

# Genetics of Paget's disease of bone-like disorders

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## Summary

Paget's disease of bone (PDB) is a common metabolic bone disease characterised by focal areas of increased bone turnover affecting one or more bones throughout the skeleton, accompanied by bone pain, bone deformity and an increased susceptibility to fracture. The cause of PDB is unclear, but there is considerable evidence for a genetic aetiology, including familial clustering and ethnic differences in frequency. A number of other skeletal disorders have also been described which are clinically quite similar to PDB, though they are usually characterised by a more severe bone phenotype. The genetic causes of several of these PDB-like disorders have been elucidated and have provided clues to the causes of PDB and prompted new avenues of investigation. In some cases, the genes that cause these PDB-like disorders have been found to have no effect on PDB risk, and in others they have been found to confer mildly increased risk. With the emergence of the *SQSTM1* gene as a common cause of PDB, common molecular pathways involved in PDB and PDB-like disorders are emerging, and these are providing intriguing new possibilities for investigating the underlying molecular defect that leads to PDB.

**KEY WORDS:** Paget's disease of bone, familial expansile osteolysis, expansile skeletal hyperphosphatasia, juvenile Paget's disease, idiopathic hyperphosphatasia, RANK, OPG, SQSTM1.

## Introduction

Paget's disease of bone (PDB) is a common condition, characterised by focal areas of increased bone turnover affecting one or more bones throughout the skeleton (1). Clinical features include bone pain, deformity, an increased susceptibility to fracture, and an increased incidence of osteosarcoma. The lesions of PDB tend to affect the axial skeleton, skull, femora and tibiae, whereas the bones of the extremities are less frequently involved. Ultrastructural studies of osteoclasts from pagetic lesions have revealed the presence of 'virus-like' nuclear inclusion bodies (reviewed in ref. 2) but no intact virus has been recovered from pagetic bone, and the role of viruses in the pathogenesis of PDB remains controversial. There

is accumulating evidence to suggest that genetic factors play a key role in the pathogenesis of PDB. There are marked ethnic differences in susceptibility to PDB (3), which persist after migration to other countries (4). Familial clustering of Paget's disease has also been documented and first degree relatives of PDB patients run a 7-fold increased risk of developing the disease compared to controls (5-7).

Recently there has been significant progress in elucidating the molecular-genetic basis of PDB. Genome-wide scans in multiplex families with autosomal dominant PDB have identified several chromosomal regions with a high probability of linkage to the disease (8-12). In one of these regions (5q35), mutations of the *SQSTM1* gene have been identified that segregate with the disease in families (13-20). Important clues to the pathogenesis of PDB have also come from the study of other bone diseases that are clinically similar to PDB. This paper will review the clinical presentation of these PDB-like bone diseases and describe progress to date in understanding their causes. Particular emphasis will be placed on the how these studies have provided insights into the cause of PDB.

## Familial Expansile Osteolysis (FEO)

In 1981, Osterberg and colleagues (21) described a bone dysplasia with many clinical features similar to PDB affecting 40 of 100 members across five generations of a large family from Northern Ireland, which they named Familial Expansile Osteolysis (FEO; MIM 174810). A clear pattern of autosomal dominant inheritance was evident and radiographs showed both generalised and focal skeletal changes associated with elevated serum alkaline phosphatase and urinary hydroxyproline values, bone pain at radiologically affected sites, tooth loss and progressive loss of hearing. Virus like inclusion bodies identical to those in PDB were also identified in the nuclei of osteoclasts from affected bone.

Many aspects of the disease in this family were dissimilar to PDB, however. The first presentation of the disease in most patients was with hearing loss, sometimes from early as four years of age. Bone pain was also apparent from a much earlier age of onset than in PDB, beginning in the 2<sup>nd</sup> decade, and was so severe in some cases as to be resistant to opiates and require limb amputation. Focal lesions developed at previously unaffected sites and progressed along the shafts of long bones at almost twice the rate of lesions in PDB patients. Lesions were frequently observed in the forearm, hand and foot bones but rarely in the axial skeleton. The most noteworthy difference between FEO and PDB is the apparent uncoupling of the rates of osteoblast and osteoclast activity in the late stages of FEO, leading to gross expansion of the medullary cavity and thinning of the cortex, with almost complete replacement of the bone with vascularised fatty tissue.

## Expansile Skeletal Hyperphosphatasia (ESH)

Whyte and colleagues (22) described a familial metabolic bone disease in a mother and daughter from Australia, which they

called Expansile Skeletal Hyperphosphatasia (ESH). Inherited as a highly penetrant autosomal dominant trait, ESH is characterised by early onset deafness, premature tooth loss and progressive hyperostotic expansion of the long bones that particularly affects the fingers. Serum alkaline phosphatase and other markers of bone turnover were considerably elevated in affected patients. However, ESH was not considered by Whyte to be a variant of either PDB or FEO because of the episodic hypercalcaemia and widespread diffuse bone involvement without the presence of focal osteolytic lesions.

