Genetic aspects of Paget’s disease of bone

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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 FE0 (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.

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 involved in the pathogenesis of PDB.

PDB2 locus encodes a member of the Tumor Necrosis Factor-\(\alpha\) Receptors Superfamily: RANK (TNFRSF11A) gene. FE0, ESH and early onset PDB phenotypes

Mutations of TNFRSF11A gene, located at human chromosome 18q21, have been reported to cause three different clin-
Mutations of SQSTM1/p62 gene seem 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 TNSFR11A 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 common PDB to a vary extent. Specifically, the mutations of SQSTM1/p62 gene, acting as a cause of PDB, it suggests that whatever the 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 turnover (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).

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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 can 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 "classical" 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 benefiting from 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 once yearly, the serum AP activity is elevated can identify Paget’s disease in 1 of 5 screened family members of patients with familial disease. In fact, the rate of detection of the disease increases further when only subjects who are older than 40 years are included in these studies" (16). Unfortunately, we have to conclude that PDB exhibits a complete 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 PBD family with known 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 mesoscale pollution 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 polygenic 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 19q21 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 family an age-linked to 18q23 loci (PDB7) associated to an early onset of PDB (27). Finally, 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 (29). This gene encodes osteoprotegerin (OPG). It has been well established that OPG plays a critical role in the regulation of osteoclast forma-
tion and osteoclast resorption. The RANK-RANKL system is critical for osteoclast superfamily acting as a physiological regulator of bone turnover by competitively binding to RANKL and preventing RANKL-induced activation of RANK on osteoclasts and osteo-
clast progenitors (30). Interestingly, mice over expressing OPG develop osteopetrosis secondary to the failure of osteoclast for-
tation (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 the TNFRSF11B gene have been identified (34, 35). Some evidences suggest that allelic variation at the TNFRSF11B locus could be associated to 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 hyperphos-
phatasia or juvenile Paget’s disease, a rare disorder presenting in infancy or childhood with increased bone turnover, progres-
itive bone deformity, bone fractures, and deafness (20, 28). Recently, a study on British descent PDB cases reveal a signif-
icant association between the TNFRSF11B G1181C polymor-
phism, at exon 1, and both sporadic and familial PDB (37). The G1181 allele is over-represented in subjects with PDB, consis-
tent 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 indi-
viduals with a first-degree relative affected with PDB (1) sug-
gesting 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 stratafa-
tion, the Authors also performed a family-based study and reported the evidence of transmission disequilibrium of TNFRSF11B 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 terminus 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. 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 binding and the signal peptide mutation, far from the hydrophobic patch, does not perturb ubiquitin chain binding. Although the clustering of the “DB”-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 deep correlation between the ability of the mutant UBA domain to bind polyubiquitin and the presence of a variant of PDB has not been demonstrated. Thus, the mutations of SQSTM1/p62 may cause PDB of independent on the polyubiquitylation properties of the mutant UBA domain. Mutations of 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 the 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 awaits 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 therapeutic strategies.

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