Genetic determinants of Paget’s disease of bone

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Paget’s disease is a common skeletal disorder with a strong genetic component, which is characterized by focal increases in disorganized bone remodeling, predominantly affecting the axial skeleton. Current evidence suggests that classical Paget’s disease of bone (PDB) is caused by a combination of rare alleles of large effect size that cause autosomal dominant inheritance of the disease and more common alleles of smaller effect size. Mutations of SQSTM1 are the most common cause of classical PDB, occurring in about 10% of patients. The causal mutations cluster in the ubiquitin-associated domain and impair its ability to bind ubiquitin. Other loci that predispose to PDB have recently been identified by genome-wide association studies, which have identified variants at seven loci that predispose to the disease. These increase the risk of PDB individually by 1.3- to 1.7-fold, but have combined effects that account for about 86% of the population-attributable risk of PDB in SQSTM1 negative patients.

Keywords: genetic; genome-wide association study; Paget’s disease; SNP

Epidemiology

Paget’s disease of bone (PDB) affects between 1% and 2% of Caucasians over the age of 55 years. The disease is characterized by focal abnormalities of increased bone turnover affecting one or more sites throughout the skeleton. The axial skeleton is preferentially affected and the most common sites of involvement are the pelvis (70% of cases), femur (55%), lumbar spine (53%), skull (42%), and tibia (32%). The prevalence of Paget’s disease increases with age, and the disease affects about 8% of men and 5% of women by the eighth decade in the UK. The UK has the highest incidence of PDB in the world, but the disease is also common in Western and Southern Europe and in British migrants to Australia, New Zealand, and South Africa. Conversely, PDB is rare in Scandinavia, the Indian subcontinent, China, Japan, and other countries in the Far East. These ethnic differences in the incidence of PDB emphasize the importance of genetic factors in the pathogenesis of the disease, but there is evidence that environmental factors also play a role. Support for this comes from the observation that there have been reductions in the prevalence and severity of PDB in the UK, some European countries, and New Zealand over the past 25 years, and a delayed onset of the disease in children of patients with SQSTM1-mediated familial PDB. However, for reasons that are currently unclear, no major changes in severity or incidence of PDB have been observed in other countries such as Italy and some regions of the United States. It is likely that there have been changes in hitherto unidentified environmental triggers for the disease in several countries over the past 25 years. However, the reduction in disease prevalence in some countries, such as the UK, may also be due in part to changes in ethnic makeup of the population due to influx of migrants from low prevalence regions such as the Indian subcontinent and the Far East.

Genetic architecture of PDB

Familial clustering is common in classical PDB and in many cases the disease is inherited as an autosomal dominant trait with a penetrance of about 80% and 90% by the seventh decade. Approximately 15% of PDB cases report having a positive family history of the condition but the proportion of familial cases is likely to be higher because PDB is
often asymptomatic.\textsuperscript{16,17} The risk of developing PDB in relatives of an affected person is approximately seven times greater than in relatives of controls.\textsuperscript{16–18} In addition, several rare syndromes have been described with a Mendelian mode of inheritance (either autosomal dominant or autosomal recessive) that share several features in common with PDB. It seems likely that the ethnic differences in incidence of PDB referred previously is due to differences between population in carriage of risk alleles for the disease, but this has not yet been specifically studied. These genes and loci that predispose to PDB are discussed in more detail later.

**Genes that cause rare PDB-like syndromes**

Several rare bone disorders have been described which exhibit clinical, radiological, or histological features in common with Paget’s disease. These include: familial expansile osteolysis (FEO),\textsuperscript{19} expansile skeletal hyperphosphatasia (ESH),\textsuperscript{20} early onset familial Paget’s disease (EoPDB),\textsuperscript{21} juvenile Paget’s disease (also known as idiopathic hyperphosphatasia),\textsuperscript{22} and the syndrome of hereditary inclusion body myopathy, Paget disease of bone, and frontal-temporal dementia (IBMPFD).\textsuperscript{23} With the exception of juvenile Paget’s disease, which is an autosomal recessive condition, all of these disorders are inherited in an autosomal dominant manner.

