Genetics of Paget’s disease of bone

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ABSTRACT

PDB (Paget’s disease of bone) is a common condition characterized by focal increases in bone turnover affecting one or more sites throughout the skeleton. Genetic factors are important in the pathogenesis of PDB and many families have been described where PDB is inherited in an autosomal-dominant fashion. Several candidate loci for susceptibility to PDB and related syndromes have been identified by genome-wide scans and recent evidence suggests that mutations in genes that encode components of the RANK (receptor activator of NF-κB) pathway play an important role in the pathogenesis of this group of diseases. Insertion mutations in the TNFRSF11A gene encoding RANK have been identified as the cause of familial expansile osteolysis, some cases of early onset PDB and expansile skeletal hyperphosphatasia. Inactivating mutations in the TNFRSF11B gene that encodes OPG (osteoprotegerin) have been found to cause the syndrome of juvenile PDB. Polymorphisms in OPG also appear to increase the risk of developing PDB. The most important causal gene for classical PDB is Sequestosome 1 (SQSTM1), which is a scaffold protein in the NF-κB signalling pathway, and mutations affecting the UBA (ubiquitin-associated) domain of this protein occur in between 20–50 % of familial and 10–20 % of sporadic PDB cases. The rare syndrome of IBMPFD (inclusion body myopathy, PDB and fronto-temporal dementia) is due to mutations in the VCP gene and these also cluster in the domain of VCP that interacts with ubiquitin, suggesting a common disease mechanism with SQSTM1-mediated PDB.

INTRODUCTION

PDB (Paget’s disease of bone) is a common condition characterized by focal areas of increased osteoclastic bone resorption and a coupled, but disorganized, increase in osteoblastic bone formation [1]. PDB is thought to occur primarily as the result of a derangement in osteoclast formation and activity, but the cause of this is not completely understood. Two principal mechanisms have been proposed for the causation of PDB. The first is that it is due to a slow virus infection of osteoclasts, and the second is that the condition is genetically determined. A unifying hypothesis has also been put forward which suggests that PDB may be caused by genetic predisposition to an infectious agent. The aim of this review is to summarize recent advances in understanding the role of genetic factors in PDB.

PDB is the second most common metabolic bone disorder in the U.K., affecting approx. 2.5 % of men and 1.6 % of women over the age of 55 years [2]. Although frequently asymptomatic, PDB is associated with significant morbidity in approx. 30 % of cases due to symptoms such as pain, bone deformity and deafness, and complications such as osteoarthritis [3,4]. Although the disease is primarily focal in nature, PDB patients have histological evidence of increased bone remodelling in...
bones that are not obviously affected [5]. This increase in bone turnover cannot be completely explained by the modest increase in circulating parathyroid hormone levels, which are found in approx. 20 % of cases [6], suggesting that PDB patients may exhibit a mild generalized increase in bone turnover, as well as focal lesions.

**EPIDEMIOLOGY**

Paget's disease is not a new condition, since evidence of the disease has been recorded in skeletal remains dating back to the Neolithic era [7]. Archaeological studies indicate that the prevalence of PDB in those aged >40 years in the U.K. was 2.5 % from 900 to 1850 A.D., compared with 2 % at present [2,8,9]. It should be noted, however, that there has been a fall in prevalence and clinical severity of PDB during the past 30 years in developed countries [2–4,10–12]. The reasons for this are not completely understood, but possibilities include changes in prevalence of an environmental trigger for the disease or changes in ethnic makeup of the populations where these studies have been carried out. There are major ethnic differences in the prevalence of PDB. Paget’s disease is common in the U.K. [13,14], Australia [15], New Zealand [12], North America [16] and parts of Western Europe [17], but is rare in Scandinavia and Japan. Migrant studies have shown that the risk of the disease remains high in subjects who move from high-prevalence regions to low-prevalence regions, which supports the importance of genetic factors in its pathogenesis [15]. Evidence in support of a genetic cause also comes from the observation that 15–40 % of patients have a positive family history of the disease [18–20]. Reflecting this fact, the risk of developing PDB is 7–10 times higher in relatives of PDB patients compared with controls [19,20]. Patients with PDB who have a positive family history are more likely to have severe disease, with an earlier age at presentation, polyostotic involvement and bone deformity, than those without a family history [18,20]. Some families have a curious preponderance for involvement of particular anatomical sites, the reason for which remains unclear [18]. In most families, the mode of inheritance is autosomal-dominant with incomplete penetrance that increases with age [18,21–24].

