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REVIEW

Genotype–phenotype correlation in Juvenile Paget disease: role of molecular alterations of the *TNFRSF11B* gene

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Abstract Juvenile Paget disease (JPD) {MIM 239000} is a rare inherited bone disease that affects children. The patients affected with JPD present an altered bone turnover, therefore, show a phenotype characterized by progressive bone deformities, fractures, and short stature. Deletions or missense mutations of the *TNFRSN11B* gene are common in these children. This gene encodes a soluble protein, the osteoprotegerin, which leads to uncontrolled osteoclastogenesis when mutated. JPD is characterized by a strong genotype–phenotype correlation, so depending on the alteration of the *TNFRSN11B* gene, the phenotype is variable. This review describes the different clinical features which are characteristic of JPD and the correspondence with the different molecular alterations of the *TNFRSN11B* gene.

Keywords Juvenile Paget disease (JPD) · Osteoprotegerin · Genotype · Phenotype

Introduction

Paget's disease of bone (PDB) is the second most common skeletal disorder after osteoporosis and affects 1–2 % of adults over 50 [1]. The disease is characterized by focal abnormalities of increased bone turnover affecting one (monostotic) or more sites (polyostotic) throughout the skeleton. Pagetic bone lesions show evidence of increased osteoclastic bone resorption, enhanced but disorganized bone formation with increased number of osteoblasts (OBs) and osteocytes (per mm^3 of bone), with a decreased grade of organization of their canalicular net, marrow fibrosis, and increased vascularity of bone [1, 2]. However, the primary cellular abnormality resides in the osteoclasts (OCs) [2, 3]. OCs in PDB have more nuclei/OC, are markedly increased in number and size, have an augmented rate of formation, and a major bone resorbing capacity per OC. These OCs also contain characteristic nuclear inclusion bodies, which are microcylindrical structures that in some respects resemble virus particles [4]. However, the true identity of the inclusions has not yet been established and remains a subject of ongoing debate [4]. In addition, OC precursors in peripheral blood and bone marrow from patients with the disease show increased sensitivity to factors that stimulate bone resorption, including 1,25 dihydroxyvitamin D and receptor activator for nuclear factor κB (NFκB) ligand (RANKL) [5, 6]. Although PBD has traditionally been regarded as a disease of the OCs, evidences suggest also the involvement of mesenchymal cells and OBs in the pathogenesis of this disease. In particular, OBs cultured from Pagetic bone lesions are abnormal since they show increased expression of genes such as interleukin 1, interleukin 6, and dickkopf 1, which could also contribute to the focal abnormalities of bone turnover that characterize the disease [7]. Although not all agree with this theory [7], cultures of bone marrow stromal cells from patients with PDB seem to show a greater ability to promote OC formation and to express increased amounts of RANKL compared to the stromal cells from healthy controls [8, 9]. In addition, patients with Paget's disease have circulating levels of RANKL greater than controls [10]. These abnormalities in the activity of bone cells lead

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to the production of bone with a disorganized architecture and reduced mechanical strength, leaving patients with Paget's disease at increased risk of developing deformities and pathological fractures [11, 12]. In most cases, PBD is asymptomatic [12]; normally, it is diagnosed at a later stage of the disease progression by radiologic findings or by elevated serum levels of alkaline phosphatase. Symptoms specifically related to the disease are bone pain and deformities of long bones [11]. Pain is usually caused by the lesion itself or by complications, such as bone deformities, fractures, osteoarthritis, or nerve compression. A rare but severe complication is the sarcomatous transformation [13–15].

