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Rare Inherited forms of Paget's Disease and related syndromes

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Abstract

Several rare inherited disorders have been described that show phenotypic overlap with Paget's disease of bone (PDB) and in which PDB is a component of a multisystem disorder affecting muscle and the central nervous system. These conditions are the subject of this review article. Insertion mutations within exon 1 of the *TNFRSF11A* gene, encoding the Receptor Activator of Nuclear Factor Kappa B (RANK) cause severe, PDB-like disorders including Familial Expansile Osteolysis, Early-onset familial PDB and Expansile Skeletal Hyperphosphatasia. The mutations interfere with normal processing of RANK and cause osteoclast activation through activation of nuclear factor kappa B (NFκB) independent of RANK Ligand stimulation. Recessive, loss of function mutations in the *TNFRSF11B* gene, which encodes osteoprotegerin, cause juvenile PDB and here the bone disease is due to unopposed activation of RANK by RANKL. Multisystem proteinopathy is a disorder characterised by myopathy and neurodegeneration in which PDB is often an integral component. It may be caused by mutations in several genes including *VCP*, *HNRNPA1*, *HNRNPA2B1*, *SQSTM1*, *MATR3*, and *TIA1*, some of which are involved in classical PDB. The mechanisms of osteoclast activation in these conditions is less clear but may involve NFκB activation through sequestration of IκB. The evidence base for management of these disorders is somewhat limited due to the fact they are extremely rare. Bisphosphonates have been successfully used to gain control of elevated bone remodeling but as yet, no effective treatment exists for the treatment of the muscle and neurological manifestations of MSP syndromes.

Keywords

familial expansile osteolysis; expansile skeletal hyperphosphatasia; juvenile Paget's disease; multisystem proteinopathy

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Conflicts of Interest

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Introduction

Paget's disease of the bone (PDB) is a common skeletal disorder caused by focal abnormalities of the bone remodelling process which leads which can present with various features including bone pain, bone deformity, deafness, pathological fractures and rarely osteosarcoma. Several rare inherited bone disorders have also been described that show phenotypic overlap with classical PDB but which have an earlier age at onset and which are more severe. They include familial expansile osteolysis (FEO) [1] expansile skeletal hyperphosphatasia (ESH) [2] Early-onset familial Paget's disease [3]. Juvenile Paget's disease [4], and panostotic expansile bone disease [5]. In addition, classical PDB is recognised to be a component of rare inherited syndromes with non-skeletal features including inclusion body myopathy, frontotemporal dementia, and amyotrophic lateral sclerosis disease [6].

The disorders discussed in this review are mostly inherited in an autosomal dominant manner in which the PDB-like component is variably penetrant, although Juvenile PDB is an autosomal recessive condition. In general, the PDB component in these disorders has an earlier age of onset than classical PDB and in the case of the syndromes caused by *TNFRSF11A* and *TNFRSF11B* mutations is much more severe. The presentation, clinical features, pathogenesis and management of classical PDB are discussed by Gennari in an accompanying article in this issue [7].

Inherited Expansile Osteolytic syndromes

These disorders are characterised clinically by early onset deafness, premature tooth loss and expansile bone lesions predominantly affecting the axial skeleton. They are caused by insertion mutations of between 12 and 27 base pairs within the first exon of *TNFRSF11A* which encodes the Receptor Activator of Nuclear Factor Kappa B (RANK) (Figure 1). Current evidence suggests that these mutations inhibit or prevent cleavage of the RANK signal peptide causing the abnormal protein to accumulate in the Golgi apparatus which in turn causes activation of NF κ B signalling through the unfolded protein response (Figure 2). There is considerable heterogeneity of clinical phenotype in subjects with different mutations and in those who carry the same mutation both between and within families. In this review, the individual disorders are discussed on the basis of clinical phenotypes which are summarised in Table 1.

Familial Expansile Osteolysis

Clinical features

In 1988, Osterberg and colleagues [1] coined the term "familial expansile osteolysis" (FEO; MIM 174810) to describe a bone dysplasia characterised by the presence of focal expansile bone lesions predominantly affecting the lower limbs, accompanied by bone pain, deformity and pathological fractures. In fact, a similar disorder affecting two brothers had been described about 10 years earlier by Enderle and Willert [8].

The initial presentation of the disorder in affected members of the family described by Osterberg was with hearing loss, initially of a conductive type during the first or second decade, which subsequently evolved into a mixed sensorineural type of deafness. The cause of the deafness was attributable to damage of the bones of the middle ear. A characteristic feature on otological examination was replacement of the incus by fibrous tissue. Musculoskeletal manifestations of the disease include deformity and bone pain predominantly affecting the appendicular skeleton. Common sites of involvement were the tibia, fibula and humerus. Pathological fractures were common and reported to occur in 12 of 97 lesions observed (12.4%). Dental abnormalities were also common, presenting with excessively mobile teeth, fractured teeth and premature tooth loss in the second decade. Further reports of FEO were later published affecting individuals from Spain [9] and the USA [10, 11]. The clinical presentation in a three-generation Spanish family reported by Palenzuela and colleagues [9] was with deafness during the first decade, followed by tooth loss and other dental problems in the second decade. It is of interest that focal osteolytic lesions were not prominent in this family and occurred in only two of the subjects studied. In contrast, expansile osteolytic lesions were a prominent feature in affected members of a five-generation family reported by Whyte [11]. The proband first presented age 63 with a pathological fracture of the humerus occurring at the site of a lytic lesion but there was no history of early onset deafness or early tooth loss. Of the eight affected family members where data were available, 6 (75%) had expansile osteolytic lesions; 6 (75%) had early-onset deafness but only 1 (12.5%) had early tooth loss. In another study, Johnson-Pais [10] reported the phenotype of two unrelated individuals with FEO, both had early onset deafness and premature tooth loss and both had osteolytic lesions.

