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Genetics of Paget's Disease of Bone

Stuart H. Ralston · Omar M. E. Albagha

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Abstract Paget's disease of bone (PDB) is a common condition, which is characterised by focal areas of increased and disorganized bone remodeling. Genetic factors play an important role in the disease. In some cases, Paget's disease is inherited in an autosomal dominant manner and the most common cause for this is a mutation in the *SQSTM1* gene. Other familial cases have been linked to the *OPTN* locus on Chromosome 10p13 and still other variants have been identified by genome wide association studies that lie within or close to genes that play roles in osteoclast differentiation and function. Mutations in *TNFRSF11A*, *TNFRSF11B* and *VCP* have been identified in rare syndromes with PDB-like features. These advances have improved understanding of bone biology and the causes of PDB. The identification of genetic markers for PDB also raises the prospect that genetic profiling could identify patients at high risk of developing complications, permitting enhanced surveillance and early therapeutic intervention.

Keywords Paget's disease of bone · Osteitis deformans · Osteoclast · Genetic · *SQSTM1* · *RIN3* · Optineurin · RANK · RANKL · Osteoprotegerin · DC-STAMP · *TM7SF4* · Macrophage colony stimulating factor · M-CSF · *CSF1* · Bone resorption · Fracture · NFκB · Osteosarcoma

Introduction

Paget's disease of bone is a common metabolic bone disease that is characterized by increased and disorganized bone remodeling [1•]. It preferentially targets the axial skeleton and may affect one or multiple skeletal sites, most commonly the pelvis, spine, skull, femur and tibia. Clinically the raised bone turnover may cause bone pain and this is the most common presenting symptom of the disease. The raised bone turnover may leads to bone expansion, which can manifest clinically as visible deformity, particularly when weight bearing long bones such as the tibia and femur are affected. When the spine and skull are affected, the bone expansion can cause nerve compression syndromes resulting in cranial nerve defects, spinal stenosis or cord compression. Deafness is a recognized complication of PDB affecting the base of skull although this is usually due to a conductive problem as the result of osteosclerosis affecting the temporal bone, rather than compression of the 8th cranial nerve [2]. Bone formation is increased in PDB but the new bone is laid down in a disorganized fashion and is mechanically weak. This can predispose to the development of pathologic fractures, in response to minimal or no trauma. Finally patients with PDB have an increased risk of osteosarcoma. Although osteosarcoma occurs in 0.5 % of patients or less, virtually all osteosarcomas that occur in adulthood do so in patients with PDB [3].

S. H. Ralston (✉) · O. M. E. Albagha
Bone and Rheumatology Research Group, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK
e-mail: stuart.ralston@ed.ac.uk

Epidemiology

Paget's disease has a very striking geographic distribution. The highest incidence in the world is in the UK and within the UK the highest incidence is in the North West of England. Paget's disease also occurs quite commonly in North West France, Spain and Italy. It also occurs in most other European countries with the exception of Scandinavia where it is quite rare. Paget's disease is also common in other areas of the world which have been colonized by British and European immigrants such as Australia, New Zealand, the USA and some regions of Canada, most notably Quebec. Paget's is rare in the Indian subcontinent, Malaysia, Indonesia, China, and

Japan. These geographic differences, combined with other evidence from archaeological studies are consistent with a genetic model of predisposition whereby predisposing genetic variants arose in people from the UK many centuries ago and then spread to Europe and the rest of the world through emigration [4, 5]. Although there is little doubt that PDB has a genetic basis, the incidence and severity of the disease has decreased over the past few decades [6, 7], even in patients who are genetically predisposed to develop the disease [8]. Although Paget's can affect both genders, men are affected more commonly than women. In the UK, the ratio of males to females affected is approximately 1.4 to 1.0 [3]. The occurrence of Paget's disease is also strongly age-dependent; the disease is unusual before the age of 50 but increases in frequency exponentially thereafter. In the UK, the incidence doubles each decade from the age of 50 onward to reach a peak incidence about 5.4/10,000 population per year among women and 7.6/10,000 population per year among men in those aged 85 and above 85 [3].

Cellular Pathology

At a cellular level the cardinal feature of PDB is increase osteoclast activity. Osteoclast numbers are increased at affected sites and the osteoclasts themselves are much larger and containing many more nuclei than normal. They contain characteristic nuclear inclusion bodies consisting of microcylindrical structures. These were originally thought to represent viral nucleocapsids [9] but it has more recently been suggested that they might represent abnormal protein aggregates due to defects of the autophagy pathway [10]. Osteoclast precursors from PDB patients show increased sensitivity to RANKL and calcitriol compared with those of control subjects [11, 12]. Bone formation is also increased. This is thought to be secondary to the increased osteoclast activity but there is evidence that cells of the osteoblast lineage are also abnormal in PDB [13]. It has been suggested that the osteoclast abnormalities in PDB are caused by paramyxovirus infection. Although over-expression of paramyxovirus proteins has been shown to increase osteoclast activity in vitro and in animal models [14], attempts to isolate paramyxovirus proteins and nucleic acids from patient samples have yielded inconclusive results [15–17].