Mutations of the \textit{TNFRSF11A} gene, which encodes RANK, are responsible for the syndromes of FEO, ESH, and EoPDB. These disorders share several features in common including the presence of focal osteolytic lesions, premature deafness, and premature tooth loss.\textsuperscript{21,22,24} The causal mutations are duplications of between 15–27 nucleotides in the first exon of \textit{TNFRSF11A}, which introduce between 5 and 9 additional amino acids residues into the RANK signal peptide and prevent it from being cleaved normally.\textsuperscript{24} This causes the abnormal RANK molecules to accumulate in the Golgi apparatus.\textsuperscript{25} Cells which have been engineered to express these mutations do not show evidence of a constitutive increase in NF-κB signaling and also fail to activate NF-κB signaling in response to RANKL.\textsuperscript{25} Despite this, overexpression of the mutants in osteoclast precursors promotes osteoclast differentiation \textit{in vitro} by mechanisms that are currently unclear. Although similar mutations of \textit{TNFRSF11A} have been excluded as a cause of classical PDB,\textsuperscript{26} there is strong evidence to suggest that common variants at the \textit{TNFRSF11A} locus predispose to the disease.\textsuperscript{27–29}

Mutations in the \textit{TNFRSF11B} gene encoding osteoprotegerin (OPG) are the cause of juvenile Paget’s disease (JPD). This is a rare disorder associated with grossly abnormal bone remodeling, bone expansion, and bone deformity which presents in childhood and adolescence. Various mutations have been described in JPD including deletions involving the whole gene\textsuperscript{30} and various missense mutations.\textsuperscript{31} These are loss-of-function mutations that result in the OPG protein not being produced at all or that result in formation of an abnormal OPG protein, which is incapable of binding to RANKL and inhibiting bone resorption.\textsuperscript{32} Mutations of \textit{TNFRSF11B} have not so far been detected in classical PDB, but there is some evidence to suggest that variants at the \textit{TNFRSF11B} locus predispose to PDB at least in women.\textsuperscript{33,34}

Mutations in the VCP gene have been identified as the cause of IBMPFD, a syndrome characterized by myopathy, and dementia, which is often accompanied by PDB.\textsuperscript{35} The predominant clinical feature of this syndrome is myopathy, which typically presents after the age of 40 and is observed in 90% of patients. About 43% of patients also develop typical PDB lesions and 37% develop dementia.\textsuperscript{23} All of the mutations affect highly conserved amino acid residues clustered in the N-terminal domain of the VCP gene product, which is known to be involved in ubiquitin binding. This finding is of interest in relation to the fact the VCP is known to regulate degradation of the IKB-α protein, which is involved in NF-κB signaling.\textsuperscript{36} Mutations of VCP have been excluded as a cause of classical PDB.\textsuperscript{37}

**Genes that cause classical Paget’s disease**

**\textit{SQSTM1}**

Mutations affecting the \textit{SQSTM1} gene cause a high penetrance form of PDB, which is inherited in an autosomal dominant manner. The causal mutations were identified as the result of a positional cloning approach following the identification of a strong susceptibility locus for PDB on chromosome 5q35 in two independent populations.\textsuperscript{14,15} Mutation screening of genes within the region identified a proline to leucine mutation at codon 392 of the \textit{SQSTM1} gene as the cause of 5q35 linked PDB in the French Canadian population.\textsuperscript{38} Soon after this, additional mutations of \textit{SQSTM1} clustering in the ubiquitin
associated (UBA) domain were identified in British patients.\(^3^9\) A large number of \textit{SQSTM1} mutations have now been identified in PDB and most of these affect the UBA domain.\(^4^0,4^1\) The \textit{SQSTM1} gene encodes p62, which is an adaptor protein in the NF-κB signaling pathway.\(^4^2\) In addition to its role in regulating downstream of the RANK, TNF, and NGF receptors,\(^4^3\) p62 appears to play a key role in regulating other cellular processes through its involvement in autophagy.\(^4^4–4^6\) Mutations of \textit{SQSTM1} occur in about 40% of patients with a family history of PDB and up to 10% of “sporadic” cases.\(^1^8,3^8–4^0,4^7\) The mechanisms by which \textit{SQSTM1} mutations lead to PDB are incompletely understood, but a common feature of virtually all mutations described so far is that they interfere with the ability of p62 to bind to ubiquitin.\(^4^8\) This leads to enhanced NF-κB signaling and increased sensitivity of osteoclast precursors to RANKL by mechanisms that remain incompletely understood.\(^4^8\) It has previously been suggested that \textit{SQSTM1} plays a permissive, rather than causal, role in PDB on the basis that the disease is incompletely penetrant and that mice carrying the P394L mutation of \textit{sqstm1} (equivalent to the P392L human mutation) did not develop PDB-like bone lesions in the spine.\(^4^9\) However, recent studies have shown that mice with the P394L mutation do develop a bone disorder with remarkable similarity to PDB, characterized by focal osteolytic and osteosclerotic lesions predominantly affecting the lower limbs, woven bone, and inclusion bodies very similar to those observed in the human disease.\(^5^0\) The difference between these studies is most likely due to technical differences in analyzing the skeletal phenotype. In one study,\(^4^9\) screening for lesions was limited to analysis of the lumbar spine and was carried out by histology, whereas in another study,\(^5^0\) screening for lesions was done by MicroCT and included analysis of the spine and lower limbs.

