Heritable disorders of the RANKL/OPG/RANK signaling pathway

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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) superfamily[1]. 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 bases[2,3]. 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 Japan[2]. 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 RANK[4]. 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)[3].

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 deformity[6-8]. 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 bones[9,10]. The pathogenesis is excessive RANK effect[2].

History

FEO was first mentioned in 1976 with a brief communication by Osterberg from Northern Ireland[11]. 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 PDB[10,12].

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* 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 kindred[8,9,13-19]. A report by Whyte et al., recounting 30 years experience with a five-generation American FEO family, was published in 2002[20].

In 1994, FEO was mapped by Hughes and co-workers to chromosome 18q21.1-q22[21]. In 2000, these investigators discovered the molecular defect using a candidate gene approach[2]. 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 osteoclastogenesis[1,22-32]. 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 RANK[2]. 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 humans[2].

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, respectively[6,9-11,12-20]. In 2003, two additional, unrelated, American cases of FEO were reported[33]. A review of FEO by Hughes and Barr (pre-dating identification of the gene defect) was published in 1996[6], and a summary by Whyte and co-workers[20] (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(Ger)

Two brothers with FEO(Ger) were reported in 1979[10,12]. The proband had expansile disease in both fibulae, radii, and patellae noted at approximately age 25 years. His brother manifested similar clinical and radiographic findings[10]. Their father was probably affected because he died at age 47 years from metastatic osteosarcoma with focal skull lesions[10] - a cancer that also occurs in FEO(NI) *(see below)*.

In FEO(Ger), there was no mention of hearing loss or dental problems[12]. 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 formation[10]. Electronmicroscopy (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" phase[10].

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 fibulae[10].

FEO(NI)

The publications beginning in 1987[8], comprising eight reports by several groups of investigators[9,13-19], 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 features[16]. First, progressive osteolytic lesions (90% prevalence) were especially common in the lower limbs and typically multifocal[6,17]. A tibia was invariably involved[17,19]. Most started at (or near) the ends of long bones[19] and then advanced relentlessly[13] 6.5–22 mm yearly[14,17,19]. Skeletal pain began between 18 and 44 years of age, sometimes leading to limb amputation[13-15]. The maximum number of such lesions was 12 in one individual[19]. 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 age[17], but more commonly was detected in the second decade[14]. The long process of the incus was typically absent or replaced by fibrous tissue[6,17,19]. Third, dental loosening and pain and/or fracture of secondary teeth troubled most FEO(NI) patients[17,19]. Deciduous teeth, however, were not involved[15,16].

Radiographic abnormalities in FEO(NI) included altered modeling of some bones – particularly the humerus, radius, ulna and tibia[13,17]. Furthermore, there was always a tightly meshed ("fish-net") trabecular pattern variably present throughout the skeleton[13], especially in metaphyses[17] and sometimes in the mandible[14]. Bone scanning showed proportionately greater uptake of isotope in tibias compared to

Figure 1. The 71-year-old proband with FEO(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(Am) showed characteristic external resorption of his teeth (black arrows). Note also that the tooth root is being destroyed.

femurs[17]. Osteopenia, however, was not mentioned as a significant feature of FEO(NI) (or FEO(Ger))[6].

Histopathology of early osteolytic lesions in FEO(NI) resembled PDB[9,17,19] and biochemical markers indicated increased rates of skeletal turnover[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[6,17,19]. Osteoclasts in lytic lesions were particularly large with increased numbers of nuclei[19]. Furthermore, all of these polykaryons studied by EM had microcylindrical nuclear inclusion bodies[9,14,19] whose ultrastructure resembled measles, canine distemper, or respiratory syncytial virus[9,17,19]. Nevertheless, iliac crest light microscopy appeared unremarkable[19]. Several individuals with FEO(NI) suffered malignant transformation of bone[6].

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

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

FEO(Am)

Our 30-year experience involving FEO(Am) confirmed that the excessive RANK/NF-κB activity discovered by Hughes, et al.[2] can cause early-onset deafness, loss of adult dentition, and expansile osteolysis of major appendicular bones in humans *(Figure 1)*[20]. However, FEO(Am) also clearly illustrates that the molecular defect involving RANK has systemic consequences. Osteopenia is a common and sometimes important fourth feature of FEO(Am) in adults – reflecting generalized, accelerated bone remodeling and a form of high-turnover osteoporosis that occasionally results in fractures. Familiarity with FEO(Am) suggests that trauma could be an inciting factor for the osteolytic lesions[20]. Furthermore, as discussed below, FEO(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[20].

In FEO(Am), early-onset hearing loss from degeneration of middle ear bones is characteristic[34]. 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(Am), although the characteristic external resorption of secondary dentition can occur suddenly[16]. Additionally, FEO(Am), in contrast to FEO(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[20]. 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[20].

In FEO$_{(Am)}$, elevated serum alkaline phosphatase (ALP) activity early in life reflects the fundamental pathogenetic disturbance, i.e., rapid turnover of the skeleton[20]. 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[20]. 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[20]. 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[20].