Focal and generalized abnormalities of bone have both been described in patients with FEO. Osterberg commented that abnormalities in the trabecular pattern of bone on x-rays could be identified affecting the humerus, radius, ulna and tibia, before osteolytic lesions develop. The focal osteolytic bone lesions which developed in Osterberg's family most commonly affected the tibia (31%), radius (11%), fibula (10%), humerus (9%) and femur (9%) [1]. In contrast to classical PDB, the pelvis, spine and skull were unaffected. The rate at which the lytic bone disease advanced in the long bones was estimated to be 10-18mm per year which is much more rapid than in classical PDB [1]. Radionuclide bone scans show increased tracer uptake at the site of focal osteolytic lesions. In addition, tracer uptake was reported to be increased in the tibiae of patients without focal bone lesions in keeping with a generalized elevation in bone turnover. Biochemical evaluation of affected patients showed elevated circulating concentrations of total alkaline phosphatase (ALP) and hydroxyproline consistent with a state of high bone turnover.

The clinical features of FEO are entirely consistent with dysregulation of osteoclast activity. Although bone formation is also increased, as reflected by the elevations in ALP concentrations this is thought to be the result of coupling between bone resorption and formation as occurs in classical PDB. Although there are many similarities between FEO with PDB, the severity and pattern of skeletal involvement in these two disorders are distinct.

Genetics

The gene responsible for FEO in the family described by Osterberg was localised to chromosome 18q21 by Hughes and colleagues [12]. Subsequently, mutations in exon 1 of the *TNFRSF11A* gene were identified as the cause of FEO [13]. The *TNFRSF11A* gene encodes Receptor Activator of NF κ B (RANK) [14] which plays a critical role in osteoclast differentiation and function when activated by its ligand RANK ligand (RANKL) [15]. The causal mutation in FEO was found to be a 18-bp duplication at position 84 of exon 1 (84dupl8) that segregated with the disease in all affected family members and in the families described by Whyte [11] and one affected individual described by Enderle and Willert [8]. Subsequently the same mutation was identified in the FEO patients described by Johnson-Pais [10] and Palenzuela [9]. This region of *TNFRSF11A* consists of a repeated GC-rich sequence (Figure 1), raising leading the possibility that slippage during the process of recombination resulted in the duplication.

Pathophysiology

The 84dupl8 mutation is predicted to elongate the RANK signal peptide by six amino-acids and bio-informatic studies coupled with studies of human embryonic kidney cells (HEK293) cells transfected with wild type and mutant RANK proteins showed that the mutations prevented cleavage of the signal peptide [13, 16]. These studies also showed that the mutant protein was expressed at a lower level than wild type. Transient expression of the 84dupl8 mutation in the same cells showed increased activation of an NF κ B reporter vector when compared with wild type RANK but only when the levels of expression were corrected for the lower amounts of protein present with the 84dupl8 mutation [13]. Crockett and colleagues subsequently performed a series of studies to further elucidate the mechanisms by which the 84dupl8 insertion mutation caused osteoclast activation [16]. At the same time Crockett investigated the functional effects of the 75dupl7 and 84dupl5 mutations which cause the related disorders of early onset familial PDB [3] and expansile skeletal hyperphosphatasia [2] (discussed later in this review). These experiments confirmed that the all three mutations prevented signal peptide cleavage and also showed that overexpression of wild type and mutant RANK proteins activated NF κ B signalling *In vitro*. Crockett further demonstrated that the mutant RANK proteins were not expressed at the cell membrane as they should be, but instead became trapped in the endoplasmic reticulum (ER). It was also found that when the mutant proteins were stably expressed as a single copy in HEK293 cells, they did not cause constitutive activation of RANK signaling. On the contrary cells expressing the mutant proteins were unable to increase NF κ B signalling when stimulated with RANKL. This led Crockett to the conclusion that these were loss-of-function mutations with regard to RANKL induced RANK signaling. Support for the concept that the insertion mutations are loss-of-function in terms of classical RANKL-RANK signaling comes from the observation presented so far in abstract form only, that mice homozygous for the 75dup27 mutation exhibit an osteopetrotic phenotype with absence of functioning osteoclasts [17]. The conundrum remains as to why these mutations are associated with osteoclast activation and high bone turnover *in vivo*. This has not yet been fully explained but the authors speculated [16] that the osteoclast activation seen in this and related mutations may be due to ligand independent activation of NF κ B as the result of the mutant proteins being resident in the ER [18].

Management

Medical management of FEO has focused on the use of osteoclast inhibitors. Affected members of the family described by Osterberg have been treated at various points with calcitonin, dichloromethylene bisphosphonate and pamidronate [19]. Calcitonin and dichloromethylene bisphosphonate were reported to give biochemical improvement as reflected by reductions in markers of bone turnover and clinical improvement in terms of bone pain but this was reported to be short lived. Intravenous pamidronate was reported to give a more sustained clinical and biochemical improvement in affected family members with evidence in some cases of remineralisation of the osteolytic lesions [19]. The long-term effects of these drugs on the natural history of FEO remains unclear

Expansile Skeletal Hyperphosphatasia

Clinical features

The term “expansile skeletal hyperphosphatasia” was coined by Whyte and colleagues to describe an inherited osteolytic disorder affecting a mother and her 13 year-old daughter, [2]. Both individuals had presented with early onset deafness and premature tooth loss. Subsequently bone involvement became more prominent with hyperostotic expansion of several long bones and particularly the phalanges of the hands. Hypercalcaemia was reported during lactation and periods of immobilization in the mother. Serum alkaline phosphatase and other markers of bone turnover were elevated in both affected individuals.

Genetics

Genetic analysis showed that the disorder was caused by a 15bp duplication at position 84 (84dup15) in *TNFRSF11A* affecting the signal peptide of RANK.

Pathophysiology

The pathophysiology of this disorder was investigated by Crockett [16] and is thought to be similar to that of FEO and early onset familial PDB; namely, failure of signal peptide cleavage of RANK with sequestration of the protein in the endoplasmic reticulum and activation of NF κ B signaling through accumulation of the abnormal protein in the ER.

Management

The mother received treatment with calcitonin, etidronate and pamidronate; all three treatments reduce biochemical markers of bone turnover and (in the case of the mother) improved hypercalcaemia but the authors reported that they had little effect on bone pain.

