Genetic Linkage of Paget Disease of the Bone to Chromosome 18q

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Summary

Paget disease is a common bone disease characterized by abnormal osteoclasts that are large, multinucleated, and overactive and that contain paramyxovirus-like nuclear inclusions. There is evidence for a major genetic component to Paget disease, with up to 40% of patients having affected first-degree relatives; however, the locus (loci) and gene(s) involved are unknown. Another bone disorder, familial expansile osteolysis (FEO), although extremely rare, also is characterized by similar osteoclast abnormalities but has an earlier age at onset and a more aggressive clinical progression. The causative gene for FEO has been localized to a region of human chromosome 18q. On the basis of the presence of similar clinical findings and of viral-like nuclear inclusions in osteoclasts, we hypothesized that FEO and Paget disease are allelic versions of the same locus. Therefore, a large kindred with a high incidence of Paget disease was examined to determine if Paget disease was linked to genetic markers in the same region of chromosome 18 as that for FEO. Our analysis yielded a two-point LOD score of 3.40, with the genetic marker D18S42, a marker tightly linked to the FEO locus. This demonstrates that the gene(s) responsible for FEO and that for Paget disease are either closely linked or the same locus.

Introduction

Paget disease is one of the most flagrant examples of disordered bone remodeling, with abnormalities in all phases of the bone-remodeling process. Paget disease has an estimated incidence of 3.3% in individuals >40 years

of age, on the basis of autopsy data (Schmorl 1932). It is typically a disease found in older persons, and diagnosis at <40 years of age is uncommon (Klein and Norman 1995). Although a high proportion of patients with Paget disease appear asymptomatic, ~5% present with pain related to the pagetic process (Klein and Norman 1995). This disease has many other manifestations, including increased fractures, deafness, and neurological complications, as well as osteosarcomas (Hamdy 1995). The pathophysiological bases for these abnormalities are not defined clearly, except that the primary cellular abnormalities appear to reside in the osteoclasts. Osteoclasts in Paget disease are increased in number and in size and contain paramyxovirus-like nuclear inclusions (Singer 1996).

Paget disease has a strong familial tendency (Jacobs et al. 1979; Sofaer et al. 1983; Mirra 1986), suggesting a genetic component to the disease. In a recent study of the frequency and characteristics of familial aggregation of Paget disease in Spain, Morales-Piga et al. (1995) found that >40% of their index cases had at least one first-degree relative affected with Paget disease. In the pedigrees examined, Paget disease appeared to be transmitted by an autosomal dominant mode of inheritance. Siris (1994) conducted an epidemiological study of Paget disease in the United States, using questionnaires completed by 864 patients with physician-diagnosed Paget disease and by 500 controls of similar ages. A history of Paget disease was noted in a first-degree relative in 12% of the patients, compared with only 2% of controls. The risk that first-degree relatives of a pagetic patient will develop Paget disease was seven times greater than that for an individual without an affected relative. The cumulative risk for development of Paget disease, up to 90 years of age, for a first-degree relative of a patient was 9%, compared with 2% for individuals with unaffected relatives. Such data provide strong evidence for genetic factors playing a role in the acquisition of the disease.

