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Address for correspondence and reprints: Dr. Terry-Lynn Young, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9. E-mail: tlyoung@morgan.ucs.mun.ca

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Autosomal Dominant (Beukes) Premature Degenerative Osteoarthropathy of the Hip Joint Maps to an 11-cM Region on Chromosome 4q35

To the Editor:

We have previously reported the clinical and radiographic features of affected individuals from a large kindred who have an autosomal dominant form of bilateral dysplasia of the hip joints with severe secondary osteoarthrosis (Cilliers and Beighton 1990). This family came to the attention of one of us (H.C.) because of the number of patients with the family name, Beukes, who presented to the Department of Orthopaedic Surgery, University of Orange Free State, South Africa, for prosthetic hip-joint replacement as a consequence of bilateral premature degenerative osteoarthropathy. Genealogical studies subsequently revealed that all the affected individuals were members of an extended family that could be traced back to a single Dutch immigrant to South Africa who arrived in 1685 (Cilliers and Beighton 1990). Our continued investigation of this family has now traced 55 individuals in eight generations who, on the basis of either their medical histories or clinical and radiographic presentation of the disorder, appear to have inherited the disorder. The disorder clearly has an autosomal dominant mode of inheritance, but there is some evidence of nonpenetrance in that apparently unaffected individuals have had affected offspring. The clinical and radiographic manifestations have been described in detail elsewhere (Cilliers and Beighton 1990). In brief, the presenting symptom is hip-joint discomfort, which usually develops during childhood at age <2 years but may develop either later in childhood or, as in one instance, as late as the age of 35 years. After onset of symptoms, the hip joints deteriorate progressively, gait is disturbed, and, by early adulthood, affected persons are crippled by degenerative arthropathy. The earliest radiological changes are broadening of the femoral necks, late appearance of the secondary ossification centers of the femoral head, and an irregular appearance of the proximal epiphyseal line of the femur. By mid childhood, the femoral heads are flat (coxa plana), with broadening of the femoral necks, adaptation of the acetabulum to the mal-