Inheritance of Paget's Disease

Familial Paget's Disease

Patients with Paget's disease often have a positive family history of the condition and it has been estimated that the risk of PDB occurring in a first degree relative of an affected subject is about a 7-fold increase compared with an unrelated individual. In many families the disease is inherited in an autosomal dominant manner with high but incomplete penetrance with increasing age. Linkage analysis has identified potential candidate loci on chromosomes 2p36, 5q31, 5q35, 10p13, and 18q21. The gene that predisposes to PDB on 5q35 is *SQSTM1* [18, 19] and mutations in this gene are the most common cause of familial PDB. Cross-sectional studies indicate that more than 80 % carriers of *SQSTM1* mutations develop Paget's disease by the eighth decade [20, 21]. However, there is evidence that age of onset in familial PDB is delayed in the current generation of people with *SQSTM1* mutations as opposed to their parents' generation [8]. This emphasizes the importance of environmental factors as triggers for the disease.

The causal gene on 10p13 has not yet been identified but families which show linkage to this region show autosomal dominant inheritance with incomplete penetrance as does 5q35-linked Paget's disease. The strongest candidate gene within the 10p13 region is *OPTN* [22, 23••].

Other loci identified by linkage in familial PDB lie on chromosome 2q36, 5q31 and 18q21 [24–26]. The 2q36 linkage peak in UK families seems likely to have been a false positive since the signal disappeared when data were reanalyzed after excluding families with *SQSTM1* mutations [22]. The status of the 5q31 locus, which was identified in the French Canadian population [24], remains unclear. No gene has yet been identified. The linkage signal previously reported on 18q21 for classical PBD [26] has not been substantiated by other linkage studies [24, 25, 27] although this locus is involved in the PDB-like disorder familial expansile osteolysis [28].

Familial PDB-Like Syndromes

Several rare syndromes with clinical and radiological similarities to PDB have also been described which are clearly inherited. Details are summarized in Table 1. The syndromes of familial expansile osteolysis [29], expansile skeletal hyperphosphatasia [30], and early onset familial Paget's disease [31] are allelic disorders caused by mutations in *TNFRSF11A* which encodes RANK. These are severe disorders with high penetrance, which present with deafness and tooth loss in childhood and adolescence followed by the development of osteolytic lesions in early adulthood. The syndrome of juvenile Paget's disease is a recessive disorder cause by mutations affecting *TNFRSF11B* which encodes osteoprotegerin [32, 33]. The presentation is during infancy with bone deformity and multiple fractures. The syndrome of inclusion body myopathy, Paget's disease and frontotemporal dementia (IBMPFD) is inherited in an autosomal dominant fashion and caused by mutations in the *VCP* gene [34]. It usually presents in the fourth decade with

Table 1 Inherited syndromes with features similar to Paget's disease of bone

Syndrome	Clinical features	Casual gene	Inheritance	Mutation type
Familial expansile osteolysis	Osteolytic lesions, and deformity affecting appendicular skeleton Onset childhood/adolescence Tooth loss, hearing loss. Severe panostotic disease and mandibular tumor with 12 bp insertion	*TNFRSF11A*	AD	Insertion mutation 18 bp, exon 1. Insertion mutation 12 bp, exon 1
Early onset familial PDB	Similar to FEO, but axial involvement more prominent. Hypercalcemia on immobilization	*TNFRSF11A*	AD	Insertion mutation, 27 bp exon 1
Expansile skeletal hypophosphatasia	Osteolytic and sclerotic lesions affecting appendicular skeleton and hands. Onset before puberty. Bone pain. Tooth loss and hearing loss. Hypercalcemia	*TNFRSF11A*	AD	Insertion mutation, 15 bp, exon 1
Juvenile PDB	Osteolytic and sclerotic lesions with bone deformity and expansion. Pathologic fractures. Deafness. Hypercalcemia. Onset during infancy or childhood. Premature death due to cardiovascular disease.	*TNFRSF11B*	AR	Missense and nonsense mutations in coding exons or gene deletions.
Inclusion body myopathy, Paget's disease and frontotemporal dementia	Proximal myopathy. Bone lesions typical of classical PDB in 40 % of cases. Dementia, usually in 5th-6th decade. Typical onset of PDB in 4th decade.	*VCP*	AD	Various missense mutations, most of which affect exons, 3, 5, and 6.

AD Autosomal dominant, *AR* autosomal recessive

myopathy but about 40 % of patients also go onto develop classical Paget's disease and 40 % go on to develop dementia.

Sporadic Paget's Disease

The majority of patients with Paget's disease are unaware of a family history of the disorder. Since Paget's disease is often clinically silent [3] it is unclear to what extent these patients truly have sporadic disease or if they may have had an affected relative in whom the diagnosis was never made. Recent studies have shown that the genetic variants which predispose to PDB have additive effects on susceptibility to the disease and on disease severity [35••, 36•]. In view of this it seems likely that patients who do not carry high penetrance susceptibility alleles such as *SQSTM1* mutations, might develop PDB when they have inherited a sufficient number of predisposing smaller effect size variants and have been exposed to the relevant environmental triggers [35••].

Genes and Loci That Predispose to PDB

The genes and loci that predispose to PDB have been identified by a combination of linkage analysis in families and genome wide association studies in unrelated individuals. The following section focuses only on genes and loci with robust evidence for association with PDB or related syndromes.

CSF1

The *CSF1* gene located on 1p13 encodes macrophage colony stimulating factor has been implicated as a candidate gene for Paget's disease by a genome wide association study [23••]. The associated most strongly SNP lay about 87 kb upstream of *CSF1*. Although the *EPS83L* gene also lies within the region it is separated from the associated SNP by 2 recombination hot spots. Macrophage colony stimulating factor is a strong candidate gene for PDB since it plays an essential role in promoting differentiation of stem cells to cells of the monocyte-macrophage and osteoclast lineage. The causal genetic variants in *CSF1* have not yet been defined. There is evidence that patients with PDB have higher circulating M-CSF levels than controls which would be consistent with the hypothesis that the variants which predispose to PDB at this locus do so by increasing M-CSF expression or altering its stability or secretion from cells.

