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Familial Paget's Disease of Bone: Nonlinkage to the *PDB1* and *PDB2* Loci on Chromosomes 6p and 18q in a Large Pedigree

DAVID GOOD,[1] FRANCES BUSFIELD,[1] DAVID DUFFY,[2] PAUL K. LOVELOCK,[1] JANINE B. KESTING,[1] DONALD P. CAMERON,[3] and JOANNE T.E. SHAW[1]

ABSTRACT

Paget's disease of bone is a common condition characterized by bone pain, deformity, pathological fracture, and an increased incidence of osteosarcoma. Genetic factors play a role in the pathogenesis of Paget's disease but the molecular basis remains largely unknown. Susceptibility loci for Paget's disease of bone have been mapped to chromosome 6p21.3 (*PDB1*) and 18q21.1-q22 *(PDB2)* in different pedigrees. We have identified a large pedigree of over 250 individuals with 49 informative individuals affected with Paget's disease of bone; 31 of whom are available for genotypic analysis. The disease is inherited as an autosomal dominant trait in the pedigree with high penetrance by the sixth decade. Linkage analysis has been performed with markers at *PDB1*; these data show significant exclusion of linkage with $\log_{10}$ of the odds ratio (LOD) scores < -2 in this region. Linkage analysis of microsatellite markers from the *PDB2* region has excluded linkage with this region, with a 30 cM exclusion region (LOD score < -2.0) centered on D18S42. These data confirm the genetic heterogeneity of Paget's disease of bone. Our hypothesis is that a novel susceptibility gene relevant to the pathogenesis of Paget's disease of bone lies elsewhere in the genome in the affected members of this pedigree and will be identified using a microsatellite genomewide scan followed by positional cloning. (J Bone Miner Res 2001;16:33–38)

Key words: genetic linkage analysis, Paget's disease of bone

INTRODUCTION

PAGET'S DISEASE of bone is a common metabolic bone disease. Ethnic and geographic clustering is apparent, and the condition is most common in Europe, North America, Australia, and New Zealand.[(1)] Prevalence rates in hospitalized patients over the age of 55 years in several European cities have been documented, and the highest prevalences have been found in England (4.6%) and France (2.4%).[(1)] In Australia and New Zealand the prevalence is between 3% and 4%, with higher prevalence rates among British immigrants.[(2)] Paget's disease of bone affects both sexes but is slightly more common in men than in women.[(3)] It is characterized by focal areas of increased bone turnover at specific sites throughout the skeleton, with clinical features of bone pain, deformity, pathological fracture, and an increased risk of osteosarcoma. All of these features suggest that Paget's disease may represent a disorder of bone cell growth and regulation, but the molecular basis of this process remains unclear. Slow virus infection has been invoked as a possible cause[(4)]; however, no intact virus has ever been recovered from pagetic bone, and studies that have sought to

[1]Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Australia.
[2]Queensland Institute of Medical Research, Brisbane, Australia.
[3]Division of Research, Princess Alexandra Hospital, Brisbane, Australia.

identify viral transcripts from affected tissue and cultured cells using molecular techniques have yielded contradictory results.[5] Although the role of viruses in the etiology of Paget's disease remains uncertain, there is no doubt that genetic factors play a significant role. Familial clustering of Paget's disease has been documented,[6,7] and the first-degree relatives of pagetic patients have a 7-fold increased risk of developing the disease as compared with controls.[6]

There has been a relative paucity of family studies in Paget's disease. This is related, at least in part, to the late age of onset of the condition and the difficulty in ascertaining informative multiplex pedigree for linkage analysis. Suggestive linkage with Paget's disease of bone was first obtained with the HLA locus (chromosome 6p21.3) by Fotino et al.[8] and Tilyard et al.[9] Fotino et al.[8] indicated a $\log_{10}$ of the odds ratio (LOD) score of 2.44 at a recombination fraction of 0.108. This locus is referred to as *PDB1*. However linkage with this region was not confirmed in a study by Moore and Hoffman[10] or in a recent report by Laurin et al.,[11] suggesting that the HLA locus is unlikely to play a major role in the majority of pedigrees with familial Paget's disease of bone.