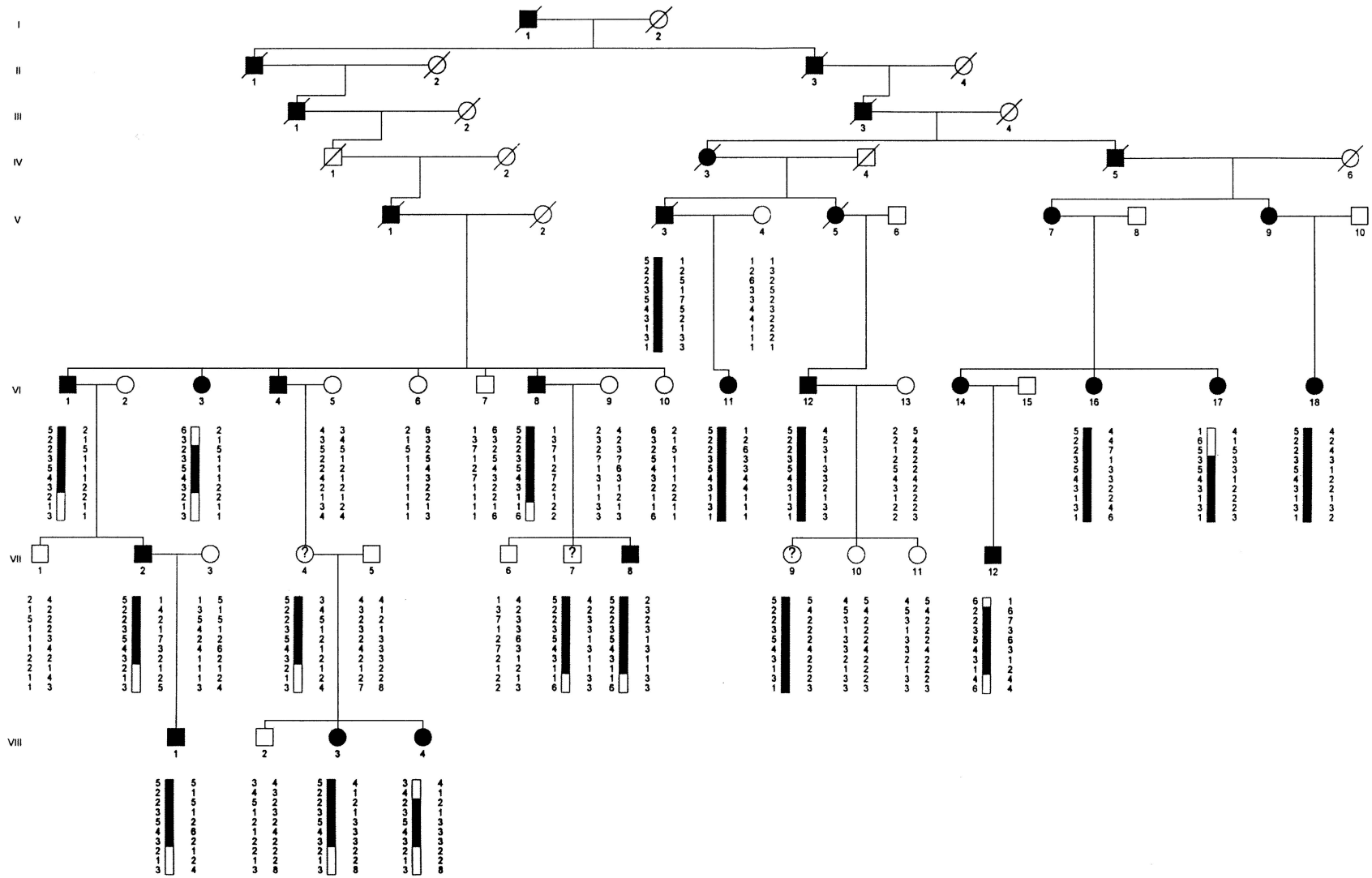

Figure 1 BHD pedigree, showing disease-linked haplotypes. Blackened circles and squares represent affected females and males, respectively. Symbols containing a question mark (?) are likely to be nonpenetrant carriers of the disease. The haplotypes for all individuals that were genotyped are given under the symbols. The DNA sample from individual V-3 was obtained before his death. The disease-linked haplotype is on the left and is indicated by a blackened box to its right. The marker order, from top to bottom, is D4S1607, D4S2951, D4S1554, D4S408, D4S2924, D4S171, D4S1540, D4S3051, D4S426, and D4S2940.

Table 1

Two-Point *Z* Values, between BHD and Chromosome 4q35 Markers

		Z AT θ =						
MARKER	Z_{max} (θ_{max})	.00	.01	.05	.10	.20	.30	.40
D4S1607	1.54 (.18)	−11.96	−2.19	.37	1.23	1.53	1.14	.48
D4S2951	1.46 (.17)	−12.20	−1.32	.63	1.26	1.43	1.07	.53
D4S1554	2.00 (.10)	−1.42	1.27	1.89	2.0	1.75	1.25	.63
D4S408	3.58 (.00)	3.58	3.55	3.37	3.05	2.23	1.30	.39
D4S2924	5.73 (.00)	5.73	5.68	5.40	4.96	3.87	2.58	1.17
D4S171	4.84 (.00)	4.84	4.79	4.55	4.15	3.16	2.01	.82
D4S1540	5.11 (.00)	5.11	5.03	4.67	4.17	3.06	1.90	.78
D4S3051	.18 (.33)	−3.14	−2.35	−1.01	−.43	.04	.17	.14
D4S426	.03 (.40)	−7.10	−2.63	−1.18	−.60	−.15	0	.03
D4S2930	.18 (.29)	−12.36	−3.64	−1.44	−.55	.07	.18	.11

NOTE.—All values are calculated under the assumption of 90% penetrance and a disease-allele frequency of .0001.

formed femoral head, superolateral displacement of the femoral head, and an irregular appearance of the greater trochanteric epiphyses. By adulthood, these features are more pronounced, and there is superior migration and superolateral displacement of the femoral head and overgrowth of the greater trochanter in a superomedial direction. In the later stages, coxa vara is a prominent finding. Signs of degenerative osteoarthrosis (periarticular cysts, periarticular sclerosis, and narrowing of the joint space) are evident in early to mid childhood and are progressive. Apart from the hip problems, the general health of affected individuals is good, their height is normal, and, other than in one instance in which a young adult had severe kyphoscoliosis that necessitated spinal fusion, involvement of the vertebral bodies and other joints is minimal. The radiographic findings, the absence of involvement of the vertebral bodies and joints other than the hip, and the normal stature of affected individuals has led to the conclusion that this disorder is distinct from other autosomal dominant forms of chondrodysplasia, in which premature degenerative osteoarthropathy of the hip joint is a major complication. It therefore seemed appropriate that this condition be categorized as a familial hip dysplasia, and it was called "Beukes hip dysplasia" (BHD [MIM 142669]), on the basis of the name of the affected family (Cilliers and Beighton 1990).