PDB is a complex disorder whose pathogenesis is determined by both environmental and genetic factors. Several potential environmental triggers for PDB have been suggested on the basis of epidemiological studies, observational data, or hypotheses, including low dietary calcium intake or vitamin D deficiency during childhood [16, 17], zoonotic or viral infections [18, 19], and occupational exposure to toxins [20]. The only environmental trigger for this disease that has been studied experimentally is the paramyxovirus infection, which was first suggested when OCs from affected patients were shown to have inclusion bodies resembling viral nucleocapsids [4]. Since this time, many investigators have attempted to detect evidence of paramyxoviruses in bone and blood samples from patients with this disease, but the results have been conflicting [21–24]. In addition, very recently, it has been reported that among Italian PDB cases, those originating from Campania region have the highest prevalence of animal contacts, showing also an increased number of affected sites than patients without animal contacts [25]. However, further studies needed to support these findings in other large affected populations from different geographical areas. On the other hand, the animal related factors may be important in the etiology of PDB and may interact with genetic factors influencing disease severity [25]. In fact, there is a strong genetic predisposition in subjects who develop PDB, and mutations in the *SQSTM1/p62* gene, encoding an important scaffold protein involved in OC differentiation, have been associated with familial and sporadic disease in up to 40 % of cases [26–28].

Several rare inherited bone diseases show phenotypic overlap with classical PDB, because they are characterized by increased bone turnover, bone deformity, bone expansion, and elevated serum levels of alkaline phosphatase. These diseases include familial expansile osteolysis [29], expansile skeletal hyperphosphatasia [30], early-onset Paget's disease [31], juvenile Paget's disease (JPD) [32], and the syndrome of hereditary inclusion-body myopathy, PDB, and frontotemporal dementia (IBMPFD) [33]. With the exception of JPD, which is an autosomal recessive condition, the other disorders are inherited in an autosomal dominant way. These diseases seem develop earlier than PDB. In this review we focalized our attention on JPD.

Juvenile Paget's disease

JPD also referred to as idiopathic hyperphosphatasia and osteoectasia with hyperphosphatasia is a rare autosomal recessive condition, of which about 50 cases have been reported worldwide [28]. It is a generalized skeletal disorder characterized by markedly increased bone turnover. This is manifested histologically by increased numbers of both OCs and OBs in bone, and is demonstrated biochemically by a major rate of excretion of type I collagen breakdown products, very high plasma alkaline phosphatase activity, from which the disorder takes its name, and elevated serum osteocalcin levels [34–36]. Woven bone predominates in the calvarium, vertebrae, and ribs [37–39]. Cortices of tubular bones can be thinned or thickened along their entire lengths from accelerated endosteal bone remodeling [36, 37, 40–42]. Increased periosteal bone formation thickens diaphyses and widens individual bones [38, 42, 43]. The clinical consequence of generalized acceleration of bone remodeling in JPD are extremities that are bowed, painful, and easily fractured [34–43]. Patients suffer short stature, kyphoscoliosis, chest wall deformity, and skull enlargement that may lead to cranial nerve deficits. There can be foci of bony sclerosis and rarefaction. In particular, short tubular bones may be wide and somewhat sclerotic [44]. Increased thickness of the skull with wide diploic spaces and focal areas of sclerosis and uneven mineralization can be seen [35, 45–47]. Vertebral bodies may be sclerotic or osteopenic and sometimes manifest compression fractures [36, 47]. In the pelvis, in addition to thick trabeculae and areas of sclerosis, there can be some areas of rarefaction [44, 46, 47]. Ribs may be sclerotic, narrowed, or widened. Radiographs may show evidence of fractures that possibly occurred without trauma, but healing is complete [35, 45]. JPD symptoms and signs are usually evident from early infancy, when the disease presents with skeletal deformity and failure to thrive. It is usually recognized in infancy or early childhood, depending on the severity of the phenotype, which could be classified as follows:*severe*, onset of deformity recognized in the first 18 months of life; walking ability is not acquired at all or is delayed and not maintained past 5 years, height is less the third percentile. The radiographs of subjects with severe phenotype of JPD showed diaphyseal widening, cortical thinning, new periosteal bone formation, and long bone deformity before the age of 15 months; *intermediate*, onset after 2 years, gained ability to walk at normal age, gradual onset of deformities with growth, height less than the third

percentile. Their radiographs showed coarse widening of the diaphyses with cortical thickening. The phenotype tends to be more severe with aging; *mild*, deformity recognized after 2 years, normal walking ability and stature [48]. Although JPD has some similarities to classical PDB, it is clearly a more severe condition as attested by the early age at onset and the development of marked bone deformity during childhood. Deletion or mutations of the *TNFRSF11B* gene, a member of the tumor necrosis factor (TNF) receptor superfamily encoding OPG [32], have been implicated in the etiology of JPD [48, 49].