Familial expansile osteolysis (FEO) is a rare, autosomal dominant bone disorder characterized by focal areas of increased bone remodeling. FEO has been described in a large Northern Irish pedigree.[12,13] Osteolytic lesions, which usually develop in the long bones during early adulthood, show increased osteoblast and osteoclast activity. Hughes et al.[14] showed linkage between FEO and a region of chromosome 18q21.2–18q21.3 flanked by microsatellite markers D18S51 and D18S64, with no recombinants between FEO and marker D18S42. Cody et al.[15] performed linkage studies in a large kindred with Paget's disease of bone using 12 microsatellite markers from the region of 18q surrounding the FEO locus. No recombinants were observed between the Paget's disease locus and D18S42, giving a maximum LOD score of +3.40. Approximately 1% of pagetic patients develop osteosarcoma, which represents an increase in risk that is several 1000-fold over that of the general population.[16] Analysis of tumor-specific loss of constitutional heterozygosity in sporadic and pagetic osteosarcomas has identified a putative tumor-suppressor locus within the region tightly linked to familial Paget's disease on chromosome 18q.[17] The gene encoding receptor activator of nuclear factor κB (RANK) TNFRSF11A maps to 18q21.2–21.3. RANK is essential in osteoclast formation. Hughes et al.[18] identified two heterozygous insertion mutations in exon 1 of TNFRSF11A in affected members of four families with FEO or familial Paget's disease of bone. One was a duplication of 18 bases and the other a duplication of 27 bases, both of which affected the signal peptide region of the RANK molecule. Expression of recombinant forms of the mutant RANK proteins revealed perturbations in expression levels and lack of normal cleavage of the signal peptide. Both mutations caused an increase in RANK-mediated nuclear factor κB (NF-κB) signaling in vitro, consistent with the presence of an activating mutation. The prevalence of mutations in TNFRSF11A in familial and sporadic cases of Paget's disease of bone remains to be defined.

Genetic linkage studies were undertaken in eight multiplex families from diverse ethnic backgrounds in which several members from successive generations suffered from Paget's disease using a panel of microsatellite markers in the 18q21–22 region.[19] Five of the families showed evidence for linkage with this region; however, the remaining three families did not show evidence for linkage at this locus. These data suggest the presence of at least one additional locus conferring susceptibility to Paget's disease, which remains to be identified.

MATERIALS AND METHODS

Clinical studies

The research protocol was approved by the Princess Alexandra Hospital Research Ethics Committee, and informed consent was obtained from all study participants. We have identified a large pedigree from a proband with Paget's disease of bone who attends the Princess Alexandra Hospital. The founder was born in Prussia in the mid-1800s and immigrated to Australia as a young man. The pedigree includes over 250 adult members, with 49 affected subjects, 31 of whom were alive and willing to participate in the family study. The pattern of disease is polyostotic, with the major sites of involvement including the spine, pelvis, skull, femur, tibia, and humerus. The earliest symptom in the majority of affected pedigree members is tinnitus followed by progressive hearing impairment. The mean age at diagnosis for the affected family members is 58 years (SD, 10 years). In the absence of effective medical therapy before the last two decades, the affected subjects developed severe progressive disease with bowing of the legs and spinal deformity. None of the affected pedigree members have developed osteosarcoma. The affected family members currently are treated with bisphosphonate therapy and have had a substantial biochemical response to therapy. The clinical features of the pedigree suggest that the affected family members have familial Paget's disease of bone rather than FEO, which rarely involves the axial skeleton.[13]

Venous blood samples have been obtained with informed consent for DNA extraction and biochemical analysis. All study participants underwent measurement of the serum total alkaline phosphatase (ALP), because ALP values usually are elevated in untreated Paget's disease.[3] Family members who reported previously diagnosed Paget's disease of bone had the diagnosis confirmed by review of their medical notes and bone scans. Individuals above the age of 60 years in whom serum ALP values were below 65 μmol/liter (reference range, 40–110 μmol/liter) were considered as unaffected, whereas those with ALP values that were in the high normal range (65–110 μmol/liter) or obviously elevated (>110 μmol/liter) were labeled as diagnosis unclear and were investigated further. These individuals were scored as affected if evidence of Paget's disease was found on radionuclide bone scan examinations and/or skeletal radiographs. Unless Paget's disease had been confirmed radiologically or scintigraphically, individuals under the age of 60 years were considered as diagnosis unclear even in the presence of normal ALP levels to take into account the fact

