Genomewide Search in Familial Paget Disease of Bone Shows Evidence of Genetic Heterogeneity with Candidate Loci on Chromosomes 2q36, 10p13, and 5q35

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Paget disease of bone (PDB) is a common disorder characterized by focal abnormalities of increased and disorganized bone turnover. Genetic factors are important in the pathogenesis of PDB, and previous studies have shown that the PDB-like bone dysplasia familial expansile osteolysis is caused by activating mutations in the \textit{TNFRSF11A} gene that encodes receptor activator of nuclear factor \(\kappa\B\) (RANK); however, linkage studies, coupled with mutation screening, have excluded involvement of RANK in the vast majority of patients with PDB. To identify other candidate loci for PDB, we conducted a genomewide search in 319 individuals, from 62 kindreds with familial PDB, who were predominantly of British descent. The pattern of inheritance in the study group as a whole was consistent with autosomal dominant transmission of the disease. Parametric multipoint linkage analysis, under a model of heterogeneity, identified three chromosomal regions with LOD scores above the threshold for suggestive linkage. These were on chromosomes 2q36 (LOD score 2.7 at 218.24 cM), 5q35 (LOD score 3.0 at 189.63 cM), and 10p13 (LOD score 2.6 at 41.43 cM). For each of these loci, formal heterogeneity testing with HOMOG supported a model of linkage with heterogeneity, as opposed to no linkage or linkage with homogeneity. Two-point linkage analysis with a series of markers from the 5q35 region in another large kindred with autosomal dominant familial PDB also supported linkage to the candidate region with a maximum LOD score of 3.47 at D5S2034 (187.8 cM). These data indicate the presence of several susceptibility loci for PDB and identify a strong candidate locus for the disease, on chromosome 5q35.

Introduction

Paget disease of bone (PDB [MIM 167250 and MIM 602080]) is a common disorder that affects as many as 3% of individuals \(\geqslant 55\) years of age, in the United Kingdom and in other white populations (Barker 1984; Kanis 1992; Cooper et al. 1999). The disease is characterized by focal areas of increased osteoclastic bone resorption, coupled with increased and disorganized formation of new bone. Although many patients are asymptomatic, as many as 30% have symptoms related to the disease, such as bone pain, bone deformity, pathological fracture, and deafness (Tiegs et al. 2000). Patients with PDB are also at an increased risk of developing osteosarcoma. Although this is a rare complication, individuals with PDB constitute more than half of all patients \(\geqslant 60\) years of age who develop osteosarcomas (Huvos 1986).

The cause of PDB is incompletely understood, but accumulating evidence suggests that there is a strong genetic component. Familial clustering is common in PDB, and 15%–40% of affected individuals have been reported to have an affected first-degree relative (Sofaer et al. 1983; Siris et al. 1991; Morales-Piga et al. 1995). Several families have been described in which PDB is inherited in an autosomal dominant manner (Morales-Piga et al. 1995; Cody et al. 1997; Haslam et al. 1998; Nance et al. 2000; Good et al. 2001), as is the rare bone dysplasia familial expansile osteolysis (FEO [MIM 174810]), which shares many clinical features with PDB (Wallace et al. 1989). The gene responsible for FEO was mapped to chromosome 18q21-22 by Hughes and colleagues (1994), and some PDB kindreds were subsequently found to be linked to the FEO region (Cody et al. 1997; Haslam et al. 1998). Recent studies have shown that FEO is caused by a 6-amino acid-insertion mutation affecting the signal peptide region of the receptor activator of nuclear factor \(\kappa\B\) (NF-\(\kappa\B\)) (RANK) (Hughes et al. 2000).
examination showed an overlap between the features of FEO and familial PDB, a 9-amino acid-insertion mutation was identified in affected individuals within the same region of the RANK molecule (Hughes et al. 2000). Subsequent work has shown that linkage to chromosome 18q21-22 is unusual in familial PDB (Hocking et al. 2000; Nance et al. 2000; Good et al. 2001), and RANK mutations have been excluded as a common cause of PDB in a large number of patients with either familial or sporadic PDB (Sparks et al. 2001; Wuys et al. 2001). These data indicate that other genes may be responsible for most cases of familial PDB. In the present study, we have used a genomewide search to attempt to identify loci that predispose to familial PDB.