**FAMILIAL PDB-LIKE SYNDROMES**

A spectrum of rare genetic bone disorders with clinical, radiological or histological features in common with classic PDB has been described. These include FEO (familial expansile osteolysis) [25], ESH (expansile skeletal hyperphosphatasia) [26], juvenile PDB (also known as idiopathic hyperphosphatasia) [27], early onset PDB [28] and a syndrome of IBMPFD (inclusion body myopathy with early onset PDB and fronto-temporal dementia) [29]. The clinical features of some of these diseases, such as ESH and FEO, overlap with classical PDB, but the age at onset is much earlier and the distribution of involvement is different. For IBMPFD, the clinical features and age at onset seem to be similar to that in non-syndromic PDB. The genes responsible for these conditions are discussed in more detail below.

**SUSCEPTIBILITY LOCI FOR PDB**

At the time of writing, seven potential susceptibility loci for classical PDB have been identified. The first locus (**PDB1**) was identified on the basis of a candidate gene linkage study [30], whereas the remaining loci (**PDB2–7**) were identified by genome-wide searches (Table 1).

None of the genome-wide searches performed so far have confirmed candidacy of the **PDB1** locus and this is currently believed to be a false-positive result. Although two studies reported evidence of linkage between familial PDB and the **PDB2** locus on chromosome 18q.21, the families investigated were too small to give definitive results, and it would now appear that the **PDB2** locus is primarily involved in the syndromes of familial expansile osteolysis, early onset PDB and expansile skeletal hyperphosphatasia, rather than typical PDB. Mutations in genes that cause PDB have been identified by positional cloning efforts for two of the loci (**PDB2** and **PDB3**) and these will be discussed in more detail below, along with other genes that have been implicated in the pathogenesis of PDB.

**GENES THAT CAUSE PDB AND RELATED SYNDROMES**

**TNFRSF11A**

Mutations in the **TNFRSF11A** gene, encoding RANK [receptor activator of NF-κB (nuclear factor κB)], have
been identified as the cause of FEO, ESH and the syndrome of severe early onset familial PDB. The TNFRSF11A gene lies within the middle of the PDB2 critical region identified by a genome-wide search, which was originally undertaken in a large FEO family by Hughes et al. [31]. Subsequently, two groups reported linkage between classical PDB and the PDB2 region, although the families were too small to conclusively confirm linkage [21,22]. RANK, a member of the TNFR (tumour necrosis factor receptor) superfamily, is highly expressed on osteoclast precursors and osteoclasts, and is essential for osteoclast differentiation and function [32]. Mutation screening of TNFRSF11A revealed three distinct insertion mutations affecting exon 1, resulting in duplication of several amino acids in the RANK signal peptide. The 84dup18 mutation (18-bp duplication at position 84) [33,34] and a closely related 83dup18 mutation [35] have been found in all cases of FEO so far described. The 75dup27 mutation was reported in a Japanese family with severe early onset familial PDB [33], but has not so far been described in other families. The 84dup15 mutation has been described in one family with ESH [36]. Functional studies have indicated that both the 84dup18 and 75dup27 mutations cause failure of RANK signal peptide cleavage and activation of NF-κB signalling in vitro, although the exact mechanisms by which they do so are unclear [33]. It seems likely that the 84dup15 mutation has similar functional effects, but this has not been specifically studied. There is no evidence that mutations or polymorphisms in TNFRSF11A contribute to the pathogenesis of classic PDB [33,37,38] and mutation screening of affected individuals in the PDB families described by Cody et al. [21] and Haslam et al. [22] that showed possible evidence of linkage to PDB2 showed no evidence of TNFRSF11A mutations, except in one Japanese family with severe early onset disease.

