Mini-review

Genetic aspects of Paget's disease of bone

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Summary

Paget's disease of bone (PDB) is a metabolic bone disease characterized by excessive bone resorption and formation due to increased osteoclasts activity. PDB mostly runs asymptomatically, although increased bone turnover can be present and in approximately 30% of patients bone abnormalities, such as bone pain and deformities, pathological fractures and deafness may occur. The existence of familial aggregation of PDB has been reported in numerous papers, describing the occurrence of disease in successive generations. It has been clearly established that PDB is genetically heterogeneous with several loci able to conferan increased susceptibility to develop this bone metabolic disorder. In particular, the PDB3 locus in chromosome 5q35-qter hosts the sequestosome 1/p62 (*SQSTM1/p62*) gene whose mutations account for most of the sporadic and familial forms of PDB reported in literature. *SQSTM1/p62* gene encodes the SQSTM1/p62 protein, component of the NF-kB signaling pathway and mediating intracellular signaling from IL-1/TNFα toward NF-kB, crucial for osteoclast differentiation and activity. A functional study suggests that the *SQSTM1* mutation may predispose to PDB affecting the interaction between SQSTM1/p62 protein and a hitherto unidentified protein(s) modulating the bone turnover, but the underlying molecular mechanism need to be elucidated. However, independently from the knowledge of the functional aspects of *SQSTM1/p62* mutation, the opportunity to perform germline mutational analysis in PDB patients may be helpful in detecting new genetic carriers in potentially familial forms of PDB and in studying the co-segregation of such DNA variants with the PDB phenotype. All together these studies could open new possibilities in the prevention and therapy of PDB and of other metabolic bone disorders.

KEY WORDS: Paget's disease of bone, genetics, SQSTM1/p62 gene, mutational analysis.

Introduction

Several evidences reported in literature support the important of genetics in the pathogenesis of Paget's disease of bone [PDB; MIM 602080]: a) the maintenance of the hereditary pattern after the emigration (1); b) a positive familial history of PDB in affected members from multigenerational pedigrees (1-3); c) approximately 15 to 40% of index cases has at least one first degree relative affected by PDB (1, 4) and in a large number of multigenerational affected pedigrees the disease exhibits a dominant autosomal pattern of inheritance, although a male to male transmission has been also described (5).
Other clinical entities have been reported to be correlated to the clinical phenotype of PDB, in particular Familial Expansile Osteolysis [FEO; MIM 602080, 174810], characterized by similar histological features, presence of viral-like inclusions in affected osteoclasts and earlier age at onset respect to the one of "classic" PDB, and Expansile Skeletal Hyperphosphatasia [ESH; MIM 602080] that differs from both PDB and FEO (6). Finally, an early onset familial PDB variant has been originally reported in one family (7, 8), and it could be regarded as a variant of common PDB, differing for some clinical parameters.
These PDB correlated syndromes are specifically treated in another chapter in this issue of the Journal.
Thus, PDB is genetically heterogeneous with at least 7 genetic loci (*PDB1-PDB7*) initially reported to be associated to a higher susceptibility's risk to develop the disease (Table I). In particular, *PDB1* and *PDB2* loci involvement seems to be restricted to only few families (9, 10), whereas mutations of *PDB3* locus has been reported to be a common cause of both sporadic and familial PDB cases in populations of different ethnic origin (11-17). Since controversial findings have been reported for the role of *PDB1* locus into PDB pathogenesis (18, 19), we consider only six possible candidate loci identified so far (*PDB2-7*) by genome-wide searches. This review will be particularly focused on the role that *PDB3* locus plays in the pathogenesis of PDB.

PDB2 locus encodes a member of the Tumor Necrosis Factorα Receptors Superfamily: RANK (*TNFRSF11A*) gene. FEO, ESH and early onset PDB phenotypes

Mutations of *TNSFR11A* gene, located at human chromosome 18q21, have been reported to cause three different distinct clin-

Table I - PDB is a genetically heterogeneous disorder.