SQSTM1

The *SQSTM1* gene located on chromosome 5q35 was identified as a candidate gene for PDB in families by genome wide linkage analysis and positional cloning. Two groups independently identified 5q35 as predisposing locus for PDB in UK and French-Canadian families [24, 25]. Mutation screening of genes in the region resulted in the identification of a proline to leucine mutation at codon 392 (P392L) of *SQSTM1* in the French Canadian population, which segregated with the disease. Subsequently this and other mutations affecting the

ubiquitin-associated domain of the protein were identified in the UK population [19] and in other populations worldwide (reviewed by Ralston and Layfield [10]). It has been estimated that between 40 %–50 % of patients with a family history of PDB carry a *SQSTM1* mutation compared with 5 %–10 % of those with "sporadic" disease [20, 37]. At the time of writing, 23 different mutations in *SQSTM1* have been identified in patients with PDB and most of these affect the ubiquitin associated (UBA) domain.

Mutations of *SQSTM1* affect severity and extent of PDB as well as predisposing to the occurrence of PDB. This was first demonstrated in a study of patients participating in the PRISM trial where patients with *SQSTM1* mutations had more extensive disease and more complications than those without mutations [38]. These results have recently been confirmed in a multinational study, which showed that the variants identified by genome wide association studies as predisposing to PDB interacted with *SQSTM1* mutations to influence disease severity and extent [36•].

Extensive research has been conducted to investigate the mechanisms by which *SQSTM1* mutations regulate osteoclast activity. Functional analysis has shown that most mutations cause loss-of-function for ubiquitin binding suggesting that this may be a unifying mechanism by which the disease occurs [39]. Having said that, a missense mutation which results in substitution of serine to threonine at position 349 (S349T) has been described in PDB which does not impair ubiquitin binding or affect NFκB signaling [40]. It has been shown that this mutation impairs the ability of the *SQSTM1* gene product p62 to interact with the protein Keap1 and it has been speculated that this might contribute to PDB by regulating expression of genes involved in oxidative stress [40].

Additional studies have shown that several expression constructs with PDB-associated mutations of *SQSTM1* activate NFκB signaling compared with wild type *SQSTM1* and that osteoclast precursors transfected with the P392L *SQSTM1* variant formed more osteoclasts in response to RANKL and M-CSF compared with wild type *SQSTM1*. The extent to which different mutants can activate NFκB in vitro has recently been shown to correlate with disease extent [41].

It is likely that the UBA domain mutations of *SQSTM1* cause osteoclast activation by a number of pathways including activation of atypical protein kinase Cξ/λ, and phosphoinoside dependent protein kinase 1 [42] but an important mechanism seems to involve the deubiquitinating enzyme CYLD [43]. Under normal circumstances, the CYLD protein is recruited to the intracellular domain of the RANK receptor by interacting with the UBA domain of *SQSTM1*. This, in turn causes deubiquitation of TNF-receptor associated factor 6 (TRAF6), thereby inhibiting signal transduction downstream of RANK. Deletion of the *SQSTM1* UBA domain [43] and the P392L mutation [44] has both been shown to prevent CYLD being recruited to the RANK receptor complex, causing activation of NFκB signaling.

Animal models have also been generated in which the P392L human mutation has been knocked into the germ-line of mice. Mice carrying this mutation have been shown to exhibit increased osteoclastogenesis in 1 study [45] and in another to develop bone lesions with remarkable similarity to those found in human PDB [46].

Chromosome 7q33

A genomic region of approximately 350 kb at this locus was identified by an extended genome wide association study. The region is bounded by 2 recombination hotspots and contains 3 genes, *NUP205*, *SLC13A,4,* and *CNOT4*. Although none of these are known to play a role in bone metabolism *CNOT4* has E3 ubiquitin ligase activity. Given the importance of the ubiquitin pathway in osteoclast function it is, therefore, plausible that *CNOT4* might be the candidate gene in this locus. However, further work will be required to confirm or refute this hypothesis, to identify the causal variants and examine the molecular mechanisms responsible for the association observed.

TM7SF4

The *TM7SF4* gene (also known as *DCSTAMP*) located on 8q22 encodes dendritic cell specific transmembrane protein (DC-STAMP). Common variants at this locus were identified as risk factors for Paget's disease following a genome wide association study. The region of association extended for about 220Kb but the region of strongest association was within a 18Kb region encompassing the *TM7SF4* gene. This gene is a strongest candidate for susceptibility to PDB since its product plays an essential role in promoting fusion of osteoclast precursors to form mature osteoclasts [47, 48]. The causal genetic variants remain to be identified but the associated SNP from the GWAS was strongly associated with *TM7SF4* expression in monocytes suggesting that the causal variants most probably influence gene expression. Recently a rare coding variant causing an amino acid change from Leucine to Phenylalanine at position 397 (L397F) was reported in the French Canadian population that was associated with PDB [49]. Using exome sequencing, we have also found the L397F variant and another 2 novel coding variants in a small number of PDB cases (unpublished data). The functional effects of these variants remain to be investigated but these observations could be consistent with a model whereby predisposition to PDB is mediated not only by changes in levels of gene expression but also by rare coding variants.