**TNFRSF11B**

The TNFRSF11B gene encodes OPG (osteoprotegerin), which is an inhibitor of osteoclastic bone resorption and a decoy receptor for RANKL (RANK ligand) [32]. Following the discovery of TNFRSF11A mutations in patients with FEO, attention turned to TNFRSF11B as a candidate gene for PDB and related syndromes. Mutation screening of the TNFRSF11B gene in two apparently unrelated Navajo individuals with juvenile PDB revealed the absence of circulating OPG and evidence of an identical homozygous 100 kb deletion involving TNFRSF11B in both cases [27]. In an independent study, Cundy et al. [39] showed evidence of linkage between juvenile PDB and chromosome 8q24 and subsequently reported a homozygous mutation affecting exon 3 of TNFRSF11B as the cause of juvenile PDB in three affected siblings with the disease. Functional studies confirmed that the mutant OPG protein was unable to inhibit bone resorption when expressed in vitro, indicating that it was an inactivating mutation. Mutations of TNFRSF11B have been excluded as a cause of classic late-onset familial and sporadic PDB [38], but there is evidence to suggest that polymorphic variation at the TNFRSF11B locus predisposes to PDB. In a small study of Belgian patients, Wuyts et al. [38] found an association between a common SNP (single nucleotide polymorphism) in intron 2 of TNFRSF11B (400 + 4 C/T or C505T) and PDB. A much larger study by Darozewksa et al. [40] involving patients of predominantly British descent showed evidence of association between a polymorphism in exon 1 of TNFRSF11B (G1181C) and both sporadic PDB and familial PDB. The G1181C SNP is a common variant that causes a lysine → asparagine amino acid change at codon 3 of the OPG signal peptide. The functional significance of this change remains to be fully investigated, but it could affect OPG trafficking and secretion and, thereby, alter local concentrations of this critical regulator of osteoclast activity.

**Sequestosome 1**

Mutations in the Sequestosome 1 gene (SQSTM1) were identified as a common cause of PDB as the result of positional cloning efforts by two groups, who independently found evidence of linkage between familial PDB and the PDB3 locus on chromosome 5q35 [24,41]. Subsequent positional cloning studies in French–Canadian subjects identified a mutation in exon 8 of SQSTM1 in affected patients, causing a proline → leucine amino acid substitution at codon 392 (P392L) of the protein [42]. The same mutation was found in approx. 20% of patients with apparently 'sporadic' PDB from the same population. It is possible, however, that many of these subjects may have had unrecognized familial PDB. Shortly after this, Hocking et al. [43] confirmed the presence of the P392L mutation as a common cause of PDB in families of British descent and reported truncating mutations at codons 390 and 396 of SQSTM1 in other families. Many groups worldwide have now reported the presence of SQSTM1 mutations in familial and sporadic PDB and, at the time of writing, 11 different missense or non-sense mutations have been described, all of which affect the UBA (ubiquitin-associated) domain of the protein [44–46]. Genotype–phenotype analysis has indicated that patients with truncating mutations tend to have more severe disease than those with missense mutations and a few patients have been described with mutations in both alleles of SQSTM1. These patients have had severe disease, although there is considerable overlap between disease severity in patients who carry identical mutations even within the same family [42,45].

The common occurrence of the P392L mutation in PDB led Laurin and co-workers [42] to speculate that this region may be a mutational hot-spot. However, new evidence has emerged to show that in the vast majority of SQSTM1-mediated PDB cases of British descent,
Figure 1  Binding of RANKL to RANK activates the NF-κB signalling cascade, which leads to increased osteoclastogenesis

SQSTM1 acts as a scaffold protein, which links the RANK/TRAF6 signalling complex to aPKC and probably regulates this step through the UBA domain interaction with polyubiquitylated (Ub) TRAF6. The activated IKK (IκB kinase) complex phosphorylates IκB, which undergoes degradation, mediated by VCP, in the ubiquitin/proteasome degradation pathway allowing for dissociation of NF-κB and its translocation to the nucleus with consequent activation of response genes. OPG, a decoy receptor for RANKL, inhibits osteoclastogenesis. The association between the mutations in genes encoding respective proteins and resulting clinical entities is indicated. Activating mutations of RANK cause FEO, ESH and early onset PDB. Mutations affecting the UBA domain of Sequestosome 1 have been identified in classic PDB. OPG deficiency results in juvenile PDB. Mutations in VCP cause the syndrome of IBMPFD.

the P392L mutation occurs on an identical haplotype, consistent with a founder mutation on an ancestral chromosome. This suggests that many so called ‘sporadic’ PDB cases may in fact have clinically occult familial disease [47].

The SQSTM1 gene encodes a protein also known as p62, which is ubiquitously expressed and contains many structural motifs [48], including a SH2 (Src homology 2) binding domain, an AID (aPKC (atypical protein kinase C)-interacting domain), a ZZ domain, a TRAF6 (TNFR-associated factor 6) binding domain, two PEST motifs and a UBA domain. There is evidence that p62 acts as a scaffold protein in the NF-κB signalling pathway, where it links molecules such as RIP (receptor-interacting protein) and TRAF6 to aPKC and downstream effectors (Figure 1) [49]. It is of interest that all PDB-causing mutations described so far affect the UBA domain of p62, indicating that the mechanisms by which they cause PDB might be related to abnormalities in ubiquitin-mediated degradation of proteins that interact with p62. In keeping with this, recent functional studies have shown that both missense and non-sense PDB-causing mutations result in loss of ubiquitin binding when studied in the context of the full-length protein [50]. This indicates that the missense mutations probably cause a subtle conformational change in the protein that renders it unable to interact with ubiquitin properly. It is currently unclear how exactly these events result in accelerated osteoclast activity, but recent studies in mice with targeted inactivation of SQSTM1 have shown defects in RANKL-induced osteoclastogenesis, emphasizing the importance of p62 in osteoclast function [51].