TABLE 1. THE CLINICAL FEATURES OF THE AFFECTED FAMILY MEMBERS WITH PAGET'S DISEASE OF BONE

Patient	*Age (years)*	*Age at diagnosis (years)*	*Current treatment*	*Duration of current treatment*	*SAP (μmol/l)*	*Distribution of disease*
V-42	59	36	APD	6 years	400	Pelvis, left femur, right humerus
1V-28	82	55	APD	2 years	353	Extensive polyostotic disease
1V-33	75	58	Alendronate	1 year	180	Humerus
1V-47	81	62	Alendronate	6 months	1697	Skull, vertebrae, pelvis, right femur & humerus
1V-38	78	58	Alendronate	2 months	673	Both femora
V-61	55	55	Newly diagnosed		98	Right calcaneum
V-67	58	58	Newly diagnosed		273	Cervical spine, L1 vertebra, sacrum, right pelvis, left femur
V-44	61	58	Alendronate	2 years	61	Skull, shoulder, pelvis, hips
V-46	62	62	Newly diagnosed		239	Skull, left humerus, right tibia, left femur, right hemipelvis, vertebrae
1V-50	88	60	APD	6 years	977	Extensive polyostotic disease
V-85	63	63	Newly diagnosed		239	Skull, left humerus, right tibia, left femur, pelvis, T11 vertebra
1V-63	73	50	Alendronate	3 months	363	Pelvis, base of skull
V-68	61	55	Alendronate	2 years	50	Skull, right femur, pelvis
V-66	48	48	Newly diagnosed		105	Skull, femora
1V-10	79	59	nil to date	20 years	2230	Femur
1V-41	74	74	Newly diagnosed		77	Skull
V-69	61	61	Newly diagnosed		147	Scapula, sacrum, pelvis, tibia, right calcaneum
V-70	53	53	Newly diagnosed		106	Right tibia, skull
1V-62	84	84	Newly diagnosed		63	Left hip, left maxilla
V-05	53	53	Newly diagnosed		114	L3 vertebra, right humeral head, left tibia
V-55	46	46	Newly diagnosed		100	Left scapula
V1-39	44	44	Newly diagnosed		70	Tibia
V-23	76	76	Newly diagnosed		71	Right hemipelvis, sacrum
V-76	64	52	Alendronate	2 years	93	Femora, pelvis, base of skull
V-77	62	60	Alendronate	2 years	92	Rib, shoulder, femora, tibiae
V-79	57	50	Alendronate	1 year	366	Vertebrae, pelvis, left femur
111-05	76	76	Newly diagnosed		125	Pelvis, right scapula
V-40	59	59	Newly diagnosed		159	Skull, left femur, left tibia, right ischium, left acetabulum
V-51	49	49	Newly diagnosed		166	Left hemipelvis, sacrum
1V-54	70	70	Newly diagnosed		126	Right femur
1V-52	72	69	nil to date		246	Left tibia, left shoulder, left femur

APD, pamidronate; SAP, serum alkaline phosphatase.

that Paget's disease may not present clinically until the fifth or sixth decade.[3] The clinical details of the affected family members are summarized in Table 1. Because of its large size and multiple affected members, the pedigree is a unique resource for the detection of susceptibility genes for Paget's disease of bone.

Molecular studies

DNA was extracted from blood samples using the salting-out method.[20] Paternity was established by the use of highly polymorphic markers in a polymerase chain reaction (PCR)–based protocol. Using sex-averaged distances from the location database (ftp://cedar.genetics.soton.ac.uk/pub), we selected markers that span the 18q21–22 susceptibility region (*PDB2*): centromere–D18S464, D18S478, D18S1102, D18S474, D18S64, D18S60, D18S68, D18S42, D18S878, D18S466, D18S61, D18S1161–telomere. We also studied the following markers that span the *PDB1* region of chromosome 6p: centromere—D6S1617, D6S1574, D6S309, D6S470, D6S289, D6S422, D6S258, D6S1571, tumor necrosis factor α (TNF-α), D6S1610, D6S257, D6S460, D6S262, D6S292, D6S308—telomere. PCR reactions were performed in 96-well plates in 10-μl volumes containing 50 ng of genomic DNA, 1$\times$ PCR buffer (Perkin Elmer Cetus, Norwalk, CT, USA), 0.5 pmol of each primer, 0.5 U of Amplitaq Gold (Perkin Elmer Cetus), 2.5 mM $MgCl_2$, 1 μCi [^{32}P]deoxycytidine triphosphate (dCTP; Geneworks, Adelaide, South Australia), 0.025 mM dCTP,

