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Review

Genetics and aetiology of Pagetic disorders of bone

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ABSTRACT

Paget's disease of bone (PDB) is a late-onset disorder characterised by focal areas of increased bone turnover containing enlarged hyperactive osteoclasts. The disease has a strong genetic predisposition and mutations in *SQSTM1* have been associated with familial and sporadic disease in up to 40% of cases. Additional genetic loci have been associated in other cases, but genes are yet to be identified. Earlier-onset conditions with similar bone pathology (familial expansile osteolysis, expansile skeletal hyperphosphatasia and early-onset PDB) are caused by mutations in *TNFRSF11A* (*RANK*). The syndrome of inclusion body myositis, Paget's disease and frontotemporal dementia is caused by mutations in *VCP*. Despite the increased knowledge about genes involved in PDB and related disorders, the etiology of the diseases remains puzzling. Presence of inclusion bodies appears to link Pagetic diseases mechanistically to diseases associated with presence of misfolded proteins or abnormalities in the ubiquitin-proteasomal, or autophagy pathways. Juvenile PDB, caused by osteoprotegerin deficiency, appears mechanistically distinct from the other Pagetic diseases. This review will discuss evidence from recent studies, including new animal models for Pagetic diseases.

Pathophysiology of PDB and related disorders

Paget's disease of bone (PDB[1], OMIM 602080) is a late-onset (sixth decade of life) disorder characterised by focal areas of increased bone turnover. Within the Pagetic foci, the osteoclasts are increased in size, in number and in nuclearity. The increased resorption by these large and highly active osteoclasts is coupled with increased osteoblast activity, such that bone formed is disorganised, architecturally weak and subsequently prone to fracture (Fig. 1). The disease is osteoclast-driven and bisphosphonate therapy inhibits the excessive resorption followed by normalisation of osteoblast function. The clinical presentation of PDB varies widely [1]. Many patients are asymptomatic whilst others experience bone pain, skeletal deformity, deafness, neurological symptoms and pathological fractures. Patients also have an increased susceptibility to osteosarcoma. The most frequently affected bones in PDB are those of the axial skeleton, including the lumbar spine, sacrum, pelvis, femur, tibia and skull. The bones of the upper extremity are less commonly involved. The disease may be monostotic (affecting only a single bone), but is more frequently polyostotic (affecting two or more bones). Progression can occur in a given bone but the appearance of a new site of involvement after initial diagnosis is unusual. Diagnosis typically occurs after the fifth decade, but disease onset likely occurs in the third decade [2]. There is anecdotal evidence to suggest that mechanical loading may influence sites involved in PDB. This is suggested by the fact that PDB predominantly affects weight-bearing bones and affects the dominant limbs more frequently than the non-dominant. Associations have also been reported between repetitive use of a limb and localisation of the disease [3]. The mechanism by which this occurs is unclear, but a possibility would be attraction of abnormal osteoclasts to repair sites of skeletal microdamage. There is a slight male preponderance to PDB with male:female ratios varying from 1.0 to 1.8 [4–11], which has been attributed to the larger mechanical loads on the bones of males.

PDB is still one of the more common bone disorders in Caucasian populations, with around 3% individuals aged over 50 affected. Recent reports however, suggest that in some countries the incidence and the severity of the disease is declining [6,12,13], although not in all populations [10].

In addition to the late-onset, common form of PDB, three extremely rare forms of earlier-onset and more severe Pagetic diseases have been described. Early-onset Paget's disease (ePDB), familial

