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Biology and Treatment of Paget's Disease of Bone

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ABSTRACT

Paget's disease of bone (PDB) is a common skeletal disorder characterized by increased and disorganized bone remodeling affecting one or more skeletal sites. Although some patients are asymptomatic others develop complications such as bone pain, deformity, nerve compression syndromes, and fragility fractures. Genetic factors play an important role in the pathogenesis of PDB and there is strong evidence that susceptibility is determined by variants within or close to genes that regulate osteoclast function. Environmental factors also play a key role but the nature of the environmental triggers is less clear. Bisphosphonates are a highly effective treatment for the elevations in bone turnover that are characteristic of PDB but it is unclear at present if they alter the natural history of the disease. Here, we review the epidemiology, clinical, cellular, and molecular abnormalities in PDB as well as environmental and genetic triggers, and current available treatment options. J. Cell. Biochem. 117: 289–299, 2016.

KEY WORDS: PAGET'S DISEASE; BONE DISEASE; BISPHOSPHONATES

Paget's disease of bone (PDB) is a common skeletal disorder which is characterized by focal abnormalities of bone remodeling, affecting one or more skeletal sites. The classical description was by Sir James Paget who referred to the condition as *Osteitis deformans* [Paget, 1877]. This reflects the fact that the acceleration of bone turnover in PDB leads to bone expansion and deformity. Almost any bone can be affected but PDB predominantly targets the axial skeleton and the most commonly involved sites are the pelvis (67%), spine (39%), femur (33%), tibia (19%), and skull (25%) [Langston et al., 2007].

EPIDEMIOLOGY

Paget's occurs most commonly in the people of British descent. Within the UK, the highest prevalence is in the north west of England. The disease is also common in British migrants to countries like Australia, New Zealand, North America, and in other countries in Europe, such as in France, Germany, Spain, or Italy. There is no information on the prevalence of Paget's in Africans but no differences in prevalence have been observed between African Americans and Caucasian Americans from two cities in the USA [Guyer and Chamberlain, 1980]. Archaeological studies of skeletal remains have suggested that the disease may have originated in England, possibly due to mutational events and subsequently spread as the result of a founder effect to the rest of Europe and other countries [Mays, 2010]. Paget's is rare in Scandinavian countries and Asia.

Paget's disease is rare in subjects under 50, but increases progressively in prevalence thereafter to affect 5.8% of women and 6.9% of men aged over 85 in the UK.. The overall prevalence in those above the age of 55 in the UK is about 2% and it is about 1.6 times more common in men than in women (Fig. 1) [Van Staa et al., 2002].

There is evidence that PDB has become less common and less severe over the past quarter of a century in the UK and many other countries [Poor et al., 2006].

CLINICAL FEATURES

It has been estimated that between 7% and 15% of patients with PDB come to medical attention [Van Staa et al., 2002; Tan and Ralston, 2014]. In a recent systematic review [Tan and Ralston, 2014], bone pain was the most common complaint occurring in about 40% of cases followed by deformity (20%), pathological fracture (10%), and deafness (6%). Other complications include nerve compression syndromes, spinal stenosis, osteoarthritis, dental problems, high output cardiac failure, and hypercalcaemia in patients who are

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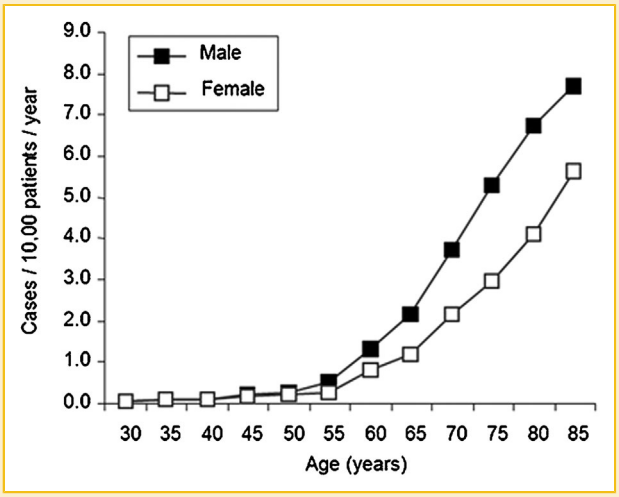

Fig. 1. Prevalence of Paget's disease of bone. The effects of age and gender on the prevalence of PDB in the United Kingdom. Adapted from [van Staa et al., 2002].

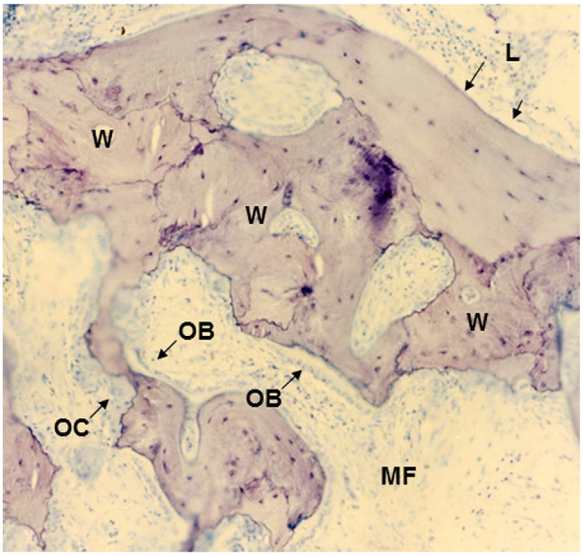

Fig. 2. Photomicrograph of Pagetic bone. The section consists almost entirely of woven bone (W) although there is an area of lamellar bone to the top left of the section (L). Osteoblast activity is clearly visible (OB) juxtaposed with areas of osteoclastic bone resorption (OCL). There is extensive marrow fibrosis (MF).

immobilised [Ralston, 2013]. Osteosarcoma and giant cell tumors are rare complications [Rendina et al., 2015]. Vascular calcification is more common in patients with PDB as compared with controls [Laroche and Delmotte, 2005] and there is evidence that the risk of cardiovascular disease is increased [Van Staa et al., 2002].

CELLULAR PATHOLOGY

Under normal circumstances bone is renewed and replaced in an orderly fashion through the process of bone remodeling. This firstly involves resorption of bone by osteoclasts which are multinucleated cells derived from hematopoietic precursors. This is followed by new bone formation which is carried out by osteoblasts, derived from mesenchymal stem cells. During bone formation, some osteoblasts become trapped within bone matrix and differentiate into osteocytes which are thought to act as mechanosensors and play an important role in regulating osteoblast and osteoclast activity by producing receptor activator of Nuclear Factor Kappa B Ligand (RANKL) and sclerostin [Bonewald, 2011].

The microstructure of bone is highly abnormal in PDB (Fig. 2). Osteoclasts are increased in number, size, and nuclearity. It has been estimated that the rate of osteoclastic bone resorption is increased sevenfold in Pagetic lesions as compared with normal bone [Meunier et al., 1980]. A characteristic feature of Pagetic osteoclasts is the presence of nuclear inclusions. These were first reported by Rebel et al. [1974] and their presence has been subsequently confirmed by many researchers [Helfrich et al., 2000]. These structures were initially thought to be paramyxoviruses although more recently it has been suggested that they could be aggregates of undegraded proteins due to defects in the autophagy pathway [Hocking et al., 2010]. Bone formation is also increased in PDB, and this is thought to be secondary to the increased bone resorption. The newly formed bone is abnormal, however, and is laid down in a chaotic fashion resulting in woven bone which is mechanically weak. Other histological features of active PDB include increase vascularity and marrow fibrosis.