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[9,10,35,36]. Dr. Barbara Mills concluded that nothing distinguished the histopathology of FEO$_{(Am)}$ from "garden variety" PDB. There was membranous bone formation, osteoclast-mediated bone resorption, and marrow fibrosis. However, "mosaic bone," a hallmark of PDB[35], was rarely seen– perhaps reflecting the extreme rates of lesional bone remodeling. Stasis lines that create this pattern were absent. Instead, woven bone seemed to be rapidly deposited, yet did not mature or become extensively remodeled into cortical bone to produce "healing" with any degree of osteosclerosis even in older areas of these lesions[20]. Osteolytic lesions were enlarged and penetrated by 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. However, the bony defects were often unlike PDB because they were also without "cutting cones" containing extensive concentrations of osteoclasts, but instead had more scattered osteoclasts. EM demonstrated that inclusion bodies were rare in FEO$_{(Am)}$[20].

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[20].
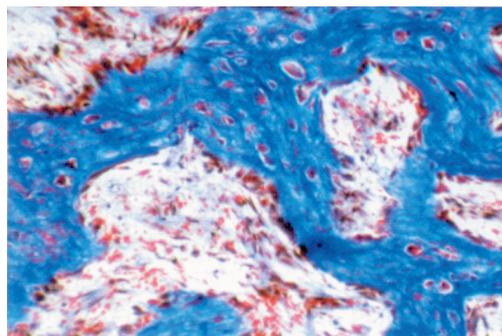
Figure 3. An undecalcified specimen from an osteolytic lesion of the distal humerus in FEO$_{(Am)}$ shows woven bone, fibrous tissue accumulation in the marrow spaces, and numerous osteoclasts (Masson trichrome stain x 80).

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.[2] 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 PDB2[2]. In 2003, detailed clinical, radiographic, and histopathologic findings were reported by Nakatsuka et al. which appeared to support this variation[37]. Furthermore, the disorder we called *expansile skeletal hyperphosphatasia (see below)* also seemed different from FEO[38]. 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[38]. In 2002, ESH was shown to be caused by a unique, yet remarkably similar 15-bp tandem duplication (84dup15) in exon 1 of *TNFRSF11A*[4] *(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$_{(NI)}$[6] and especially FEO$_{(Ger)}$, which is most severe[10,12], despite identical molecular defects in *TNFRSF11A*[2]. 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[20]. Although malignant transformation of the skeleton occurs in PDB[35,39] and troubled one patient with FEO$_{(Ger)}$ and several with FEO$_{(NI)}$, 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 conditioning 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 FEO$_{(Am)}$[20].

Treatment

The medical history of one FEO$_{(Am)}$ patient suggested that pregnancy (a time when skeletal turnover accelerates) can exacerbate the osteolytic disease[13]. Conversely, the mother with *expansile skeletal hyperphosphatasia (*see below*)* experienced worsening of her disease during lactation[4,38]. 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 option[6].

FEO$_{(Am)}$ patients received anti-resorptive pharmaceuticals as they became available in the United States at various times during the past three decades[20]. 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 lesions[20]. Such responses also occur in PDB[39,40]. 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 treatment[20].

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 ineffective[20].

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 lesions[20]. 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 completely[20]. 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 bone[20]. 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 PDB[9,10], 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 years[35,39,42]. PDB features focal accelerated skeletal remodeling (monostotic or polyostotic) in axial and/or appendicular bone[35,39,43]. 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 described[35,39].

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 FEO[35,39]. Additionally, however, PDB2 in the Japanese family is associated with axial bone lesions like in PDB[37]. 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$_{(Am)}$ 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 FEO[2].

In fact, increasing evidence (now primarily from genetic studies) indicates that PDB should continue to be viewed as a metabolic bone disease. McKusick recognized the familial predisposition and sometimes multigenerational occurrence of PDB in 1960 and proposed autosomal dominant inheri-

tance[44] although the gene defect(s) was unknown (MIM #167250, #602080)[5]. Nevertheless, until recently, reports of large families or kindreds with PDB had been rare[35,36]. Now the prevalence of PDB in first-degree relatives of affected individuals is appreciated to be 12-40%, representing a 7-fold enhanced risk[42,44,45]. In 1977, investigation of small PDB families suggested genetic predisposition involving chromosome 6, near the HLA loci (PDB1, MIM #167250)[5,46]. Subsequently, the similarity of FEO to the osteolytic phase of PDB and its autosomal dominant inheritance understandably re-activated interest in a genetic basis for PDB[6,47]. 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)[21,48,49]. Initially, linkage studies of further PDB families were consistent[48,50] or inconsistent[51,52] 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)[37]. 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 18q21[49]. Nevertheless, FEO is not a variant of "garden variety" PDB (MIM #167250). PDB has been shown not to involve *TNFRSF11A* mutations[53,54]. 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 inheritance[52].

Now, PDB is understood to be genetically heterogeneous[5], and considerable efforts are underway to identify additional causal genes[47,51]. In 2001, PDB was also mapped to chromosome 5q35-qter and 5q31 in French Canadian families (i.e., PDB3 and PDB4, respectively)[55,56] and this led to the discovery of mutations in the gene encoding sequestosome (SQSTM1) in at least 29 families and 33 sporadic cases[57,58]. Subsequently, several additional mutations were identified in *SQSTM1* for a large number of familial and sporadic PDB cases[58-61]– 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 PDB[57,59-61]. 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 PDB[63] was mapped to chromosome 9p13.3-p12 in 2001[64]. In 2004, this disorder was shown to involve mutations in the gene encoding *valosin-containing protein* (VCP) in 13 families[65]. SQSTM1 and VCP are both involved in the intracellular process of "ubiquination"[65]. Further, VCP may regulate NF-κB signaling by binding to Inhibitor-κBa (IκBa) and affecting ubiquitin-dependent proteosome degradation of IκBa[66]. However, this syndrome – caused by VCP mutation in muscle, brain, and bone – also seems to be genetically heterogeneous[65].