Familial expansile osteolysis (FEO) is an extremely rare bone dysplasia that is known to be transmitted in an autosomal dominant fashion in a single large kindred
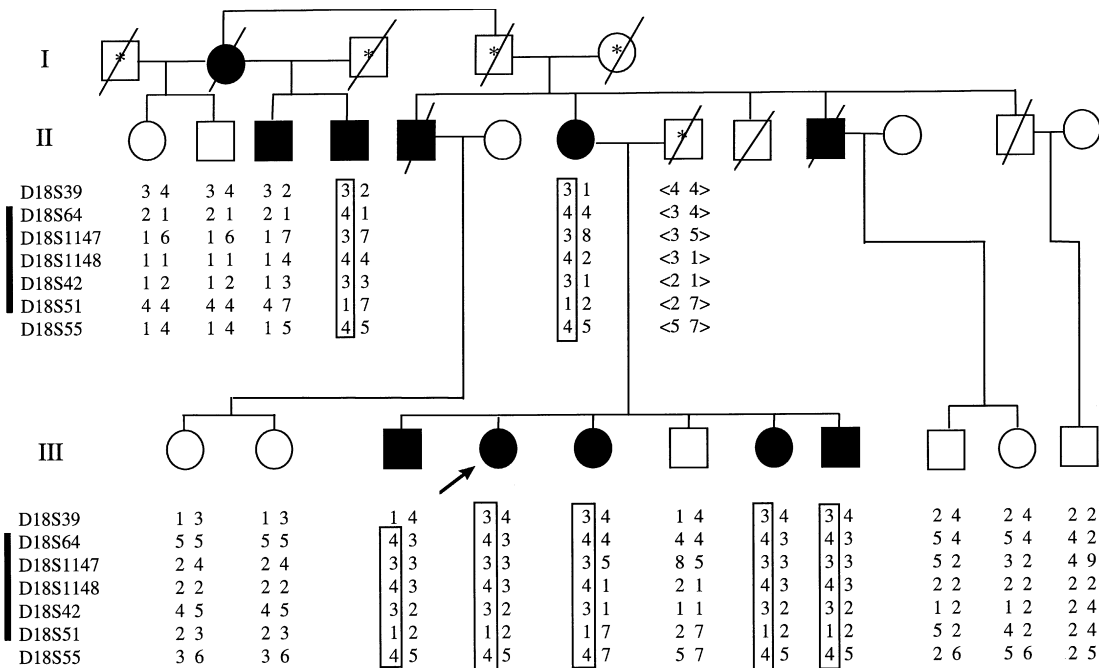
Figure 1   Pedigree of kindred with Paget disease. Blackened symbols represent affected individuals. An asterisk (*) indicates those individuals for whom the phenotype is unknown. The disease haplotype is indicated by a rectangle. The FEO critical region is denoted by a vertical boldface line to the left of the marker names.

from northern Ireland (Wallace et al. 1989). The pathogenesis of FEO is related to Paget disease in several ways. The histological bone lesions of FEO appear to be similar to pagetic lesions, although they occur at a much earlier age and are more severe. Furthermore, as in Paget disease, the osteoclasts in patients with FEO contain paramyxoviral-like nuclear inclusions (Dickson et al. 1991). These similarities suggest that FEO may be a rare and exaggerated form of Paget disease.

Recently, Hughes et al. (1994) have utilized genetic linkage analysis of a large kindred in northern Ireland, to localize FEO to chromosome 18q. The disease shows tight linkage to several polymorphic markers on chromosome 18q, with a maximum two-point LOD score of 11.53, with marker D18S64. Using the approach of linkage analysis, we determined that Paget disease is linked to the same region of chromosome 18 as that for FEO, in a single large kindred with Paget disease. This supports the hypothesis that Paget disease and FEO may be allelic versions of the same disorder.

Patients and Methods

Patient Population

We evaluated a large kindred (fig. 1) from Los Angeles that had migrated from Belize to the United States, over the past 30 years. This family is of Mayan, Spanish, and Scottish descent. It is thought that the disease was in-

herited from an ancestor who migrated to Belize from Scotland, where the incidence of Paget disease is high (Barker and Gardner 1974). We collected blood samples from 16 individuals in this kindred, who are ≥35 years of age, 8 of whom are diagnosed with Paget disease.

Clinical Evaluation

This study was approved by the Institutional Review Board of the St. John's Hospital and Health Center, Santa Monica, and all participants provided informed consent. All living participants were evaluated clinically by one of the authors (F.R.S.), who is an acknowledged expert in the clinical presentation of Paget disease. Individuals II-4, II-7, III-3, and III-4 were diagnosed with Paget disease before initiation of this study. All other living family members were assessed for their disease status first by measurement of serum alkaline phosphatase levels. Those individuals with a clinical indication of possible Paget disease, such as the presence of a high-normal or an elevated alkaline phosphate level (>90 IU/liter) or of symptoms that are suggestive of a musculoskeletal disorder, were evaluated further by an x-ray or a bone scan. Since these techniques involve radiation exposure, albeit at low levels, these procedures were performed only on those patients with a clinical indication of possible Paget disease. The deceased members of the kindred were diagnosed with Paget disease, after ex-

amination of photographs and from detailed descriptions provided by family members.