**Genome-wide significant loci for classical PDB**

Recently, additional variants that predispose to PDB have been identified by genome-wide association studies (GWAS), which showed that single nucleotide polymorphisms at the \textit{CSF1}, \textit{OPTN}, \textit{TNFRSF11A}, \textit{TM7SF4}, \textit{NUP205}, \textit{PML}, and \textit{RIN3} loci were significant risk factors for the development of PDB \(^2^7,2^9\) (Table 1). The \textit{CSF1}, \textit{TNFRSF11A}, and \textit{TM7SF4} loci contain strong functional candidate genes for PDB susceptibility. The \textit{CSF1} gene is situated on chromosome 1p13 and encodes macrophage colony stimulating factor (M-CSF), a cytokine that is essential for osteoclast and macrophage differentiation.\(^5^1\) The \textit{TM7SF4} gene is situated on chromosome 8q22 and encodes dendritic cell–specific transmembrane protein (DC-STAMP), a cell surface protein that is essential for the formation of multinucleated osteoclasts and macrophage polykaryons.\(^5^2\) The \textit{TNFRSF11A} gene is situated on chromosome 18q21 and encodes RANK, a receptor that is essential for osteoclast differentiation and bone resorption.\(^5^3\) The remaining four loci contain genes that have not previously been implicated in bone metabolism. The 7q33 locus contains three genes (\textit{CNOT4}, \textit{NUP205}, and \textit{SLC13A4}) and two predicted coding transcripts (\textit{PL-5283} and \textit{FAM180A}). Any could be responsible for the association observed, but the strongest signal was within \textit{NUP205}, which encodes the nucleoporin 205kd protein, a component of the nuclear pore complex.\(^5^4\) The only

| Locus | Nearest gene(s) | SNP          | \(P\) value   | Odds ratio (95% CI) |
|-------|----------------|--------------|---------------|---------------------|
| 1p13  | \textit{CSF1}  | rs10494112   | \(7.06 \times 10^{-35}\) | 1.72 (1.57–1.87)    |
| 7q33  | \textit{CNOT4}, \textit{NUP205}, \textit{SLC13A4} | rs4294134 | \(8.45 \times 10^{-10}\) | 1.45 (1.29–1.63)    |
| 8q22  | \textit{TM7SF4} | rs2458413   | \(7.38 \times 10^{-17}\) | 1.40 (1.29–1.51)    |
| 10p13 | \textit{OPTN}  | rs1561570    | \(4.37 \times 10^{-38}\) | 1.67 (1.54–1.80)    |
| 14q32 | \textit{RIN3}  | rs1049635   | \(2.55 \times 10^{-11}\) | 1.44 (1.29–1.60)    |
| 15q24 | \textit{PML}, \textit{GOLGA6A} | rs5742915 | \(1.6 \times 10^{-14}\) | 1.34 (1.25–1.45)    |
| 18q21 | \textit{TNFRSF11A} | rs3018362 | \(7.98 \times 10^{-21}\) | 1.45 (1.34–1.56)    |