Despite considerable recent progress, the precise pathogenesis of PDB is still unknown[43,67]. Increasing evidence supports the name given to PDB by Sir James Paget in 1876, i.e., *osteitis deformans*[68]. Viral infection involving the cellular lineage of osteoclasts seems important for developing the skeletal manifestations of PDB[43,53,67,69,70]. In PDB, the marrow microenvironment is especially osteoclastogenic[43]. Paramyxovirus infection has been investigated as a necessary pathogenetic insult[43,67,70,71]. In fact, osteoclasts in FEO$_{(NI)}$ contain nuclear inclusions similar to PDB[9,14,43]. 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 factor[20]. Nevertheless, FEO illustrates how focal bone lesions can be a major finding in an autosomal dominant skeletal disease[20].

The poorer skeletal repair of FEO compared to PDB (osteolytic expansion of the entire long bone without osteosclerosis or hyperostosis) is not understood[35,39]. 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 adipocytes[9,10]. Understandably, advanced expansile lesions are then unresponsive to bone anti-resorptive therapy[20].

We speculate that skeletal injury sometimes explains the focal and lytic nature of the appendicular bone defects in FEO. Indeed, trauma has been postulated to initiate PDB lesions[35,39]. Several FEO$_{(Am)}$ patients gave histories consistent with this possibility[20]. 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 involved[35,39]. 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 age[20]. Only he among his kindred had undergone this procedure that moves teeth by activating osteoclasts to cause osteolysis within alveolar bone[16]. 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 teeth[15,16].

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 fractured[35,39]. Additionally, axial lesions occurred in the Japanese family with PDB2[37].

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.

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[38]. 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[38]. 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)*[4]. 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[4].

Clinical features

ESH was characterized after investigation of the affected individuals at 36 and 11 years of age, respectively[38]. Like in

FEO[20], deafness is the earliest clinical problem[38,72]. 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[38]. In fact, the mother reportedly had absence of middle ear bones[38], perhaps like the necrotic degeneration of these structures with other abnormalities causing deafness in FEO[6,34]. Alternatively, hearing loss was also frequent in JPD *(see below)*, where conductive deficits had been reported[5]. Finally, deafness from a variety of mechanisms was common when PDB involves the temporal bone[35,39].

Premature tooth loss during childhood or early adult life also seems to characterize ESH[73]. The pathogenesis initially seemed unclear[38]. We did not observe radiographic abnormalities in the mandible of the daughter[38]. Her mother's X-rays showed edentia. However, the daughter subsequently lost adult teeth. Notably, early tooth loss is also a feature of FEO[20] and JPD[74,75] and sometimes occurs in PDB[35].

Skeletal symptoms in ESH began before puberty, at about 10 years of age[38]. 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[38,78]. Possibly, pain is due to periosteal stretching or joint distortion[38]. 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[38].

Radiographic survey of the mother showed that ESH eventually disturbs the entire skeleton[38]. 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[38,72]. However, the most remarkable changes involved her fingers where phalangeal widening could be extraordinary *(Figure 4)*. Additionally, bowing of long bones, particularly the femurs, was noted. The daughter's radiographic changes were similar, although less severe[38].

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 *(see below)*[38]. Reportedly[72], the mother's skeletal disturbance was influenced by intercurrent illnesses and pregnancies[4]. Her episodic hypercalcemia occurred spontaneously during childhood as well as later during intercurrent illnesses and lactation. Chosich and colleagues[72] 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[72]. Episodic hypercalcemia, that was initially documented only

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 resorptive 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[20]. 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) *(Figure 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*[2], 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-$\varkappa$B, etc., effects within the skeleton although transfection studies of the 84dup15 mutation have not been performed[4]. Of course, with few patients

Figure 5. The distal tibia of this 36-year-old woman with ESH is expanded with irregular cortical thickening and a disorganized (coarse and radiolucent) trabecular pattern. Similar changes are seen in the fibula.

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$_{(Am)}$[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[4]. 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 isoenzyme of ALP[78]. Examples are severe polyostotic fibrous dysplasia, including the McCune-Albright syndrome, and JPD[78]. FEO and ESH could now be considered among these disorders.

JPD [MIM # 23900], also called idiopathic or hereditary hyperphosphatasia[5], is typically diagnosed in infants or young children[77,79]. Approximately 40 case reports have been published[5,77,79]. Skeletal remodeling is greatly accelerated, often causing extreme hyperphosphatasemia[3,78,80]. Exceptionally, JPD is more mild and the skeletal disease does not manifest until later in childhood[80]. Accordingly, severe and especially rare mild forms of JPD have been described[1,80]. There also appears to be a particularly unusual type associated with significant mental retardation[80]. All forms of JPD are considered autosomal recessive conditions[5].

JPD affects the entire skeleton[81]. 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[5,78,81]. Radiographs show marked expansion (undertubulation) of long bones with osteopenic cortices[81]. These changes reflect extremely rapid rates of skeletal remodeling inferred by markedly elevated biochemical markers of bone turnover[78] and supported by histopathologic findings in any area of the skeleton[80]. In fact, severe JPD will respond to anti-resorptive therapy, including human calcitonin[78] and bisphosphonates[82]. Premature loss of teeth[74,75] and deafness[83] are also typical manifestations.