There is evidence to suggest that the autophagy pathway is abnormal in PDB. Autophagy is responsible for the degradation and recycling of damaged protein and is involved in the regulation of cell differentiation, cell death, and responses to cellular stress [Ravikumar et al., 2010]. The abnormalities of autophagy might be explained by the fact that many of the proteins that have been implicated in the pathogenesis of PDB also play a role in autophagy. For example, p62 plays a critical role in autophagy by recruiting ubiquitinated proteins to the autophagosome by interacting with the light chain 3 (LC3) protein [Ravikumar et al., 2010]. It has been shown that levels of LC3 and p62 are both increased in osteoclasts precursors from mice with the P394L mutation (equivalent to the human P392L mutation) [Daroszewska et al., 2011]. Also clinical studies have shown that p62 levels are increased in immortalized B-cells from PDB patients [Collet et al., 2007]. These findings are both in keeping with an upregulation of autophagic flux in PDB. The optineurin protein which has been implicated as a mediator of PDB linked with the 10p13 locus [Lucas et al., 2008; Albagha et al., 2010] is also known to play a role in autophagy [Korac et al., 2013]. Finally the Vasolin-Containing Protein (VCP) protein which plays a role in the pathogenesis of inclusion body myopathy, Paget's disease, and frontotemporal dementia also plays a role in autophagy [Johnson et al., 2010]. Specifically, VCP is involved in regulating the activation of NFκB by binding to and regulating ubiquitin mediated proteasome degradation of phosphorylated IκBα [Dai et al., 1998]. Although all the evidence points to dysregulation of the autophagy pathway in PDB, it remains unclear at present whether the defects in autophagy are directly related to the increased

osteoclastogenesis or whether it is a bystander effect due to involvement of proteins such as p62, OPTN, and VCP which themselves play a role in autophagy.

ENVIRONMENTAL TRIGGERS

There is strong evidence that environmental factors contribute to the pathogenesis of Paget's disease. This comes in part, from the observation that the disease has become less common in many countries over recent years, particularly in regions of high prevalence [Poor et al., 2006] although this has not been observed in all regions [Gennari et al., 2005; Corral-Gudino et al., 2013]. Further evidence in support of environmental influence on the disease comes from the observation that carriers of *SQSTM1* mutations develop PDB at a later age than their parents [Cundy et al., 2015]. The potential environmental triggers are unclear, but several have been suggested as discussed in more detail below.

DIET

One epidemiological study proposed that low calcium intake during childhood is associated with an increased risk of PDB [Siris, 1994] and it was also suggested that vitamin D deficiency may contribute based on the fact that the prevalence of PDB was increased in regions of the UK where vitamin D deficiency is common [Barker and Gardner, 1974]. The proposed mechanism by which a low calcium diet and vitamin D deficiency could influence severity is by inducing secondary hyperparathyroidism which would be expected to cause osteoclast activation and increase bone turnover. In this regard it has been reported that primary hyperparathyroidism can increase severity and activity of PDB [Kanis, 1992].

ENVIRONMENTAL TOXINS

It has been suggested that PDB might be caused by environmental toxins due to environmental pollution from cotton mills in the North West of England—an area of high PDB prevalence of PDB [Lever, 2002]. This association is circumstantial however, with no experimental evidence to support or refute it.

BIOMECHANICAL FACTORS

There is evidence that biomechanical factors influence susceptibility to PDB. This is supported by the predilection for the axial skeleton and weight bearing limbs. Sparing of Pagetic involvement in a patient with extensive disease has been reported in a limb that was paralysed due to polio [Barry, 1969]. There have also been case-reports of PDB originating in bones which have been subject to over-use [Solomon, 1979]. A further observation in keeping with the biomechanical theory is that PDB usually originates at the site of muscle insertions into bone although it remains unclear why PDB can target a bone on one side of the body yet spare the other side.

INFECTIONS

It has long been considered that PDB might be caused by exposure to an infectious agent and the most widely studied theory is that PDB might be due to a slow virus infection by one of the paramyxoviruses. The initial observation in support of this was the finding of nuclear inclusion bodies in Pagetic osteoclasts by electron microscopy which were thought to represent measles virus (MV) [Rebel et al., 1974, 1980]. Subsequently an epidemiological study revealed a higher frequency of dog ownership in PDB patients than controls leading to the suggestion that the inclusions might represent canine distemper virus (CDV) rather than MV [O′ Driscoll et al., 1990]. However, the association between dog ownership and PDB could not be confirmed in two other studies [Siris et al., 1990; Khan et al., 1996]. Other epidemiological studies have revealed evidence of associations between PDB and living in rural areas and being exposed to livestock early in life, again raising the possibility of an infectious trigger [Lopez-Abente et al., 1997; Gennari et al., 2006].

Following on from these observations a great deal of research has been performed to find evidence of Paramyxovirus infection in Pagetic tissue using various techniques including immunohisto-chemistry, in situ hybridization, Reverse-Transcription-Polymerase Chain Reaction (RT-PCR), and *In Situ*-Reverse Transcriptase-Polymerase Chain Reaction (IS-RT-PCR). Early studies using IHC showed evidence of staining for Measles Virus (MV) and also Respiratory Syncytial Virus (RSV) in Pagetic tissue [Mills et al., 1984]. In some samples staining for both MV and RSV was observed [Mills et al., 1984]. Studies using *in situ* hybridisation have revealed evidence of MV in some studies, CDV in other studies [Gordon et al., 1991] and no evidence of viral RNA in other studies [Helfrich et al., 2000]. The results of RT-PCR have similarly been mixed with some laboratories reporting the presence of MV [Reddy et al., 1996], others CDV [Gordon et al., 1992] and others reporting no evidence of viral RNA [Helfrich et al., 2000].

One blinded multicenter study using a highly specific quantitative PCR-based technique showed no evidence of viral transcripts in blood samples from patients with PDB and evidence that PCR contamination was an issue in one laboratory [Ralston et al., 2007]. Another independent study using the same technique similarly failed to detect viral mRNA in cultured osteoblast-like cells from PDB patients [Naot et al., 2007]. Some authors have reported that MV transcripts amplified from peripheral blood of PDB patients revealed point mutations that differed between patients [Friedrichs et al., 2002]. There has been debate about whether these might represent PCR artefacts or indicate that PDB may occur in association with strains of MV that carry certain mutations in the nucleocapsid sequence [Rima et al., 2002]. This issue remains unresolved.

GENETICS

Paget's disease has a strong heritable component. First degree relatives of affected patients have a seven to 10-fold risk of developing the disease as compared with controls [Sofaer et al., 1983; Siris et al., 1991]. In a proportion of cases, PDB can be inherited within families in an autosomal dominant manner with high but incomplete penetrance [Haslam et al., 1998]. The proportion of patients with familial PDB differs in different countries and ranges from about 5% in Belgium, to 15% in the UK and up to 40% in some regions of Spain and Québec [Morissette et al., 2006]. This most probably reflects a founder effect of high penetrance variants in some populations but not in others. Several rare syndromes with

similarities to PDB have also been described which are inherited in a Mendelian manner as reviewed previously [Lucas et al., 2006].

Current evidence suggests that predisposition to PDB is mediated by a combination of rare high penetrance variants and more common variants which predispose to the disease but which in isolation are not sufficient to cause the disease. These variants have been identified by a combination of linkage studies and genome wide association studies as summarised in Figure 3. In many cases, the identified loci are close to genes that play a key role in the regulation of osteoclast differentiation and function (Fig. 4).

LINKAGE ANALYSIS

Linkage analysis in families with dominantly inherited PDB identified three susceptibility loci for the disease that reach statistical significance. One locus on chromosome 5q35 was identified independently in the French-Canadian [Laurin et al., 2001] and UK populations [Hocking et al., 2001]. Another locus was identified on chromosome 5q31 in the French-Canadian population [Laurin et al., 2001] but this has not yet been replicated in other populations. Two other loci, at 2q36 and 10p13 showed only suggestive linkage with PDB [Hocking et al., 2001] but a subsequent study confirmed significant linkage with 10p13 and refuted the association with 2q36 [Lucas et al., 2008].

Positional cloning studies identified mutations in the *SQSTM1* gene as the cause of 5q35 linked PDB. A recurrent mutation resulting in a Proline to Leucine amino acid change at codon 392 (P392L) was first identified in the French-Canadian population [Laurin et al., 2002] and soon after this other mutations in *SQSTM1* were identified in the UK population [Hocking et al., 2002] and other populations [Morissette et al., 2006].