Genotypic Analysis

High-molecular-weight DNA was isolated from peripheral blood samples, in accordance with the methods of Bell et al. (1981). The DNA samples were genotyped by use of highly polymorphic PCR-based markers developed and mapped by Généthon (Dib et al. 1996). In addition, several other microsatellite markers in the region were utilized (LeBeau et al. 1993). The PCR reactions were performed as described elsewhere (Cody et al. 1997), by use of a total of 12 markers on 18q. The markers were D18S39, D18S64, D18S38, D18S1134, D18S499, D18S1147, D18S1148, D18S862, D18S60, D18S42, D18S51, and D18S55. Genotypic determinations were made without knowledge of disease status.

Linkage Analyses

For linkage analysis, it was assumed that the disease was inherited in an autosomal dominant mode, and the disease allele frequency was determined to be 1.5%, on the basis of a prevalence of 3% in the population (Schmorl 1932). Individuals were considered to be unaffected if they had a serum alkaline phosphatase level <90 IU/liter, had no evidence of a musculoskeletal disease, and were ≥35 years of age. On the basis of the observation of affected-to-unaffected ratios of individuals at risk in this kindred, we have assumed high penetrance (95%) for the disease allele. Allele frequencies were determined by the typing of 15–25 unrelated individuals of northern European descent, for all markers except D18S42. Because the D18S42 allele segregating with the disease in the family was a very rare allele (0.96% frequency), 104 chromosomes were used to determine the allele frequencies in unrelated individuals. All allele frequencies were determined on gels that contained samples from the affected family, which were run in lanes adjacent to the samples from the unrelated individuals. The LOD-score calculations were performed by use of the computer program LINKAGE, version 5.1 (Lathrop et al. 1984).

Results

To determine if a gene for Paget disease of bone resides on chromosome 18q, we studied a large kindred in which the disease appears to be inherited in an autosomal dominant fashion. This kindred has affected individuals, in multiple generations, who exhibit transmission through both males and females. All participants were evaluated clinically, with the results summarized in table 1. Included in table 1 are the results for four family members (individuals I-2, II-5, II-10, and II-12) who are deceased and who were diagnosed retrospectively, by

analysis of their history. The clinical severity of Paget disease in the family ranged from clinically asymptomatic but with radiological findings (individual III-7) to the extensive and crippling disabilities of the propositus (individual III-4, the sister of individual III-7), who had clinically apparent involvement of several bones, at an early age.

Since FEO previously had been localized to human chromosome 18q (Hughes et al. 1994), the DNA from these individuals was genotyped by use of markers from 18q. For the genotypic analysis performed for their study, Hughes et al. (1994) used the following markers in the following order (proximal to distal): D18S35, D18S64, D18S60, D18S55, D18S42, D18S61, MBP, D18S43, and D18S70. The FEO gene was flanked by the recombinant markers D18S35 and D18S61. These markers, as well as additional polymorphic markers in the region, were used to analyze the Paget disease kindred. In figure 1, the results of the genotypic analysis with the seven most informative markers are shown directly beneath each individual. The disease haplotype is indicated by a rectangle, in figure 1. However, one individual (II-3) had a clear diagnosis of Paget disease but did not possess the disease haplotype.

We obtained a maximum two-point LOD score of 3.40 with genetic marker D18S42, assuming 95% penetrance (table 2). Two-point LOD-score analysis also was performed with the assumption of a penetrance rate of 90% (data not shown). This analysis also generated a LOD score >3 with marker D18S42. However, the D18S42 allele segregating with the disease in this family was very rare. A significant LOD score (3.04) also was obtained with adjacent marker D18S1148. The allele frequency (21%) for this marker segregating with the disease indicated that this marker was not rare. The Paget disease region is bounded proximally by a recombination event at D18S39 (individual III-3). The distal boundary of the region remains undefined in this family, so we therefore were unable to determine the size of the critical region.