It is tempting to speculate that UBA domain SQSTM1 mutations impair the ability of p62 to shuttle ubiquitylated proteins within the NF-κB pathway for degradation, whilst leaving the scaffold function intact. This would be expected to lead to prolonged activation of the NF-κB pathway and enhanced osteoclastogenesis. However, further work will be required to investigate this hypothesis.

VCP (valosin-containing protein)

Mutations in the VCP gene were identified as the cause of IBMPFD by a positional cloning effort of the 9p21 locus identified by Kovach et al. [29]. Analysis of individuals from 16 families identified several different missense mutations in exons 3, 5 and 6 of the VCP gene, which segregated with the disease in affected family
members [52]. VCP encodes valosin-containing protein (VCP or p97), a member of the type II AAA (ATPases Associated with a variety of Activities) proteins, whose fundamental role is one of a molecular chaperone in the ubiquitin–proteasome degradation pathway [53]. All of the mutations involve the N-terminal domain of VCP, which is responsible for binding to ubiquitylated proteins, including IkBα (inhibitor of NF-κB), thus targeting these proteins for degradation by the proteasome [54]. This suggests that the mechanisms of disease for PDB associated with VCP mutations may be similar in some respects to that associated with SQSTM1 mutations, in that both types of mutations are likely to result in impaired degradation of target proteins, some of which are involved in NF-κB signalling. Presumably, the additional features of inclusion body myopathy and frontotemporal dementia in IBM/PF occur as a result of differences in the range of proteins that VCP and SQSTM1 interact with. The role of VCP in the pathogenesis of non-syndromic PDB not associated with dementia and myopathy is unclear, but is under investigation at present.

**CLINICAL IMPLICATIONS**

Although many patients with PDB remain asymptomatic, a number will develop complications, which can be particularly severe and occur at an early age in familial PDB. Currently, the treatment of choice, specifically reducing bone turnover, is the use of bisphosphonates. However, it is unclear whether bisphosphonates affect the clinical outcome of PDB other than reducing bone pain [57]. It is expected that the PRISM trial (Paget’s Disease: a Randomized Trial of Intensive vs. Symptomatic Management), which is close to completion now, will provide clues as to the best line of management of affected individuals not only according to the presence or absence of symptoms, but also according to the presence or absence of the SQSTM1 gene or other mutations. Until then, it will be difficult to advocate routine screening of patients with PDB for mutations, as the presence of one would not alter the management. On the other hand, such a test would not only provide an explanation of the aetiology of the condition to the patient, but would also be valuable to the relatives in their risk assessment. Identification of other causal genes in the PDB4–6 loci and the understanding of the mechanism of SQSTM1, VCP, TNFRSF11A and TNFRSF11B mutations causing PDB and related conditions are the fundamental steps for future development of novel targeted therapy, which is likely to involve modulation of the RANK/NF-κB and the ubiquitin/proteasome pathways.

**CONCLUSIONS**

Over the last 5 years, tremendous advances have been made in elucidating the role of genetic factors in PDB and related syndromes with the discovery of mutations in four causal genes. In each case, however, the exact mechanisms which link these genetic mutations to the clinical picture of PDB and related syndromes remains to be fully understood. It is unclear, for example, why PDB targets specific bones in the skeleton and what the factors are which determine the distribution of lesions. The wide variation in severity and distribution of disease in patients who share the same mutation is also puzzling and suggests that other factors such as gene–gene interactions or environmental influences play an important role in disease severity. There are many anecdotal case reports of PDB targeting specific regions of the skeleton that are subject to repetitive mechanical loading and this could be one explanation. Alternatively other factors such as diet or infectious agents could similarly act as a trigger for the disease in genetically susceptible individuals. Further research of the pathophysiology of PDB and related conditions will help develop novel targeted therapy, which is likely to involve modulation of the RANK/NF-κB and the ubiquitin/proteasome pathways.

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