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[1] *Abbreviations used*: ALFY, autophagy-linked FYVE-domain containing protein; ePDB, early-onset Paget's disease of bone; EM, electron microscopy; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; ESH, expansile skeletal hyperphosphatasia; FEO, familial expansile osteolysis; IBM, inclusion body myositis; IBMPFD, inclusion body myositis with Paget's disease and frontotemporal dementia; IκB, inhibitor of NFκB; IKK, IκB kinase; JPD, juvenile Paget's disease; Lys, lysine; MTOC, microtubule organising centre; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa B; OMIM, online Mendelian inheritance in man; OPG, osteoprotegerin; OSER, organised smooth ER; PDB, Paget's disease of bone; RANK, receptor activator of NFκB; RANKL, RANK ligand; sIBM, sporadic inclusion body myositis; TNFα, tumour necrosis factor alpha; TNFRSF11A, tumour necrosis factor receptor superfamily member 11A (aka RANK); TNFRSF11B, tumour necrosis factor receptor superfamily member 11B (aka OPG); TRAF6, tumour necrosis factor receptor associated family member 6; UBA, ubiquitin associated; UPS, ubiquitin-proteasomal system; vit D3, 1,25-dihydroxy vitamin D_3.

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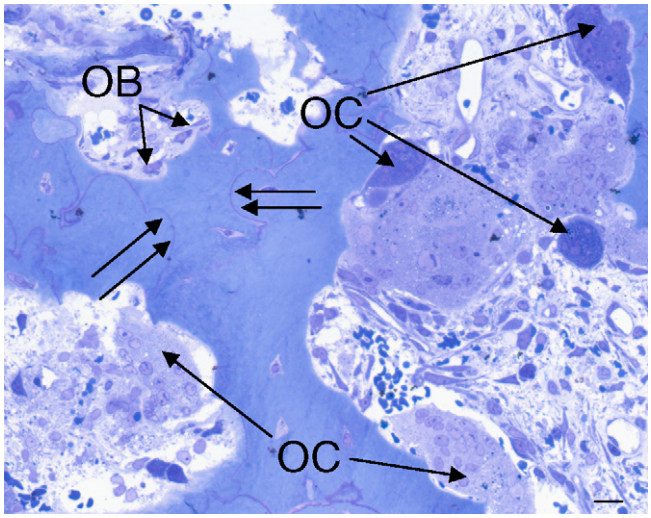

Fig. 1. Section of trabecular bone from a patient with late-onset Paget's disease of bone. Osteoclast activity is evident. Many large osteoclasts are visible (arrows) and the bone surface shows characteristic scalloped surfaces, indicative of active resorption. In this small region of the tissue section osteoblasts are less prominent, although some bone formation can be seen in the top left hand area. Double arrows indicate prominent cement lines, indicating rapid bone turnover. OC, osteoclast; OB, osteoblast. Semi-thin (1 μm thick) Epon-embedded section of demineralised bone stained with toluidine blue. Bar is 20 μm.

expansile osteolysis (FEO OMIM 174810) and expansile skeletal hyperphosphatasia (ESH) share features with late-onset PDB as well as with each other, including focal areas of increased bone resorption, enlarged osteoclasts and the presence of woven bone. Although all three syndromes are clinically similar, some phenotypic variations exist, such as in the age of onset (typically around the second decade) and in the different bones that are affected [14]. In all diseases early tooth loss and deafness occur. In ePDB, osteolytic and sclerotic lesions affect the pelvis, skull, mandible and maxilla, and in some cases, the small bones of the hands [15]. FEO features focal areas of expansile osteolytic bone lesions causing severe deformity [16,17]. ESH is characterised by hyperostotic long bones, including phalanges, and biochemical evidence of increased bone remodelling [18].

Two more very rare genetic diseases of increased bone turnover are known. Hereditary inclusion body myopathy (IBM) with early-onset PDB (P) and frontotemporal dementia (FD) is a complex dominantly inherited disorder (abbreviated as IBMPFD, OMIM 167320) where PDB combines with muscle weakness and early dementia. The myopathy is the main clinical phenotype, but in about half of the patients an "early-onset" (in the fourth decade) PDB is seen [19] and in 30% of the patients frontotemporal dementia is found [20]. The main morbidity and mortality in this syndrome is caused by cardiorespiratory failure due to myopathy or by dementia [21,22]. This intriguing combination of pathologies may provide clues about the pathogenesis of PDB.