TABLE 2. TWO-POINT LINKAGE ANALYSIS LOD SCORES FOR THE MICROSATELLITE MARKERS SPANNING THE *PDB2* LOCUS (CHROMOSOME 18q21-22) VERSUS AFFECTION STATUS IN THE PEDIGREE

Markers	*Position (cM)*	*Recombination fraction*							
		0.00	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*	*0.40*	
D18S464	36.58	−2.26	−2.01	−1.36	−0.90	−0.48	−0.31	−0.16	
D18S478	55.77	−3.20	−2.81	−1.79	−1.06	−0.30	−0.01	0.06	
D18S1102	63.13	−8.47	−7.36	−5.06	−3.58	−1.84	−0.80	−0.23	
D18S474	71.30	−9.20	−8.15	−5.58	−3.78	−1.75	−0.67	−0.14	
D18S64	86.37	−3.96	−3.33	−2.10	−1.32	−0.48	−0.07	0.05	
D18S60	94.83	−4.20	−3.70	−2.45	−1.55	−0.60	−0.14	0.04	
D18S68	98.73	−6.37	−5.35	−3.24	−1.85	−0.48	0.06	0.18	PDB2 region
D18S42	98.99	−4.87	−4.42	−3.24	−2.42	−1.46	−0.81	−0.31	
D18S878	100.93	−4.39	−3.87	−2.57	−1.64	−0.64	−0.15	0.04	
D18S466	104.83	−3.04	−2.60	−1.82	−1.27	−0.59	−0.27	−0.10	
D18S61	105.43	−5.93	−5.34	−3.89	−2.67	−1.06	−0.29	0.00	
D18S1161	114.17	−2.99	−2.40	−1.06	−0.18	0.52	0.56	0.31	

and 0.25 mM each of deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP) (Pharmacia Biotech, Uppsala, Sweden). After a 10-minute denaturing step at 94°C in a 96-well thermal cycler (Corbett Research, Sydney, Australia), samples were subjected to 30 cycles of 15 s at 55°C for annealing and 72°C for 30 s and 94°C for 15 s for denaturing; a final 5-minute incubation took place at 72°C. After amplification, the products were denatured by the addition of 9 μl of formamide loading buffer followed by heating at 95°C for 5 minutes. Next, 2 μl of each sample were loaded onto a 6% denaturing polyacrylamide gel in 1× Tris-borate-EDTA (TBE) buffer and resolved electrophoretically at 80–100 W. Gels were fixed, dried, and exposed to autoradiographic film. After film development, microsatellite genotypes were scored without knowledge of the clinical or pedigree data. Consistency within and between gels was maintained by using positive controls and Centre d′Etude du Polymorphisme Humain standard DNA.

Statistical analysis

The relationship between Paget's disease of bone and the candidate loci on 18q and 6p was studied by genetic linkage analysis, which was performed using the VITESSE algorithm for rapid exact multilocus linkage analysis.[21] Based on the pedigree data, we assumed an autosomal dominant mode of inheritance with disease penetrance set at 90%. We assumed trait allele frequency of 1% and phenocopy rate of 2.3%, which gives a population prevalence of 4% and a sibling recurrence risk of 0.225 ($\lambda_s = 5.6$). These are consistent with the Australian epidemiological data for Paget's disease of bone.[2] The allelic frequencies of the pedigree were used in the analysis to avoid error from using published frequencies, which may not be appropriate for this pedigree. Simple counting estimates have been used.

RESULTS

Two-point LOD scores resulting from linkage analysis between the disease and the markers spanning the *PDB2* and *PDB1* loci are shown in Tables 2 and 3. Figure 1 shows the multipoint LOD scores plotted against genetic distance for the *PDB2* locus on chromosome 18q and shows the 30 cM exclusion region (LOD score < -2.0) centered on D18S42. Figure 2 shows the multipoint LOD scores plotted against genetic distance for the *PDB1* region of chromosome 6p, confirming significant exclusion of linkage with this region.

