Inheritance of Osteosarcoma and Paget's Disease of Bone

A Familial Loss of Heterozygosity Study

Julie D. K. McNairn,* Timothy A. Damron,[†]
Steve K. Landas,* J. Lee Ambrose,[‡] and
Antony E. Shrimpton*

From the Departments of Pathology,* Orthopedic Surgery,[†] and
Radiology,[‡] SUNY Upstate Medical University, Syracuse, New York

Pagetoid osteosarcoma is a complication of Paget's disease of bone. Sarcomatous transformation is most often seen in severe, long-standing Paget's disease. Familial clustering of Paget's disease has been described with apparent autosomal dominant inheritance with high penetrance by the sixth decade. Although definitive proof of the specific gene involved remains elusive, some researchers have shown loss of heterozygosity in a region of chromosome 18q in a relatively high percentage of studied patients affected with either Paget's disease alone, in Pagetoid osteosarcoma, and in uncomplicated osteosarcoma. Our patient was diagnosed with Pagetoid osteosarcoma and had a first-degree relative with history of the same. We hypothesized that our patient's tumor samples might contain a similar genetic abnormality. Our analysis of several polymorphic markers from the chromosome 18q21–22 region showed loss of maternally inherited alleles throughout the region. This finding is similar to those described previously⁴ and provides further evidence of a susceptibility region relating to this disease. This report describes a father and son, their young ages at diagnosis of Pagetoid sarcoma, the identical sites of disease involvement, and a loss of heterozygosity study illustrating the inheritance of the presumed defective gene. (*J Mol Diag* 2001, 3:171–177)

Paget's disease of bone is a disorder with a predilection for older individuals.^{1–9} Current data suggest that the disease affects 2 to 3% of the population over the age of fifty and 10% over the age of eighty.⁵ The symptoms of the disease vary from virtually asymptomatic to widespread bone involvement with significant morbidity.¹⁰ The most serious complication of Paget's disease is sarcomatous transformation of affected bone.¹

An uncoupling of bone remodeling, the underlying cause of Paget's disease of bone, has been explored

extensively. Some have proposed a viral etiology given the bizarre morphology and increased number of nuclei in giant cells.^{11–13} Others have ascribed a genetic character to the disease.^{3,6,9,14–16} Still others have suggested a genetic predisposition to a viral infection as a likely cause of this abnormal bone remodeling.^{11,14,17} A combined hypothesis is a multifactorial model, which includes both sporadic and familial forms of the disease.⁹

Although Paget himself failed to recognize a familial tendency of Paget's disease, familial clustering has been observed. Likewise, a familial form of osteosarcoma affecting bone involved by Paget's disease is documented by reports in the literature. The details of these cases are varied; only one describes a direct lineage in which Pagetoid osteosarcoma developed in affected family members.^{15,16,18–23}

This genetic study describes a son and father; both affected with Pagetoid osteosarcoma at identical anatomical sites. Both were young men at the time of diagnosis, and both met untimely deaths from the osteosarcoma.

Case Presentations

A 33-year-old man with a 9-year history of Paget's disease presented with a mass of the distal right femur with worsening pain, a recent 25-pound weight loss, and drenching night sweats. Several months before this, he had reported stiffness in his knees. The stiffness had been attributed to chronic osteomyelitis and leg length discrepancy and was treated with non-steroidal anti-inflammatory drugs and braces.

His past medical history was significant for multiple long bone fractures. At age 18 he fractured his femurs bilaterally in a motorcycle accident. At age 24 he suffered a left mid-femur fracture while jogging. Work-up at that time led to a diagnosis of Paget's disease involving both femurs and proximal tibiae. Physical examination re-

Supported by funding from the Pathology Medical Service Group and the Marvin A. Damron Memorial Cancer Research Fund.

Accepted for publication July 27, 2001.

Address reprint requests to Timothy A. Damron, M.D., Department of Orthopedics, Suite 100, 550 Harrison Center, Syracuse, NY 13202. E-mail: damront@mailbox.upstate.edu.