Chromosome	Gene
6p	*HLA-PDB1*
18q21.2-21.3	*TNFSR11A-PDB2*
5q35	*SQSTM1-PDB3*
5q31	*PDB4*
2q36	*PDB5*
10q13	*PDB6*
18q23	*PDB7*
9p21.1-q12	(?)

ical familial syndromes, although partially overlapping, resembling common PDB to a vary extent. Specifically, the mutations consist of three different insertions (duplications), located at exon 1 of the gene, affecting the signal peptide of RANK: a) 84dup18 bp accounts for FEO phenotype (7, 20, 21); b) 84dup15 bp for ESH phenotype (20, 22); and c) 75dup27 causes early onset familial PDB (7). *In vitro* analysis demonstrated that 84dup18 bp and 75dup27 insertions affect the normal proteolytic cleavage of the RANK signal peptide with consequent reduction in the amount of RANK protein and similar degree of the transcription factor nuclear factor κB (NFκB) activation (23). NFκB is essential in the molecular pathway to osteoclastogenesis and/or osteoclast activation (12).
Mutations of *TNSFR11A* gene seems to be restricted to only few affected families and several reports failed to detect both positive linkage and mutations in sporadic and familial forms of PDB. In fact, linkage to 18q21 loci is not frequent in familial PDB (12, 24, 25) and no significant association between *TNS - FR11A* gene polymorphisms and sporadic PDB has been described (7).

PDB3 locus encodes the SQSTM1/p62 protein: an ubiquitin-binding protein

SQSTM1/p62 protein has been shown to play an important role as a scaffold protein leading to the activation of NFκB (26), important factor for recruitment of pre-osteoclasts and maturation of osteoclastic cells. Mutational analyses of *SQSTM1/p62* gene, located at human chromosome 5q35, originally performed in French-Canadian and British pedigrees, identified it as the PDB3 locus, accounting for the most of both sporadic and familial PDB cases (11-17). All the *SQSTM1/p62* mutations reported in literature, either in sporadic or familial PDB cases, are within exons 7 and 8 encoding the ubiquitin-binding associated domain (UBA) (26). *SQSTM1/p62* protein has the ability to non covalently bind multi-ubiquinated chains through amino acids 386-434 in the C terminus of the protein (Figure 1), maybe storing ubiquinated proteins. As reported above, sequestosome1 is a scaffold protein in both the TNFα and interleukin 1 pathway leading to a selective activation of NFκB (26).
More than ten different *SQSTM1/p62* gene mutations, acting through a dominant negative manner, have now been widely reported in PDB affected subjects from ethnically different populations, strongly confirming its role into pathogenesis of sporadic and familial form of PDB (Figure 2). In particular, amino acidic substitution of a proline residue with a leucine residue at codon 392 (P392L), at exon 8, is the most frequently *SQSTM1/p62* described mutation in PDB cases (11-17) (Table II). In general, SQSTM1 missense mutations are mostly represented than nonsense mutations and, although the latter determine a truncated protein, no clear difference in genotype/phenotype correlation, in term of age at onset and skeletal extent of PDB, has been established (14). In fact, recently, Hocking et al. performed an elegant functional and structural analysis of three widely report missense mutations at UBA domain of *SQSTM1/p62* gene (14): P392L, M404V (methionine to valine substitution at codon 404) and G425R (glycine to arginine substitution at codon 425). They found *SQSTM1/p62* mutations in approximately 38% of the PDB families analyzed: 30% missense mutations and approximately 8% truncating mutations. No *SQSTM1/p62* mutations were reported in the remaining 62% of PDB families. In general, these Authors observed that PDB exhibiting *SQSTM1/p62* gene mutations could have a precocious age at onset and a more extent disease than nonmutant PDB subjects. More specifically, their statistical approach, although not significant, revealed a tendency to a more extent skeletal involvement and an earlier onset of PDB in affected subjects with truncating mutations (14). However, although this work confirms the importance of UBA domain-specific mutations of *SQSTM1/p62* as a cause of PDB, it suggests that whatever the *SQSTM1/p62* mutation it predisposes to PDB independently from affecting the binding properties to ubiquitin, but rather it may involve interaction between *SQSTM1/p62* protein and a hitherto unidentified protein(s) modulating the bone

Table II - *SQSTM1/p62* P392L mutation is the most frequent.