Early-onset familial Paget’s disease

Clinical features

This autosomal dominant disorder has so far been reported in one family of Japanese descent [3]. The initial presentation in affected family members was with hearing loss in the first to second decade followed by premature tooth loss in the second to third decade, Musculoskeletal symptoms including bone pain and deformity developed subsequently. This

disease affected both the axial and appendicular skeleton. Radionuclide bone scan examination showed increased tracer uptake in affected bones and radiographs showed mixed osteolytic and osteosclerotic lesions reminiscent of classical PDB. Two affected individuals had striking involvement of the maxilla and the mandible. Biochemical investigations showed markedly elevated levels of ALP and one patient developed an episode of hypercalcaemia during a period of immobilization. A second family of Chinese origin with a similar disorder was described by Ke and colleagues [20]. In this family the presentation was with expansile bone lesions in a distribution similar to that reported in the Japanese family accompanied by deformity, pain and pathological fracture. In one patient however, the only manifestation was evidence of increased bone turnover. The authors did not mention whether the affected patients had suffered deafness or premature tooth loss.

Genetics

The cause of the disorder in the Japanese family [3] was found to be a 27bp duplication within exon 1 of the *TNFRSF11A* gene [13]. In the Chinese family [20] there was a similar 27bp duplication but starting at position 78 (78dup27) instead of 75.

Pathophysiology

As in the case of the FEO mutation, this duplication was found to prevent cleavage of the signal peptide of RANK, causing accumulation of the abnormal protein in the ER [16].

Management

Affected patients in the Japanese family were treated with various antiresorptive drugs including etidronate, pamidronate, risedronate and calcitonin. Etidronate treatment given to three patients reduced biochemical markers of bone turnover but did not normalize them. Pamidronate and risedronate were reported to be more effective than etidronate at reducing markers of bone turnover. One patient treated with pamidronate reported an improvement in bone pain. There was no discernable effect of treatment on deafness or bone deformity in any patient. One patient in the Chinese family was treated with intravenous ibandronate which normalized ALP although the effects on pain were not reported.

Juvenile PDB

A female individual with an early onset PDB like syndrome due to a *TNFRSF11A* mutation has been described [21]. The presentation was with tooth loss and hearing loss and low trauma fractures of the tibia and fibula at aged 2 but the patient was also noted to have femoral deformity by ultrasound *In utero*. At aged 4 years the patient was immobilized following a fracture and developed hypercalcaemia. Biochemical markers of bone turnover were markedly elevated. Radionuclide bone scan at aged 3 revealed increased tracer uptake affecting several bones and radiographs showed changes that were thought to be consistent with juvenile PDB. However, genetic analysis excluded the presence of mutations in *TNFRSF11B* and instead the patient was found to have a heterozygous 15bp insertion mutation at position 87 in exon 1 of *TNFRSF11A* (87dup15). Although the mutation is the same size as the 15bp mutation causing FEO, the sequence change is slightly different as is the site of insertion (Figure 1). The pathogenesis is likely to be the same as in the other

conditions with *TNFRSF11A* insertion mutations. The patient was treated pamidronate between the age of 4 and 11 years. This reduced biochemical markers of bone turnover this didn't appear to modify progression of deafness or tooth loss. The patient wasn't reported to have pain either before or after pamidronate.

Panostotic expansile bone disease

The term “panostotic expansile bone disease” was coined to describe the clinical phenotype in a 32-year-old man who presented with an expansile bone disease affecting virtually the whole skeleton accompanied by a massive benign tumour affecting the mandible and benign tumours of the maxilla [5].

The initial presentation was with congenital deafness but subsequently the patient developed bone deformity of the lower limbs from the age of about 4 years. The patient was edentulous and his mother reported that his deciduous teeth were never replaced by permanent teeth. From the age of 12 years he suffered multiple fragility fractures after minimal trauma. The mandibular tumour developed during his teenage years and he was hospitalised on several occasions for anaemia secondary to bleeding from this tumour which was found on histological examination to show features of a benign fibro-osseous tumour. The patient also developed a tumour affecting the maxilla and there was a history of recurrent renal stones. The patient suffered widespread bone pain. Biochemical studies showed greatly elevated levels of total serum alkaline phosphatase as well as bone specific alkaline phosphatase. He had hyperuricaemia in the presence of normal renal function. Urinary calcium excretion was not elevated.

Genetics

Genetic analysis showed that the disorder was caused by a 12bp duplication at position 84 (84dup12) in *TNFRSF11A* affecting the signal peptide of RANK. (Figure 1). The pathophysiology of this disorder has not been investigated but the authors of the report speculated that it was likely to be failure of signal peptide cleavage of RANK as had been described for other mutations in this region.

Management

The patients jaw lesions were treated successfully by surgery. Prior to this he was treated with alendronate which reduced biochemical markers of bone turnover to near normal levels but which also caused hypocalcaemia despite the fact that calcium and vitamin D supplements were given. Pamidronate therapy at low dose (10mg, intravenously) was subsequently used to suppress bone turnover along with calcium and vitamin D supplements. Although the patient responded well in terms of biochemical markers, his clinical response was disappointing with little or no improvement in bone pain (personal communication, Dr Anne Schafer)

Juvenile Paget's disease

Juvenile Paget's disease (JPD) (MIM 239000) is a rare, autosomal recessive disorder which was originally described in the 1950's [4]. Other names for the condition include Idiopathic

Hyperphosphatasia and Familial Hyperphosphatasemia. The disease is characterised by markedly increased bone turnover; skeletal deformities reminiscent of classical PDB, bone expansion, bone pain, and an increased risk of pathological fractures.

Clinical features

The presentation is during infancy or childhood with skeletal deformity, growth retardation and failure to thrive. Other clinical features include; enlargement of the skull, immobility or difficulty in walking, progressive sensorineural deafness, kyphosis and acetabular protrusion.

The phenotype is variable, depending on the type of mutation. Chong and colleagues [22] suggested a simple clinical classification for the disorder based on age at onset, mobility and height (Table 2).