Multipoint analysis was performed by use of two adjacent markers in the map order, taken two at a time with the disease locus. All markers shown in table 2 were utilized, except for marker D18S51. This marker was excluded from multipoint analysis because its genetic location is not well established. The multipoint analysis places the disease locus at D18S42 (maximum LOD score of 3.1).

Discussion

We have performed linkage analysis on a large kindred in which Paget disease of bone was segregating in an autosomal dominant fashion. Because of the similarities between Paget disease and FEO, we postulated that these

Table 1

Phenotypic Data for Paget Disease Kindred

			PHENOTYPIC ASSESSMENT		
PATIENT	PRESENT AGE (years)	AFFECTED STATUS[a]	Serum Alkaline Phosphatase before Treatment[b] (IU/liter)	Skeletal Survey/ Bone Scan Performed	Clinical Diagnosis
I-2	Deceased	+	ND	No/no	Skull diagnosed retrospectively, through history and photographs
II-1	76	−	83	No/no	Not affected
II-2	64	−	84	No/no	Not affected
II-3	60	+	218	Yes/no	Pelvis, right and left femur
II-4	55	+	1,000	Yes/yes	Pelvis, right femur, sacrum, thoracic and lumbar spine, left humerus
II-5	Deceased	+	ND	No/no	Skull diagnosed retrospectively, through history and photographs
II-7	82	+	25[c]	Yes/no	Pelvis, lumbar spine, right clavicle, left humerus
II-10	Deceased	+	ND	No/no	Diagnosed retrospectively, through photographs and family report
II-13	Deceased	−	ND	No/no	Not affected
III-1	53	−	84	Yes/no	Not affected
III-2	35	−	55	No/no	Not affected
III-3	61	+	220	Yes/yes	Skull, left scapula, sacrum
III-4 (propositus)	60	+	2,000	Yes/yes	Skull, right and left humerus, right ilium, left patella, right femur, right and left tibia, thoracic spine, lumbar spine
III-5	58	+	ND	Yes/yes	Skull, right scapula, left calcaneus, lumbar spine, cervical spine
III-6	54	−	64	Yes/yes	Not affected
III-7	50	+	97	Yes/yes	Right and left ischium
III-8	48	+	329	Yes/no	Right femur
III-9	42	−	77	No/no	Not affected
III-10	35	−	67	No/no	Not affected
III-11	51	−	70	No/no	Not affected

[a] A plus sign (+) denotes that the patient was affected; a minus sign (−) denotes that the patient was unaffected.

[b] Normal level <126 IU/liter. ND = not determined, because the test was not performed.

[c] Measured at a different laboratory, where normal levels are <3 IU/liter.

two diseases may be allelic, caused by different mutations of the same gene. We therefore genotyped the individuals in our kindred, with markers for chromosome 18q that were in the region previously determined to be linked to FEO (Hughes et al. 1994). Hughes et al. (1994) obtained a maximum LOD score of 11.53 with D18S64, at 0 recombination, and a LOD score of 10.90 with D18S42, at 0 recombination, for a large kindred from northern Ireland (Hughes et al. 1994). We have obtained a maximum two-point LOD score of 3.40 with D18S42, demonstrating that Paget disease is linked to the same region of chromosome 18q as that for FEO. In addition, Haslam et al. (1997) recently reported, in abstract form, of the linkage of Paget disease to D18S42, with a max-

Table 2

Two-Point LOD Scores for Paget Disease Kindred, Obtained with Chromosome 18 Markers

MARKER	LOD SCORE AT RECOMBINATION FRACTION OF						
	.001	.01	.05	.10	.20	.30	.40
D18S39	−.13	.14	.48	.56	.50	.36	.18
D18S64	.45	.61	.87	.91	.75	.48	.20
D18S1147	1.89	2.01	2.13	2.00	1.50	.90	.36
D18S1148	3.04	2.99	2.76	2.47	1.83	1.15	.50
D18S42	3.40	3.34	3.07	2.27	2.01	1.28	.59
D18S51	2.11	2.24	2.37	2.26	1.79	1.20	.57
D18S55	1.63	1.77	1.91	1.81	1.37	.81	.29

imum LOD score of 5.7, determined by use of four kindreds from the United Kingdom. Thus, the combined LOD score for linkage of Paget disease to marker D18S42 is 9.1.