Described in
French-Canadian
Italy
Australia / Geelong
Australia / Perth
Hungary / Budapest
New Zeland / Auckland
UK / North and SouthEngland
UK /Scotland
USA
The Netherlands

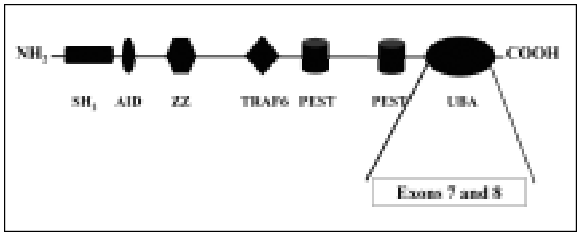

Figure 1 - Domains organization of the *SQSTM1/p62* protein. All the *SQSTM1/p62* mutations reported in literature are within exons 7 and 8 encoding the ubiquitin-binding associated domain (UBA). For further details see Geetha T, Wooten MW.J Biol Chem. 2003 [ref. 26].

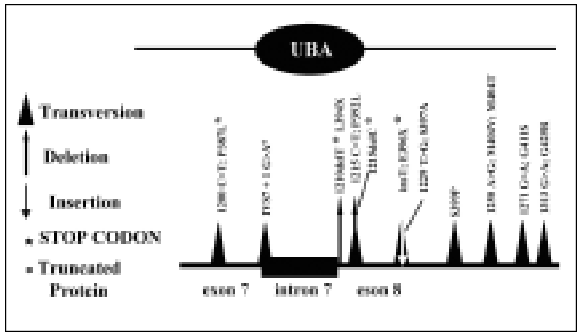

Figure 2 - UBAmutations reported in literature.See references 11-17.

turnover.
Considering the isolated UBA domain functioning as a compact monomer it may explain the hypothetical effects of the mutations on polyubiquitin binding. Both the P392L and G411S mutations seem to have light local effects on secondary structure of UBA that could be particularly relevant in full-length *SQSTM1/p62* protein. Further studies to identify the *in vivo* ubiquitylated substrates of *SQSTM1/p62* will add important information to assess the functional significance of the SQSTM1/p62-ubiquitin interaction and, consequently the disease-associated mutations.

Importance of the *SQSTM1/p62* gene mutational analysis in clinical practice

All the reports appeared in international literature clearly indicate an autosomal pattern of inheritance of familial PDB (11-17) with most of the affected subjects having a *SQSTM1/p62* mutation and several asymptomatic gene mutant carriers in last generations. Thus, mutational analysis in sporadic index case of PDB may be helpful to detect new PDB families, especially in small size pedigrees and/or in family without an apparent familial aggregation of PDB. In particular, in generations with young members (below the threshold age of onset of 55 years commonly established for clinical expression of "classic" PDB) asymptomatic gene mutant carrier(s) could be detected, providing the opportunity to perform an accurate clinical follow-up of these subjects in order to precociously detect the onset of the first PDB-related sign/symptom. Good et al. and Eekhoff et al. suggest to evaluate the circulating levels of alkaline phosphatase (AP) in asymptomatic carriers, also beneficing the low cost and the wide availability of such test (15, 16). More specifically, Eekhoff et al. wrote that "initial screening for serum AP activity followed by bone scintigraphy only when the serum AP activity is elevated can identify Paget's disease in 1 of 5 screened family members of patients with familial disease. This rate of detection of the disease increases further when only subject who are older than 40 years undergo imaging studies" (16). Unfortunately, we have to consider that PDB exhibits an incomplete penetrance that may limit the efficacy and the advantages of both genetic and biochemical tests.