DISCUSSION

The pedigree described in this study shows familial Paget's disease and a pattern of inheritance consistent with an autosomal dominant trait. To clarify the molecular basis of the disease, we conducted linkage analysis using microsatellite markers spanning the defined candidate susceptibility loci on chromosomes 6p21.3 (*PDB1*) and 18q21–22 (*PDB2*). Analysis of our pedigree excluded linkage with PDB1 and PDB2. These data confirm and expand the results of Haslam et al.[19] who studied the relationship between 18q21–22 and Paget's disease in eight large multiplex families from diverse ethnic backgrounds with inherited Paget's disease. There was evidence of genetic heterogeneity, with evidence for linkage in five families and evidence against linkage in three families. Three of four Spanish pedigrees with familial Paget's disease of bone showed no evidence of linkage to this locus, and in one of these pedigrees linkage to this region was excluded with an LOD score < -2. A recent report documented negative linkage results at *PDB1* and *PDB2* in French Canadian pedigrees.[11] Our pedigree arose from a Central European founder, and supports the hypothesis that there may be geographic differences in the susceptibility genes responsible for Paget's disease.

Inclusions that resemble viral nucleocapsids have been described in the nuclei and cytoplasm of osteoclasts at pagetic sites, but not in nonpagetic osteoclasts from the same patients or from normal subjects.[22,23] The viruslike particles resemble members of the paramyxovirus family. In situ hybridization studies have reported the detection of

TABLE 3. TWO-POINT LINKAGE ANALYSIS LOD SCORES FOR THE MICROSATELLITE MARKERS SPANNING THE *PDB1* LOCUS (CHROMOSOME 6p21.3) VERSUS AFFECTION STATUS IN THE PEDIGREE

Markers	*Position (cM)*	*Recombination fraction*							
		0.00	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*	*0.40*	
D6S1617	13.51	−2.93	−2.65	−1.87	−1.24	−0.49	−0.10	0.04	
D6S1574	16.11	−4.01	−3.34	−1.94	−1.04	−0.01	0.36	0.29	
D6S309	19.37	−3.79	−3.30	−2.01	−1.00	0.09	0.43	0.32	
D6S470	24.1	−4.96	−4.35	−2.91	−1.84	−0.53	0.06	0.16	
D6S289	38.57	−2.91	−2.40	−1.25	−0.53	0.10	0.25	0.15	
D6S422	45.54	−2.18	−1.71	−0.75	−0.26	0.09	0.15	0.10	
D6S258	53.27	−3.36	−2.95	−1.81	−1.00	−0.22	0.03	0.02	
D6S1571	53.41	−2.22	−2.03	−1.49	−1.04	−0.43	−0.11	0.01	PDB1 region
TNF-A	56.58	−6.65	−6.00	−4.26	−2.88	−1.25	−0.44	−0.08	
D6S1610	60.49	−6.28	−5.41	−3.32	−1.86	−0.40	0.15	0.21	
D6S257	86.05	−7.09	−5.72	−3.42	−1.96	−0.44	0.18	0.28	
D6S460	94.86	−8.54	−5.75	−3.12	−1.89	−0.63	0.02	0.18	
D6S262	126.89	−2.65	−2.36	−1.54	−0.92	−0.27	−0.02	0.04	
D6S292	132.72	−4.39	−3.72	−2.07	−0.95	0.15	0.51	0.43	
D6S308	138.78	−6.19	−5.54	−3.88	−2.63	−1.18	−0.42	−0.06	

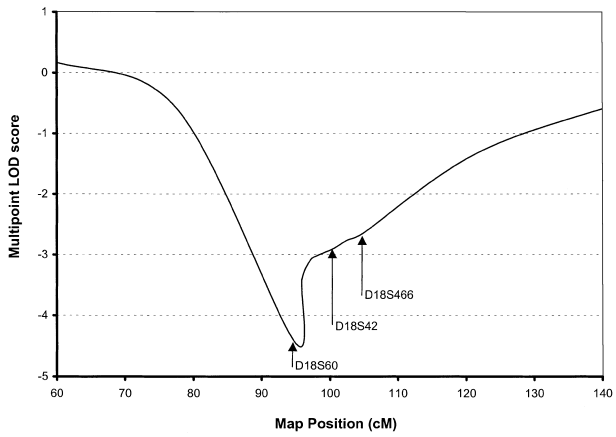

FIG. 1. Multipoint location score curve for markers spanning the *PDB2* region on chromosome 18q. The multipoint LOD scores are shown on the vertical axis and estimated genetic distance (cM) is shown on the horizontal axis. The approximate positions of the markers are indicated.