Mutations in the genes encoding components of the extracellular matrix of cartilage have been identified in families with forms of chondrodysplasia with secondary osteoarthritis (see Kuivaniemi et al. 1997; Briggs et al. 1998, and references therein). We commenced our studies to locate the gene responsible for BHD, by performing analyses of linkage to polymorphic markers within or near cartilage candidate genes. We found, however, no evidence of linkage to COL2A1 (Beighton et al. 1994), COL9A1, COL9A2, COL11A1, COL11A2, COL10A1, CRTL-1, CRTM, AGC1, or COMP (Al-Ali et al. 1994; G. Wallis, P. Roby, and S. Eyre, unpublished data). We therefore performed a genomewide screen with a panel of 290 markers with an average spacing of 11 cM (Davies et al. 1994). For this purpose, genotype data were obtained from 32 individuals from the BHD kindred, including 15 affected individuals, 11 unaffected related individuals, and 6 unrelated spouses. The affected status of the 15 individuals who were genotyped had been established on the basis of their clinical and radiological presentation of the disorder. The BHD pedigree shown infigure 1 has been condensed and includes only those individuals who were genotyped and those who were required for the linkage analysis. Despite the fact that the pedigree has been condensed, the relationships between the members of the pedigree have been retained. Genotyping was done with an ABI 373 sequencer and GENESCAN 1.2.2-1 and GENOTYPER 1.1.1 software. Two-point LOD score (Z) values were computed by the LINKAGE package (Lathrop and Lalouel 1984), for various recombination fraction (θ) values, with penetrance values of 90%, 95%, and 100%, and a disease frequency of .0001. One marker on chromosome 4, D4S408, had a maximum Z (Z_{max}) value of 3.58 at a maximum θ (θ_{max}) of .00, at 90% penetrance (see table 1). Further analysis with markers from this region, with penetrance values of 85%, 90%, and 95%, gave a two-point Z_{max} value of 5.73 for marker D4S2924, at θ = .00 and at a penetrance of 90% (see table 1). Haplotypes were constructed with the map order D4S1607–1.5 cM–D4S2951–1 cM–D4S1554–4 cM–D4S408–3.2 cM–D4S2924–0.5 cM–D4S171–0.5 cM–D4S1540–3 cM–D4S3051–1 cM–D4S426–1.3 cM–D4S2930. The order of the markers was derived from on-line genetic mapping data at the Center for Medical Genetics, Marshfield Medical Research Foundation Website. As judged on the basis of an examination of the marker haplotypes segregating with BHD (fig. 1), the closest recombinants involving affected family