TNFRSF11B

The *TNFRSF11B* gene located on 8q24 encodes osteoprotegerin, which is an endogenously produced inhibitor of osteoclast differentiation and activity. Mutations in *TNFRSF11B* have been identified as the cause of juvenile Paget's disease (JPD) by family based studies. The first report was that of Whyte and colleagues who described 2 individuals with JPD with an identical deletion in chromosome 8q24 encompassing the whole of the *TNFRSF11B* gene [32]. Circulating levels of OPG were undetectable and markers of bone turnover greatly elevated. Around the same time linkage analysis in families with JPD had gained suggestive evidence of linkage to the 8q24 region and subsequent mutation screening in patients with JPD identified several homozygous loss of function mutations as the cause of the disease [33, 50]. Candidate gene association studies have suggested that common polymorphism at the *TNFRSF11B* locus might also predispose to PDB [51, 52] but this locus has not emerged as a candidate for predisposition to PDB in genome wide association studies [23••, 35••]. In view of the role of *TNFRSF11B* variants as a predisposing factor for classical PDB remains uncertain.

VCP

Mutations affecting the *VCP* gene located on chromosome 9p13 have been identified as the cause of the autosomal dominantly inherited syndrome of inclusion body myopathy, Paget's disease and frontotemporal dementia [53]. The *VCP* gene encodes valosin-containing protein, which has been shown to regulate a variety of cellular processed including cell division, membrane fusion, stress responses, and protein degradation. The most common mutation is an arginine to histidine amino acid change at position 155 (R155H), although several other mutations have been described (reviewed by Metha [54]). Most of the mutations affect the CDC48 domain 2, which is located in the N-terminus of the protein. This is a substrate recognition domain, which binds polypeptides, prevents protein aggregation, and catalyzes refolding of permissive substrates. Although 9p13 did not emerge as a candidate locus for classical PDB either in linkage studies or GWAS studies, 2 groups of investigators have sought to determine if common variants in *VCP* were associated with PDB. In 1 study [55] mutation screening of *VCP* was conducted in 44 families with a history of PDB and an association study conducted in 197 sporadic PDB cases and a similar number of controls. No coding mutations were found in the families and there was no association between common *VCP* variants and PDB in the association study [56]. In another study of Belgian patients, a common SNP within *VCP* was associated with susceptibility to the disease [57]. In view of this, further studies to investigate the role of *VCP* as a predisposing gene for classical PDB would be of interest.

OPTN

The *OPTN* gene, which is located on 10p13 was implicated as a candidate gene for PDB by linkage analysis in families [22] and genome wide association studies [23••, 35••]. Genome wide linkage in UK families identified a candidate locus on 10p13 with the highest LOD score close to the position of *OPTN* and subsequently GWAS studies narrowed the region to a ~200Kb interval encompassing *OPTN*. The optineurin protein is involved in regulating multiple cellular processes relevant to the pathogenesis of PDB including autophagy and NFκB signaling although it has no known role in bone metabolism. Our preliminary data showed that knock down of optineurin in mouse bone marrow derived macrophages enhances osteoclast formation suggesting that optineurin acts as a negative regulator of osteoclast differentiation [58]. The most strongly associated SNP from the GWAS was associated with *OPTN* expression [23••] implying that the causal variant most probably affects regulatory elements involved in controlling transcription of *OPTN*. At the present time, the identity of the causal variant(s) remains to be discovered.

TNFRSF11A

The *TNFRSF11A* gene located on Chromosome 18q21 encodes receptor activator of nuclear factor kappa B (RANK), which plays a critical role in osteoclast differentiation and activity. Variants in the *TNFRSF11A* gene have been identified as genetic risk factors for PDB by studies in familial expansile osteolysis and associated disorders as well as by genome wide association studies in classical PDB. Mutations of *TNFRSF11A* were identified as a cause of familial expansile osteolysis (FEO) and early onset familial PDB by linkage analysis and positional cloning studies [29]. The causal mutations are duplications, which result in the addition of 6 or 9 amino acids to the signal peptide. This in turn prevents signal peptide cleavage and causes activation of RANK signaling by mechanisms that are still incompletely understood [59•]. Similar mutations were subsequently identified in expansile skeletal hyperplasia a related disorder [30]. Furthermore a distinct 12 bp duplication in the signal peptide region of the RANK gene was recently reported in a patient with a severe panostotic expansile bone disease associated with multiple bone deformities and a massive mandibular tumor, which was comprised of woven bone and fibrous tissue [60••]. The *TNFRSF11A* locus was also identified as predisposing to classical Paget's by genome wide association studies. Although protein coding mutations similar to those implicated in FEO have not been identified in classical PDB, two relatively common coding variants have been identified (H141Y and V192A), which have been shown to activate NFκB signaling in vitro, but only when co-transfected with *SQSTM1* mutations [61•]. Other researchers who investigated the effects of

the H141Y and V192A coding variants in reporter assays could not find evidence of an effect on NFκB signaling [62].