Figure 1. Anteroposterior radiograph of the right distal femur shows severe deformity due both to the underlying Paget's disease and a healed distal femoral fracture. A soft tissue mass is shown extending posteromedially (arrows). Pagetoid involvement of the adjoining proximal tibia is also seen.

vealed tenderness, palpable fullness, and increased warmth through the distal right thigh.

Radiographs of the right knee and distal femur demonstrated severe bone deformity consistent with Paget's disease and his prior healed fracture, but a soft tissue mass was identified posterior and medial to the distal femur emanating from the bone (Figure 1). Chest radiographs identified two discrete pulmonary nodules. A bone scan revealed markedly increased flow to the right knee and right distal femur (Figure 2).

A magnetic resonance imaging study demonstrated Paget's disease involving the epiphysis and distal diame-

Figure 2. Total skeleton bone scan shows increased uptake in the medial and lateral condyles and distal medial cortex of the right femur, with a negative image created by the tumor mass (arrow).

taphyseal region of the right femur. A large soft tissue mass was appreciated posteromedially adjacent to the thickened periosteum and cortex of the distal diaphysis with distal extension (Figure 3). The soft tissue mass was approximated to be 10 centimeters in greatest dimension.

The clinical and radiological data supported a presumptive diagnosis of Pagetoid osteosarcoma. An open biopsy of the right distal thigh mass showed a storiform, collagenized neoplasm with focal osteoid production. The cells were very pleomorphic, forming sheets in several foci. There were scattered tumor giant cells with a

Figure 3. Magnetic resonance imaging coronal T1 weighted spin echo (A) and coronal T2 weighted fast spin echo (B) show a large lobulated tumor mass arising from the epiphysis and distal diaphysis. The mass (arrows) shows low signal on T1-weighted images and hyperintensity on T2-weighted images.

semicircular configuration of nuclei at the cell periphery. Mitotic figures were seen at a rate of approximately 60/10 high power field. The diagnosis of Grade 4 osteosarcoma, fibroblastic type, was made (Figure 4).

The patient was treated with neoadjuvant chemotherapy, including high dose methotrexate, as per modified POG protocol 9450. A magnetic resonance imaging study performed following the neoadjuvant therapy showed a large, lobulated mass of the distal femur with soft tissue extension. The mass was similar to, but slightly larger than that described in previous studies. Additional lung nodules were identified at this time.

The patient underwent a right above-the-knee amputation, as the tumor involvement was too extensive to

consider limb-sparing surgery. Examination of the amputation specimen showed Grade 4 osteosarcoma similar to that in the biopsy specimen. Gross examination of the specimen showed destruction of the metaphysis and tibial plateau. The tumor mass was thirteen by nine by eight centimeters and appeared to enter the knee joint space. Microscopic examination showed marginal necrotic bone and approximately 50% tumor necrosis identified. The resection margins were free from tumor, as confirmed by frozen section. Despite additional chemotherapy, including high-dose methotrexate, etoposide, ifosfamide, adriamycin, and platinum, with resection of multiple lung nodules, the patient succumbed to his disease almost 2 years following diagnosis.

Figure 4. Histological sections of the tumor mass (**A**, **B**) demonstrate the hypercellular storiform tumor mass. Mitotic figures are extensive. Focal scant osteoid production is identified in **A** (arrows) diagnostic of osteosarcoma. Histological sections of bone away from the sarcoma within the resected distal femoral specimen (**C**) illustrate haphazardly organized, thickened trabeculae and irregular cement lines consistent with bone involved by Paget's disease after neoadjuvant chemotherapy. **A:** Trichrome stain, $\times 20$; **B:** Hematoxylin and eosin, $\times 40$; **C:** Hematoxylin and eosin, $\times 20$.