The FEO region recently has been narrowed to a chromosome 18 region of ~4.5 Mb, between markers D18S64 and D18S51 (Hughes and Barr 1996). There are several genes that have been identified in this region; however, none of them are obvious candidate genes for this disease.

The data in figure 1 show that individual II-3 does not carry the disease haplotype but meets the disease criteria for the study. There are at least three possible explanations for this finding. First, his Paget disease could be the result of a new mutation of a gene involved in Paget disease. Second, individual II-3 may have inherited the disease gene from his father (individual I-3), whose phenotype is unknown. If inherited from his father, his Paget disease could be due to either a gene at this locus or, if the disease is genetically heterogeneous, a gene at another locus. This possibility seems likely, since the incidence of Paget disease in the general population is relatively high (~3%). Third, the patient could have a form of Paget disease that does not have a genetic cause. It is not possible to determine the relative probability of these explanations, because no gene(s) has been identified for Paget disease, and, thus, the rate of new mutation is unknown.

Individuals who had a high-normal alkaline phosphatase level or signs of musculoskeletal disease were evaluated further by an x-ray or a bone scan. One individual (III-7) in this kindred had no external signs of Paget disease, but a bone scan revealed the presence of two small pagetic lesions in her pelvis. This finding indicates that, although a high serum alkaline phosphatase level often is associated with the disease, a high-normal serum alkaline phosphatase level does not exclude the presence of the disease. This finding also suggests that the familial nature of Paget disease may not be appreciated fully, since some individuals may have occult pagetic lesions.

Owing to the late-onset nature of Paget disease, it is possible that individuals, in this kindred, who were <45 years of age actually carried the disease gene and were asymptomatic owing to their age. However, in this family, several members had early onset of the disease (<30 years of age). Early onset commonly is observed in genetic forms of common sporadic diseases (Hall et al. 1990).

A viral etiology has been suggested for Paget disease. In 1974, Rebel et al. (1974) described organized groups of microcylinders within the nuclei and cytoplasm of osteoclasts in pagetic bone lesions from patients with Paget disease and suggested that these may be viral inclusions (Rebel et al. 1976; Harvey et al. 1982). These histological findings were substantiated by Mills and Singer (1976) and by others (Gherardi et al. 1980; Vacher-Lavenu et al. 1981), who reported viral inclusions in the osteoclasts from essentially all Paget disease patients. However, these inclusions are not limited to or diagnostic of Paget disease. Similar findings of inclusion bodies have been reported rarely in bone specimens from patients with giant-cell tumors of the bone (Welsh and Meyer 1970) and in a few cases of pychodysostosis (Beneton et al. 1987), osteopetrosis (Mills et al. 1988), and primary oxalosis (Bianco et al. 1992). These viral-like inclusions appear to be related to the paramyxovirus group of viruses, for several reasons. First, the viral-like inclusion bodies are closely similar in structure to paramyxoviruses. Second, these inclusions cross-react with antibodies against paramyxoviral nucleocapsid antigens. Third, osteoclasts and their precursor cells from patients with Paget disease express the measles virus (Reddy et al. 1995) or the canine-distemper-virus nucleocapsid mRNA (Gordon et al. 1990).

At present, the gene(s) responsible for Paget disease is unknown. One possible function of this gene(s), which may be mutated both in Paget disease and in FEO patients, is to confer an increased susceptibility, to osteoclasts and their precursors, to viral infection by paramyxovirus. Alternatively, this gene(s) may be involved in enhanced osteoclast activity or formation.

The identification of a locus on 18q for Paget disease is the first step toward identification of the gene involved. The identification of a gene(s) will give important insights into the mechanism underlying the abnormal osteoclast activity in Paget disease and the normal bone-remodeling process. In addition, identification of the gene(s) for Paget disease could have important therapeutic implications for the estimated 2–3 million patients in the United States who have Paget disease.

Acknowledgments

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