Functions of OPG protein, the encoded product of *TNFRSF11B* gene

OPG is normally secreted into the marrow space by cells derived from mesenchyme, such as preosteoblasts and OBs [50]. OPG acts as a decoy receptor for RANKL to its receptor RANK, the crucial osteoclast differentiation and survival receptor. Normally, the binding of RANKL to RANK on OC precursors leads to activation of specific transcription factors, which in turn regulate the osteoclastogenesis. Consequently, OPG can block OC formation and bone resorption in vivo by binding to RANKL and reducing its ability to interact with RANK. Therefore, OPG is a critical regulator of osteoclastogenesis. The gene which encodes for this protein is the *TNFRSF11B,* is located on chromosome 8q23–24. Mice knockout for *TNFRSF11B* gene have osteoporosis, show numerous OCs and rapidly remodeling woven bone [51], thus showing similarity to JPD.

OPG is initially synthesized as a ~55 kDa monomer within the cell, after converted to a disulfide-linked dimer of ~110 kDa, which is then secreted into the marrow space. While smaller amounts of monomeric OPG are also secreted, the predominant extracellular form of OPG is a disulfide-linked dimer [52]. This protein contains two domains: N-terminal half, which harbors four tandem cysteine-rich TNFR motifs, and C-terminal half, which is unrelated to any known protein sequences but appears to function in the association of OPG monomers as they are processed within the secretory pathway. The presence of 2–6 cysteine-rich domains in the extracellular ligand-binding region is the classical signature of the TNFR family, of which OPG is a member. Each domain has cysteine residues that form two or three disulfide bridges: SS1, SS2, and SS3.

Genetic and clinical studies in patients with JDP

Previous studies have reported alterations of the *TNFRSF11B* gene in JPD patients. Whyte and coworkers have suggested that JPD would be more appropriately named as "OPG deficiency" [53]. The patients show different phenotypes on the basis of gene alterations. The Table 1 shows the genotype–phenotype correlations in JPD patients.

The loss of the entire gene, the 20-bp deletion and the two missense mutations Cys65Arg and Cys87Tyr are responsible for a severe phenotype, even though they alter gene function in a different way from each other.

The first two patients diagnosed with severe phenotype of JPD showed the following phenotype: the one-year-old patient was small, deaf, and weak and with a disproportionately large head, short humeri, laterally bowed femora, anteriorly curved tibiae, markedly delayed gross motor skills, and poor muscle tone. The 26-year-old woman was deaf, severely deformed, and incapacitated [32]. In both the patients the alkaline phosphatase activity was elevated. Both patients had a homozygous deletion of *TNFRSF11B* gene spanning ~30 kb [32]. The OPG was undetectable in the serum sample from patients.

The 20-bp deletion of the *TNFRSF11B* gene begins in the exon 3 and includes the splice donor site GT residue of the conserved AGGT exon–intron junction sequence. This mutation produces a truncated, unstable protein and causes a severe phenotype [48].

The T287C (Cys65Arg) and G354A (Cys87Tyr) missense mutations disrupt the disulfide bridge, thus leading to loss of the native OPG structure and disruption of binding of OPG to its ligand, RANKL [48].

The intermediate phenotype is caused by two gene alterations: a missense mutation T443C (Phe117Leu) and the 638_640delGAC (Asp182del).