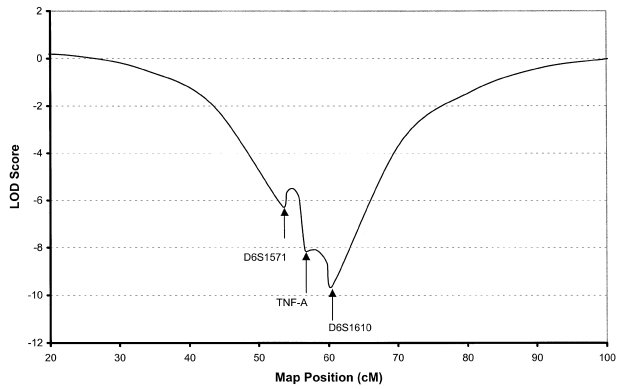

FIG. 2. Multipoint location score curve for markers spanning the *PDB1* region on chromosome 6p. The multipoint LOD scores are shown on the vertical axis, and estimated genetic distance (cM) is shown on the horizontal axis. The approximate positions of the markers are indicated.

measles virus or canine distemper virus transcripts in pagetic osteoclasts.[24,25] Studies of osteoclast precursors from Paget's disease patients show evidence of measles virus messenger RNA (mRNA), including mutations of a specific region of the viral nucleocapsid gene.[26] There have been studies suggesting that interleukin-6 may play a role in regulating the behavior of the osteoclast line in Paget's disease.[27] In the current study we did not examine the expression of paramyxoviral transcripts in affected family members because we do not have access to osteoclast tissue from pedigree members at present. A current "unifying" hypothesis is that the large, functionally hyperactive osteoclasts in pagetic bone are a product of a virus-mediated increase in cell fusion between osteoclasts and osteoclast progenitor cells that migrate to pagetic sites. It is possible that a common viral infection in a genetically susceptible host predisposes to an osteoclast lesion that is manifest in adulthood as Paget's disease.[28]

The identification of genetic markers for Paget's disease is fundamental for understanding the cause of the disease, for identifying subjects at risk at a preclinical stage, and for the development of more effective preventive and therapeutic strategies for the management of the condition. We propose to undertake linkage analysis with microsatellite markers distributed across the genome to localize a novel susceptibility locus for Paget's disease of bone, which can be followed by positional cloning to identify the gene. These studies will result in a better understanding of the causes of Paget's disease, and in the longer term will lead to the development of more effective strategies for prevention and treatment.