GENOME WIDE ASSOCIATION ANALYSIS

Genome wide association studies have yielded important insights into the genetic basis of PDB. Two genome wide association studies performed by Albagha et al. [2010, 2011] on PDB patients who were negative for *SQSTM1* identified seven susceptibility loci for the disease (Fig. 3). Strong candidate genes for susceptibility within these loci include *CSF1* (1p13) which encodes macrophage colony stimulating factor (M-CSF), a cytokine that is required for osteoclast differentiation [Cecchini et al., 1997], *RIN3* (14q32) which encodes a guanine exchange factor called Rab and Ras interactor 3 [Kajiho et al., 2003b], *OPTN* (10p13) which is involved in regulating NFκB signalling [Zhu et al., 2007], *TNFRSF11A* (18q21) which encodes Receptor Activator of Nuclear Factor κ B (RANK), a receptor that is essential for osteoclast differentiation [Boyle et al., 2003] and *TM7SF4* which encodes Dendritic Cell-Specific Transmembrane Protein (DC-STAMP), a molecule that is essential for fusion of osteoclast precursors [Kukita et al., 2004].

For the other identified loci, the candidate genes are less clear. At the 7q33 locus the strongest signal was for the SNP rs4294134 which is located within an intron of *NUP205*, encoding Nucleoporin 205 [Albagha et al., 2011]. This protein is involved in the regulation of cellular trafficking of macromolecules between the cytoplasm and nucleus via Nuclear Pore Complexes (NPC) [Albagha et al., 2011]. Another gene at this locus is *CNOT4* which encodes a protein showing E3 ubiquitin ligase activity. This could be potentially relevant in PDB given the importance of ubiquitination in osteoclast function [van Wijk et al., 2009].

Another susceptibility gene called *CTHRC1* in locus 8q22 (which also contains DC-STAMP), is also interesting for PDB as studies have suggested that it may play a role in osteoblast differentiation and bone formation [Kimura et al., 2008].

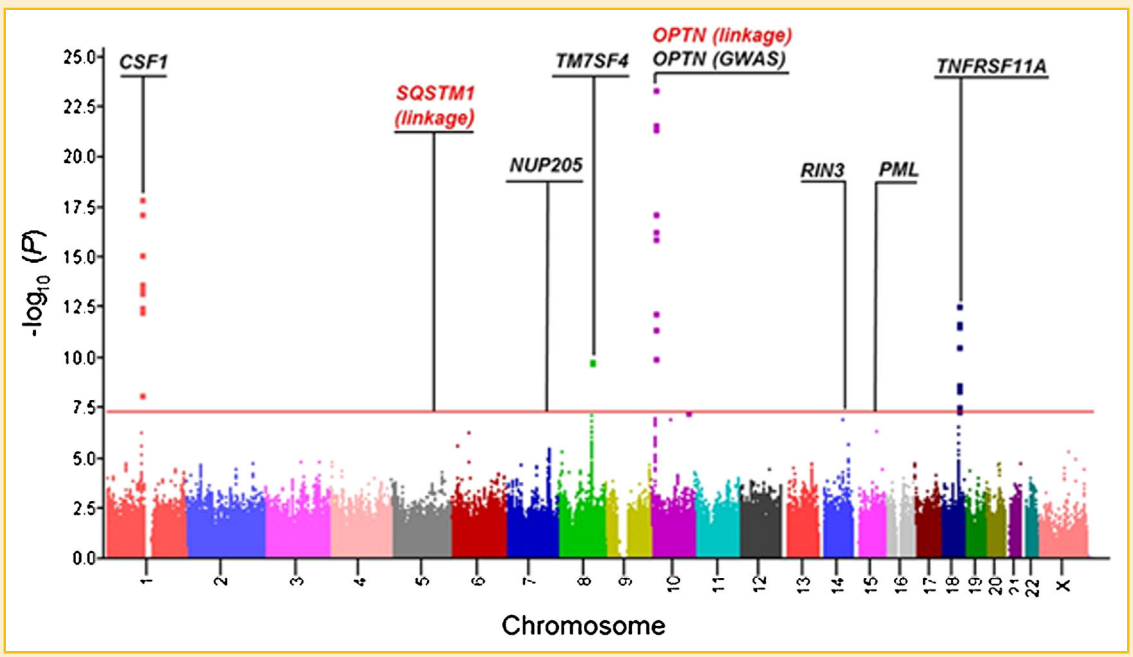

Fig. 3. Susceptibility loci for PDB. Confirmed susceptibility loci identified by linkage or association are shown. The chromosomal locations are shown on the *x*-axis and the log10 *P*-values for the genome wide significant hits are shown on the *y*-axis. Selected candidate genes for the candidate loci are indicated. Adapted from [Albagha et al., 2010, 2011].

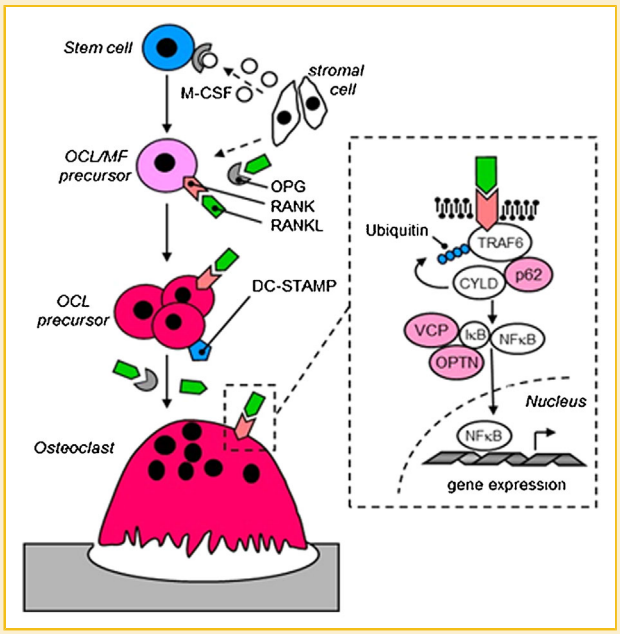

Fig. 4. Regulators of osteoclast differentiation and function. Key players in the regulation of osteoclast differentiation and function are shown with particular reference to the genes implicated in the pathogenesis of PDB. Osteoprotegerin (OPG), Receptor activator of nuclear factor kappa B (RANK), RANK ligand (RANKL), macrophage colony stimulating factor (MCSF), and dendritic cell specific transmembrane protein (DC-STAMP) all play key roles in the regulation of osteoclast differentiation. Within the osteoclast, p62, valosin containing protein (VCP), and optineurin (OPTN) act as regulators of NFκB signalling downstream of the RANK receptor. MF, macrophage; OCL, osteoclast. Adapted from Ralston, Cecil Goldman Textbook of Medicine (with permission).

At the 15q24.1 locus there are two susceptibility genes. One of them is the Promyelocytic Leukemia (PML) gene. The top GWAS hit for this locus (rs5742915) encodes for a missense variant (pF645L) within PML although the functional significance of this variant is unclear. Seven isoforms of PML have been described, called PMLI to PMLVIIb, and all are from alternative splicing from the *PML* gene. They are classified by size with PML1 being the longest, and PMLVII the shortest isoform. Most are found in the nucleus. All share the N-terminal region (exons 1–3), containing the RBCC/TRIM motif, which is composed of zinc binding motifs (a RING-finger and two B-boxes called B1 and B2) and an α-helical coiled-coil domain. A SIM hydrophobic core (exon 7a) is seen in PMLI to PMLV only, and a nuclear export signal (exon 9) is only present in PMLI [Nisole et al., 2013]. The PML protein plays a role in the regulation of TGF-β signalling. Although TGF-β is known to be involved in regulating bone resorption and bone formation the role of PML in the bone microenvironment is yet to be established [Albagha et al., 2011].