Finally, juvenile Paget's disease (JPD, OMIM 239000), also known as familial idiopathic hyperphosphatasia, and first described in 1956 [23], presents in early childhood characterised by both increased bone resorption and formation. In JPD, most bones are affected and like other forms of Pagetic bone disease, this increase in bone turnover results in woven bone, which is structurally weak. Trabeculae in the iliac crest from JPD patients exhibit a parallel plate organisation rather than the usual meshwork appearance of trabecular bone [24], a phenomenon as yet not understood. The discovery that JPD is caused by loss of function of osteoprotegerin (OPG) makes this a disease that is primarily endocrine in nature and, although driven by osteoclast dysfunction, it is not an osteoclast-intrinsic disease. As we feel that the other Pagetic diseases may be more mechanistically related (even though precise mechanisms are not known as yet), we will first describe the genetics and pathogenesis of JPD before focusing our attention on the genetics of late-onset PDB, IBMPFD and the early-onset Pagetic disorders and discussing their possible pathogenic mechanisms.

Genetics and pathogenesis of Juvenile Paget's disease, or "osteoprotegerin deficiency

JPD has been shown to be caused by autosomal recessive mutations in the gene for OPG (*TNFRSF11B*), a member of the tumour necrosis factor (TNF) receptor superfamily, resulting in either complete deletion of the OPG gene [25] or mutations within the gene [26]. OPG is a soluble receptor secreted by osteoblasts [27] and discussed in more detail in the article by Boyce in this issue [137]. It acts as a decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL) to its receptor RANK, the crucial osteoclast differentiation and survival receptor. Normally, binding of RANKL to RANK acts via the ligand-initiated formation of signalling complexes leading to activation of the transcription factors NFκB, AP-1 and NFATc1. These in turn regulate the transcription of osteoclast-specific gene expression. In JPD patients where the entire OPG gene is absent, serum levels of OPG are undetectable and, as expected, levels of RANKL elevated [25]. The *in silico* predicted effects of the six different reported mutations within the OPG gene [28] on protein expression, secretion, function and disease severity have been confirmed experimentally. For example, OPGΔ182, causing deletion of an aspartate residue, was predicted to result in an unstable loop structure and reduced affinity for RANKL, an effect confirmed in binding studies with synthetic WT and mutated OPG and further verified by reduced inhibitory activity of OPGΔ182 in osteoclast formation studies [29]. The deletion/insertion resulting in a premature stop codon at amino acid 325 (also known as the "Balkan mutation" [30]), does not directly affect the RANKL binding domain of OPG, but instead leads to impaired homodimerisation [31]. Since this monomeric OPG is less able to bind to RANKL than the homodimeric form [32] it is able to enter the circulation and, paradoxically, increased levels of immunoreactive OPG are found [30] whilst RANKL is not sufficiently inhibited and hence also persistently elevated. Monomeric OPG, however, is not completely ineffective in inhibiting RANKL and hence the disease in the patients with the truncated OPG appears less severe, albeit there is heterogeneity in disease severity even in patients with the same mutations [28,30,31], suggesting that factors other than the *OPG* mutation are also involved in the pathogenesis.

In addition to bisphosphonate therapy to inhibit osteoclast function, reconstitution of OPG has now emerged as a treatment option and successful use of recombinant OPG in treatment of JPD has been reported [33]. However, for patients with mutated OPG, rather than complete absence, treatment with neutralizing antibodies to RANKL has been suggested [30].

JPD is clearly an endocrine disorder and Whyte and coworkers [30] have suggested the disease would be more appropriately named as "OPG deficiency".

Genetics of late-onset Paget's disease

A genetic component to PDB has been long-recognised [34,35], and many families have been documented where PDB is transmitted with an autosomal dominant mode of inheritance. Linkage studies in these families have identified a number of susceptibility