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REFERENCES

1. Barker DJ 1984 The epidemiology of Paget's disease of bone. Br Med Bull **40:**396–400.
2. Gardner MJ, Guyer PB, Barker DJ 1978 Radiological prevalence of Paget's disease of bone in British migrants to Australia. BMJ **6128:**1655–1657.
3. Ooi CG, Fraser WD 1997 Paget's disease of bone. Postgrad Med J **73:**69–74.
4. Siris ES 1996 Seeking the elusive etiology of Paget's disease: A progress report. J Bone Miner Res **11:**1599–1601.
5. Ralston SH, Helfrich MH, Hobson RP, Pennington TH 1997 Does Paget's disease really have a viral etiology? J Bone Miner Res **12:**863–864.
6. Siris ES, Ottman R, Flaster E, Kelsey JL 1991 Familial aggregation of Paget's disease of bone. J Bone Miner Res **6:**495–500.
7. Morales-Piga AA, Rey-Rey JS, Corres-Gonzales J, Garcia-Sagredo JM, Lopez-Abente G 1995 Frequency and characterisation of familial aggregation of Paget's disease of bone. J Bone Miner Res **10:**663–670.
8. Fotino M, Haymovits A, Falk CT 1977 Evidence for linkage between HLA and Paget's disease. Transplant Proc **9:**1876–1878.
9. Tilyard MW, Gardner RJ, Milligan L, Cleary TA, Stewart RD 1982 A probable linkage between familial Paget's disease and the HLA loci. Aust N Z J Med **12:**498–500.
10. Moore SB, Hoffman DL 1988 Absence of HLA linkage in a family with osteitis deformans (Paget's disease of bone). Tissue Antigens **31:**69–70.
11. Laurin N, Brown J, Duchesne A, Brousseau C, Huot D, Lacourciere Y, Drapeau G, Verreault J, Raymond V, Morissette J 1999 Genetic heterogeneity of Paget's disease of bone: Exclusion of linkage to the PDB1 and PDB2 loci in French-Canadian pedigrees. Am J Hum Genet **65**(Suppl):1442 (abstract).
12. Osterberg PH, Wallace RG, Adams DA, Crone RS, Dickson GR, Kanis JA, Mollan RA, Nevin NC, Sloan J, Toner PG 1988 Familial expansile osteolysis: A new dysplasia. J Bone Joint Surg **70:**255–260.
13. Wallace RG, Barr RJ, Osterberg PH, Mollan RA 1989 Familial expansile osteolysis. Clin Orthop **248:**265–277.
14. Hughes AE, Shearman AM, Weber JL, Barr RJ, Wallace RG, Osterberg PH, Nevin NC, Mollan RA 1994 Genetic linkage of familial expansile osteolysis to chromosome 18q Hum. Mol Genet **3:**359–361.
15. Cody JD, Singer FR, Roodman GD, Otterund B, Lewis TB, Leppert M, Leach RJ 1997 Genetic linkage of Paget's disease of the bone to chromosome 18q. Am J Hum Genet **61:**1117–1122.
16. Hadjipavlou A, Lander P, Srolovitz H, Enker IP 1992 Malignant transformation in Paget's disease of bone. Cancer **70:**2802–2808.
17. Nellissery MJ, Padalecki SS, Brkanac Z, Singer FR, Roodman GD, Unni KK, Leach RJ, Hansen MF 1998 Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18 region genetically linked with Paget's disease of bone. Am J Hum Genet **63:**817–824.
18. Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, van Hul W, Whyte MP, Nakatsuka K, Hovy L Anderson DM 2000 Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet **24:**45–48.
19. Haslam SI, Van Hul W, Morales-Piga A, Balemans W, San-Millan JL, Nakatsuka K, Willems P, Haites NE, Ralston S 1998 Paget's disease of bone: Evidence for a susceptibility locus on chromosome 18q and for genetic heterogeneity. J Bone Miner Res **13:**911–917.
20. Miller SA, Dykes DD, Polesky HF 1988 A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res **16:**1215.
21. O'Connell JR, Weeks DE 1995 The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set recoding and fuzzy inheritance. Nat Genet **11:**402–408.
22. Rebel A, Bregeon C, Basle M, Malkani K, Patezour A, Filmon R 1975 Osteoclastic inclusions in Paget's disease of bone. Rev Rhum Mal Osteoartic **42:**637–641.
23. Mills BG, Singer FR 1976 Nuclear inclusions in Paget's disease of bone. Science **194:**201–202.
24. Gordon MT, Mee AP, Anderson DC, Sharpe PT 1992 Canine distemper virus transcripts sequenced from pagetic bone. Bone Miner **19:**159–174.
25. Cartwright EJ, Gordon MT, Freemont AJ, Anderson DC, Sharpe PT 1993 Paramyxovirus and Paget's disease. J Med Virol **40:**133–141.
26. Reddy SV, Singer FR, Roodman GD 1995 Bone marrow mononuclear cells from patients with Paget's disease contain measles virus nucleocapsid messenger ribonucleic acid that has mutations in a specific region of the sequence. J Clin Endocrinol Metab **80:**2108–2111.
27. Roodman GD, Kurihara N, Ohsaki Y, Zukita A, Hosking D, Demulder A, Smith JF, Singer FR 1992 Interleukin-6: A potential autocrine/paracrine factor in Paget's disease of bone. J Clin Invest **89:**46–52.
28. Siris ES 1996 Paget's disease of bone. In: Favus MJ (ed.) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. Lippincott-Raven, Philadelphia, PA, USA, pp. 409–419.

Address reprint requests to:
Dr. Joanne Shaw
Department of Diabetes and Endocrinology
Princess Alexandra Hospital
Ipswich Road
Woolloongabba, Brisbane Q 4102, Australia

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