A second gene at this locus is GOLGA6A (Golgin A6 family, member A). This is a coiled-coil protein able to interact with the Golgi apparatus which plays a role membrane fusion. Although the role of GOLGA6A in bone is unknown studies have shown mutations in another member of the Golgin family, called Golgi microtubule-associated protein 210 (GMAP-210), cause a lethal skeletal dysplasia [Smits et al., 2010]. Mutations in the Golgin SCY1-like 1 binding protein 1 (SCYL1BP1) were also associated with severe form of osteoporosis [Hennies et al., 2008].

MOLECULAR PATHOLOGY OF PDB

A great deal of research has been conducted to identify possible mediators and mechanisms which underlie the increased osteoclast activity that is characteristic of PDB. At a cellular level, it has long been established that bone marrow samples from PDB patients show increased sensitivity to the effects of calcitriol and RANKL as compared with control cultures such that the number and size of osteoclasts generated is increased [Demulder et al., 1993; Neale et al., 2000]. This indicates that osteoclast precursors from PDB patients seem to be hypersensitive to agents that promote osteoclast differentiation and bone resorption. There is evidence that this hypersensitivity is due to intrinsic abnormalities in osteoclasts or their precursors that are genetically determined as well as increased production of osteoclastogenic cytokines in the bone marrow compartment [Menaa et al., 2000]. The potential mediators and mechanisms are discussed in more detail below.

INTERLEUKIN 6

Interleukin-6 (IL-6) has been implicated as a possible mediator of increased osteoclast activity in PDB. IL-6 is a pro-inflammatory cytokine that is produced by macrophages, osteoclasts, osteoblasts, and T-cells. The effects of IL-6 on osteoclastogenesis and bone resorption are complex and studies on the effects of IL-6 on bone resorption have yielded conflicting results. It has been shown in some studies to increase osteoclast differentiation activity and bone resorption but in most studies no stimulatory effects have been found unless IL-6 is administered in combination with soluble IL-6 receptor [Tamura et al., 1993]. Indeed, there is evidence that IL-6 may actually act to directly inhibit osteoclast formation [Duplomb et al., 2008]. It is currently considered that the stimulatory effects of IL-6 on osteoclast activity are indirect and mediated by an increase in RANKL expression by osteoblasts [Duplomb et al., 2008]. In the context of PDB, levels of IL-6 have been reported to be increased in bone marrow plasma and blood samples from PDB patients as compared with controls [Roodman et al., 1992]. Evidence for increased expression of IL-6, IL-6 receptor, and nuclear factor IL-6 have also been reported using in situ hybridisation in PDB bone samples as compared with control (osteoarthritis) bone samples [Hoyland et al., 1994]. However, another study using RT-PCR of whole bone samples showed that trancripts for IL-6 were only detected in about 60% of cases with no evidence of increased expression of IL-6 in PDB versus control samples [Ralston et al., 1994]. It has also been shown that neutralising antibodies to IL-6 inhibit osteoclastogenesis in bone marrow cultures from PDB patients but not control cultures [Menaa et al., 2000]. In the same studies, IL-6 was found to potentiate RANKL induced osteoclastogenesis in normal marrow cultures [Menaa et al., 2000]. Finally over-expression of measles virus nucleocapsid protein (MVNP) in mice has been reported to markedly induce production of IL-6 [Kurihara et al., 2011]. Also when MVNP over-expressing mice were crossed with IL-6 deficient mice, the increased bone turnover and osteoclastogenesis were abrogated. These observations suggest that

IL-6 is a mediator of the increased osteoclast activity and bone resorption that occurs as the result of MVNP overexpression. Interestingly in the same study [Kurihara et al., 2011] mice with a knock-in of the P394L mutation did not show over-expression of IL-6, suggesting that activation of IL-6 may be specific to the MNVP over-expressing model of PDB. There is no evidence that genetic variation at the IL-6 locus or variation at loci that encode other components of the IL-6 signalling pathway play a role in susceptibility to PDB.

TRANSCRIPTION ACTIVATING FACTOR 12

The Transcription Activating Factor 12 (TAF12) protein (also known as TAFII-17) is a component of the TFIID transcription complex. It was implicated in PDB as the result of studies which sought to identify proteins that bound to the vitamin D receptor, based on the observation that PDB-derived bone marrow cells have increased sensitivity to calcitriol, as compared with marrow cells from controls. Cell lysates from osteoclast precursors derived from PDB patients and MVNP-transduced osteoclast precursors were incubated with a GST-Vitamin D receptor fusion protein and TAF12 was identified as an-interacting protein. Subsequent studies showed that an antisense oligonucleotide to TAF12 significantly decreased osteoclast formation in response to calcitriol in PDB-derived but not normal marrow cultures. It was also shown that over-expression of TAF12 in NIH3T3 cells increased VDR transcriptional activity [Kurihara et al., 2004]. Interestingly, osteoclast precursors from patients with the P392L mutation of *SQSTM1* do not show increased levels of expression for TAF12 or hypersensitivity to the osteoclastogenic effects of calcitriol [Kurihara et al., 2007]. This suggests that like IL-6, involvement of TAF12 may be specific to the increase in bone turnover induced by over-expression of MVNP, rather than that which accompanies the P394L mutation of *SQSTM1*.

MACROPHAGE COLONY STIMULATING FACTOR

Macrophage Colony-Stimulating Factor (MCSF) encoded by the *CSF1* gene is a key cytokine which is required for the differentiation of hematopoietic stem cells to the macrophage-osteoclast lineage [Cecchini et al., 1997]. The MCSF protein is secreted by osteoblasts and interacts with the CSF1R membrane receptor which is a tyrosine kinase linked receptor that is expressed on haematopoietic stem cells and macrophage and osteoclast precursors [Lacey et al., 1998].

Several lines of evidence support a role of MCSF in the pathogenesis of PDB. Serum levels of MCSF were found to be significantly higher in Paget's samples compared to controls and PDB patients under treatment, which indicates that there is an association between MCSF and the occurrence and activity of PDB [Neale et al., 2002]. In addition CSF1 lies within the PDB susceptibility locus on chromosome 1p13 and the most strongly associated SNP lies 87Kb upstream of the gene itself within a conserved region of Histone 3 acetylation at the lysine 27 position in several cell lines. Acetylation of histone at this site is associated with upregulation of gene expression and so it is possible that the variant identified by GWAS might be acting as a mark for differences in transcription of the CSF1 gene. Nonetheless, allelic variants at this locus are not associated with differences in CSF1 expression in the cell lines that have been investigated so far. This does not exclude the possibility that the 1p13 variants might be associated with differences in CSF1 expression in osteoblasts or bone marrow, but further work will be required to explore this possibility.

OPTINEURIN

Optineurin, encoded by the *OPTN* gene was identified as a potential mediator of PDB by a linkage study and by a genome wide association study. The *OPTN* gene lies in the middle of the PDB susceptibility locus identified on chromosome 10p13 by Lucas [Lucas et al., 2008] and in the middle of the locus identified by Albagha et al. [2010]. In the GWAS study, *OPTN* was the only gene within the region of susceptibility and was flanked by two recombination hot spots. Prior to these observations protein coding mutations had been identified in patients with glaucoma and amyotrophic lateral sclerosis [Maruyama et al., 2010]. No protein coding mutations of OPTN have been identified as yet in PDB (Albagha and Ralston, unpublished data). However, the predisposing variant at the top hit in the GWAS study (rs1561570) was found to be very strongly related to reduce expression of *OPTN* mRNA in monocytes suggesting that reduced levels of *OPTN*, rather than a specific mutation in *OPTN* predispose to PDB.