In all except the very mildest cases of JPD bone deformity increases during adolescence and this is often accompanied by loss of mobility. Interestingly, fractures occur more commonly in patients with a mild or intermediate phenotype, presumably because they are more mobile [22]. Extra-skeletal manifestations may become prominent later in life. Most patients develop a retinopathy by the second decade. This is detectable on fundoscopy by the appearance of angioid streaks, pallor of the optic disc, peripapillary atrophy and evidence of choroidal neovascularization. Vascular calcification is another recognised feature which becomes more penetrant with increasing age. Aneurysms of the internal carotid artery have also been reported [23]. Other features which have been reported include contractures, which seem most likely to be secondary to deformity, sinus aplasia, hormone deficiencies and hypertension [24]. Difficulties with intubation have been reported due to calcification of tracheal cartilage [25]. Auricular calcification may also occur [26]. Concentrations of serum alkaline phosphatase, and other markers of bone turnover are greatly elevated in JPD, reflecting the generalised increase in the rate of bone remodelling. The bones are enlarged with radiological features reminiscent of PDB. Serum concentrations of OPG are not helpful in diagnosis; while circulating OPG concentrations are undetectable in some patients; others have normal values and elevated values have been reported in patients that carry the so-called Balkan mutation which causes a frameshift in exon 5 [27].

Genetics

Juvenile PDB is most commonly caused by recessive loss-of-function mutations in the *TNFRSF11B* gene which encodes osteoprotegerin, although a similar phenotype has been reported in a patient with a mutation of *TNFRSF11A* [21] as discussed in the previous section of this article. Osteoprotegerin is a member of the TNF-receptor superfamily, which acts as a soluble decoy receptor for RANK-ligand, blocking osteoclast activation and bone resorption [15]. Deficiency of OPG in JPD was first reported by Whyte and colleagues who described two Navajo patients with JPD with undetectable circulating concentrations of OPG. Genetic analysis revealed a homozygous deletion of the *TNFRSF11B* gene as the cause of the disorder [28]. Subsequently, Cundy and colleagues described a family of Iraqi origin, in which three of nine siblings had JPD. They found suggestive evidence of linkage to the *TNFRSF11B* locus on chromosome 8q24. Mutation screening of *TNFRSF11B* in this family revealed a homozygous 3bp deletion in all three affected siblings, which was

predicted to result in the loss of an aspartate residue that is highly conserved in members of the TNF-receptor superfamily [29]. To-date several disease-causing mutations in *TNFRSF11B* have been so far identified in JPD, as summarised in Table 3

Pathophysiology

The cause of JPD is deficiency or absence of a fully functional osteoprotegerin which causes unopposed activation of RANK signaling in bone and other tissues, most notably the cardiovascular system. Most of the JPD-associated *TNFRSF11B* mutations have only been evaluated by in silico techniques for their predicted effects on OPG function. However, Cundy and colleagues conducted a detailed analysis of the functional consequences of the DelArg182 in vitro [29, 35]. They showed that the mutant form of OPG was over-glycosylated; failed to inhibit osteoclastic resorption in organ cultures confirming that the mutation causes loss of OPG function [29, 35]. Secretion of the mutant protein was also found to be defective in various cell culture models leading the authors to conclude that abnormal protein may be retained within the cell and degraded [35]. Subsequent studies by an International collaborative group evaluated nine families with clinical features of JPD and identified homozygous missense mutations of *TNFRSF11B* in six families with variations in severity depending on the site of the mutations [22]. Within this study, mutations affecting cysteine residues that were predicted to cause disruption of the ligand-binding region, were associated with a severe phenotype; those not affecting cysteine residues in the ligandbinding domain an intermediate phenotype, and an insertion/deletion mutation at the C-terminus a mild phenotype.

Management

Medical management of JPD has mainly focused on the use of the osteoclast inhibitors calcitonin and bisphosphonates but replacement therapy with osteoprotegerin has also been investigated. Calcitonin was one of the first drugs to be used in the management of JPD [36] although the evidence base its use stems exclusively from case reports. These reports indicate that calcitonin can reduce serum ALP concentrations and improve bone pain in JPD [37, 38]. However, calcitonin does not restore normal levels of bone turnover and there is a rapid relapse when treatment is stopped. Because of this, and the availability of bisphosphonates, calcitonin has now been superseded. Various bisphosphonates have been used in JPD including etidronate [39], alendronate [40], pamidronate [41, 42], ibandronate [43], risedronate [27, 44] and zoledronic acid [44]. All of these drugs have been shown to reduce biochemical markers of bone turnover to varying extent and to improve bone pain. Since the evidence base consists of case reports or small case series, it's difficult to reach firm conclusions except to note that nitrogen-containing bisphosphonates have given more complete suppression of biochemical markers of bone turnover than non-nitrogen containing bisphosphonates and that higher cumulative doses may be required to control the marked elevations in bone turnover that are observed in this condition. For example, Cundy and colleagues reported that high doses of ibandronate gave effective biochemical control in PDB when standard doses of pamidronate had not worked adequately. Zoledronic acid has also been successfully used to treat JPD but an important adverse event that has emerged with this agent is severe hypocalcaemia [44] which reflects the very potent inhibitory effects that this bisphosphonate has on osteoclastic bone resorption. It is thought that high dose

bisphosphonates might help to prevent progression of bone deformity in JPD but there isn't any firm evidence as yet to show that this strategy is effective in altering the natural history of JPD.

In recent years, and the RANKL inhibitor denosumab have been used and found to suppress markers of bone turnover in JPD [30, 45]. Severe hypocalcaemia has been reported as an adverse effect with denosumab in this situation [30].

Another treatment approach has been the use of recombinant osteoprotegerin [46]. This has the theoretical advantage of addressing the deficiency of osteoprotegerin which underlies JPD [46]. Treatment with osteoprotegerin was successful in inhibiting bone turnover in JPD but the effect wore off soon after treatment was stopped [46]. Currently, this drug is not available for clinical use.