**Patients, Material, and Methods**

**Family Recruitment and Disease Ascertainment**

Families were recruited by a combination of routine clinic referrals and by advertisements in the Newsletter of the National Association for Relief of Paget's Disease (a patient self-help group based in the United Kingdom). The study group comprised 319 individuals, of predominantly British descent, from 62 kindreds in which we were able to recruit a proband and at least one living relative affected by PDB. Twenty-eight (45%) families were recruited from the United Kingdom, 17 (27%) from Australia, 14 (23%) from New Zealand, and 1 (2%) each from the United States, Canada, and Hungary. The mean (SD) family size was 5.4 (3.7) individuals, with a range of 2–24. Clinical features and patterns of inheritance for 50 of the 62 families included in the present study have been described in more detail elsewhere (Hocking et al. 2000). In agreement with the findings of numerous other investigators (McKusick 1976; Morales-Piga et al. 1995; Cody et al. 1997; Haslam et al. 1998; Nance et al. 2000; Good et al. 2001), we found that the pattern of inheritance in larger families was consistent with that of an autosomal dominant gene. Although it was not possible to unequivocally determine the inheritance pattern in smaller families, such as affected sib pairs, we found that 56% of at-risk individuals in the present study had developed PDB by the age of 55 years, with an equal frequency in men and women, in keeping with hypothesis that the disease is inherited in an autosomal dominant manner. As in one study reported elsewhere (Hocking et al. 2000), we found no significant difference in the frequency of disease inheritance from paternal and maternal sources in subjects for whom parental data were available (data not shown). A full set of pedigree drawings for families included in the present study is available at the University of Aberdeen Web site. We screened for the presence of PDB by measurement of serum total alkaline phosphatase (AP), because AP values are almost invariably elevated in untreated patients with PDB (Kanis 1992). Individuals >55 years old in whom serum AP values were <65 U/liter (reference range 40–105 U/liter) were considered unaffected, whereas those with AP values in the high normal range (65–105 U/liter) or with frankly elevated values (>105 U/liter) were considered to have an unclear diagnosis and were investigated further. These individuals were defined as affected if evidence of PDB was found through radionuclide bone-scan examinations and/or skeletal radiographs. Unless PDB had been confirmed radiologically or scintigraphically, individuals <55 years of age were considered to have an unclear diagnosis, even in the presence of normal AP levels (<65 U/liter), to take account of fact that PDB does not usually present clinically until the fifth or sixth decade (Kanis 1992).

Limited genotyping, for one candidate locus identified by the genomewide search, was performed in an additional large U.S. family that had autosomal dominant inheritance of PDB. This family comprised 18 individuals, of whom 11 were affected with the disease.

All subjects gave informed consent to being included in the studies, which were approved by the Grampian research ethics committee and the research ethics committees of the other contributing centers.