Optineurin is a multifunctional protein, involved in membrane vesicle trafficking, signal transduction, autophagy, cell survival, Golgi ribbon formation, and mitosis. Among many structural domains, Optineurin has an ubiquitin-binding domain (UBD) and a NFκB essential modulator (NEMO)-like domain and it has been shown to compete with NEMO in binding to poly ubiquitinated Receptor Interacting Protein (RIP). Optineurin has also been shown to interact with CYLD [Nagabhushana et al., 2011] which is thought to be involved in mediating the effects of p62 mutations on NFκB signalling and osteoclastogenesis [Jin et al., 2008]. Over-expression of OPTN in 293 cells causes inhibition of NFκB signalling [Maruyama et al., 2010]. OPTN is also known to play a role in autophagy [Korac et al., 2013] but until recently was not known to play a role in bone metabolism. However recent studies published so far in abstract form, have shown that silencing of *OPTN* mRNA increases osteoclast formation in vitro [Obaid et al., 2012]. Taken together these observations would be consistent with a model whereby *OPTN* negatively regulates osteoclast differentiation and bone resorption and that the mechanism by which genetic variants at 10p13 predispose to PDB is by reducing *OPTN* expression.

SEQUESTOSOME 1

The Sequestosome 1 (SQSTM1) gene was identified as a mediator of PDB by positional cloning. This gene encodes p62, a protein that is involved in the NFκB signalling cascade downstream of the RANK receptor and nerve growth factor receptors [Geetha and Wooten, 2002]. The p62 protein has numerous structural motifs, including (from the N-terminal to the C-terminal) a SH2 domain, an acidic interaction domain (AID) which to binds to atypical PKCs (aPKCs), a ZZ finger, a binding site for Tumour Necrosis Factor (TNF) receptor-associated factor 6 (TRAF6), two PEST sequences rich in Proline, Glutamic acid, Serine, and Threonine, a LC3 Interacting region (LC3) and an ubiquitin associated (UBA) domain. The UBA domain localised on the C-terminal end of the protein allows p62 to bind to ubiquitinated proteins, which confers a role for p62 in autophagy (discussed below). The different domains of p62 permit interaction

with various proteins that are involved in signal transduction. For example the TRAF6 domain permits interaction with TRAF6 which is involved in regulation NFκB downstream of several receptors. The AID domain permits interaction with aPKCs that are involved in cell survival, also through control of the NFκB pathway. The ZZ finger domain interacts with aPKCs recruiting Receptor Interaction Protein (RIP) [Sanz et al., 2000].

Nearly 30 mutations in the *SQSTM1* gene have been identified and most affect the UBA domain. Most of the causal mutations cause loss of function for ubiquitin binding and this seems to be an important mechanism by which the mutations cause osteoclast activation [Layfield et al., 2004]. In addition, several mutations have been shown to cause increased activation of the NFκB pathway in vitro and to stimulate osteoclast differentiation and activity [Layfield et al., 2004]. There is experimental evidence to suggest that an important mechanism by which these mutations cause osteoclast activation is because the mutant p62 protein is unable to recruit the deubiquitinating molecule CYLD to TRAF6 downstream of the RANK receptor (Fig. 4). This causes an increase in TRAF6 ubiquitination, enhanced RANK signaling, and osteoclast activation [Jin et al., 2008].

The effects of the P392L *SQSTM1* mutation have also been studied in mice where the UBA region is highly conserved but where the Proline residue is situated at codon 394. One study has reported that mice expressing the P394L mutation of *SQSTM1* in osteoclasts driven by the Tartrate Resistant Acid Phosphatase (TRAP) promoter have increased osteoclastogenesis but do not have osteolytic bone lesions [Kurihara et al., 2007]. In contrast, mice with a germ line mutation of P394L exhibited increased osteoclastogenesis and lesions characteristic of PDB [Daroszewska et al., 2011]. The differences between these studies are presumably due to the different patterns of expression of the P394L mutation in the difference strains of mice. Specifically, the observations suggest that for the osteoclastogenic effects of the P394L mutation to be fully penetrant it needs to be present in the germ line as well as TRAP expressing mature osteoclasts. Taken together with the in vitro findings these observations indicate that p62 is a key mediator of increased osteoclast activity in PDB with effects that directly impact on NFκB signalling and osteoclastogenesis.

DENDRITIC CELL SPECIFIC TRANSMEMBRANE PROTEIN

The dendritic cell specific transmembrane protein (DC-STAMP) was identified as a potential mediator of PDB by GWAS which showed a strong association between the rs2458413 variant on 8q22 and PDB. This variant lies close to the Transmembrane 7 Superfamily Member 4 gene (*TM7SF4*) [Albagha et al., 2011]. Subsequently another potentially damaging coding mutation—a substitution of Leucine to a Phenylalanine at position 397, was detected in a patient with PDB however the significance was borderline [Beauregard et al., 2014]. DC-STAMP is a 470 amino acids protein with seven transmembrane domains. An immunoreceptor tyrosine-based inhibitory motif (ITIM) has been recently detected on the cytoplasmic tail of the protein [Chiu et al., 2012]. The ligand for DC-STAMP ligand has yet to be identified. DC-STAMP is expressed in dendritic cells and osteoclasts but not in macrophages. It plays an important role in osteoclastogenesis since targeted activation of the mouse homolog of *TM7SF4* causes defective osteoclastogenesis and completely prevents multinucleated osteoclast formation. A role in cytoplasmic membrane fusion has also been suggested [Yagi et al., 2005].

RAB AND RAS INTERACTOR 3

The Rab and Ras Interactor 3 (*RIN3)* gene has been implicated in the pathogenesis of Paget's disease by GWAS as previously discussed. RIN3 is part of the RIN family of proteins, along with RIN1 and RIN2. The RIN3 protein has numerous domains, shared with RIN1 and RIN2: (from N-terminal to C-terminal) a Src-homology 2 (SH2), a Proline rich region, a Ras-Association (RA) domain, a Vacuolar Sorting Protein 9 (Vps9) domain conferring guanine exchange factor (GEF) activity, and an ubiquitin like domain [Kajiho et al., 2003c]. Studies in HeLa cells have shown that RIN3 is involved in vesicular trafficking to early endosomes and interacts with amphiphysin II, a protein involved in the regulation of endocytosis [Kajiho et al., 2003a]. RIN3 also participates in the internalization of receptor tyrosine kinase KIT in mast cells [Janson et al., 2012]. Although the role of RIN3 in bone cells has not been studied it is an interesting candidate for PDB given the crucial role that small GTPases play in osteoclast function [Itzstein et al., 2011]. Recent studies have shown that *RIN3* is expressed in macrophages and osteoclasts at the mRNA and protein level. Although *RIN3* mRNA is also expressed in osteoblasts, the levels are low [Vallet et al., 2015]. At present, the mechanisms by which RIN3 regulates osteoclast activity are unclear but genetic analysis has identified a large number of missense variants within the gene that are over-represented in PDB cases as compared with controls. These are situated throughout the gene product, particularly within the 5′ region of the proline-rich domain. Others were also found in the SH2 domain and VPS9 domain. Most of these are rare variants but there is one common variant (R279C) predicted to be functional by *in silico* studies [Vallet et al., 2015]. At the present time, it seems likely that the predisposing variants in PDB cause loss-of-function of RIN3, although it is currently unclear which component of RIN3 function is affected.