RIN3

The *RIN3* gene, which is located on 14q33, was implicated as a candidate gene for PDB by an extended genome wide association study [35••]. The strongest association lay within an interval of 62Kb flanked by recombination hot spots containing only the *RIN3* gene. The *RIN3* gene encodes Rab and Ras interactor protein 3, which acts as a guanine nucleotide exchange (GEF) for the Rab5 family of proteins including Rab5 itself and RAb31. The RIN family of proteins comprises RIN1, RIN2, and RIN3 [63]. They share in common several functional domains including SH2 and proline rich domains at the amino terminus and RH, VPS9, and RA domains at the C-terminus. Through these domains, members of the RIN family interact with various proteins and play a role in endocytosis, vesicular trafficking and signal transduction. The role of RIN3 in bone metabolism has not specifically been studied, although it is known that Rab proteins play a role in regulating osteoclast function through their effects on vesicular trafficking [64]. The causal variants and mechanism underlying the association remain to be discovered but unlike *OPTN* and *TM7SF4*, the associated variants were not associated with expression of *RIN3*, possibly implying an effect on the coding region.

Chromosome 15q24

A genomic region of approximately 200 kb at the 15q24 locus was identified by an extended genome wide association study. The region contains 2 genes (*GOLGA6* and *PML*) but the strongest association lay within *PML* at a SNP which actually codes for a phenylalanine to leucine amino acid substitution at codon 645 (F645L). Neither *PML* nor *GOLGA6* are known to play a role in bone metabolism so further work will need to be done to identify the causal variants and examine the molecular mechanisms responsible for the association observed.

Somatic Mutations

The focal nature of PDB has led to speculation that somatic mutations might be responsible for localization of the disease to specific bones and indeed this was first suggested as a mechanism of disease by one of the authors more than 20 years ago [65]. The issue of whether PDB is associated with somatic mutations has been investigated in 2 studies. In one study, 2 of 5 patients were reported to have a somatic P392L mutation in *SQSTM1* in affected bone but not blood samples [66]. Similar analysis in 5 osteosarcomas from PDB patients showed that 3 tumors were positive for the P392L mutation whereas blood samples were negative. In both the bone samples and the tumor samples the mutant allele was present at much lower abundance than found in peripheral blood samples from patients heterozygous for the P392L mutation. This led the authors to conclude that there may be mosaicism for the mutation in affected tissue [66]. Another study which looked for the P392L mutation in osteoblasts and bone marrow cells cultured from affected bone showed no evidence of the P392L mutation in 28 samples from 23 patients [67]. Accordingly, the contribution of somatic mutations to the pathogenesis of PDB requires further study and needs to be investigated not only for *SQSTM1* but also for other candidate genes and genomic regions.

Genetic Testing for PDB

In view of the fact that many of the genetic variants that predispose to PDB are of large effect size there is a prospect of using genetic testing to identify patients at risk of developing the disease or of developing complications [36•]. In the rare PDB-like syndromes of familial expansile osteolysis, early onset familial PDB, expansile skeletal hyperphosphatasia, and inclusion body myopathy, Paget's disease and frontotemporal dementia predictive genetic testing is already employed in clinical practice. So far as the authors are aware, this has not yet been implemented for Juvenile PDB, but certainly could be given the high penetrance of the mutations. It has also been suggested that genetic testing should be considered in classical PDB with the aim of identifying carriers at an early stage before complications had developed [68]. This would most easily be implemented for *SQSTM1* mutations which have a high penetrance and indeed a clinical trial is currently in progress (the ZiPP study, ISRCTN 11616770) in which children of patients with PDB are offered testing for *SQSTM1* mutations and entered into a randomized trial of prophylactic zoledronic acid or placebo. It would also be possible to test for other susceptibility alleles with the aim of developing a genetic risk score for the disease [36•]. Further work would have to be carried out to evaluate how best to do this in clinical practice, but a possible starting point would be in children of patients who are diagnosis with PDB. This would allow carriers to be entered into a program of enhanced surveillance for early signs of disease and treatment where appropriate.

Conclusions

Huge advances have been made in understanding the genetic determinants of PDB over the past fifteen years. Current evidence suggests that genetic susceptibility to PDB is

mediated by a combination of rare variants of large effect size and common variants of moderate effect size. These act in an additive manner to influence susceptibility to the disease and disease severity. Several questions remain unanswered however. One of the key issues is to gain better understanding of why the disease has such a curious focal distribution. A possible explanation for this would be somatic mutations and there is some evidence that this might occur in some patients, but the data are inconclusive. Another unanswered question is with regard to the changes in incidence and severity that have occurred over recent years. This suggests that key environmental trigger factors have altered in frequency over recent years but the nature of these factors and mechanisms by which they interact with the genes that predispose to PDB remain to be determined. From a clinical perspective, there is a real prospect that genetic testing for susceptibility could be employed to identify people at risk of the disease for enhanced surveillance and intervention although much work needs to be done to evaluate how such as program would best be implemented.