The father of our patient presented at age 39 to Roswell Park Cancer Center in Buffalo, NY in early 1962 with abnormal x-ray findings of both distal femurs. A presumptive diagnosis of osteogenic sarcoma of both femurs was rendered. He underwent biopsies of both right and left femurs to confirm this presumptive diagnosis. Both biopsies illustrated Paget's disease of bone. However, only the specimen from the right femur contained osteogenic sarcoma arising in the Paget's disease. He underwent a right hip disarticulation. Nearly one year later, severe pain prompted x-rays of the chest, pelvis, and left leg. These all failed to demonstrate metastases or disease progression. Approximately six months later, the patient presented again with lesions of the left femur and tibia including a pathological fracture through the upper left tibia. An open biopsy performed at this time showed osteogenic sarcoma of the left leg. X-rays of the chest demonstrated two discrete pulmonary nodules, believed to be metastatic involvement. The patient was treated with BCNU (bis-chloronitrosourea, carmustine) for 3 days but suffered severe toxicity. No further chemotherapy or surgery was initiated. The patient succumbed to his osteosarcoma 3 months later, less than 2 years after his initial diagnosis.

Materials and Methods

Linkage Analysis

Microsatellite markers located in 18q21.2–18q23 were selected for polymerase chain reaction, based on their

heterozygosity. Primers were obtained from Research Genetics (Huntsville, AL) and amplified using the manufacturer's conditions. DNA isolated from peripheral blood and tumor samples were genotyped at these markers by running PCR products on 8% polyacrylamide gels.

Patient Samples

Formalin-fixed post-surgical osteosarcoma samples were obtained from the primary and metastatic tumor sites from our patient. A matched control sample was obtained as adjacent normal tissue from the post-surgical specimen. Tumor samples from the father of our patient were unavailable. A blood sample was obtained from the mother and siblings of our patient to better elucidate the inheritance at the 18q loci in question. This research was done under the approval of the Institutional Review Board, and informed consent was obtained before sample acquisition.

DNA Isolation

DNA was isolated from the formalin-fixed post-surgical specimens as described previously.²⁴ DNA was isolated from the peripheral blood sample of the living relatives using a non-organic extraction kit, Intergen Company, (Q-Biogene, Carlsbad, CA) catalog number S4520.

Figure 5. Pedigree microsatellite genotype results. The index case is represented by post-surgical non-tumor specimen (II:4) and post-surgical tumor specimen (II:5).

Loss of Heterozygosity Analysis

Several polymorphic microsatellite loci were used in this analysis, including D18S858, D18S1144, D18S1129, D18S38, D18S1147, D18S60, D18S68, D18S1142, D18S42, D18S55, D18S1113, D18S878, D18S43, D18S844, and myelin basic protein. Additional tumor markers in the 18q region were uninformative. PCR amplified products from the unaffected mother (I:2), three unaffected siblings (II:1, II:2, II:3), the affected son (II:4), and his tumor (II:5) were run on polyacrylamide gels and detected by silver staining.²⁵

Results

Results from the 18q microsatellite marker genotyping are shown in Figures 5 and 6. Unfortunately no material was available from the affected father (I:1), though his genotype could be partially reconstructed from his offspring. Loss of heterozygosity involving the maternally inherited alleles was detected in the tumor (II:5) as compared to the blood (II:4). Markers in 18q21.2 (D18S858), 18q22.1 (D18S68, D18S1113, D18S878) were detected in reduced amounts indicating a chromosomal deletion. The reduced amounts rather than complete absence of

A.

D18S858

Allele

1

2

Lane 1

2

B.

MBP

Allele

1

2

Lane

3

4

Figure 6. A: D18S858 PCR product from blood (lane 1) and from tumor of the index case (lane 2). Run on an 8% polyacrylamide gel for 11,000- volt hours. Note that allele 1 (•) of the tumor is present in reduced amounts compared to allele 1 in blood (x). B: In contrast, in lane 3, MBP PCR product from blood, and lane 4, from tumor of the index case show no evidence of loss of heterozygosity.

the maternal allele indicated that the tumor sample included non-tumor tissue. The maternally inherited MBP microsatellite allele in 18q23 did not appear to be deleted in the tumor. Thus the distal breakpoint appears to lie between D18S878 and MBP. The proximal breakpoint was not defined but appears to lie proximal to D18S858. All other markers were uninformative.