**Genotyping and Linkage Analysis**

Genotyping was carried out on DNA extracted from venous blood using the Nucleon II kit (Scotlab), according to the manufacturer’s instructions. The genomewide screen was performed using 366 polymorphic microsatellite markers spaced, on average, at 9-cM intervals throughout the autosomes (ABI Prism Linkage Mapping Set MD-10). The order of markers and the distance between markers were obtained from the Marshfield database. Markers were genotyped by standard techniques on a 377 automated DNA sequencing system (Applied Biosystems). Alleles were scored manually, using ABI Genescan and Genotyper software, and were binned using the Linkage Designer program. Parametric linkage analysis was performed using Genehunter version 1.3 (Kruglyak et al. 1996), under models of both homogeneity and heterogeneity, assuming an autosomal dominant mode of inheritance, with penetrance set at 90% for individuals aged >55 years. The disease gene frequency was set at .00465, and the phenocopy rate was set at .02635, to reflect the estimated prevalence of familial and sporadic PDB in the U.K. population (Sofaer et al. 1983; Cooper et al. 1999). Genehunter 1.3 was also used to calculate nonparametric linkage (NPL) statistics. For linkage analysis under a model of heterogeneity, the fraction of pedigrees linked (α) was allowed to vary until a maximized LOD score was reached. Loci
in which maximized LOD scores exceeded the threshold for suggestive linkage (>1.9) were also analyzed for linkage heterogeneity, using the HOMOG program. The power of the family set to detect linkage was simulated using the SLINK program, in which genotypes were simulated for a marker with eight alleles of equal frequency. This showed that the cohort had 100% power to detect significant linkage (i.e., LOD score ≥3.3) under a model of homogeneity and that 64% had power to detect significant linkage under a model of heterogeneity (50% of families linked). Corresponding power values for suggestive linkage (LOD score ≥1.9) were 100% and 89%, respectively. Two-point linkage analysis of a candidate region on chromosome 5q35 was conducted with the use of the MLINK and ILINK programs from the Linkage Analysis Package version 5.1 (Lathrop and Lalouel 1984), in one large family that was not included in the genome search. Positional candidate genes from the regions of interest were identified using the Ensembl Database.

**Results**

Linkage analysis under a model of homogeneity identified only one locus where the LOD score was above the threshold for suggestive linkage, and this was on chromosome 5q35 between the markers D5S400 and D5S408 (LOD score 1.9). Linkage analysis under a model of heterogeneity identified three loci where maximized LOD scores exceeded the threshold for suggestive linkage (fig. 1). These were on chromosome 5q35 at 189.63 cM between the markers D5S400 and D5S408 (LOD score 3.0 at α = .637), on chromosome 2q36 at 218.24 cM between the markers D2S126 and D2S396 (LOD score 2.7 at α = .470), and on chromosome 10p13 at 41.43 cM between the markers D10S1653 and D10S548 (LOD score 2.6 at α = .542). NPL analysis showed the following maximum scores at these loci: 2.18 (P = .008) at 193.77 cM on chromosome 5q35, close to D5S408; 2.38 (P = .004) at 225.3 cM on chromosome 2q36, between D2S126 and D2S396; and 2.40 (P = .004) at 38.2 cM on chromosome 10q13, close to D10S1653.

To investigate the candidate loci further, heterogeneity testing was also performed using the HOMOG program on multipoint LOD scores from the regions of interest generated by Genehunter (table 1). For the candidate locus on chromosome 2q36, there was no evidence to support linkage under a model of homogeneity, but there was evidence of linkage under a model of heterogeneity, between the markers D2S126 and D2S396 at α = .680. Hypothesis testing showed significant evidence of linkage with heterogeneity (χ² = 12.5, 2 df, P = .002). For the candidate locus on chromosome 5q35, there was also evidence of linkage under a model of heterogeneity between the markers D5S400 and D5S408 at α = .680. There was also some evidence of linkage under a model of homogeneity at this locus, but hypothesis testing favored a model of linkage...
Table 1

Heterogeneity Testing of Candidate Loci for Familial PDB

<table>
<thead>
<tr>
<th>Locus and Hypotheses</th>
<th>Max LnL</th>
<th>α</th>
<th>Map Position (cM)</th>
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<td>2q36:</td>
<td></td>
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<tr>
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<tr>
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<td>.466</td>
<td>218.24</td>
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<tr>
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</tr>
<tr>
<td>H0</td>
<td>(0)</td>
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<td></td>
</tr>
<tr>
<td>df</td>
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<tr>
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<td>510.029</td>
</tr>
<tr>
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<td>0</td>
<td>1.000</td>
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<tr>
<td>H2 vs. H0</td>
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<td>12.469e</td>
<td>510.029</td>
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<td>12.128e</td>
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</table>

NOTE.—Numbers in parentheses show values that are set by definition.