RECEPTOR ACTIVATION OF NUCLEAR FACTOR KAPPA B

The receptor activator of nuclear kappa B protein (RANK), encoded by the *TNFRSF11A* gene is known to play a key role in osteoclastogenesis and bone resorption as well as in lymph node formation and dendritic cell function [Dougall et al., 1999; Boyle et al., 2003]. RANK is a transmembrane receptor in the TNF receptor superfamily. It is a glycoprotein of 616 amino acids long, with (from the N terminal to the C terminal) an extracellular domain of 184 amino acids followed by a 28 amino acids region made of four cysteine-rich domain (CRDs) and two N-glycosylation sites, a transmembrane domain of 21 amino acids, and an intracellular domain of 383 amino acids. The RANK receptor self-associates to form trimers and this process is essential for activation of the receptor and signal transduction [Zhang et al., 2009]. Reference has already been made to the fact that osteoclast precursors from patients with PDB exhibit increased sensitivity to various osteoclastogenic stimuli including calcitriol and RANKL [Neale et al., 2000]. It seems likely that this increase in sensitivity is mediated in part by abnormalities of RANK expression or function. The rare PDB like syndromes of familial expansile osteolysis, early onset familial

Paget's and expansile skeletal hyperplasia are all known to be caused by insertion mutations in the signal peptide [Lucas et al., 2006]. These result in osteoclast activation by mechanisms that are still incompletely understood [Crockett et al., 2011]. Common variants at the *TNFRSF11A* locus on chromosome 18q21 also predispose to classical PDB. However, the predisposing variants do not appear to influence *RANK* mRNA expression, at least as judged by eQTL analysis [Albagha et al., 2011]. One common protein coding variant, V192A localized within exon 6 of *TNFRFS11A*, has been found in study to influence NFκB signalling in reporter assays. However, other investigators were unable to significantly detect an effect of this variant on NFκB signalling using the same in vitro assay [Chung et al., 2010]. The V192A variant has been associated with severity of PDB in patients who are also carriers of the *SQSTM1* mutation [Gianfrancesco et al., 2012]. At present, it seems very likely that genetic variants at the *TNFRSF11A* locus predispose to PDB by altering RANK expression or activity but the mechanisms by which this occurs is incompletely understood.

MANAGEMENT OF PAGET'S DISEASE OF BONE

The clinical presentation of PDB is highly variable in that some patients are asymptomatic or have few symptoms, whereas others develop various complications such as bone pain, deformity, fracture, and deafness.

TREATMENT GOALS AND STRATEGIES

The main indication for treatment of PDB is bone pain localised to an affected site which is thought to be due to increased metabolic activity. There is strong evidence to show that inhibitors of bone resorption such as bisphosphonates can improve bone pain in PDB.

An issue that has long been debated is whether treatment can favourably alter the natural history of PDB. Some experts believe that it would be advantageous to try and restore bone turnover to normal in active PDB in the hope that this will prevent complications [Singer et al., 2014] but there is no evidence as yet to suggest that normalising bone turnover can prevent complications [Ralston et al., 2015]. The question of whether long-term "intensive" bisphosphonate treatment had favorable effects on complications was addressed by the Paget's disease of bone: a randomized trial of intensive versus symptomatic management (PRISM) study [Langston et al., 2010]. This investigated the effects of intense bisphosphonate treatment aimed at restoring normal ALP levels with the effects of treatment given primarily because of bone pain (which included bisphosphonates if patients did not respond to analgesics). The PRISM study showed that levels of ALP were significantly lower with intensive bisphosphonate therapy as compared with symptomatic treatment. However, no beneficial effects were observed on the quality of life, fractures, requirement for orthopaedic surgery of hearing loss [Langston et al., 2010]. An extension of this study, reported recently in abstract form also showed no benefit of intensive treatment for up to 7 years [Tan et al., 2015].

Therefore, there is no evidence at present to demonstrate that inhibitors of bone resorption can prevent complications of PDB in the long-term and so management is probably best focused on improving the patients' symptoms [Ralston, 2013].

CLINICAL ASSESSMENT

In deciding whether a patient might need treatment for PDB, it is customary to identify the bones that are affected and to take blood tests to assess metabolic activity. The best screening test for affected bones is a radionuclide bone scan. Although radionuclide uptake on bone scan is not entirely specific, a diagnosis can often be made with a high degree of certainty based on the appearances. Bone scanning is usually followed by X-ray examination of affected sites which can almost always confirm the diagnosis. Next, blood tests are taken for measurement of total Alkaline Phosphatase (ALP) activity. Although ALP can be derived from other tissues such as liver, kidney, and placenta, a high ALP level in the presence of bone scan and X-ray evidence of PDB usually indicates that the disease is metabolically active. If here is any doubt—such as in patients with co-existing liver disease—is possible to measure bone specific ALP which is elevated in active PDB [Selby et al., 2002]. While patients with active PDB usually have an elevated ALP (total or bone specific), it is possible to have active disease with normal ALP levels if only one site is affected.

From a clinical perspective, if one encounters a patient with PDB who has pain localized to an effected site which cannot be attributed to another cause, this would represent an indication for treatment. The treatments that can be given in this situation as discussed below.

ANALGESICS

Patients who have mild or intermittent pain can be managed by analgesic therapy with paracetamol or non-steroidal anti-inflammatory drugs, although there has not been a formal comparison of these measures compared with bisphosphonates, the most commonly used treatments for PDB. Most often analgesic therapy is used as an adjunct to bisphosphonate therapy.

BISPHOSPHONATES

These are the most commonly used treatments for PDB. Bisphosphonates are structural analogs of inorganic pyrophosphate which have a high affinity for binding to hydroxyapatite. They are powerful inhibitors of bone resorption and act primarily on osteoclasts but also have an inhibitory effect on bone formation [Russell et al., 2008]. This is thought to be due mainly to be secondary to the inhibition of bone resorption, but there is also evidence that bisphosphonates directly inhibit bone formation [Idris et al., 2008].

Bisphosphonates can be divided into two groups on the basis of potency and mechanism of action [Russell et al., 2008]. Non-nitrogen-containing bisphosphonates such as clodronate, etidronate, and tiludronate are internalized by the osteoclast during bone resorption and become incorporated into ATP analogs, which accumulate in the cytoplasm and cause apoptosis. Nitrogen containing bisphosphonates (aminobisphosphonates) such as pamidronate, alendronate, zoledronate, and risedronate are currently the most widely treatments for Paget's disease because of their greater potency. They inhibit farnesyl diphosphate (FPP) synthase, which plays a central role in the mevalonate pathway. This, in turn, prevents the post-translational prenylation of small GTPases leading

to a disruption of osteoclast function. Although macrophages and osteoclasts are the most sensitive cells to nitrogen-containing bisphosphonates, FFP is an ubiquitous enzyme and such treatment also have an impact on other cells [Roelofs et al., 2010].

Placebo controlled randomized trials have shown that bisphosphonates are superior to placebo in the management of bone pain in PDB [Ralston et al., 2008]. Comparison between bisphosphonates has shown that aminobisphosphonates are more effective at supressing bone turnover than non-nitrogen-containing bisphosphonates, although they have not generally been shown to be more effective at controlling bone pain. There are few comparative studies of different amino-bisphosphonates, but those that have been performed suggest that zoledronate is superior to risedronate and pamidronate in reducing bone turnover and improving bone pain [Singer et al., 2014].

CALCITONIN

Calcitonin is an osteoclast inhibitor which has been found to be effective at improving bone pain and reducing biochemical markers of bone turnover in PDB. It is now seldom used due to the fact that bisphosphonates are more convenient to administer, cheaper, and have fewer side effects.

DENOSUMAB

Denosumab is an osteoclast inhibitor which is a monoclonal antibody that neutralize RANKL. It is a powerful inhibitor of bone resorption. Although Denosumab is not licensed for PDB it has been found to be effective in isolated cases and might represent a treatment option if other drugs are contraindicated [Wat, 2014].

SURGERY

Orthopedic surgery is frequently required in PDB patients for fracture fixation, treatment of spinal stenosis, and for the treatment of co-existing osteoarthritis. Clinical experience indicates that the outcome of orthopaedic surgery is good in PDB, although the complexity and risk of complications is may be increased due to deformity and increased blood flow through affected bones. Delayed union has also been observed in Pagetic bone, particularly in patients with active disease [Parvizi et al., 2003]. Cochlear implantations for patients with affected temporal bone have proven to be effective in reversing hearing loss at least in some cases [Takano et al., 2014]. It has been suggested that bisphosphonates should be given pre-operatively to prepare patients for surgery in the hope that this prevents complications but it's not clear if this is effective at reducing the risk of postoperative complications.

MONITORING AND RETREATMENT

Many patients require repeated courses of treatment for PDB. The most common reason for retreatment is recurrence of bone pain thought to be due to PDB. This would be manifest clinically by return of pain to an affected site and an elevation in ALP. Because of this most clinicians keep PDB patients under review periodically, every 6–12 months. It should be noted that bone pain can occur as the result of coexisting problems such as osteoarthritis so it is important that patients are carefully evaluated to make sure their pain is due to PDB and not another cause.