Compliance with Ethics Guidelines

Conflict of Interest SH Ralston and OME Albagha both declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.• Ralston SH. Clinical practice. Paget's disease of bone. N Engl J Med. 2013;368:644–50. *Up-to-date review on clinical aspects of Paget's disease, including clinical features and management.*
2. Monsell EM, Bone HG, Cody DD, Jacobson GP, Newman CW, Patel SC, et al. Hearing loss in Paget's disease of bone: evidence of auditory nerve integrity. Am J Otol. 1995;16:27–33.
3. van Staa TP, Selby P, Leufkens HG, Lyles K, Sprafka JM, Cooper C. Incidence and natural history of Paget's disease of bone in England and Wales. J Bone Miner Res. 2002;17:465–71.
4. Mays S. Archaeological skeletons support a northwest European origin for Paget's disease of bone. J Bone Miner Res. 2010;25:1839–41.
5. Lucas GJ, Hocking LJ, Daroszewska A, Cundy T, Nicholson GC, Walsh JP, et al. Ubiquitin-associated domain mutations of SQSTM1 in Paget's disease of bone: evidence for a founder effect in patients of British descent. J Bone Miner Res. 2005;20:227–31.
6. Poor G, Donath J, Fornet B, Cooper C. Epidemiology of Paget's disease in Europe: the prevalence is decreasing. J Bone Miner Res. 2006;21:1545–9.
7. Cundy HR, Gamble G, Wattie D, Rutland M, Cundy T. Paget's disease of bone in New Zealand: continued decline in disease severity. Calcif Tissue Int. 2004;75:358–64.
8. Bolland MJ, Tong PC, Naot D, Callon KE, Wattie DJ, Gamble GD, et al. Delayed development of Paget's Disease in offspring inheriting SQSTM1 mutations. J Bone Miner Res. 2007;22:411–5.
9. Rebel A, Malkani K, Basle M, Bregeon C, Patezour A, Filmon R. Ultrastructural characteristics of osteoclasts in Paget's disease. Rev Rhum Mal Osteoartic. 1974;41:767–71.
10. Ralston SH, Layfield R. Pathogenesis of Paget disease of bone. Calcif Tissue Int. 2012;91:97–113.
11. Menaa C, Reddy SV, Kurihara N, Maeda H, Anderson D, Cundy T, et al. Enhanced RANK ligand expression and responsivity of bone marrow cells in Paget's disease of bone. J Clin Invest. 2000;105:1833–8.
12. Demulder A, Takahashi S, Singer FR, Hosking DJ, Roodman GD. Abnormalities in osteoclast precursors and marrow accessory cells in Paget's disease. Endocrinology. 1993;133:1978–82.
13. Naot D, Bava U, Matthews B, Callon KE, Gamble GD, Black M, et al. Differential gene expression in cultured osteoblasts and bone marrow stromal cells from patients with Paget's disease of bone. J Bone Miner Res. 2007;22:298–309.
14. Kurihara N, Zhou H, Reddy SV, Garcia Palacios V, Subler MA, Dempster D, et al. Expression of measles virus nucleocapsid protein in osteoclasts induces Paget's disease-like bone lesions in mice. J Bone Miner Res. 2006;21:446–55.
15. Matthews BG, Afzal MA, Minor PD, Bava U, Callon KE, Pitto RP, et al. Failure to detect measles virus RNA in bone cells from patients with Paget's disease. J Clin Endocrinol Metab. 2008;93:1398–1401.
16. Ralston SH, Afzal MA, Helfrich MH, Fraser WD, Gallagher JA, Mee A, et al. Multicenter blinded analysis of RT-PCR detection methods for paramyxoviruses in relation to Paget's disease of bone. J Bone Miner Res. 2007;22:569–77.
17. Helfrich MH, Hobson RP, Grabowski PS, Zurbriggen A, Cosby SL, Dickson GR, et al. A negative search for a paramyxoviral etiology of Paget's disease of bone: molecular, immunological, and ultrastructural studies in UK patients. J Bone Miner Res. 2000;5:2315–29.
18. Laurin N, Brown JP, Morissette J, Raymond V. Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget Disease of bone. Am J Hum Genet. 2002;70:1582–8.
19. Hocking LJ, Lucas GJA, Daroszewska A, Mangion J, Olavesen M, Nicholson GC, et al. Domain specific mutations in Sequestosome 1 (SQSTM1) cause familial and sporadic Paget's disease. Hum Mol Genet. 2002;11:2735–9.
20. Morissette J, Laurin N, Brown JP. Sequestosome 1: mutation frequencies, haplotypes, and phenotypes in familial Paget's Disease of bone. J Bone Miner Res. 2006;21 Suppl 2:38–44.
21. Hocking LJ, Lucas GJA, Daroszewska A, Cundy T, Nicholson GC, Donath J, et al. Novel UBA domain mutations of SQSTM1 in Paget's disease of bone: genotype phenotype correlation, functional analysis and structural consequences. J Bone Miner Res. 2004;19:1122–7.
22. Lucas G, Riches P, Hocking L, Cundy T, Nicholson G, Walsh J, et al. Identification of a major locus for Paget Disease on chromosome 10p13 in families of British descent. J Bone Miner Res. 2008;23:58–63.
23.•• Albagha OM, Visconti MR, Alonso N, Langston AL, Cundy T, Dargie R, et al. Genome-wide association study identifies variants at CSF1, OPTN and TNFRSF11A as genetic risk factors for Paget's disease of bone. Nat Genet. 2010;42:520–4. *Genome wide association study reporting the identification of three new loci for predisposition to PDB. All three loci contain genes that are involved in osteoclast function supporting the hypothesis that PDB is a genetic disorder of osteoclast function.*