Incomplete penetrance and variable expression of PDB: major pitfalls in genetic analysis of both sporadic and familial forms of PDB

Several Authors have reported an incomplete penetrance of PDB clinical expression in members from PDB families with known *SQSTM1/p62* mutation. We briefly summarize four major conditions of incomplete penetrance: 1) affected individuals from PDB family with know *SQSTM1/p62* mutation do not exhibit the segregating mutation (11-15); 2) subjects from a PDB family with known *SQSTM1/p62* mutation share the same mutation of affected members but they do not have PDB clinical expression, although they are older than 55 years of age (13); 3) *SQSTM1/p62* mutant individuals from a PDB family with known *SQSTM1/p62* mutation carry the mutation but they do not have PDB clinical expression. It may be due to their younger age (13, 16). Moreover, it should be also considered that, differently from older PDB affected, youngest carriers may have not had a long term exposure to rural environment and/or measles infection because of massive vaccination campaigns (started in 1963 in the United States of America); and 4) PDB is a genetically heterogeneous disorder with many families exhibiting incomplete penetrance and variable expression. All that indicates PDB to be a poligenic trait, with a possible second hit occurring in a modifying gene, partly explaining the lack of penetrance observed in carrier members from affected families. In particular, the possible role of modifying genes, able to control the PDB clinical expression in *SQSTM1/p62* mutant carriers, has been recently postulated for *PDB2-RANK* gene and the still unknown PDB7 locus (13-15). Johnson-Pais et al. describe a family with positive linkage to chromosome 18q21 region including *RANK* gene co-segregating with a *SQSTM1/p62* mutation in affected subjects (13). Thus, they hypothesized the existence of a possible dual modifying interaction among *RANK* and *SQSTM1/p62* genes. Conversely, Good et al. previously reported in a branch of a large PDB pedigree a positive linkage to 18q23 loci (PDB7) associated to an early onset of PDB (27). Lately, they found that this large family also exhibited a significant linkage to the 5q35 region that harbors the *SQSTM1/p62* gene. Subsequently, *SQSTM1/p62* mutational analysis revealed the presence of L394X truncating mutation segregating with all, but three, PDB affected members and within the branch exhibiting early PDB onset (PDB7). Thus, Authors hypothesized that the still unknown PDB7 locus may harbor a gene modulating the age at onset of PDB (27).

Other PDB susceptibility's gene(s)

Another rare syndrome related to PDB, named juvenile Paget's disease, has been described to be caused by inactivating mutations of the *TNFRSF11B* gene on 8q24 (28). This gene encodes osteoprotegerin (OPG). It has been well established that OPG plays a critical role in the regulation of osteoclast formation and bone resorption (29). OPG is a member of the TNF receptor superfamily acting as a physiological regulator of bone turnover by competitively binding to RANKL and preventing RANKL-induced activation of RANK on osteoclasts and osteoclast progenitors (30). Interestingly, mice over expressing OPG develop osteopetrosis secondary to the failure of osteoclast formation (31), whereas mice with targeted inactivation of OPG develop osteoporosis and bone fractures caused by increased bone turnover (32). In previous studies, mutations of both *OPG* and *RANK* genes have been excluded as a common cause of PDB (33, 34) but several polymorphisms of *TNFRSF11B* gene have been identified (34, 35). Some evidences suggest that allelic variation at the *TNFRSF11B* locus could be associated with PDB (34, 36). In a small case-control study on Belgian PDB patients an association between a single nucleotide polymorphism (SNP) in intron 2 and sporadic PDB has been described (34).
In literature, individuals with loss-of-function mutations and deletions affecting the *TNFRSF11B* gene have been described. They develop the syndrome of idiopathic hyperphosphatasia or juvenile Paget's disease, a rare disorder presenting in infancy or childhood with increased bone turnover, progressive bone deformity, bone fractures, and deafness (20, 28). Recently, a study on British descent PDB cases reveal a significant association between the *TNFRSF11B* G1181C polymorphism, at exon 1, and both sporadic and familial PDB (37). The G1181 allele is over-represented in subjects with PDB, consistent with a dominant effect of G1181 on PDB susceptibility. In a previous study the same "G" allele at position 1181 was over-represented in Danish osteoporotic fracture patients when compared with controls (35). All these findings strongly suggest that the "G" allele may predispose to an increased bone turnover. The increased risk for PDB reported in the paper by Daroszewska et al. was not as high as the one described in individuals with a first-degree relative affected with PDB (1) suggesting that the G1181C polymorphism may represent only one of the several factors contributing to the susceptibility to PDB. In order to reduce the possibility of false positive results that in association studies are caused by population stratifica-