This result is consistent with the paternal transmission of a mutation in a putative tumor suppressor gene that is uncovered by an acquired "second hit" deletion including 18q22.1 in the tumor. The carrier status of this putative tumor suppressor mutation in the three unaffected siblings is uncertain given the lack of an exact location of the tumor suppressor and the lack of a paternal sample to genotype.

Discussion

Worsening pain, pain resistant to analgesics, or changes in the pain pattern often signals the development of osteosarcoma in Paget's disease.^{26,27} Swelling, fracture, weight loss, night sweats, and neurological complaints may accompany these symptoms.^{2,28} The typical presentation of an affected patient with sarcomatous transformation is a 65-to-70 year-old man with a 15-to-25 year history of polyostotic Paget's disease.^{2,27-29} The sarcoma itself is usually high grade with a poor prognosis.²⁹ Some researchers have attributed the low survival in Pagetoid osteosarcoma to effects of advanced age: a weakened immune system, a decreased ability to battle the disease, and decreased tolerance of treatment,²⁷ for example. Other factors, such as environmental exposure, may also prove significant though proof is lacking. However, both patients described here were significantly younger than those typically affected by these disease processes, though they did exhibit some of the classic symptoms. The x-ray findings in our patient clearly show involvement by the sclerotic phase of Paget's disease, suggesting a long disease course.

Both of these patients had involvement of the distal femur, a common site of involvement for both the diseases.^{2,27,30,31} One previous report of a father and son affected in identical locations describes a father, affected at age 78, and son, affected at age 60, with Pagetoid osteosarcoma. Both men were affected at the proximal tibia, had long histories of Paget's disease leading up to the sarcomatous transformation, and died of their disease within months of diagnosis with evidence of pulmonary metastases.¹⁸ Previous to that report, Paget's disease was considered to be transmitted as an incompletely dominant X-linked gene. That case was the first to give credence to male-to-male transmission, initiating research to explore the possibility of involvement of an autosomal gene. Since this time, evidence has pointed to several genes linked to Paget's disease, some as yet unidentified. Linkage analysis studies first uncovered an apparent link to the HLA locus on chromosome 6, now termed PDB1.¹¹

Further study provided evidence of linkage between Paget's disease and a susceptibility locus on chromo-

some 18q21-22. This locus harbors the gene identified in a family afflicted with familial expansile osteolysis, a rare bone disorder with features very similar to Paget's disease.^{6,13} A subsequent study has also identified a putative tumor suppressor gene on chromosome 18q. In that study, all tumor samples examined from individuals affected with Pagetoid osteosarcoma showed some loss of heterozygosity in the 18q21-22 region studied. A lower percentage of tumor samples from sporadic osteosarcoma, those not superimposed on Paget's disease, also showed loss of heterozygosity in this same region.⁴ This is in contrast to the more conventional high-grade osteosarcomas occurring in children affected with retinoblastoma, as part of the Li-Fraumeni syndrome, or in so-called cancer families.³²⁻³⁴ Another study used seven polymorphic loci in this region of chromosome 18q21-22 in eight genetically diverse families affected by Paget's disease only. Five of eight showed linkage to the region, while two showed no linkage and the last family was inconclusive. Interestingly, three of the families were of Spanish descent, yet one showed linkage to this region.⁵

Our genetic analysis does support the idea of a susceptibility region on chromosome 18 in the region described previously.⁴⁻⁶ Work to definitively determine the associated gene would potentially be helpful in further understanding the disease etiology. The gene encoding the receptor activator of nuclear factor- κ B (*RANK*, TNFRSF11A) lies within the critical region of 18q that has been deleted. The *RANK* gene represents a strong candidate gene since it has been found to be mutated in familial expansile osteolysis.³⁵ Although thus far the *RANK* gene has not been found to be mutated in Paget's disease of bone, Wuyts did find a statistical association between a *RANK* polymorphism and Paget's disease of bone, indicating that it may represent a susceptibility factor.^{36,37} Sequencing of this gene in the presented family, as well as examination of other regions, including the PDB1 locus may uncover more information.