CONCLUSION AND FUTURE PROSPECTS

Paget's disease of bone is a complex and fascinating disorder. Huge advances have been made in recent years in advancing understanding of the pathogenesis of the disease and in developing effective treatments for the high bone turnover that is characteristic of the condition. Unfortunately many patients present at a late stage in disease evolution with irreversible skeletal deformity and complications. The PRISM study demonstrated that at this stage, therapy with potent bisphosphonates has little impact on complications and quality of life. A future challenge will be to develop ways of diagnosing PDB at an earlier stage and intervening before the condition has progressed too far. In this regard, a study has commenced in children of PDB patients who carry SQSTM1 mutations (the ZIPP study ISRCTN 11616770) to determine if predictive genetic testing coupled to targeted intervention with bisphosphonates can prevent the development of new lesions and improve long-term outcome.

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REFERENCES

Albagha OM, Visconti MR, Alonso N, Langston AL, Cundy T, Dargie R, Dunlop MG, Fraser WD, Hooper MJ, Isaia G, Nicholson GC, Del Pino MJ, Gonzalez-Sarmiento R, Di SM, Tenesa A, Walsh JP, Ralston SH. 2010. Genome-wide association study identifies variants at CSF1, OPTN and TNFRSF11A as genetic risk factors for Paget's disease of bone. Nat Genet 42:520–524.

Albagha OME, Wani S, Visconti MR, Alonso N, Goodman K, Cundy T, Brandi ML, Chung PY, Dargie R, Devogelaer JP, Falchetti A, Fraser WD, Gennari L, Gianfrancesco F, Hooper MJ, Van Hul W, Isaia G, Nicholson GC, Nuti R, Del Pino MJ, Ratajczak T, Rea SL, Rendina D, Gonzalez-Sarmiento R, Di SM, Ward L, Walsh JP, Ralston SH. 2011. Genome-wide association identifies three new susceptibility loci for Paget's disease of bone. Nat Genet 43:685–689.

Anderson AP. 1992. Canine distemper transcripts sequenced from pagetic bone. Bone Miner 19:159–174.

Barker DJ, Gardner MJ. 1974. Distribution of Paget's diease in England, Wales and Scotland and a possible relationship with vitamin D deficiency in childhood. Br J Prev Soc Med 28:226–232.

Barry HC. 1969. Paget's disease of bone.

Chung PY, Beyens G, Riches PL, Van WL, de FF, Jennes K, Daroszewska A, Fransen E, Boonen S, Geusens P, Vanhoenacker F, Verbruggen L, Van OJ, Goemaere S, Zmierczak HG, Westhovens R, Karperien M, Papapoulos S, Ralston SH, Devogelaer JP, Van HW. 2010. Genetic variation in the TNFRSF11A gene encoding RANK is associated with susceptibility to Paget's disease of bone. J Bone Miner Res 25:2316–2329.

Collet C, Michou L, Audran M, Chasseigneaux S, Hilliquin P, Bardin T, Lemaire I, Cornelis F, Launay JM, Orcel P, Laplanche JL. 2007. Paget's disease of bone in the French population: Novel SQSTM1 mutations, functional analysis, and genotype-phenotype correlations. J. Bone Miner Res 22:310–317.

Corral-Gudino L, Garcia-Aparicio J, Sanchez-Gonzalez MD, Miron-Canelo JA, Blanco JF, Ralston SH, Del Pino-Montes J. 2013. Secular changes in Paget's disease: Contrasting changes in the number of new referrals and in disease severity in two neighboring regions of Spain. Osteoporos Int 24:443–450.

Cundy T, Rutland MD, Naot D, Bolland M. 2015. Evolution of Paget's disease of bone in adults inheriting SQSTM1 mutations. Clin Endocrinol (Oxf) DOI: 10.1111/cen.12741

Daroszewska GJA, Mangion A, Olavesen J, Nicholson M, Ward GC, Bennett L, Wuyts ST, Van Hul W. 2002. Domain specific mutations in Sequestosome 1 (SQSTM1) cause familial and sporadic Paget's disease. Hum Mol Genet 11:2735–2739.

Demulder A, Takahashi S, Singer FR, Hosking DJ, Roodman GD. 1993. Abnormalities in osteoclast precursors and marrow accessory cells in Paget's disease. Endocrinology 133:1978–1982.

Geetha T, Wooten MW. 2002. Structure and functional properties of the ubiquitin binding protein p62. FEBS Lett 512:19–24.

Guyer PB, Chamberlain AT. 1980. Pagets-disease of bone in 2 American-cities. Br Med J 280:985.

Hennies HC, Kornak U, Zhang H, Egerer J, Zhang X, Seifert W, Kuhnisch J, Budde B, Natebus M, Brancati F, Wilcox WR, Muller D, Kaplan PB, Rajab A, Zampino G, Fodale V, Dallapiccola B, Newman W, Metcalfe K, Clayton-Smith J, Tassabehji M, Steinmann B, Barr FA, Nurnberg P, Wieacker P, Mundlos S. 2008. Gerodermia osteodysplastica is caused by mutations in SCYL1BP1, a Rab-6 interacting golgin. Nat Genet 40:1410–1412.

Idris AI, Rojas J, Greig IR, van't Hof RJ, Ralston SH. 2008. Aminobisphosphonates cause osteoblast apoptosis and inhibit bone nodule formation in vitro. Calcif Tissue Int 82:191–201.

Janson C, Kasahara N, Prendergast GC, Colicelli J. 2012. RIN3 is a negative regulator of mast cell responses to SCF. PLos ONE 7:e49615.

Kajiho H, Saito K, Tsujita K, Kontani K, Araki Y, Kurosu H, Katada T. 2003. RIN3: A novel Rab5 GEF interacting with amphiphysin II involved in the early endocytic pathway. J Cell Sci 116:4159–4168.

Kajiho H, Saito K, Tsujita K, Kontani K, Araki Y, Kurosu H, Katada T. 2003. RIN3: A novel Rab5 GEF interacting with amphiphysin II involved in the early endocytic pathway. J Cell Sci 116:4159–4168.

Kajiho H, Saito K, Tsujita K, Kontani K, Araki Y, Kurosu H, Katada T. 2003. RIN3: A novel Rab5 GEF interacting with amphiphysin II involved in the early endocytic pathway. J Cell Sci 116:4159–4168.

Kanis JA. 1992. Pathophysiology and treatment of Paget' s disease of bone (second edition) Martin Dunitz, London. p 1–293.

Korac J, Schaeffer V, Kovacevic I, Clement AM, Jungblut B, Behl C, Terzic J, Dikic I. 2013. Ubiquitin-independent function of optineurin in autophagic clearance of protein aggregates. J Cell Sci 126:580–592.

Langston AL, Campbell MK, Fraser WD, Maclennan G, Selby P, Ralston SH. 2007. Clinical determinants of quality of life in Paget's disease of bone. Calcif Tissue Int 80:1–9.

Laroche M, Delmotte A. 2005. Increased arterial calcification in Paget's disease of bone. Calcif Tissue Int 77:129–133.

Laurin N, Brown JP, Lemainque A, Duchesne A, Huot D, Lacourciere Y, Drapeau G, Verreault J, Raymond V, Morissette J. 2001. Paget disease of bone: Mapping of two loci at 5q35-qter and 5q31. Am J Hum Genet 69:528–543.

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

Layfield R, Ciani B, Ralston SH, Hocking LJ, Sheppard PW, Searle MS, Cavey JR. 2004. Structural and functional studies of mutations affecting the UBA domain of SQSTM1 (p62) which cause Paget's disease of bone. Bio Chem Soc Trans 32:728–730.

Lever JH. 2002. Paget's disease of bone in Lancashire and arsenic pesticide in cotton mill wastewater: A speculative hypothesis. Bone 31:434–436.