Multisystem proteinopathy syndromes

Paget's disease can rarely be inherited as part of a wider syndrome in which other neuromuscular features occur in association with defects in the autophagy pathway and/or accumulation of abnormal protein aggregates in affected tissues. The syndrome can occur in association with mutations in several genes and has been referred to as multisystem proteinopathy (MSP). The first reference to PDB occurring in association with muscle disease that the authors of this review could find was that of McBride [47] who in 1966 reported a Scottish family in which PDB had been inherited in an autosomal dominant manner, in association with what was considered to be a form of muscular dystrophy. Subsequently, Tucker [48] and then Kimonis [49] described families in which in which Paget's disease and a limb girdle type of muscular dystrophy were inherited together in an autosomal dominant fashion. The research group of Kimonis extended these observations when they described four families in which myopathy and Paget's disease had been inherited in association with frontotemporal dementia, thereby delineating, a syndrome which they named inclusion body myopathy, Paget's disease and frontotemporal dementia (IBMPFD; now known as known as MSP1) (MIM 605382) [50]. The IBMPFD syndrome was localised to chromosome 9p13 by linkage analysis [50] and subsequent positional cloning studies revealed that missense mutations in the *VCP* gene, encoding Valosin containing protein were the cause of the disorder [51]. As early as 2002 it was recognised that not all families with this syndrome were linked to 9p13, indicative of genetic heterogeneity [52]. Subsequent genetic analysis of families affected by PDB, myopathy and neurodegenerative disease in whom VCP mutations were not identified showed that mutations in the *hnRNPA2B1* and *hnRNPA1* genes may also cause the syndrome [6] and that a syndrome with overlapping features could be caused by mutations in the *SQSTM1* and *OPTN* genes, both of which are implicated in classical PDB [53–55].

Multisystem Proteinopathy

This rare syndrome was first described as "inclusion body myopathy, Paget's disease and frontotemporal dementia" (IBMPFD) by Kimonis and colleagues in a series of families from the USA where the disease was inherited in an autosomal dominant fashion [MIM 605382].

Subsequent appreciation of the broader phenotypic spectrum (for example motor neuron disease and parkinsonism) associated with this syndrome led to adoption of a new term, “multisystem proteinopathy” (MSP), to describe these and similar families [56].

Clinical features

The initial description was with PDB and myopathy [49], but it was soon extended to encompass the additional feature of frontotemporal dementia (FTD) [50] and amyotrophic lateral sclerosis (ALS). Myopathy is the most frequent feature of IBMPFD which occurs in about 90% of individuals. The age at presentation varies widely with a mean age of 42 years and a range of 3-66 years. Involvement of the proximal limb girdle muscles is almost universal but the distal limb muscles and facial muscles may also be involved. Muscle biopsies show evidence of myopathic changes, and prominent intracellular rimmed vacuolar inclusion bodies. Motor neuron involvement is frequent in MSP, affecting more than half of the patients in a one clinical series [57]. About 43% of individuals also have PDB with an average age at onset of 42 years with a range of 29-61 years. The clinical and radiographic features are similar to classical Paget’s disease but with an earlier age at onset. As is the case in classical PDB, patients may or may not be symptomatic. The third component of dementia affects about 37% of individuals with an average age at onset of 54 years and a range of 39 to 62 years. Patients with MSP have a reduced life expectancy with death occurred at an average age of 58 years due to respiratory and cardiac failure.

Genetics

Mutations of the VCP gene encoding Valosin-Containing Protein were discovered to be a cause of MSP1 by linkage analysis [50] followed by positional cloning [51]. In the original report, six different disease-causing mutations in VCP were identified. However, at the time of writing, 45 different pathogenic VCP mutations have now been identified in patients with MSP (Table 4) [58]. Whilst mutations have been identified throughout the gene, the majority are situated in exons 3-5 which encode the N-terminal domain which is involved in ubiquitin binding and binding of co-factors essential for VCP’s function. Mutations in VCP are estimated to account for about half of all cases of MSP [59], with additional MSP mutations identified in the genes encoding hnRNPA2B1 (MSP2), hnRNPA1 (MSP3), SQSTM1 (MSP4) and Matrin-3 (MSP5) [56].

Pathophysiology

VCP is a member of the AAA+ (ATPase Associated with diverse cellular Activities) family of proteins. VCP governs an ever-expanding spectrum of ubiquitin-dependent cellular processes, ranging from protein quality control and cellular signaling to organelle biogenesis and elimination [60]. The primary function of VCP is that of a ubiquitin-dependent segregase that uses a variety of adaptor proteins to engage ubiquitinated clients and physically disengage these proteins from multimeric complexes, pull proteins out of membranes, and in some cases unfold client proteins to facilitate proteasomal degradation. This activity of VCP is essential for at least three ubiquitin-dependent modes of protein degradation, including endoplasmic reticulum-associated degradation (ERAD) [61] ubiquitin-dependent autophagy [62] and ribosome quality control [63]. VCP activity is also required for the assembly of the Golgi apparatus and ER [60] as well as mitochondrial

fission and clearance of defective mitochondria by mitophagy [64]. Among the nuclear functions dependent upon VCP activity are regulation of DNA damage responses [65] and chromatin-associated protein degradation [60]. Of potential relevance to the PDB component of the disease, VCP is involved in signaling pathways, including degradation of I κ B [66]. Another essential VCP function, which is particularly interesting in light of the genetic heterogeneity of MSP, is autophagic degradation of stress granules, a type of RNP granule that forms in the cytoplasm in response to translation inhibition [67]. Not only is VCP (linked to MSP1) found in stress granules, but so are at least three additional MSP-linked gene products, including hnRNPA2B1 (MSP2), hnRNPA1 (MSP3) and SQSTM1 (MSP4).