* H2 = linkage, under the model of heterogeneity; H1 = linkage, under the model of homogeneity; and H0 = no linkage.
* Max LnL = natural logarithm of maximized LOD score.
* P = .002.
* P = .003.
* P = .001.

Discussion

In the present study, we performed an autosomal genome screen for chromosomal regions that contain susceptibility genes for familial PDB. When the data were analyzed under a model of homogeneity, we identified only one possible candidate locus for the disease, on chromosome 5q35, where the LOD score (1.9) just reached the threshold for suggestive linkage. Analysis of the data under a model of heterogeneity, however, identified three loci where LOD scores exceeded the threshold for suggestive linkage. The highest LOD score was on chromosome 5q35, where a maximum value of 3.0 was found at 189.36 cM between the markers D5S400 and D5S548. A second region was found on chromosome 2q36, where the LOD score was 2.7 at 218.24 cM, between the markers D2S126 and D2S396. A third region was on chromosome 10p13, where the LOD score was 2.6 at 41.43 cM, between the markers D10S1653 and D10S548. In agreement with previous reports, we found no evidence of linkage to the FEO locus (PDB2) on chromosome 18q21-22 (Hocking et al. 2000; Nance et al. 2000; Good et al. 2001), nor did we find evidence of linkage to the human leukocyte antigen (HLA) (PDB1) locus on chromosome 6p21 (Nance et al. 2000; Good et al. 2001). Other candidate genes for PDB include RANK ligand (RANKL), which has been mapped to chromosome 13q14, and osteoprotegerin (OPG), which has been mapped to chromosome 8q24. RANKL—which is expressed on the surface of bone marrow stromal cells, osteoblasts, and activated T-cells—stimulates osteoclast formation and activity by binding to RANK and activating NF-κB and c-Jun N-terminal kinase–signaling pathways (Hofbauer et al. 2000). OPG is an inhibitor of osteoclastogenesis, which acts as a soluble decoy receptor for RANKL (Hofbauer et al. 2000). In a study reported elsewhere, we found a positive association between a polymorphism in intron 2 of the OPG gene and sporadic PDB, but we did not detect any disease-specific mutations of OPG in familial or sporadic PDB (Wuyts et al. 2001). We have similarly failed to detect any disease-specific mutations of RANKL in familial and sporadic PDB (L.J.H. and S.H.R., unpublished data). In keeping with these observations, we found no evidence of linkage to chromosomes 8q24 or 13q14 in this cohort of patients with familial PDB.

The strongest candidate locus for PDB found in the present study was a 24-cM region on chromosome 5q35 between D5S400 and D5S2006. Two-point linkage

with heterogeneity (χ² = 13.7; 2 df; P = .001; likelihood ratio (LR) of 975.75) over the model of linkage with homogeneity (χ² = 9.1, 1 df; P = .003; LR = 93.4). For the locus on chromosome 10p13, there was evidence of linkage under a model of heterogeneity between the markers D10S1653 and D10S548 at α = 0.546. Hypothesis testing showed significant evidence of linkage with heterogeneity (χ² = 12.1; 2 df; P = .002).