Other siblings within this family may have inherited the same alleles as our patient. However, these siblings, ranging in age from 39 to 44, do not show any signs of Paget's disease or Pagetoid osteosarcoma to this point. Although they are younger than the typical age for diagnosis with either disease, they are both older than their family members were at initial presentation. Why only one offspring was affected by severe Paget's disease and Pagetoid osteosarcoma is not easily explained. The similarity of his disease to that of his father is curious. Perhaps elucidation of the gene involved will clarify the mechanism. This may be the key to improving the dismal prognosis associated with this disease.

Acknowledgments

The authors are grateful for the consultative input of Dr. Krishnan Unni of the Mayo Clinic, Rochester, Minnesota. We thank S.O. Sanderson, M.D. for his assistance in photographing the histological sections.

References

- Barry HC: Paget's Disease of Bone. London, E & S Livingstone, Ltd., 1969
- Huvos AG, Butler A, Bretsky SS: Osteogenic sarcoma associated with Paget's disease of bone: a clinicopathologic study of 65 patients. *Cancer* 1983, 52:1489-1495
- Siris ES, Ottman R, Flaster E, Kelsey JL: Familial aggregation of Paget's disease of bone. *J Bone Miner Res* 1991, 6:495-500
- Nellisery MJ, Padalecki SS, Brkanac Z, Singer FR, Roodman GD, Unni KK, Leach RJ, Hansen MF: Evidence for a novel osteosarcoma tumor-suppressor gene in the chromosome 18q region genetically linked with Paget disease of bone. *Am J Hum Genet* 1998, 63:817-824
- Haslam SI, Van Hul W, Morales-Piga A, Balemans W, San-Millan JL, Nakatsuka K, Willems P, Hautes NE, Ralston SH: Paget's disease of bone: evidence for a susceptibility locus on chromosome 18q and for genetic heterogeneity. *J Bone Miner Res* 1998, 13:911-917
- Cody JD, Singer FR, Roodman GD, Otterund B, Lewis TB, Leppert M, Leach RJ: Genetic linkage of Paget disease of the bone to chromosome 18q. *Am J Hum Genet* 1997, 61:1117-1122
- Hansen MF, Nellisery MJ, Bhatia P: Common mechanisms of osteosarcoma and Paget's disease. *J Bone Miner Res* 1999, 14:39-44
- Horstmann MA, Posl M, Scholz RB, Anderegg B, Simon P, Baumgaer HK, Dellling G, Kabisch H: Frequent reduction or loss of DCC gene expression in human osteosarcoma. *Br J Cancer* 1997, 75:1309-1317
- Van Hul W: Paget's disease from a genetic perspective. *Bone* 1999, 24:29S-30S
- Siris ES: Extensive personal experience Paget's disease of bone. *J Clin Endocrinol Metab* 1995, 80:335-338
- Ooi CG, Fraser WD: Paget's disease of bone. *Postgrad Med J* 1996, 73:69-74
- Bone HG, Kleerekoper M: Clinical review: 39 Paget's disease of bone. *J Clin Endocrinol Metab* 1992, 75:1179-1182
- Roodman GD: Paget's disease and osteoclast biology. *Bone* 1996, 19:209-212
- Siris E: Epidemiological aspects of Paget's disease: family history and relationship to other medical conditions. *Semin Arthritis Rheum* 1994, 23:222-225
- Greenspan A, Norman A, Sterling AP: Precocious onset of Paget's disease: a report of three cases and review of the literature. *J Can Assoc Radiol* 1977, 28:69-72
- Irvine R: Familial Paget's disease with early onset. *J Bone Joint Surg Br* 1953, 35:106-112
- Swaney J: Familial osteogenic sarcoma. *Clin Orthop Relat Res* 1973, 97:64-68