Mild JPD is characterized by fewer fractures and bony deformities[80]. Radiographs show diffuse, acquired hyperostosis and osteosclerosis associated with biochemical and histological evidence of rapid skeletal turnover[80]. Understandably, Chosich and colleagues suggested that the mother with ESH had a mild form of JPD (see before)[72].

A mosaic pattern in bone, characteristic of PDB, is not found in JPD (or FEO). Measles virus transcripts, commonly identified in PDB[71], were not detected in peripheral blood leukocytes in mild[84] 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[1] and reported complete deletion of the gene encoding OPG (TNFRSF11B) in two apparently unrelated Navajos with JPD[3]. Both patients had identical break points on chromosome 8q24.2[3]. 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[3].

Discovery of homozygous deletion of TNFRSF11B in two native Americans with JPD provided both a cause and a mechanism for this osteopathy[3]. OPG is normally secreted into marrow spaces by cells derived from mesenchyme.
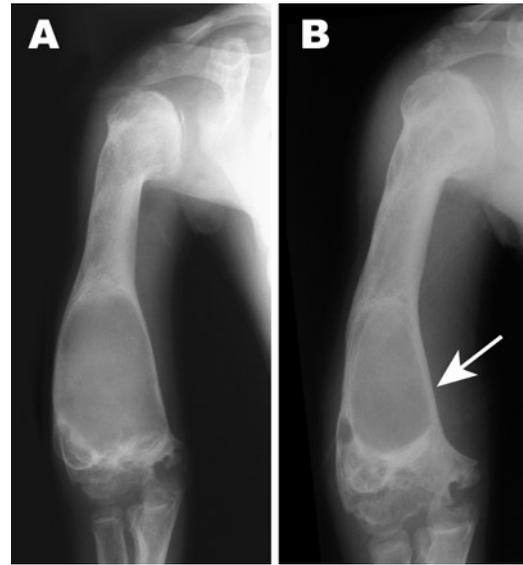
Figure 6. A. At age 7½ years, this Navajo boy with JPD has a remarkable "soap bubble" expansile lesion with cortical thinning in his distal humerus 9 months after the lesion was packed with autologous bone and 2 months after a single course of intravenous pamidronate therapy. **B.** At age 8-2/3 years, following additional courses of pamidronate, the humerus has narrowed considerably, showing improved modeling, and a bony cortex (arrow) has appeared in the osteolytic lesion.

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[85]. Accordingly, OPG knockout animals manifest JPD. Although mice heterozygous for OPG deficiency could be osteopenic[85], 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[86,87]. The tnfrsf11b knockout mouse model implicated OPG deficiency in this process because of aortic and renal artery calcification detected by histopathological studies[85]. 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[3]. Our proband initially had microscopic hematuria and renal ultrasound revealed nephrocalcinosis and several tiny echogenic foci in his kidneys, perhaps representing small calculi[3]. These calculi could have formed from his marked hypercalciuria due to impaired skeletal growth[88] 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 boy[89]. Striking changes consistent with *pseudoxanthoma elasticum* (MIM #s 177850 and 264800)[5], 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[90].

We detected no splice-site or exon mutations in *TNFRSF11B* in two unrelated women with relatively mild JPD[3]. However, also in 2002, Cundy and colleagues reported an Iraqi sibship with a homozygous 3-bp *TNFRSF11B* deletion[91], and in 2003 a variety of deactivating *TNFRSF11B* mutations in additional JPD patients in whom the nature of the gene defect seemed to correlate with the severity of the skeletal disease[92]. 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 metabolism[93]. 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 1868[94] and re-expanded to approximately 225,000 by 1990[95]. 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 carrier[96]. 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 FEO, ESH, and PDB2 in Japan[3,5,91,92], 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" PDB[53].

Our observations bolster the rationale for using antiresorptive 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 deficiency[3]. Alternatively, antibody-mediated neutralization of RANKL might be therapeutic.
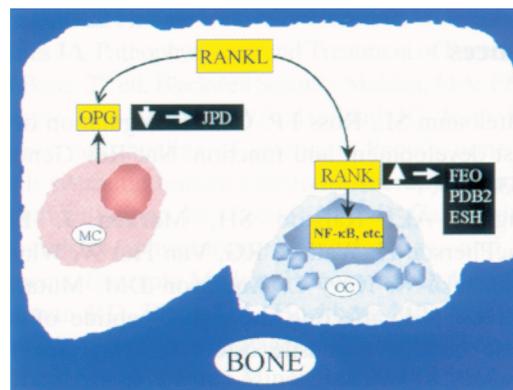
Figure 7. Heritable disorders of the RANKL/OPG/RANK signaling pathway in humans. In familial expansile osteolysis (FEO), expansile skeletal hyperphosphatasia (ESH), and early-onset PDB in Japan (PDB2), constitutive activation of RANK, conditioned by lengthening from different tandem duplications in exon 1 of *TNFRSF11A*, causes enhanced NF-κB signaling and osteoclastogenesis. In juvenile Paget disease (JPD), OPG deficiency causes enhanced RANK activity, etc., by failing to decoy RANKL from RANK. There is no human disorder known from defects in *TNFSF11* encoding RANKL.

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.