24. Laurin N, Brown JP, Lemainque A, Duchesne A, Huot D, Lacourciere Y, et al. Paget disease of bone: mapping of two loci at 5q35-qter and 5q31. Am J Hum Genet. 2001;69:528–43.
25. Hocking LJ, Herbert CA, Nicholls RK, Williams F, Bennett ST, Cundy T, et al. Genome wide search in familial Paget disease of bone shows evidence of genetic heterogeneity with candidate loci on chromosomes 2q36, 10p13, and 5q35. Am J Hum Genet. 2001;69:1055–61.
26. Cody JD, Singer FR, Roodman GD, Otterund B, Lewis TB, Leppert M, et al. Genetic linkage of Paget disease of the bone to chromosome 18q. Am J Hum Genet. 1997;61:1117–22.
27. Hocking L, Slee F, Haslam SI, Cundy T, Nicholson G, Van Hul W, et al. Familial Paget's disease of bone: patterns of inheritance and frequency of linkage to chromosome 18q. Bone. 2000;26:577–80.
28. Hughes AE, Shearman AM, Weber JL, Barr RJ, Wallace RG, Osterberg PH, et al. Genetic linkage of familial expansile osteolysis to chromosome 18q. Hum Mol Genet. 1994;3:359–61.
29. Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, et al. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet. 2000;24:45–8.
30. Whyte MP, Hughes AE. Expansile skeletal hyperphosphatasia is caused by a 15-base pair tandem duplication in TNFRSF11A encoding RANK and is allelic to familial expansile osteolysis. J Bone Miner Res. 2002;17:26–9.
31. Nakatsuka K, Nishizawa Y, Ralston SH. Phenotypic characterization of early onset Paget's disease of bone caused by a 27-bp duplication in the TNFRSF11A gene. J Bone Miner Res. 2003;18:1381–5.
32. Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, et al. Osteoprotegerin deficiency and juvenile Paget's disease. N Engl J Med. 2002;347:175–84.
33. Chong B, Hegde M, Fawkner M, Simonet S, Cassinelli H, Coker M, et al. Idiopathic hyperphosphatasia and TNFRSF11B mutations: relationships between phenotype and genotype. J Bone Miner Res. 2003;18:2095–104.
34. Watts GD, Wyme J, Kovach MJ, Mehta SG, Mumm S, Darvish D, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet. 2004;36:377–81.
35.•• Albagha OME, Wani S, Visconti MR, Alonso N, Goodman K, Cundy T, et al. Genome-wide association identifies three new susceptibility loci for Paget's disease of bone. Nat Genet. 2011;43:685–9. *Extended genome wide association study building on observations in reference 23 to identify additional additional loci for predisposition to PDB, one of which contains a gene known to be involved in regulating fusion of osteoclast precursors*.
36.• Albagha OM, Visconti MR, Alonso N, Wani S, Goodman K, Fraser WD, et al. Common susceptibility alleles and SQSTM1 mutations predict disease extent and severity in a multinational study of patients with Paget's disease. J Bone Miner Res. 2013;28:2238–46. *Multinational study demonstrating that both SQSTM1 mutations and genetic variants identified from GWAS studies predict severity and complications of PDB with quite strong effects. The work raises the possibility that genetic profiling could be used to identify high risk patients for enhanced surveillance and early intervention*.
37. Langston AL, Campbell MK, Fraser WD, MacLennan GS, Selby PL, Ralston SH. Randomised trial of intensive bisphosphonate treatment vs symptomatic management in Paget's disease of bone. J Bone Miner Res. 2010;25:20–31.
38. Visconti MR, Langston AL, Alonso N, Goodman K, Selby PL, Fraser WD, et al. Mutations of SQSTM1 are associated with severity and clinical outcome in Paget's disease of bone. J Bone Miner Res. 2010;25:2368–73.
39. Cavey JR, Ralston SH, Sheppard PW, Ciani B, Gallagher TR, Long JE, et al. Loss of ubiquitin binding is a unifying mechanism by which mutations of SQSTM1 cause Paget's disease of bone. Calcif Tissue Int. 2006;78:271–7.
40. Wright T, Rea SL, Goode A, Bennett AJ, Ratajczak T, Long JE, et al. The S349T mutation of SQSTM1 links Keap1/Nrf2 signaling to Paget's disease of bone. Bone. 2013;52:699–706.
41. Goode A, Long JE, Shaw B, Ralston SH, Visconti MR, Gianfrancesco F, et al. Paget disease of bone-associated UBA domain mutations of SQSTM1 exert distinct effects on protein structure and function. Biochim Biophys Acta. 2014;1842:992–1000.
42. Chamoux E, Couture J, Bisson M, Morissette J, Brown JP, Roux S. The p62 P392L mutation linked to Paget's disease induces activation of human osteoclasts. Mol Endocrinol. 2009;23:1668–80.
43. Jin W, Chang M, Paul EM, Babu G, Lee AJ, Reiley W, et al. Deubiquitinating enzyme CYLD negatively regulates RANK signaling and osteoclastogenesis in mice. J Clin Invest. 2008;118:1858–1866.
44. Sundaram K, Shanmugarajan S, Rao DS, Reddy SV. Mutant p62P392L stimulation of osteoclast differentiation in Paget's disease of bone. Endocrinology. 2011;152:4180–9.
45. Kurihara N, Hiruma Y, Zhou H, Subler MA, Dempster DW, Singer FR, et al. Mutation of the sequestosome 1 (p62) gene increases osteoclastogenesis but does not induce Paget disease. J Clin Invest. 2007;117:133–42.
46. Daroszewska A, van't Hof RJ, Rojas JA, Layfield R, Landao-Basonga E, Rose L, et al. A point mutation in the ubiquitin associated domain of SQSMT1 is sufficient to cause a Paget's disease like disorder in mice. Hum Mol Genet. 2011;20:2734–44.
47. Yagi M, Miyamoto T, Sawatani Y, Iwamoto K, Hosogane N, Fujita N, et al. DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. J Exp Med. 2005;202:345–51.
48. Kukita T, Wada N, Kukita A, Kakimoto T, Sandra F, Toh K, et al. RANKL-induced DC-STAMP is essential for osteoclastogenesis. J Exp Med. 2004;200:941–6.
49. Beauregard M, Gagnon E, Guay-Belanger S, Morissette J, Brown JP, Michou L. Identification of rare genetic variants in novel loci associated with Paget's disease of bone. Hum Genet. 2013;133:755–768.
50. Middleton-Hardie C, Zhu Q, Cundy H, Lin JM, Callon K, Tong PC, et al. Deletion of aspartate 182 in OPG causes juvenile Paget's disease by impairing both protein secretion and binding to RANKL. J Bone Miner Res. 2006;21:438–45.
51. Beyens G, Daroszewska A, de Freitas F, Fransen E, Vanhoenacker F, Verbruggen L, et al. Identification of sex-specific associations between polymorphisms of the osteoprotegerin gene, TNFRSF11B, and Paget's disease of bone. J Bone Miner Res. 2007;22:1062–71.
52. Daroszewska A, Hocking LJ, McGuigan FEA, Langdahl BL, Stone MD, Cundy T, et al. Susceptibility to Paget's disease of bone is influenced by a common polymorphic variant of Osteoprotegerin. J Bone Miner Res. 2004;19:1506–11.
53. Watts GD, Thomasova D, Ramdeen SK, Fulchiero EC, Mehta SG, Drachman DA, et al. Novel VCP mutations in inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia. Clin Genet. 2007;72:420–6.
54. Mehta SG, Khare M, Ramani R, Watts GD, Simon M, Osann KE, et al. Genotype-phenotype studies of VCP-associated inclusion body myopathy with Paget disease of bone and/or frontotemporal dementia. Clin Genet. 2013;83:422–31.
55. Lucas GJ, Mehta SG, Hocking LJ, Stewart TL, Cundy T, Nicholson GC, et al. Evaluation of the role of Valosin-containing protein in the pathogenesis of familial and sporadic Paget's disease of bone. Bone. 2006;38:280–5.
56. Lucas GJ, Mehta SG, Hocking LJ, Stewart TL, Cundy T, Nicholson GC, et al. Evaluation of the role of Valosin-containing protein in the pathogenesis of familial and sporadic Paget's disease of bone. Bone. 2006;38:280–5.