Although excessive numbers of osteoblasts and osteoclasts were seen on bone biopsy, they were not enlarged to the same extent as seen in PDB. Unfortunately, no osteoclasts were observed in the specimens which were subject to electron microscopy so the Authors were unable to determine whether they contained nuclear inclusions. However, the paramyxovirus gene transcripts reported in blood cells from PDB patients by some authors (23) were not detected in circulating mononuclear cells from the ESH patients.

### Early onset PDB

Nakatsuka and colleagues (24) reviewed the clinical presentation of affected individuals from a Japanese family with a severe form of PDB, whose symptoms emerged in the 2<sup>nd</sup> or 3<sup>rd</sup> decade. The affected individuals had serum alkaline phosphatase levels between 2 and 17 times elevated above the normal range, and affected patients had involvement of the skull, axial skeleton, small bones of the hands, early onset deafness and premature tooth loss. In one patient, hypercalcaemia occurred in association with an episode of immobilisation. This syndrome had some features in common with PDB, including axial involvement, skull involvement, and osteosclerotic lesions, but differed from PDB in terms of the young age of onset, and premature deafness and tooth loss. Other features reminiscent of ESH were found including involvement of the fingers and hypercalcaemia. Linkage analysis in this family showed allelic sharing of markers on chromosome 18q21 in affected individuals, and a positive LOD score, but the family was too small to confirm or refute the presence of linkage at this locus. It was concluded that the PDB-like phenotype in this Japanese family was distinct from classical PDB, but overlapped with FEO and ESH.

### RANK mutations cause FEO, ESH and early onset PDB

To search for the gene responsible for FEO, Hughes et al. (25) performed a genome wide screen in 61 members of the FEO family described by Osterberg et al. (21) using 200 restriction fragment length polymorphisms (RFLPs) and 100 highly informative microsatellite polymorphisms. They found highly significant evidence of linkage on chromosome 18q21.1-q22 (<5cM; maximum LOD score of 11.53 at D18S64). This region contains the gene encoding the Receptor Activator of NF- $\kappa$ B (RANK), *TNFRSF11A*, which is known to be expressed on osteoclast precursor cells (26, 27). RANK is the sole receptor for RANKL (RANKL), which is expressed on the surface of osteoblasts and is essential and sufficient (in the presence of small amounts of Macrophage Colony Stimulating Factor, MCSF) for the differentiation of osteoclast precursors into mature, bone-resorbing osteoclasts (reviewed in 28). The interaction between these two membrane-bound factors is central to the regulation of bone resorption.

Considering the importance of the RANKL-RANK interaction as a regulator of osteoclast activity, Hughes et al. (29) screened the coding regions, proximal promoter and intron-ex-

on boundaries of *TNFRSF11A* in all members of a Northern Irish (21) and two other FEO families and identified an 18-bp duplication (84dup18) affecting the RANK signal peptide that segregated with the disease in all affected members of these families. They did not find the mutation in 158 healthy controls. Palenzuela et al. (30) and Johnson-Pais et al. (15) subsequently confirmed that the 84dup18 mutation is a cause of FEO in families from Spain and the US respectively. Following this discovery, Whyte and Hughes (31) performed mutation screening of the RANK gene in two ESH patients (22). In both cases, they found a 15-bp duplication that was allelic to the FEO mutation (84dup15), and which was not present in 70 unaffected controls. The 84dup18 FEO and 84dup15 ESH mutations are predicted to elongate the RANK signal peptide by six and five amino-acids respectively, and expression of the recombinant form of the 84dup18 mutant in a mammalian cell system showed increased constitutive activation of RANK, possibly resulting from lack of normal cleavage of the signal peptide (29). A different duplication mutation also affecting the RANK signal peptide (75dup27) was found in the Japanese family previously described with the phenotype of early onset PDB (24), which again segregated with the disease in affected individuals. In common with the mutations that cause FEO and ESH mutations, this duplication elongated the signal peptide, preventing cleavage and was shown to activate NF- $\kappa$ B signalling.

These observations naturally led other groups to search for RANK mutations in individuals with classical PDB. Whyte et al. (22) failed to find any RANK mutations in 10 sporadic PDB cases and Hughes et al. (29) reported the same result in 90 sporadic PDB cases. Confirming these observations, Kormas et al. (32) performed mutation screening of the RANK gene in 82 sporadic and 23 familial cases of PDB from Australia but did not find any mutations. Marco-Mingot et al. (33) also failed to find disease-causing mutations in 18 PDB patients from Spain. Sparks et al. (34) found no RANK mutations in sporadic or familial PDB cases, or in osteosarcomas, a well known complication of PDB. This work and other reports of non-linkage of PDB to chromosome 18q21 (9, 35) have led to the general conclusion that mutations in the RANK gene are not a common cause of PDB.