The \(P\) values shown and odds ratios for association with PDB are from the strongest associated SNP in each locus. Data are from Albagha et al.\(^2^7\)
The most likely candidate gene for PDB susceptibility within the 14q32 locus is RIN3, which encodes Ras and Rab interactor 3 protein. The RIN3 protein plays a role in vesicular trafficking by interacting with small GTPases and could conceivably affect osteoclast function through this mechanism, but its role in bone cell function has not been studied. Two candidate genes are contained within the 15q24 locus; PML and GOLGA6A. The strongest associated SNP lies within PML and codes for a phenylalanine to leucine change at amino acid 645. The PML protein is involved in regulating cell growth, apoptosis, and senescence and also has been shown to regulate TGF-β signaling. However, PML has not previously been implicated in the regulation of bone metabolism. The other gene within this locus encodes a member of the golgin family of proteins, which are thought to play a role in membrane fusion and as structural supports for the Golgi cisternae. Mutations in other members of the golgin family have been shown to cause a lethal skeletal dysplasia and the syndrome of Gerodermia osteodysplastica, which is characterized by abnormal skin and severe osteoporosis. The role of GOLGA6A gene in bone has not yet been studied.

The seven genes identified from the GWAS studies mentioned earlier showed independent association with PDB consistent with a multiplicative model for susceptibility. Indeed, together the identified genetic variants accounted for approximately 86% of the population-attributable risk of PDB. In this context, the population-attributable risk provides an estimate of the proportion of PDB cases that are associated with carriage of the risk alleles described earlier, taking into account their frequency in the population and combined effect size. Furthermore, the risk of developing PDB increased with the number of risk alleles carried, such that patients carrying the greatest number of risk alleles had a 10-fold increase in PDB risk compared to those carrying the smallest number (Fig. 1).

**Other candidate genes for PDB**

In addition to the genes and loci mentioned earlier, polymorphisms in various other candidate genes and loci have been studied in patients with PDB. These have resulted in the identification of nominally significant associations between PDB and polymorphisms in the CASR, ESR1, and TNFRSF11B genes. A positive association has been observed in one study between polymorphisms in the VCP gene and PDB, whereas in another study, no association was observed. Other candidate genes that have been studied with negative results include, IL1B, IL1RA, IL6, IL8, TNFA, TNFSF11, and VDR. The association between TNFRSF11B polymorphisms and PDB is of some interest because current evidence suggests that these variants predispose to PDB only in women. Because TNFRSF11B did not emerge as a candidate locus for PDB overall in recent GWAS studies, further analysis of this locus as a determinant of PDB in women would be of interest.
Environmental factors and PDB

The reducing prevalence of PDB in some countries over recent years and delayed onset in offspring of patients who carry SQSTM1 mutations\(^1\) indicate that environmental triggers also play a significant role in regulating disease occurrence and severity.\(^2\) Viral infection was the first suggested environmental trigger of PDB rooted in the observation of inclusion bodies in osteoclasts from affected patients that were thought to be viral nucleocapsids.\(^3\) This led to the suggestion that PDB may be caused by slow paramyxovirus infection of osteoclast precursors. Subsequent studies into the role of viral infection in PDB have been inconclusive; however, with some studies reporting positive results\(^4\) and others reporting negative results\(^5\)\(^6\)\(^7\). The most comprehensive study investigating this issue showed no evidence of paramyxovirus transcripts in PDB patients and indicated that PCR contamination could be the reason for previously reported positive findings.\(^8\) Experimental studies have shown that paramyxoviruses enhance osteoclast formation in vitro\(^9\) and that bone turnover is enhanced in mice overexpressing measles virus nucleocapsid protein in osteoclasts.\(^10\) However, these effects are not specific to paramyxoviruses because other viral proteins, such as HLTV1 Tax, have been found to enhance bone turnover in mice. Other possible environmental triggers, such as childhood dietary calcium intake,\(^11\) mechanical loading of the skeleton,\(^12\) zoonotic infections,\(^13\) and pesticides and toxins\(^14\) have also been suggested as triggers for PDB. The evidence supporting these observations is drawn from isolated reports or anecdotal observations, however, and further studies will be required to confirm or refute these observations.

Conclusion

There have been tremendous advances in knowledge and understanding of the pathogenesis of PDB over the past decade, driven to a large extent by the identification of genes that predispose to the disease. Although these studies have shown that genetic factors play a key role in the pathogenesis of PDB, the molecular and cellular mechanisms by which these genes cause the disease remain incompletely understood. Further work will therefore be required to identify the causal genetic variants; the cellular and molecular mechanisms by which they regulate bone cell function; and to determine how these genetic factors interact with environmental triggers to influence the occurrence and severity of this fascinating disease.

Conflicts of interest

The authors declare no conflicts of interest.

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