tion, the Authors also performed a family-based study and reported the evidence of transmission disequilibrium of *TN - FRSF11B* alleles in subjects with a positive family history of PDB. Such polymorphism determines the lysine to asparagine substitution. Thus, Authors (37) speculated that the change from a positively charged lysine to an uncharged, polar asparagine in the hydrophilic N-region of the signal peptide of OPG may affect targeting and membrane insertion during OPG transport through the cytosol, potentially leading to an imbalance in availability of OPG in bone microenvironment, especially in locally stimulated increased bone turnover conditions, such as repetitive mechanical loading, trauma, viral infections, reduced calcium intake, or a combination of these factors (38-40). However, such hypothesis need further deep evaluation to be confirmed.

Conclusions

PDB is a quite common condition with a strong heterogeneous genetic component. Mutations affecting the UBA domain of *SQSTM1/p62* gene have been demonstrated to be an important cause of the disease (11, 12). However, in a functional study none of the analyzed missense mutations showed to affect the folding of the UBA domain (14). In particular, the M404V and G425R mutations have been predicted to affect the hydrophobic patch of the UBA domain binding ubiquitin in subtly different ways by either modifying the van der Waals surface contours (M404V) or by placing a highly polar side chain in the middle of the hydrophobic patch (G425R) consistent with the loss of ubiquitin chain-binding (14). Moreover, the G411S mutation, far from the hydrophobic patch, does not perturb ubiquitin chain binding. Although the clustering of the PDB-causing mutations in the UBA domain of *SQSTM1/p62* suggest that the mutant proteins cause PDB by affecting the ability of *SQSTM1/p62* to bind ubiquitin, a clear correlation between the ability of the mutant UBA domain to bind polyubiquitin and the presence or extent of PDB has not been demonstrated. Thus, the mutations of *SQSTM1/p62* may cause PDB not dependent upon the polyubiquitin binding properties of the mutant UBA domain. Mutations in the UBA domain may determine a selective loss of binding to a specific ubiquitylated substrate as suggested by the ubiquitin binding experiments conducted with unanchored ubiquitin chains (14). However, it cannot be excluded that *SQSTM1/p62* mutations may affect the overall structure of the related holoprotein, thereby affecting its half-life or other protein-protein interactions. Finally, the *SQSTM1/p62* UBA domain may interact with non-ubiquitylated substrates affecting the bone cell activity and its mutations may alter this interaction.

The OPG polymorphism found to be associated with both sporadic and familial PDB indicates the *TNFRSF11B* gene as a susceptibility gene for PDB, as well as the causal gene for the rare PDB-like syndrome of idiopathic hyperphosphatasia/juvenile Paget's disease (37). However, more association studies on large PDB populations from different Countries need to be performed in order to confirm the role of this gene in conferring susceptibility to PDB. Moreover, both wide genome linkage analysis in multigenerational PDB pedigrees and candidate gene approach will be extremely important to discover and/or confirm the existence of new PDB susceptibility genes.

Although genetic factors have been thoroughly investigating, it is important to take in mind that environmental factors, such as rural environment or viral infections, have also been implicated in the pathogenesis of PDB. In fact, the possibility that PDB arises as the result of a chronic infection of osteoclast precursors with paramyxoviruses still await for further elucidations (38-40). According to recent findings in the field of molecular genetics of PDB it can be speculated that viral proteins might interact with the mutant forms of *SQSTM1/p62* to stimulate osteoclast formation.

New molecular strategies, such as the ones offered by the "omic" technologies, will be helpful to genetically dissect the PDB pathogenesis. A better knowledge on the role that gene mutations and polymorphisms may play in the pathophysiology of PDB and bone cells function will be extremely important in order to: a) identify their effects on skeletal metabolic disorders; b) develop new therapeutical strategies.

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