### Juvenile Paget's Disease (JPD)

JPD [MIM 239000; also known as Idiopathic Hyperphosphatasia, or Familial Hyperphosphatasemia] is a rare autosomal recessive condition with a severe phenotype, of which about 50 cases have been reported worldwide. The disease is characterised by elevated rates of bone turnover, skeletal deformity, bone pain, and an increased risk of pathological fracture. Symptoms are evident from early infancy, when the disease presents with skeletal deformity and failure to thrive. This is followed by the development of skull enlargement, walking difficulty, progressive sensorineural deafness, kyphosis and acetabular protrusion. Disease severity generally increases during adolescence, but a mild form been described in some patients (36).

Levels of serum alkaline phosphatase and other bone turnover markers are greatly elevated in JPD, reflecting the generalized increase in bone turnover. The bones are enlarged and the normal trabecular architecture of healthy bone is replaced with an unusual, but characteristic pattern of abnormal parallel trabecular plates that are contribute to the reduced bone strength (37, 38). Whilst JPD has certain similarities to classical PDB, it is clearly a more severe condition as attested by the early age at onset and marked bone deformity developing during childhood.

#### OPG mutations cause JPD

Recent studies have clarified the molecular basis of JPD. Whyte and colleagues described two apparently unrelated Navajo patients with JPD in whom they postulated that the disease might be due to a defect in osteoprotegerin (OPG) function (39). OPG is a member of the TNF-receptor superfamily, which acts as a soluble decoy receptor for RANK-ligand, blocking osteoclast activation and bone resorption (28). Mice lacking OPG develop severe osteoporosis due to excessive osteoclast activity (40, 41), and overexpression of OPG leads to osteopetrosis due to inhibition of osteoclast formation (42). Whyte and colleagues first excluded mutations in the RANK gene as a cause of the JPD and then attempted to perform mutation analysis of the OPG gene, *TNFRSF11B*, in these patients. In so doing, they discovered that the entire OPG gene, along with a 100 kb stretch of flanking chromosome 8q24, had been homozygously deleted (39). In another study, Cundy et al. (43) described a family of Iraqi origin, in which three of nine siblings had JPD. They performed a genome-wide scan and found evidence of suggestive linkage on chromosome 8q24 (LOD score 2.21). Sequencing *TNFRSF11B* in this family revealed a homozygous 3bp deletion in all three affected siblings, which was predicted to result in the loss of an aspartate residue from the OPG protein. This residue is highly conserved in members of the TNF-receptor superfamily, suggesting that it is essential for normal function, and these Authors found that the mutant OPG was unable to prevent osteoclastic resorption in a bone culture system.

To evaluate the role of OPG in classical PDB, Wuyts et al. (44) looked for evidence of mutations or polymorphisms in OPG in 24 sporadic and 4 familial PDB cases. They identified several single nucleotide polymorphisms (SNPs) in the coding regions and whilst none of these were found to cause PDB, a common SNP was found in intron 2 that was over-represented in PDB patients compared with controls. In a similar study, Trainor et al. (45) described an association between PDB and a polymorphic marker close to the gene. Recently, Dąbrowska et al. (46) confirmed the link between polymorphic variation in OPG and PDB by finding a significant association between a missense polymorphism affecting exon 1 of OPG and PDB. Therefore, it appears that subtle variation in OPG can also increase risk of classical PDB, possibly by affecting OPG regulation or secretion.

Another strong candidate gene for PDB and related syndromes is RANKL since mice deficient in this protein have severe osteopetrosis, with complete lack of osteoclasts (47,48). Conversely, mice injected subcutaneously with recombinant RANKL develop severe hypercalcaemia and a reduction in bone volume due to an increase in osteoclast size and multinuclearity (48). Despite the importance of RANKL in osteoclast biology, mutations and polymorphisms of RANKL have not yet been identified in association with PDB or related disorders.

#### Inclusion body myopathy, Paget's disease and frontotemporal dementia

An unusual syndrome of inclusion body myopathy, Paget's disease and frontotemporal dementia (IBMPFD) was described by Kimonis et al. (49) and Kovach et al. (50) in a series of families from the US where the disease was inherited in an autosomal dominant fashion [MIM 605382]. Myopathy was the most prominent symptom, presenting with weakness, muscle atrophy and occasionally pain. Affected patients often experienced difficulty raising the arms and climbing stairs, and in some cases, complete immobility occurred. The mean age at onset of

symptoms was in the fifth decade, similar to that in classical PDB. Muscle biopsies revealed variation in muscle fibre size, grouped regions of muscle fibre atrophy, and intracellular blue-rimmed vacuoles with punctate staining debris and cytoplasmic protein accumulations (51). Whilst Kimonis and colleagues were first to describe the specific syndrome of IBMPFD, it is interesting to note that PDB has also been reported to be associated with myopathy in the absence of dementia by other authors (52-54).