References

1. Teitelbaum SL, Ross FP. Genetic regulation of osteo-clast development and function. Nat Rev Genet 2003; 4:638-649.
2. Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, Van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM. Mutations in *TNFRSF11A*, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet 2000; 24:45-48.
3. Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, Mumm S. Osteoprotegerin deficiency and juvenile Paget's disease. N Engl J Med 2002; 347:174-184.
4. Whyte MP, Hughes AE. Expansile skeletal hyperphos-phatasia is caused by a 15-base pair tandem duplication in *TNFRSF11A* encoding RANK and is allelic to familial expansile osteolysis. J Bone Miner Res 2002; 17:26-29.
5. McKusick VA. Mendelian Inheritance in Man: Catalogs of Human Genes and Genetic Disorders, 12th ed. Johns Hopkins University Press, Baltimore and Online 1998; Mendelian Inheritance in Man. OMIM (TM) McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University, Baltimore and National Center for Biotechnology Information, National Library of Medicine, Bethesda; 2000.
6. Hughes AE, Barr J. Familial expansile osteolysis: a genetic model of Paget's disease. In: Sharpe PT (ed) The Molecular Biology of Paget's Disease. RG Landes, Heidelberg; 1996:179-199.
7. Nance MA, Nuttall FQ, Econs MJ, Lyles KW, Viles KD, Vance JM, Pericak-Vance MA, Speer MC. Heterogeneity in Paget disease of the bone. Am J Med Genet 2000; 92:303-307.
8. Wallace RGH. A study of a familial bone dysplasia. MD thesis. The Queen's University of Belfast, Northern Ireland; 1987.
9. Dickson GR, Shirodria PV, Kanis JA, Beneton MNC, Carr KE, Mollan RA. Familial expansile osteolysis: a morphological, histomorphometric and serological study. Bone 1991; 12:331-338.
10. Enderle A, Willert HG. Osteolytic-expansive type of familial Paget's disease (osteitis deformans). Pathol Res Pract 1979; 166:131-139.
11. Osterberg PH. An unusual familial disorder of bone: response to calcitonin. In: Kanis JA (ed) Bone Disease and Calcitonin. Armour Pharmaceutical Company, London, England; 1976:181-185.
12. Enderle A, Von Gumppenberg S. Osteitis deformans (Paget) – or a tarda-type of a hereditary hyperphos-phatasia. [German]. Arch Orthop Trauma Surg 1979; 94:127-134.
13. Barr RJ, Hughes AE, Mollan RA, Nevin NC. Idiopathic multicentric osteolysis: Report of two new cases and a review of the literature. [Letter]. Am J Med Genet 1989; 32:556.
14. Crone MD, Wallace RG. The radiographic features of familial expansile osteolysis. Skeletal Radiol 1990; 19:245-250.
15. Mitchell CA, Kennedy JG, Wallace RG. Dental abnor-malities associated with familial expansile osteolysis: A clinical and radiographic study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1990; 70:301-307.
16. Mitchell CA, Kennedy JG, Owens PD. Dental histology in familial expansile osteolysis. J Oral Pathol Med 1990; 19:65-70.
17. Osterberg PH, Wallace RG, Adams DA, Crone RS, Dickson GR, Kanis JA, Mollan RA, Nevin NC, Sloan J, Toner PG. Familial expansile osteolysis: a new dyspla-sia. J Bone Joint Surg Br 1988; 70:255-260.
18. Wallace R. McCabe's disease: hereditary expansile polyostotic osteolytic dysplasia (Abstract). J Med Genet 1988; 25:276.
19. Wallace RG, Barr RJ, Osterberg PH, Mollan RA. Familial expansile osteolysis. Clin Orthop 1989; 248:265-277.
20. Whyte MP, Reinus WR, Podgornik MN, Mills BG. Familial expansile osteolysis (excessive RANK effect) in a 5–generation American kindred. Medicine (Baltimore) 2002; 81:101-121.
21. Hughes AE, Shearman AM, Weber JL, Barr RJ, Wallace RG, Osterberg PH, Nevin NC, Mollan RA. Genetic linkage of familial expansile osteolysis to chro-mosome 18q. Hum Mol Genet 1994; 3:359-361.
22. Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A. Tumor necrosis factor-α induces differentiation of and bone resorption by osteoclasts. J Biol Chem 2000; 275:4858-4864.
23. Dunstan CR. Osteoprotegerin and osteoprotegerin lig-and mediate the local regulation of bone resorption. Endocrinologist 2000; 10:18-26.
24. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000; 15:2-12.
25. Hofbauer LC, Heufelder AE. Osteoprotegerin and its cognate ligand: a new paradigm of osteoclastogenesis. Eur J Endocrinol 1998; 139:152-154.
26. Hofbauer LC, Heufelder AE. The role of receptor acti-vator of nuclear factor-κB ligand and osteoprotegerin in the pathogenesis and treatment of metabolic bone dis-eases. J Clin Endocrinol Metab 2000; 85:2355-2363.
27. Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HL, Elliott G, Kelley MJ, Sarosi I, Wawng L, Xia XA, Elliott R, Chiu L. Black T. Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 1999; 96:3540-3545.

28. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998; 93:165-176.

29. Lories RJU, Luyten FP. Osteoprotegerin and osteoprotegerin-ligand balance: a new paradigm in bone metabolism providing new therapeutic targets. Clin Rheumatol 2001; 20:3-9.

30. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998; 95:3597-3602.

31. Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci USA 2000; 97:1566-1571.

32. Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Yano K, Morinaga T, Higashio K. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Biochem Biophys Res Commun 1998; 253:395-400.