In order to investigate the mechanisms by which VCP mutations cause IBMFPD, Custer *et al* [68] generated transgenic mice with overexpression of the R155H and A232E mutations in VCP under the control of a cytomegalovirus enhanced beta-actin promoter which drives transgene expression in all tissues. When compared with wild type littermates, the mutant mice developed muscle weakness by about 4 months of age and this was accompanied by accumulation of cytoplasmic aggregates of the TDP-43 protein in combination with ubiquitin in muscle. Behavioural abnormalities were also noted accompanied by accumulation of cytoplasmic aggregates of TDP-43 and ubiquitin in neurons. Skeletal phenotyping of 13-month-old mice showed evidence of osteopenia with reduced bone volume/total volume, reduced trabecular number and reduced trabecular thickness when compared with wild type littermates. The authors also noted occasional osteosclerotic lesions within the internal cavity of long bones and areas of reduced bone density beneath the growth plate of the distal femur. Histological analysis was not performed to evaluate the nature of these lesions. In order to investigate the effects of the A232E mutation on NF κ B signaling, wild type and mutant VCP was over expressed in C2C12 myoblastic cells stimulated with TNF α . These experiments showed increased NF κ B activation following TNF α stimulation in A232E transfected cells as compared with wild type and more rapid clearance of I κ B. This suggests that the mutant VCP may have an enhanced ability to bind I κ B and target it for degradation. Wehl and colleagues generated transgenic mice in which the R155H mutation of VCP was driven by a muscle specific promoter [69]. The mutant mice developed muscle weakness from about 6 months of age in association with accumulation of inclusion bodies consisting of ubiquitin and high molecular weight ubiquitinated proteins. The animals did not show the rimmed vacuoles that are characteristic of IBMFPD but did show what the authors referred to as “rimmed cracks” in the sarcoplasm and sarcolemmal regions. In another study Badadani *et al* generated mice with a knock-in of the R155H mutation [70]. The mice developed muscle weakness by 6 months of age. Histological analysis of muscle revealed vacuolization of the myofibrils with accumulation of TDP-43 and ubiquitin containing cytoplasmic inclusion bodies. The authors also reported finding increased staining of LC3-N in muscle, providing evidence of impaired autophagy. Cytoplasmic TDP-43 and ubiquitin containing inclusions were also found in neurons but the mutant mice did not show evidence of memory impairment. However about 15% had developed seizures by the age of 9 months. Skeletal phenotyping showed no evidence of osteopenia on MicroCT analysis but cortical thickness was increased. There wasn't clear evidence of PDB-like bone lesions in R155H knock-in mice but evidence of increased osteoclast formation

was found in M-CSF and RANKL stimulated bone marrow cultures from the mutant as compared with wild type mice.

The mechanisms of disease in patients with VCP mutations are incompletely understood, but is likely to involve some but not all the ubiquitin-dependent processes that are regulated by VCP. In cell-based, *Drosophila* and mouse model systems, disease-causing mutations in VCP do not detectably impair ERAD or organelle assembly [62, 64]. An early report of ERAD impairment in response to over-expression of mutant VCP relied upon a reporter system later learned to be sensitive to autophagy impairment [71]. Indeed, subsequent analyses determined that disease-causing mutations in VCP impair ubiquitin-dependent autophagy [62, 72]. Accumulation of abnormal mitochondria has been observed consistently in animal models harboring mutant VCP mutations [64, 68, 70] and this is likely due to impaired mitophagy [64]. Interestingly, an unbiased screen for genetic modifiers of mutant VCP-related degeneration in *Drosophila* identified RNA-binding proteins, homologs of hnRNPA1 and hnRNPA2B1 in particular, as strong suppressors [73]. The intersection of these three gene products and SQSTM1 in stress granules, and the impairment of stress granule dynamics observed with defects in these proteins, has drawn particular attention to the role of abnormal stress granules in disease pathogenesis [74]. How mutations in VCP impair some functions but not others is incompletely understood, but quite likely reflects a biased effect on the interaction of VCP with various adaptors [75]. Specifically, the molecular bases of the PDB component of MSP is unknown, but likely reflects hyperactivation of NF κ B at least in part.

Management

At the present time management of this disorder involves supportive care only. Bisphosphonates are likely to be of value in managing the PDB component although there is little published information on how well this type of PDB responds to bisphosphonate therapy.

hnRNP associated multisystem proteinopathy

This syndrome was described by Kim and colleagues [6] following genetic analyses of three families with autosomal dominant inheritance of neurodegenerative disease accompanied in some cases by PDB with features that were indistinguishable from VCP-related MSP [6]. However, all patients had been screened for VCP mutations and none were found, triggering the search for an alternative causal gene.

Clinical features

Affected members of one family (Family 1) exhibited autosomal dominant inheritance of myopathy, PDB, cognitive impairment and amyotrophic lateral sclerosis (ALS) in various combinations. Clinical evaluation of families 1 and 2 showed evidence of a myopathy characterised by atrophic myofibrils, central nuclear and rimmed vacuoles, typical of inclusion body myopathy. More detailed analysis of some affected patients showed redistribution of hnRNPA2B1, hnRNPA1 and TPD-43 proteins from their normal location in the nucleus to the cytoplasmic inclusion bodies which also contained ubiquitin and p62 in

limited amounts. Similar findings had previously been reported in sporadic inclusion body myopathy (IBM) [76] and in IBM associated with VCP mutations.

Genetics

The affected members of family 1 were found to share a missense mutation in the *hnRNPA2B1* gene causing an amino acid change from aspartic acid to valine at position 290 (D290V) and 302 (D320V) in different isoforms of the protein generated by differences in splicing. In a second family (Family 2), where PDB and myopathy were inherited in an autosomal dominant manner, a missense mutation in the *hnRNPA1* gene as found causing an amino acid change from aspartic acid to valine at position 262 (D252V) and 214 (D314V) in different isoforms. Affected members of another family with autosomal dominant inheritance of ALS (Family 3) were found to have an amino acid substitution to N at the same residue (D262N/D314N). The terms MSP2 and MSP3 have been used to describe the syndromes caused by mutations in *hnRNPA2B1* and *hnRNPA1* respectively [77].