57. Chung PY, Beyens G, de Freitas F, Boonen S, Geusens P, Vanhoenacker F, et al. Indications for a genetic association of a VCP polymorphism with the pathogenesis of sporadic Paget's disease of bone, but not for TNFSF11 (RANKL) and IL-6 polymorphisms. Mol Genet Metab. 2011;103:287–92.
58. Obaid R, Wani S, Ralston SH, Albagha OME. OPTN negatively regulates osteoclast formation in vitro. Bone. 2012;50:S92–3.
59.• Crockett JC, Mellis DJ, Shennan KI, Duthie A, Greenhorn J, Scott DI, et al. Signal peptide mutations in rank prevent downstream activation of NFkappaB. J Bone Miner Res. 2011;26:1926–38. *An interesting study which shows that the insertion mutations in TNFRSF11A that cause FEO, ESH and early onset PDB actually inhibit NFkB signaling in vitro. This illustrates that further work is required to fully understand why they cause osteoclast activation in vivo*.
60.•• Schafer AL, Mumm S, El-Sayed I, McAlister WH, Horvai AE, Tom AM, et al. Panostotic expansile bone disease with massive jaw tumor formation and a novel mutation in the signal peptide of RANK. J Bone Miner Res. 2014;29:911–21. *Report of extraordinarily severe PDB-like phenotype in patient with insertion mutation in TNFRSF11A illustrating that subtle differences in mutations in exon 1 of this gene can have major clinical consequences*.
61.• Gianfrancesco F, Rendina D, DiStefano M, Mingione A, Esposito T, Merlotti D, et al. A nonsynonymous TNFRSF11A variation increases NFkappaB activity and the severity of Paget's disease. J Bone Miner Res. 2012;27:443–52. *A phenotype-genotype analysis which reports an association between TNFRSF11A variants and severity of PDB as well as providing evidence that two common coding variants of TNFRSF11A might have functional significance in altering NFkB signaling*.
62. Chung PY, Beyens G, Riches PL, Van Wesenbeeck L, de Freitas F, Jennes K, et al. Genetic variation in the TNFRSF11A gene encoding RANK is associated with susceptibility to Paget's disease of bone. J Bone Miner Res. 2010;25:2316–29.
63. Janson C, Kasahara N, Prendergast GC, Colicelli J. RIN3 is a negative regulator of mast cell responses to SCF. PLoS One. 2012;7:e49615.
64. Taylor A, Mules EH, Seabra MC, Helfrich MH, Rogers MJ, Coxon FP. Impaired prenylation of Rab GTPases in the gunmetal mouse causes defects in bone cell function. Small GTPases. 2011;2:131–42.
65. Ralston SH. Paget's disease of bone. Br Med J. 1993;306:332–3.
66. Merchant A, Smielewska M, Patel N, Akunowicz JD, Saria EA, Delaney J. Somatic mutations in SQSTM1 detected in affected tissues from patients with sporadic Paget's disease of bone. J Bone Miner Res. 2009;24:484–94.
67. Matthews BG, Naot D, Bava U, Callon KE, Pitto RP, McCowan SA, et al. Absence of somatic SQSTM1 mutations in Paget's disease of bone. J Clin Endocrinol Metab. 2009;94:691–4.
68. Michou L, Collet C, Laplanche JL, Orcel P, Cornelis F. Genetics of Paget's disease of bone. Joint Bone Spine. 2006;73:243–8.