Lopez-Abente G, Morales-Piga A, Elena-Ibanez A, Rey-Rey JS, Corres-Gonzalez J. 1997. Cattle, pets, and Paget's disease of bone. Epidemiology 8:247–251.

Lucas GJ, Daroszewska A, Ralston SH. 2006. Contribution of genetic factors to the pathogenesis of Paget's disease of bone and related disorders. J Bone Miner res 21(Suppl2):31–37.

Lucas G, Riches P, Hocking L, Cundy T, Nicholson G, Walsh J, Ralston SH. 2008. Identification of a major locus for paget disease on chromosome 10p13 in families of british descent. J Bone Miner Res 23:58–63.

Mays S. 2010. Archaeological skeletons support a northwest european origin for Paget's disease of bone. J Bone Miner Res 25:1839–1841.

Menaa C, Reddy SV, Kurihara N, Maeda H, Anderson D, Cundy T, Cornish J, Singer FR, Bruder JM, Roodman GD. 2000. Enhanced RANK ligand expression and responsivity of bone marrow cells in Paget's disease of bone. J Clin Invest 105:1833–1838.

Merlotti SM, Giordano D, Martini N, Tamone G, Zatteri C, De R, Baldi LR, Vattimo C, Capoccia A, Burroni S, Geraci L, De S, Avanzati A, Isaia A. 2005. Prevalence of Paget's disease of bone in Italy. J Bone Miner Res 20:1845–1850.

Meunier PJ, Coindre JM, Edouard CM, Arlot ME. 1980. Bone histomorphometry in Paget's disease. Quantitative and dynamic analysis of pagetic and nonpagetic bone tissue. Arthritis Rheum 23:1095–1103.

Mills BG, Singer FR, Weiner LP, Suffin SC, Stabile E, Holst P. 1984. Evidence for both respiratory syncytial virus and measles virus antigens in the osteoclasts of patients with Paget's disease of bone. Clin Orthop Rel Res 183:303–311.

Morissette J, Laurin N, Brown JP. 2006. Sequestosome 1: Mutation frequencies, haplotypes, and phenotypes in familial Paget's disease of bone. J Bone Miner Res 21(Suppl2):38–44.

Nagabhushana A, Bansal M, Swarup G. 2011. Optineurin is required for CYLD-dependent inhibition of TNFalpha-induced NF-kappaB activation. PLoS ONE 6:e17477.

Neale SD, Schulze E, Smith R, Athanasou NA. 2002. The influence of serum cytokines and growth factors on osteoclast formation in Paget's disease. QJM 95:233–240.

Nisole S, Maroui MA, Mascle XH, Aubry M, Chelbi-Alix MK. 2013. Differential roles of PML isoforms. Front Oncol 3:125.

O'Driscoll JB, Buckler HM, Jeacock J, Anderson DC. 1990. Dogs, distemper and osteitis deformans: A further epidemiological study. Bone Miner 11:209–216.

Obaid R, Wani S, Ralston SH, Albagha OME. 2012. OPTN negatively regulates osteoclast formation in vitro. Bone 50:S92–S93.

Paget J. 1877. On a form of chronic inflammation of bones (Osteitis Deformans). Med Chir Trans 60:37–64.

Parvizi J, Frankle MA, Tiegs RD, Sim FH. 2003. Corrective osteotomy for deformity in paget disease. J Bone Joint Surg 85A:697–702.

Paul M, Babu EM, Lee G, Reiley AJ, Wright W, Zhang A, You M. 2008. Deubiquitinating enzyme CYLD negatively regulates RANK signaling and osteoclastogenesis in mice. J Clin Invest 118:1858–1866.

Poor G, Donath J, Fornet B, Cooper C. 2006. Epidemiology of Paget's disease in Europe: The prevalence is decreasing. J Bone Miner Res 21:1545–1549.

Ralston SH, Afzal MA, Helfrich MH, Fraser WD, Gallagher JA, Mee A, Rima B. 2007. Multicenter blinded analysis of RT-PCR detection methods for paramyxoviruses in relation to Paget's disease of bone. J Bone Miner Res 22:569–577.

Ralston SH, Langston AL, Reid IR. 2008. Pathogenesis and management of Paget's disease of bone. Lancet 372:155–163.

Ralston SH, Corral-Gudino L, Fraser WD, Gennari L, Guanabens N, Selby PL. 2015. Letter to the Editor: The endocrine society clinical practice guidelines on Paget's disease: many recommendations are not evidence based. J Clin Endocrinol Metab 100:L45–L46.

Ralston SH. 2013. Clinical practice. Paget's disease of bone. N Engl J Med 368:644–650.

Ravikumar B, Sarkar S, Davies JE, Futter M, Garcia-Arencibia M, Green-Thompson ZW, Jimenez-Sanchez M, Korolchuk VI, Lichtenberg M, Luo S, Massey DC, Menzies FM, Moreau K, Narayanan U, Renna M, Siddiqi FH, Underwood BR, Winslow AR, Rubinsztein DC. 2010. Regulation of mammalian autophagy in physiology and pathophysiology. Physiol Rev 90:1383–1435.

Rebel A, Malkani K, Basle M. 1974. Nuclear anomalies in osteoclasts in Pagets bone-disease. Nouvelle Presse Medicale 3:1299–1301.

Rebel A, Pouplard A, Filmon R, Basle M, Kouyoumdjian S, Lepatezour A. 1980. Viral antigens in osteoclasts from Paget's disease of bone. Lancet ii:344–346.

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

Rima BK, Gassen U, Helfrich MH, Ralston SH. 2002. The pro and con of measles virus in Paget's disease: Con. J Bone Miner Res 17:2290–2292.

Roelofs AJ, Thompson K, Ebetino FH, Rogers MJ, Coxon FP. 2010. Bisphosphonates: Molecular mechanisms of action and effects on bone cells, monocytes and macrophages. Curr Pharm Des 16:2950–2960.

Selby PL, Davie MWJ, Ralston SH, Stone MD. 2002. Guidelines on the management of Paget's disease of bone. Bone 31:366–373.

Singer FR, Bone HG, III, Hosking DJ, Lyles KW, Hassan MM, Reid IR, Siris, ES. 2014. Paget's disease of bone: An endocrine society clinical practice guidelinel. J Clin Endocrinol Metab jc20142910.

Siris ES, Ottman R, Flaster E, Kelsey JL. 1991. Familial aggregation of Paget's disease of bone. J Bone Miner Res 6:495–500.

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

Smits P, Bolton AD, Funari V, Hong M, Boyden ED, Lu L, Manning DK, Dwyer ND, Moran JL, Prysak M, Merriman B, Nelson SF, Bonafe L, Superti-Furga A, Ikegawa S, Krakow D, Cohn DH, Kirchhausen T, Warman ML, Beier DR. 2010. Lethal skeletal dysplasia in mice and humans lacking the golgin GMAP-210. N Engl J Med. 362:206–216.

Sofaer JA, Holloway SM, Emery AE. 1983. A family study of Paget's disease of bone. J Epidemiol Community Health 37:226–231.

Solomon LR. 1979. Billiard-player's fingers: An unusual case of Paget's disease of bone. Br Med J 1:931.

Takano K, Saikawa E, Ogasawara N, Himi T. 2014. Cochlear implantation in a patient with Paget's disease. Am J Otolaryngol 35:408–410.

Tan A, Ralston SH. 2014. Clinical presentation of Paget's disease: Evaluation of a contemporary cohort and systematic review. Calcif Tissue Int 95:385–392.

Van Staa TP, Selby P, Leufkens HG, Lyles K, Sprafka JM, Cooper C. 2002. Incidence and natural history of Paget's disease of bone in England and Wales. J Bone Miner Res 17:465–471.

Zhang S, Liu C, Huang P, Zhou S, Ren J, Kitamura Y, Tang P, Bi Z, Gao B. 2009. The affinity of human RANK binding to its ligand RANKL. Arch Biochem Biophys 487:49–53.