Pathophysiology

In order to gain an insight into the mechanisms by which these mutations were pathogenic, the authors conducted a series of bioinformatic and functional studies. Disease-causing mutations in *hnRNPA2B1* and *hnRNPA1* were found to impact intrinsically disordered protein segments that are common to many members of the hnRNP family, including TDP-43, TIA1, hnRNPDL and FUS [6]. Indeed, ALS-, FTD- and myopathy-causing mutations were subsequently identified in these hnRNPs [78]. The intrinsically disordered protein segments found in members of the hnRNP family were found to support higher order assembly into amyloid-like fibrils in vitro and, indeed, this property is consistently enhanced by disease-causing mutations [78]. In living cells, higher order assembly of these disordered segments is important in the formation and liquid properties of membrane-less organelles that arise through phase transitions – most notably RNA granules [79]. Indeed, impairment of the dynamics of RNA granules appears to be a unifying principle underlying the degeneration observed in MSP downstream of all genetic etiologies. At the present time, the mechanisms by which these mutations cause the PDB component of the syndrome remains to be established although it is intriguing to speculate that impaired stress granule dynamics may also impact signaling through proteins like NF κ B [18].

Management

At the present time management of this disorder involves supportive care only. Bisphosphonates are likely to be of value in managing the PDB component although there is little published information on how well this type of PDB responds to bisphosphonate therapy.

PDB-associated genes and multisystem proteinopathy

Although most patients with *SQSTM1* mutations develop PDB in the absence of neurological symptoms, it has become apparent that missense mutations in *SQSTM1* can also cause neuromuscular degeneration with features of multisystem proteinopathy (termed MSP4) either in the presence or absence of PDB [77].

Clinical presentation

The clinical presentation of *SQSTM1*-associated MSP is wide and may include sporadic and familial amyotrophic sclerosis [80], frontotemporal dementia with or without ALS [81], isolated inclusion body myopathy [77] and inclusion body myopathy with PDB [82].

Genetics

At the present time it is unclear why some patients develop PDB, whereas others develop myopathy, ALS or frontotemporal dementia. A plausible reason for the differences in clinical presentation of patients with *SQSTM1* mutations suggested by Buccelli [77] is that inheritance of susceptibility alleles of other genes that predispose to individual components of the syndrome may determine which phenotypes are expressed. The strongest evidence for the importance of a gene-gene interaction in determining which phenotype is predominant in MSP comes from the observations by Lee and colleagues [83] who reported that inheritance of the N357S variant of the *NIA1* gene in combination with various mutations affecting the UBA domain of *SQSTM1* result in a distal myopathy whereas the same *NIA1* variant is not pathogenic in the absence of *SQSTM1* mutations. Furthermore, *SQSTM1* mutations in the absence of *NIA1* mutations do not cause a distal myopathy.

Pathophysiology

In order to investigate the potential mechanisms underlying the interaction between *NIA1* and *SQSTM1* mutations Lee et al [83] performed functional studies which showed that overexpression of N357S mutant form of *TIA1* in mouse embryonic fibroblasts (MEF) had increased propensity to form cytoplasmic stress granules in vitro and that repeating these experiments in MEF lacking *sqstm1* further increased stress granule formation. Finally, coexpression of the N357S variant of *TIA1* and the A390X (truncating) mutation of *SQSTM1* in myoblasts led to impaired stress granule clearance and myotoxicity. These observations led the authors to conclude that the combination of increased stress granule formation mediated by N357S *TIA1* combined with defective autophagy mediated protein degradation as the result of *SQSTM1* mutations were instrumental in causing the disease phenotype in muscle. Softer evidence in support of the importance of gene-gene interactions in the phenotypic presentation of MSP comes from the observation that inheritance of the APOE4 allele may modify development of the dementia component of IBMPFD [84]. Genome wide association studies have shown that variants at the *OPTN* locus predispose to PDB [85]. The likely mechanism is through an inhibitory effect on optineurin expression [55] which causes osteoclast activation, although at the present time, the causal variant(s) that regulate *OPTN* expression remain to be identified. Protein coding mutations in *OPTN* have also been reported to cause ALS [86] and glaucoma [87] but to date, no protein coding mutations in *OPTN* have been identified in PDB (Ralston & Albagha, unpublished) and to the knowledge of these reviewers, *OPTN* mediated ALS has not been reported to occur in association with PDB.

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	72	75	78	81	84	87	90	93													
WT	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	CTG	CTC	GCC										
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala										
	75 (dup27)																				
EoPDB	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	CTG	GCT	GCG	CTG	CTG	CTC	TGC	GCG	CTG	CTC	GCT	GCC	
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala	
	78 (dup27)																				
EoPDB	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	GCT	GCT	GCC	CTG	CTG	CTC	TGC	GCG	CTG	CTC	GCT	GCC	GCC
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala	Ala
	84 (dup18)																				
FEO	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	CTG	CTC	CTG	CTC	TGC	GCG	CTG	CTC	GCC				
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala				
	84 (dup15)																				
ESH	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	CTG	CTG	CTC	TGC	GCG	CTG	GCC						
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Cys	Ala	Leu	Leu	Ala						
	87 (dup15)																				
JPD	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	CTG	GCT	CTC	TGC	GCG	CTG	GCT	GCC					
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Ala					
	90 (dup 12)																				
POEBD	TTC	GCG	CTG	CTG	CTG	CTC	TGC	GCG	CTG	GCT	TGC	GCG	CTG	CTC	GCC						
	Phe	Ala	Leu	Leu	Leu	Leu	Cys	Ala	Leu	Leu	Cys	Ala	Leu	Leu	Ala						

Figure 1: Insertion mutations of TNFRSF11A

The insertion mutations in exon 1 of *TNFRSF11A* that have been described so far are illustrated in relation to the normal sequence (WT). The position on the mRNA at the site of each insertion mutation is indicated with the duplicated section of the mRNA shown in blue and red. Abbreviations: FEO – familial expansile osteolysis; EoPDB – Early onset familial Paget’s disease; ESH – Expansile skeletal hyperphosphatasia; JPD – Juvenile Paget’s disease; POEBD – Panostotic expansile bone disease

Normal

TNFRSF11A
Mutations

Figure 2. Disease mechanisms in familial expansile osteolysis and related disorders

Under normal circumstances the RANK signal peptide is cleaved as it emerges from the endoplasmic reticulum (ER) and the receptor migrates to the cell surface where it can be activated by RANKL causing NFκB activation through the classical pathway (left side of figure). Insertion mutations affecting the signal peptide of RANK result in failure of signal peptide cleavage causing the abnormal protein to accumulate in the ER with activation of NFκB through the unfolded protein response (UPR) (right side of figure).