33. Johnson-Pais TL, Singer FR, Bone HG, McMurray CT, Hansen MF, Leach RJ. Identification of a novel tandem duplication in exon 1 of the TNFRSF11A gene in two unrelated patients with familial expansile osteolysis. J Bone Miner Res 2003; 18376-18380.

34. Esselman GH, Goebel JA, Wippold FJ II. Conductive hearing loss caused by hereditary incus necrosis: a study of familial expansile osteolysis. Otolaryngol Head Neck Surg 1996; 114:639-641.

35. Hamdy RC. Paget's Disease of Bone: Assessment and Management. Praeger Publishers, New York; 1981.

36. Kim GS, Kim SH, Cho JK, Park JY, Shin MJ, Shong YK, Lee KU, Han H, Kim TG, Teitelbaum SL, Reinus WR, Whyte MP. Paget bone disease involving young adults in 3 generations of a Korean family. Medicine (Baltimore) 1997; 76:157-169.

37. Nakatsuka K, Nishizawa Y, Ralston SH. Phenotypic characterization of early onset Paget's disease of bone caused by a 27-bp duplication in the TNFRSF11A gene. J Bone Miner Res 2003; 18:1381-1385.

38. Whyte MP, Mills BG, Roodman GD, Reinus WR, Podgornik MN, Eddy MC, Gannon FH, McAlister WH. Expansile skeletal hyperphosphatasia: a new familial metabolic bone disease. J Bone Miner Res 2000;

39. Kanis JA. Pathophysiology and Treatment of Paget's Disease of Bone, 2nd ed. Blackwell Science, Malden, MA; 1998.

40. Whyte MP, Murphy WA, Haddad JG Jr, Avioli LV. Arrest and healing of osteolytic Paget's bone disease with synthetic human calcitonin therapy: radiographic documentation. In: Pecile A (ed) Calcitonin 1980: Chemistry, physiology, pharmacology, and clinical aspects. Excerpta Medica, Princeton, NJ; 1981:217-224.

41. Altman RD, Bloch DA, Hochberg MC, Murphy WA. Prevalence of pelvic Paget's disease of bone in the United States. J Bone Miner Res 2000; 15:461-465.

42. Siris ES. Epidemiological aspects of Paget's disease: Family history and relationship to other medical conditions. Semin Arthritis Rheum 1994; 23:222-225.

43. Reddy SV, Menaa C, Singer FR, Demulder A, Roodman GD. Cell biology of Paget's disease. J Bone Miner Res 1999; 14:3-8.

44. McKusick VA. Heritable Disorders of Connective Tissue. 2nd ed. Mosby, St. Louis, MO; 1960.

45. Morales-Piga AA, Rey-Rey JS, Corres-Gonzalez J, Garcia-Sagredo JM, Lopez-Abente G. Frequency and characteristics of familial aggregation of Paget's disease of bone. J Bone Miner Res 1995; 10:663-670.

46. Leach RJ, Singer FR, Roodman GD. Genetics of endocrine disease – the genetics of Paget's disease of the bone. J Clin Endocrinol Metab 2001; 86:24-28.

47. Singer FR, Leach RJ. Genetics of Paget's disease of bone. In: Econs MJ (ed). The Genetics of Osteoporosis and Metabolic Bone Disease. Humana Press, Totowa, NJ; 2000:309-318.

48. Cody JD, Singer FR, Roodman GD, Otterund B, Lewis TB, Leppert M, Leach RJ. Genetic linkage of Paget disease of the bone to chromosome 18q. Am J Hum Genet 1997; 61:1117-1122.

49. Haslam SI, Van Hul W, Morales-Piga A, Balemans W, San-Millan JL, Nakatsuka K, Willems P, Haites NE, Ralston SH. Paget's disease of bone: evidence for a susceptibility locus on chromosome 18q and for genetic heterogeneity. J Bone Miner Res 1998; 13:911-917.

50. Leach RJ, Singer FR, Cody JD, Roodman GD. Variable disease severity associated with a Paget's disease predisposition gene. J Bone Miner Res 1999; 14:17-20.

51. Good D, Busfield F, Duffy D, Lovelock PK, Kesting JB, Cameron DP, Shaw JTE. Familial Paget's disease of bone: Non-linkage to the PDB1 and PDB2 loci on chromosomes 6p and 18q in a large pedigree. J Bone Miner Res 2001; 16:33-38.

52. Johnson-Pais TL, Wisdom JH, Weldon KS, Cody JD, Hansen MF, Singer FR, Leach RJ. Three novel mutations in SQSTM1 identified in familial Paget's disease of bone. J Bone Miner Res 2003; 18:1748-1753.

53. Wuyts W, Van Wesenbeeck L, Morales-Piga A, Ralson S, Hocking L, Vanhoenacker F, Westhovens R,

15:2330-2344.

Verbruggen L, Anderson D, Hughes A, Van Hul W. Evaluation of the role of RANK and OPG genes in Paget's disease of bone. Bone 2001; 28:104-107.

54. Sparks AB, Peterson SN, Bell C, Loftus BJ, Hocking L, Cahill DP, Frassica FJ, Streeten EA, Levine MA, Fraser CM, Adams MD, Broder S, Venter JC, Kinzler KW, Vogelstein B, Ralston SH. Mutation screening of the *TNFRSF11A* gene encoding receptor activator of NFκB (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. Calcif Tissue Int 2001; 68:151-155.

