequently, in a preliminary report, we emphasized that homozygous defects in TNFRSF11B are found in nearly all JPD patients with OPG deficiency - illustrating the importance of consanguinity as a cause for this inborn error of metabolism96. Homozygous deletion of TNFRSF11B causing JPD in our two Navajo patients is likely a founder effect emerging in this "bottleneck" population which had decreased to approximately 6,000 people in 186894 and re-expanded to approximately 225,000 by 199095. Three JPD patients from apparently separate Navajo families have been identified since the 1960s. Although the precise prevalence of the deletion among Navajos is not known, we have estimated that 1 in 100 is a carrier96. Now, this can be directly assessed. Pre-natal diagnosis of JPD in this population is also possible.

JPD belongs among the disorders characterized by excessive signaling along the RANKL/OPG/RANK pathway leading to increased osteoclast action and accelerated rates of osseous tissue turnover. Nevertheless, autosomal recessive JPD has some clinical and radiographic features that seem different compared to autosomal dominant EFO, ESH, and PDB2 in Japan95,99,100, showing that additional factors modify the effects of this pathway when activated. However, we have recently encountered a further similarity between JPD and PDB and the disorders caused by activated TNFRSF11A. A remarkable, focal, expansile, osteolytic defect has appeared in the humerus of our proband with JPD (Figure 6A, B).

Chromosome 8q24.2 contains TNFRSF11B, but has not been reported as one of the susceptibility loci for PDB. In fact, the coding sequence of TNFRSF11B is not mutated in "garden variety" PDB93.

Our observations bolster the rationale for using anti-resorptive treatment for JPD. Furthermore, if OPG is not rejected as a foreign protein because of the complete deletion of TNFRSF11B, OPG replacement therapy might be especially effective for Navajo patients with OPG deficiency3. Alternatively, antibody-mediated neutralization of RANKL might be therapeutic.

Finally, although similarities continue to emerge for PDB and disorders of the RANKL/OPG/RANK signaling pathway, it might now be best to refer to JPD caused by TNFRSF11B defects as "osteoprotegerin deficiency."

Summary

Figure 7 summarizes the heritable disorders identified to date that directly involve the RANKL/OPG/RANK signaling pathway in humans. Activating mutations in TNFRSF11A encoding RANK and deactivating mutations in TNFRSF11B encoding OPG cause systemic bone disease (FEO, PDB2, ESH and JPD) featuring accelerated bone turnover, low bone mass, deafness early in life, and loss of dentition by enhancing signaling. No human disease has been identified involving defects in the TNFSF11 gene encoding RANKL. Despite genetic bases for these autosomal dominant and recessive conditions involving bone cell receptors, focal expansile osteolytic lesions are common and can occur perhaps from further local activation of osteoclast-mediated bone resorption following trauma. These disorders resemble PDB which can be inherited as an autosomal dominant trait with focal osteolytic disease, sometimes with deafness and tooth loss, and increasingly associated with mutations, but in other genes.

Acknowledgements

This work reflects the dedication and skill of the nursing, laboratory, and dietary staff of the Center for Metabolic Bone Disease and Molecular Research, Shriners Hospitals for Children, St. Louis, MO. Becky Whitener, CPS, provided expert secretarial help. Supported by Shriners Hospitals for Children and The Clark and Mildred Cox Inherited Metabolic Bone Disease Research Fund.
References


77. Caffey J. Familial hyperphosphatasemia with ateliosis


Note Added In Proof

A fourth kindred with FEO, involving 20 affected individuals in 4 generations in Spain, was reported in 2002 (97). FEO(Sp) is caused by the same TNFRSF11A 18 bp tandem duplication found in the other three FEO kindreds (2). FEO(Sp) features deafness, tooth loss, and low bone mass ("osteoporosis") with variable penetrance, but osteolytic lesions are a distinctly unusual finding. Characterization of FEO(Sp) documents further interesting phenotypic variation for FEO despite an identical defect in TNFRSF11A.