- Nassar VH, Gravanis MB: Familial osteogenic sarcoma occurring in Pagetoid bone. *Am J Clin Pathol* 1981, 76:235-239
- Brenton DP, Isenberg DA, Bertram J: Osteosarcoma complicating familial Paget's disease. *Postgrad Med J* 1980, 56:238-243
- Eisman JA, Martin TJ: Osteolytic Paget's disease: recognition and risks of biopsy. *J Bone Joint Surg* 1986, 68:112-117
- Jacobs P: Osteolytic Paget's disease. *Clin Radiol* 1974, 25:137-144
- Wu RK, Trumble TE, Ruwe PA: Familial incidence of Paget's disease and secondary osteosarcoma: a report of three cases from a single family. *Clin Orthop Relat Res* 1991, 306-309
- Kim GS, Kim SH, Cho JK, Park JY, Shin MJ, Shong YK, Lee K, Han H, Kim T, Teitelbaum SL, Reinus WR, Whyte MP: Paget bone disease involving young adults in 3 generations of a Korean family. *Medicine* 1997, 76:157-169
- Friedman H, Sanderson S, Stein C, Shrimpton A, Gonchoroff NJ, Zamkoff KW, Loughran TP Jr: Extramedullary granulocytopoiesis mimicking recurrent lymphoma after prolonged administration of human recombinant granulocyte-colony stimulating factor. *Ann Hematol* 1998, 77:79-83
- Wallace A, Williamson P, Mountford R, Ramsden S: Non-isotopic detection of microsatellite repeats using silver staining. *J Med Genet* 1993, 30:346-347
- Mirra JM, Brien EW, Tehranzadeh J: Paget's disease of bone: review with emphasis on radiologic features, part I. *Skeletal Radiol* 1995, 24:163-171
- Wick MR, McLeod RA, Siegal GP, Greditzer HG, Unni KK: Sarcomas of bone complicating osteitis deformans. *Am J Surg Pathol* 1981, 5:47-59
- Jattiot F, Goupille P, Azais I, Roulet B, Alcalay M, Jeannou J, Bontoux D, Valet J: Fourteen cases of sarcomatous degeneration in Paget's disease. *J Rheumatol* 1999, 26:150-155
- Mirra JM, Brien EW, Tehranzadeh J: Paget's disease of bone: review with emphasis on radiologic features, part II. *Skeletal Radiol* 1995, 24:173-184
- Haibach H, Farrell C, Dittich FJ: Neoplasms arising in Paget's disease of bone: a study of 82 cases. *Am J Clin Pathol* 1985, 83:594-600
- Greditzer HG, McLeod RA, Unni KK, Beabout JW: Bone sarcomas in Paget disease. *Radiology* 1983, 146:327-333
- Colyer RA: Osteogenic sarcoma in siblings. *Johns Hopkins Med J* 1979, 145:131-135
- Porter DE, Holden ST, Steel CM, Cohen BB, Wallace MR, Reid R: A significant proportion of patients with osteosarcoma may belong to Li-Fraumeni cancer families. *J Bone Joint Surg Br* 1992, 74:883-886
- Epstein LI, Bixler D, Bennett JE: An incident of familial cancer including 3 cases of osteogenic sarcoma. *Cancer* 1970, 25:889-891
- Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM: Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. *Nat Genet* 2000, 24:45-48
- Wuyts W, Van Wesenbeeck L, Morales-Piga A, Ralston S, Hocking L, Vanhoenacker F, Westhovens R, Verbruggen L, Anderson D, Hughes A, Van Hul W: Evaluation of the role of RANK and OPG genes in Paget's disease of bone. *Bone* 2001, 28:104-107
- Sparks AB, Peterson SN, Bell C, Loftus BJ, Hocking L, Cahill DP, Frassica FJ, Streeten EA, Levine MA, Fraser CM, Adams MD, Broder S, Venter JC, Kinzler KW, Vogelstein B, Ralston SH: Mutation screening of the TNFRSF11A gene encoding receptor activator of NF kappa B (RANK) in familial and sporadic Paget's disease of bone and osteosarcoma. *Calcif Tissue Int* 2001, 68:151-155