55. Laurin N, Brown JP, Lemainque A, Duchesne A, Huo D, Lacourcière Y, Drapeau G, Verreault J, Raymond V, Morissett J. Paget disease of bone: mapping of two loci at 5q35-qter and 5q3. Am J Hum Genet 2001; 69:528-543.

56. Hocking LH, Herbert CA, Nicholls RK, Williams F, Bennett ST, Cundy T, Nicholson GC, Wuyts W, Van Hul W, Ralston SH. Genome-wide search in familial Paget disease of bone shows evidence of genetic heterogeneity with candidate loci on chromosomes 2q36, 10p13, and 5q35. Am J Hum Genet 2001; 69:1055-1061.

57. Laurin N, Brown JP, Morissette J, Raymond V. Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. Am J Hum Genet 2002; 70:1582-1588.

58. Hocking LJ, Gucas GJA, Daroszewksa A, Mangion J, Olavesen M, Cundy T, Nicholson GC, Ward L, Bennett ST, Wuyts W, Van Hul W, Ralston SH. Domain-specific mutations in sequestosome 1 (SQSTM1) cause familial and sporadic Paget's disease. Hum Mol Genet 2002; 11:2735-2739.

59. Falchetti A, Di Stefano M, Marini F, Del Monte F, Mavilia C, Strigoli D, De Feo ML, Isaia G, Masi L, Amedei A, Cioppi F, Ghinoi V, Bongi SM, Di Fede G, Sferrazza C, Rini GB, Melchiorre D, Matucci-Cerinic M, Brandi ML. Two Novel Mutations at Exon 8 of the Sequestosome 1 (SQSTM1) Gene in an Italian Series of Patients Affected by Paget's Disease of Bone (PDB). J Bone Miner Res 2004; 19:1013-1017.

60. Hocking LJ, Lucas GJ, Daroszewska A, Cundy T, Nicholson GC, Donath J, Walsh JP, Finlayson C, Cavey JR, Ciani B, Sheppard PW, Searle MS, Layfield R, Ralston SH. Novel UBA domain mutations of SQSTM1 in Paget's disease of bone: genotype phenotype correlation, functional analysis, and structural consequences. J Bone Miner Res 2004; 19:1122-1127.

61. Good DA, Busfield F, Fletcher BH, Lovelock PK, Duffy DL, Kesting JB, Andersen J, Shaw JT. Identification of SQSTM1 mutations in familial Paget's disease in Australian pedigrees. Bone 2004; 35:277-282.

62. Good DA, Busfield F, Fletcher BH, Duffy DL, Kesting JB, Anderson J, Shaw JTE. Linkage of Paget disease of bone to a novel region on human chromosome 18q23. Am J Hum Genet 2002; 70:517-525

63. Kimonis VE, Kovach MJ, Waggoner B, Leal S, Salam A, Rimer L, Davis K, Khardori R, Gelber D. Clinical and molecular studies in a unique family with autosomal dominant limb-girdle muscular dystrophy and Paget disease of bone. Genet Med 2000; 2:232-241.

64. Kovach MJ, Waggoner B, Leal SM, Gelber D, Khardori R, Levenstien MA, Shanks CA, Gregg G, Al-Lozi MT, Miller T, Rakowicz W, Lopate G, Florence J, Glosser G, Simons Z, Morris JC, Whyte MP, Pestronk A, Kimonis VE. Clinical delineation and localization to chromosome 9p13.3-p12 of a unique dominant disorder in four families: hereditary inclusion body myopathy, Paget disease of bone and frontotemporal dementia. Mol Genet Metab 2001; 74:458-475.

65. Watts GDJ, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, Pestronk A, Whyte MP, Kimonis VE. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 2004; 10:1-5.

66. Asai T, Tomita Y, Nakatsuka S, Hoshida Y, Myoui A, Yoshikawa H, Aozasa K. VCP (p97) regulates NFκB signaling pathway, which is important for metastasis of osteosarcoma cell line. Jpn J Cancer Res 2002; 93:296-304.

67. Singer FR. Update on viral etiology of Paget's disease of bone. J Bone Miner Res 1999; 14:29-33.

68. Paget J. On a form of chronic inflammation of bones (osteitis deformans). Med Chir Trans 1877; 60:37-63.

69. Reddy SV Kurihara N, Menaa C, Landucci G, Forthal D, Koop BA, Windle JJ, Roodman GD. Osteoclasts formed by measles virus-infected osteoclast precursos from hCD46 transgenic mice express characteristics of pagetic osteoclasts. Endocrinology 2001; 142:2898-2905.

70. Ross FP. RANKing the importance of measles virus in Paget's disease. J Clin Invest 2000; 105:555-558.

71. Reddy SV, Singer FR, Mallette L, Roodman GD. Detection of measles virus nucleocapsid transcripts in circulating blood cells from patients with Paget disease. J Bone Miner Res 1996; 11:1602-1607.

72. Chosich N, Long F, Wong R, Topliss DJ, Stockigt JR. Post-partum hypercalcemia in hereditary hyperphosphatasia (juvenile Paget's disease). J Endocrinol Invest 1991; 14:591-597.

73. Olsen CB, Tanghaitrong K, Chippendale I, Graham HK, Dahl HM, Stockigt JR. Tooth root resorption associated with a familial bone dysplasia affecting mother and daughter. Pediatr Dent 1999; 21:363-367.