The T443C (Phe117Leu) mutation alters the amino acid directly adjacent to a cysteine residue involved in the SS1

Table 1 Mutation of the *TNFRSF11B* gene

Article	Mutation	Region	Phenotype
Whyte et al. [32]	Gene deletion	Whole gene	Severe
Chong et al. [48]	20pb deletion 686delG IVS3+19	Junction exon3/ intron3	Severe
Chong et al. [48]	T287C Cys65Arg	Exon 2	Severe
Chong et al. [48]	G354A Cys87Tyr	Exon 2	Severe
Chong et al. [48]	638_640delGAC delAsp182	Exon 3	Intermediate
Chong et al. [48]	T443C Phe117Leu	Exon 2	Intermediate
Chong et al. [48]	965_967delTGA 969_970insTT	Exon 5	Mild/ Intermediate

bond of cysteine-rich domain III of OPG. There are several possible consequences of this mutation. One is that it could affect the ability of the adjacent Cys118 to form the SS1 bond, which is critical in domain III of OPG (especially as there is no SS2 bond in this domain). On the other hand, Phe117 could be critical for stacking against other aromatic residues within these domains. Aromatic residues are important in stabilizing cysteine-rich domains of TNFR family members. Moreover, Phe117 is not conserved among other TNFR family members, so it may contribute to ligand-binding specificity of OPG. The substitution of leucine for phenylalanine could disrupt the tight packing that is characteristic of hydrophobic interactions at the receptor/ligand-binding surface; therefore, OPG protein could have a reduced RANKL-binding affinity [48].

The 638_640delGAC (Asp182del) mutation is also located within one of the cysteine-rich domains (IV). The deleted residue is part of a highly conserved consensus motif preceding the last cysteine of each cysteine-rich domain. This motif is involved in the formation of hydrogen bonds that anchor the loop structure and stabilize the disulfide bond [48]. Therefore, the deletion of aspartate 182 in this site leads to a structural instability and abnormal intracellular secretion of the mutant protein (OPGΔD182) [48]. Decreased levels of OPG in the extracellular environment, together with reduced binding of OPGΔD182 to RANKL, are likely to explain the dysregulated osteoclastogenesis observed in JPD affected patients. Moreover, OPGΔD182 is hyperglycosylated leading to altered folding and decreased structural stability of the protein [54].

Mild phenotype is caused by a single mutation: the insertion/deletion mutation (965_967delTGA 969_970insTT) that occurs in exon 5 causes a premature stop codon resulting in a truncated protein. [48]. This mutation causes the inability of the mutant protein to homodimerize and to bind RANKL [55]. The 965_967delTGA 969_970insTT mutation was also found in a patient with intermediate phenotype of JPD who was affected by muscle atrophy or weakness, mild respiratory problems as a result of kyphosis, and who had a disproportionately large head with a broad mandible and maxilla and a huge cranial vault, curved tibia, and short humeri [55].

Conclusions

JPD is a rare autosomal recessive disease characterized by increased bone turnover that occurs in infancy or early childhood. The patients with JPD show clinical features characterized by skeletal deformity, bone expansion, bone pain, and an increased risk of pathological fractures. In recent years, genetic studies demonstrated that mutations in the gene *TNFRSN11B*, encoding the protein OPG, could determine the phenotype of the JPD. Defective OPG is likely to cause accelerated bone resorption through the unimpeded interaction of RANKL with RANK, leading to enhanced OC formation and resorption. Approximately 50 cases of JPD have been reported worldwide. In this review the most important clinical cases have been described. The *TNFRSN11B* gene mutations described are associated to JPD, and the phenotypic variability shown by patients appears to be closely related to the degree of disruption of the OPG molecule. In fact, the most severe phenotype is caused by the loss of the entire gene and then by the lack of the protein, while the mildest phenotype is caused by an amino acid change which produces a truncated protein with reduced activity. The role of OPG in JPD has been recently confirmed by a clinical study on two adult siblings affected with JPD resulting from homozygous inactivating mutations in the *TNFRSN11B* gene, in which remarkable responses to experimental treatment with recombinant OPG has been reported [56].

Conflict of interests None.

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