To test candidacy of the chromosome 5q35 region further, we analyzed a series of markers from the 5q35 locus in a large family from the United States that had autosomal dominant inheritance of PDB (fig. 2). This showed positive LOD scores for several markers across the candidate region, with a maximum LOD score of 3.47 at D5S2034 (table 2).
studies in a large family from the United States supported linkage to the 5q35 region, but two individuals (subjects IV.14 and V.2) who had signs of PDB did not share the same haplotype as other affected individuals in the family. There are three possible explanations for this observation. First, the disease could have occurred as a result of a new mutation. The contribution of this explanation to the findings observed is difficult to determine, because the genes responsible for late-onset PDB are unknown and because the mutation rate has not been determined. A second explanation is that individuals IV.14 and V.2 could have had a nongenetic cause of PDB. This is clearly possible, because 60%–85% of PDB cases appear to be sporadic (Sofaer et al. 1983; Siris et al. 1991; Morales-Piga et al. 1995). It has been suggested that sporadic PDB may occur as a result of a slow-virus infection of osteoclasts with paramyxoviruses, such as measles and canine distemper (Siris 1996; Kurihara et al. 2000), although this issue remains controversial (Helfrich et al. 2000). Other environmental factors that have been identified as triggers of the disease include repetitive mechanical loading of affected bones (Solomon 1979) and low dietary calcium intake (Siris 1994). The third explanation is that individuals IV.14 and V.2 could have inherited a different disease gene from the grandmother (individual III.4), whose phenotype was unknown. This is certainly possible, in view of the fact that PDB is a common condition that shows evidence of locus heterogeneity, as demonstrated by the present study and by studies done by other groups (Haslam et al. 1998; Hocking et al. 2000; Nance et al. 2000; Good et al. 2001). Further work will be required to define the relative contribution of these three possible explanations to the findings observed in this family.

Several genes within the 5q35 region are known to be expressed in bone and/or to play a role in signaling mechanisms that may be relevant to the pathogenesis of PDB. They include GPRK6, a G-protein–coupled receptor kinase; MAPK9, a mitogen-activated kinase; DUSP1, a protein phosphatase involved in negative reg-
ulation of MAP kinase signaling; MSX2, a gene involved in skull development; ATP6H, a subunit of the vacuolar protein pump; and BNIP1, a protein involved in the regulation of apoptosis. Further studies will be required to define the role that these and other genes play in the pathogenesis of PDB in families that show 5q35 linkage. Although the candidate locus on chromosome 10p13 does not contain any obvious candidate genes for PDB, the chromosome 2q36 locus contains several, including members of the insulin-like growth factor–binding protein family (IGFBP2 and IGFBP5); the Indian Hedgehog (IHH) protein; and C-type natriuretic peptide precursor (NPPC).

In summary, we have identified three potential susceptibility loci for PDB on chromosomes 2q36, 5q35, and 10p13; and, for each of these loci, we found evidence of genetic heterogeneity, indicating that the phenotype of PDB may occur as a result of mutations or polymorphisms in several different genes.

At present, the relative contribution of these loci to the pathogenesis of familial PDB in general is difficult to assess. For the chromosome 5q35 locus, maximized LOD score analysis, with the use of Genehunter, coupled with formal heterogeneity testing using HOMOG, indicated that disease-related genes in 63.7%–68.0% of families may have been linked to the region of interest. Corresponding figures for the chromosome 2q36 locus were 46.6%–47.0%, and those for the chromosome 10p13 locus 54.0%–54.6%. The total for these three loci is >100%, because many of the smaller families showed evidence of linkage to more than one locus. This is a limitation of our study and simply reflects the fact that most of the families were too small to confirm or exclude linkage to a specific locus.

Positional cloning studies are in progress to try to identify the genes that are responsible for the occurrence of PDB within these chromosomal regions. The identification of these genes will give important insights into the mechanisms that underlie the abnormal bone remodeling that characterizes PDB, and this, in turn, should lead to better strategies for prevention and treatment.

**Note added in proof.**—Since the submission of the present manuscript, Laurin et al. (2001) have reported evidence of candidate loci for familial Paget disease on chromosome 5q35 and 5q31.

**Acknowledgments**

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**Electronic-Database Information**

Accession numbers and URLs for data in this article are as follows:

Ensembl database, http://www.ensembl.org (for identification of candidate genes in loci of interest)


Marshfield database, http://research.marshfieldclinic.org/genetics (for marker order and genetic distance between markers)

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim (for PDB [MIM 167250 and MIM 602080] and FEO [MIM 174810])

University of Aberdeen, http://www.abdn.ac.uk/medicine _therapeutics/bone/bone.hlt (for full details of pedigrees included in the present study)
References