Like classical PDB, bone lesions in IBMPFD typically affect the spine, pelvis and skull, and biochemical evaluation shows increased serum levels of alkaline phosphatase and elevated urinary markers of bone resorption. On radiological examination, there is coarse trabeculation of the affected bone, cortical thickening and focal lesions consistent with PDB, and individuals treated with bisphosphonates or calcitonin show clinical improvement. Dementia typically follows the symptoms of myopathy and PDB and is characterised by language difficulties and changes in personality, including apathy, increased agitation, anomia (inability to remember names). In many cases, auditory or visual hallucinations occur. These clinical features are associated with atrophy of the frontal cortex. Although detailed brain histology has not been performed to determine if the dementia in IBMPFD is also associated with inclusion bodies, protein aggregation in neurons is thought to be a feature of all neurodegenerative disorders (reviewed in 55).

#### Mutations in the VCP gene cause IBMPFD

Linkage analysis in IBMPFD families initially excluded loci involved in limb girdle muscular dystrophy, PDB, cardiomyopathy and amyotrophic lateral sclerosis. A genome-wide screen identified significant linkage to chromosome 9p13 spanning a region of 5.3 Mb (maximum LOD score 3.64) (50). Having excluded several genes in this region that are involved in muscle function (56), Watts et al. (57) screened 13 IBMPFD families for mutations in Valosin-Containing Protein (VCP), which is involved in several intracellular signalling pathways, including ubiquitin (UB)-mediated protein degradation (58) (reviewed in 59). They identified six different disease-segregating mutations affecting the highly conserved CDC48 domain, which is involved in UB-binding (60, 61). They propose that IBMPFD mutations in VCP are relatively subtle, and their impact only reaches a critical disease threshold in response to oxidative stress and old age. This may also apply to the question of why PDB only emerges in later life.

The discovery of mutations in the UB-binding domain of VCP is interesting because of the fact that mutations affecting the ubiquitin-associated (UBA) domain of *SQSTM1* are a common cause of late onset PDB. In addition to its role as a required scaffold protein for NF- $\kappa$ B-mediated osteoclast activation (62, 63), *SQSTM1* is thought to be involved in trafficking UB-tagged proteins to the proteasome through its UBA domain (64, 65). Abnormal UB-mediated catabolism is known to cause a number of diseases in several tissues, particularly the brain (66), and such disease are frequently characterised by UB-containing inclusion bodies. These inclusion bodies are thought to be composed of accumulations of undegraded protein, and Watts et al. (57) found that VCP localised to protein aggregates in muscle cells from IBMPFD patients. These findings are relevant to PDB because pagetic osteoclasts have long been shown to contain unidentified cytoplasmic or nuclear inclusion bodies, which have to date been interpreted as paramyxovirus fragments (67-71). Work is ongoing to clarify whether these inclusions contain the *SQSTM1* protein, p62, but it is interesting to note that VCP and p62 have individually been found to co-localise with UB-containing nuclear inclusions in several neurodegenerative disorders (72-76). It is currently unclear

whether VCP mutations or polymorphisms contribute to the pathogenesis of late onset PDB.

### Concluding remarks

Over the last five years, major advances have been made in understanding the genetic basis of PDB and related disorders. Analysis of candidate genes that lie in regions with strong linkage to PDB is ongoing but in *SQSTM1* we now have an important clue to the underlying defect which may help us select candidates more purposefully. While the involvement of *SQSTM1* in UB-mediated proteolysis and the possible implications this may have for the discovery of new PDB genes is a hot topic at the moment, it is worth noting that *SQSTM1*'s role in the RANK-NF- $\kappa$ B pathway has still not been fully worked out. Perhaps it is too much of a coincidence that the only gene known to cause PDB is also an indispensable member of bone's most critical osteoclastogenic signaling cascade. It has been established for some time that TRAF6, another indispensable member of the RANK-NF- $\kappa$ B pathway, has important E3 ubiquitin ligase activity and is a substrate of lysine-63-linked polyubiquitin chains, which are required for signal transduction through this pathway. The relationship between TRAF6 and *SQSTM1* in the RANK pathway is not yet clear, but they are known to interact in a way that is required for activation of the IL-1 pathway, which is capable of supporting lower levels of osteoclastogenesis in the absence of RANKL-RANK stimulation. It may turn out that there is a critical interaction between TRAF6-polyUB and the UBA domain of *SQSTM1*, but we must wait for this to be clarified before we can select new candidates for PDB from this system. We can conclude that PDB and a number of related disorders are due to mutations in various parts of the RANKL-RANK-NF- $\kappa$ B system and it may turn out that other members of this pathway also cause PDB.

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