Figure 3. Disease mechanisms in multisystem proteinopathy

Under normal circumstances, proteins such as VCP and p62 bind ubiquitylated proteins and target them for degradation through the proteasome (left side of figure). Disease-causing mutations in *VCP* and *SQSTM1* impair their ability to transport proteins to the proteasome for degradation causing these to accumulate in the cytoplasm and form inclusion bodies. Additionally, disease associated mutations in *VCP* increase avidity for I κ B, allowing NF κ B to translocate to the nucleus and activate gene expression, causing osteoclast activation. Disease causing mutations in *hnRNPA2B1* and *hnRNPA1* enhance the ability of both molecules to polymerase and form fibrils, resulting in increased stress granule formation, which combine with proteins such as ubiquitin, and TPD to form inclusion bodies. Accumulation of these inclusion bodies is thought to underlie the pathogenesis of neurological and muscle degeneration in MSP and may contribute to the PDB component by mechanisms that remain to be established (right side of figure).

Table 1.Spectrum of phenotypes associated with insertion mutations in *TNFRSF11A*.

Disorder	FEO	Early Onset PDB	Juvenile onset PDB	ESH	Panostotic expansile bone disease
<i>Mutation</i>	<i>84dup18</i>	<i>75dup27</i>	<i>87dup15</i>	<i>84dup15</i>	<i>90dup12</i>
Bone deformity	Yes	Yes	Yes	Yes	Yes
Early Onset Deafness	Yes	Yes	Yes	Yes	Yes
Premature tooth loss	Yes	Yes	Yes	Yes	Yes
Hypercalcaemia	No	Yes	No	Yes	No
Pathological Fractures	Yes	Yes	Yes	No	Yes
Disease onset	Childhood	Adolescence	Infancy	Adolescence	Infancy
<i>Pattern of involvement</i>		–			
Skull	+	+	+	–	+
Facial bones	+	++	+	–	+
Spine	–	++	–	+	+
Scapula/Clavicle	–	+	–	+	+
Pelvis	–	++	+	+	+
Femur	++	++	+	+	+
Tibia/fibula	++	+	+	+	+
Humerus	+	+	–	+	+
Radius / ulna	++	+	+	++	+
Carpal/Tarsal	–	+		+	+
Phalanges	–	+	+	++	+

Frequency of involvement is indicated by: seldom/never involved (–), sometimes involved (+), often involved (++).

Table 2.

Clinical classification of severity in JPD

	Mild	Moderate	Severe
Deformity < 2 years	No	No	Yes
Delayed walking	No	No	Yes
Height <3 rd centile	No	Yes	Yes

Table 3.

Reported mutations of TNFRSF11B in Juvenile PDB

Mutation	Predicted functional consequences	Clinical phenotype	Reference
Deletion of 100Kb encompassing entire gene	Deletion of all exons. No functional OPG produced.	Severe	Whyte [28]
Deletion affecting TNFRSF11B and 3 other genes	Deletion of all exons. No functional OPG produced.	Severe	Grasemann[30]
Met1Arg	Start codon disrupted. No functional OPG produced	Severe	Grasemann[30]
Insertion CTCG exon 1	Frameshift with truncated protein at codon 9.	Severe	Grasemann[31]
Deletion affecting exon 3/intron 3	Truncated protein	Severe	Chong [22]
Intragenic deletion of 250kb from intron 1 to downstream of exons	Deletion of exons 2-5. No functional OPG produced	Severe	Naot [32]
Deletion of 20bp affecting exon 3 / intron 3 splice junction	Truncated protein with deletion of exons 4 and 5	Severe	Chong [22]
Cys44Arg	Disruption of OPG structure through impairment of disulphide bridges	Severe	Saki [33]
Cys65Arg	Disruption of OPG structure through impairment of disulphide bridges	Severe	Chong [22]
Cys87Tyr	Disruption of OPG structure through impairment of disulphide bridges	Severe	Chong [22]
Thr76Pro	Impaired ligand binding	Mild/intermediate	Naot [32]
Phe117Leu	Impaired ligand binding	Intermediate	Chong [22]
DelArg182	Impaired ligand binding; reduced secretion	Intermediate	Cundy [29]
Insertion TT/deletion TGA exon 5.	Frameshift Truncation of exon 5	Mild/Intermediate	Chong [22] Janssens[34] Whyte [27]

Table 4.

Pathogenic mutations in VCP

Mutation	Exon	Domain	Mutation	Exon	Domain
I27V	2	N-terminal	R191Q, R191G	5	Linker 1
N91Y	3	N-terminal	L198W	6	Linker 1
R93C, R93H	3	N-terminal	I206F	6	Linker 1
R95G, R95C, R95H	3	N-terminal	A232E	6	D1
G97E	3	N-terminal	T262A	7	D1
T127A	4	N-terminal	K386E	10	L2
G128A, G128C	4	N-terminal	N387H, N387S, N387T	10	L2
P137L	4	N-terminal	N401S	11	L2
I151V	5	N-Terminal	A439S, A439G, A439P	11	L2
R155H, R155P, R155C, R155G, R155S, R155L	5	N-terminal	R487H	12	L2
G156S, G156C	5	N-terminal	D592N	12	L2
G157R	5	N-terminal	D592H	14	D2
M158V, M158I	5	N-terminal	R662C	14	D2
R159H, R159L, R159C, R159G	5	N-terminal	N750S	16	C-terminal
A160P	5	N-terminal			

Pathogenic mutations in VCP that have been described in MSP's are shown (adapted from Al-Tahan et al [58]).