74. Eyring EJ, Eisenberg E. Congenital hyperphosphatasia: a clinical, pathological, and biochemical study of two cases. J Bone Joint Surg Am 1968; 50:1099-1117.

75. Blanco O, Stivel M, Mautalen C, Schajowicz F. Familial idiopathic hyperphosphatasia: a study of two young siblings treated with porcine calcitonin. J Bone Joint Surg Br 1977; 59:421-427.

76. Recker RR Biopsies. In: Bilezikian JP, Raisz LG, Rodan GA (eds) Principles of Bone Biology. Academic Press, San Diego, USA; 1996:1333-1342.

77. Caffey J. Familial hyperphosphatasemia with ateliosis

and hypermetabolism of growing membranous bone; review of the clinical, radiographic and chemical features. Bull Hosp Jt Dis 1972; 33:81-110.

78. Cole DEC, Whyte MP. Hyperphosphatasia syndromes. In: Cohen MM Jr, Baum BJ (eds) Studies in Stomatology and Craniofacial Biology. IOS Press, Amsterdam; 1996:245-272.

79. Cassinelli HR, Mautalen CA, Heinrich JJ, Miglietta A, Bergada C. Familial idiopathic hyperphosphatasia (FIH): response to long-term treatment with pamidronate (APD). Bone Miner 1992; 19:175-184.

80. Golob DS, McAlister WH, Mills BG, Fedde KN, Reinus WR, Teitelbaum SL, Beeki S, Whyte MP. Juvenile Paget disease: life-long features of a mildly affected young woman. J Bone Miner Res 1996; 11:132-142.

81. Resnick D. Diagnosis of Bone and Joint Disorders, 3rd ed. WB Saunders, Philadelphia; 1995.

82. Pettifor JM, Daniels ED, Schnitzler CM, Moodley GP. Hereditary hyperphosphatasia: Response to oral alendronate in a 5 year old child. [Abstract]. J Bone Miner Res 1996; 11(Suppl.1):S254.

83. Iancu TC, Almagor G, Friedman E, Hardoff R, Front D. Chronic familial hyperphosphatasia. Radiology 1978; 129:669-676.

84. Whyte MP, Leelawattana R, Reddy SV, Roodman GD. Absence of paramyxovirus transcripts in juvenile Paget bone disease. [Letter]. J Bone Miner Res 1996; 11:1041.

85. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998; 12:260-268.

86. Wallin R, Wajih N, Greenwood GT, Same DC. Arterial calcification: a review of mechanism, animal models, and the prospects for therapy. Med Res Rev 2001; 21:274-301.

87. Tintut Y, Demer LL. Recent advances in multifactorial regulation of vascular calcification. Curr Opin Lipidol 2001; 12:555-560.

88. Chines A. Peterson DJ, Schranck FW, Whyte MP. Hypercalciuria in children severely affected with osteo-

genesis imperfecta. J Pediatr 1991; 119:51-57.

89. Silve C, Grosse B, Tau C, Garabedian M, Fritsch J, Delmas PD, Cournot-Witmer G, Balsan S. Response to parathyroid hormone and 1.25-dihydroxyvitamin D3 of bone-derived cells isolated from normal children and children with abnormalities in skeletal development. J Clin Endocrinol Metab 1986; 62:583-590.

90. Mitsudo SM. Chronic idiopathic hyperphosphatasia associated with pseudoxanthoma elasticum. J Bone Joint Surg Am 1971; 53:303-314.

91. Cundy T, Hegde M, Naot D, Chong B, King A, Wallace R, Mulley J, Love DR, Seidel J, Fawkner M, Banovic T, Callon KE, Grey AB, Reid IR, Middleton-Hardie CA, Cornish J. A mutation in the gene TNFRSF11B encoding osteoprotegerin causes an idiopathic hyperphosphatasia phenotype. Hum Mol Genet 2002; 11:2119-2127.

92. Chong B, Hegde M, Fawkner M, Simonet S, Cassinelli H, Coker M, Kanis J, Seidel J, Tau C, Tuysuz B, Yuksel B, Love D, Cundy T. International Hyperphosphatasia Collaborative Group. Idiopathic hyperphosphatasia and TNFRSF11B mutations: relationships between phenotype and genotype. J Bone Miner Res 2003; 18:2095-2104.

93. Mumm S, Banze S, Pettifor J, Tau C, Schmitt K, Ahmed A, Whyte MP. Juvenile Paget's disease: molecular analysis of TNFRSF11B encoding osteoprotegerin indicates homozygous deactivating mutations from consanguinity as the predominant etiology. J Bone Miner Res 2003; 18(Suppl.2):S388.

94. Gipin L. The Enduring Navaho. University of Texas Press, Austin; 1968.

95. 1990 Census of population and housing: characteristics of American Indians by tribe and language. Washington, DC: Bureau of the Census, 1994.

96. Whyte MP, Podgornik MN, Mumm S. Osteoprotegerin deficiency and juvenile Paget's disease (reply letter) N Engl J Med 2002; 347:1622-1623.

97. Palenzuela L, Vives-Bauza C, Fernandez-Cadenas I, Meseguer A, Font N, Sarret E, Schwartz S, Andreu AL. Familiar expanxile osteolysis in a large Spanish kindred resulting from an insertion mutation in the TNFRSF11A gene. J Med Genet 2002; 39:e67.

Note Added In Proof

A fourth kindred with FEO, involving 20 affected individuals in 4 genarations 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.