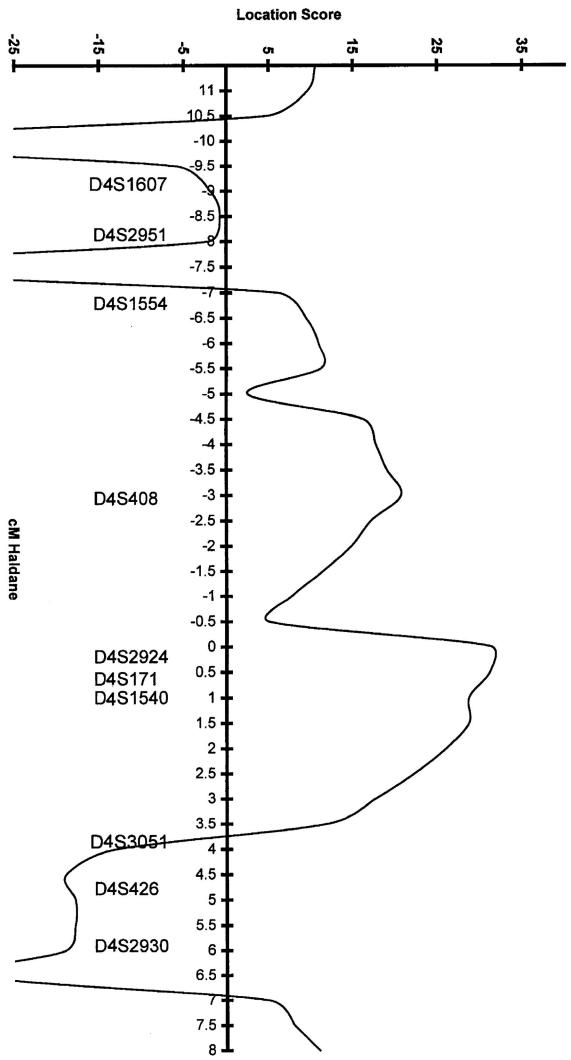

Figure 2 Graphic representation of three-point location scores based on genotype data for chromosome 4 markers and members of the BHD family. A location score is equivalent to *Z* multiplied by 4.6.

members were at D4S1554 proximally and D4S3051 distally, which limits the BHD gene locus to an interval of ~11 cM. Three apparently clinically unaffected individuals (VII-4, age 49 years; VII-7, age 33 years; and VII-9, age 33 years) were found to have inherited the disease-linked haplotype. Individual VII-4 transmitted the disorder and the disease-linked haplotype to her two affected offspring, demonstrating that she is a nonpenetrant carrier of the mutated gene. However, to date, her only potential clinical symptom of the disorder has been hip-joint pain during pregnancy. The remaining two individuals have not reported any symptoms of the disorder. Attempts are currently underway to obtain recent radiographs of these three individuals, to determine whether they have any radiological evidence of the disorder.

Multipoint analysis was done with the LINKAGE 5.1 LINKMAP program and the marker order given above. Multipoint analysis with the complete set of markers spanning the disease interval was not possible, because of both the high number of alleles per marker and the large number of individuals in the pedigree, so sequential three-point analyses were done. The combined results are shown in figure 2. The multipoint location score for the chromosome 4 markers was 30.05 (equivalent to $Z = 6.5$), and the likely location of the BHD gene was confirmed to be the 11-cM interval between D4S1554 and D4S3051. Currently, within the linked region there are no known or obvious potential candidate genes for the disease, and no other forms of familial osteochondrodysplasia are known to map to this region. Physical mapping data for this region include a single YAC contig, WC4.7, to which a number of expressed sequence tags (ESTs) have been mapped (Whitehead Institute/MIT Genome Sequencing Project).

Our finding that BHD does not map to any of the loci that have previously been identified for other forms of autosomal dominant chondrodysplasia with associated osteoarthropathy (notably, spondyloepiphyseal dysplasia [MIM 184100 and MIM 183900], multiple epiphyseal dysplasia [MIM 226900], and pseudoachondroplasia [MIM 177170]) supports the clinical and radiographic data suggesting that this disorder is a distinct form of familial hip dysplasia. Identification of the BHD gene within the linked region on 4q35 could have implications for the investigation of other, more common forms of idiopathic hip osteoarthritis.

Acknowledgments

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PHILIP ROBY,[1,2] STEPHEN EYRE,[1,2]
JANE WORTHINGTON,[2] RAJKUMAR RAMESAR,[4]
HENDRIK CILLIERS,[5] PETER BEIGHTON,[4]
MICHAEL GRANT,[1] AND GILLIAN WALLIS[1,3]

[1]*The Wellcome Trust Centre for Cell-Matrix Research, School of Biological Sciences,* [2]*Arthritis Research Campaign Epidemiology Research Unit, and* [3]*Department of Medicine, University of Manchester, Manchester;* [4]*Department of Human Genetics, University of Cape Town, Cape Town; and* [5]*University of Bloemfontein, Bloemfontein, South Africa*

Electronic-Database Information

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

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/ (for marker order)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nim.nih.gov/Omim (for BHD [MIM 142669], pseudoachondroplasia [MIM 177170], spondyloepiphyseal dysplasia [MIM 183900 and MIM 184100], and multiple epiphyseal dysplasia [MIM 226900])
Whitehead Institute/MIT Genome Sequencing Project, http://carbon.wi.mit.edu (for mapping of ESTs)

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Address for correspondence and reprints: Dr. Gillian A. Wallis, School of Biological Sciences, University of Manchester, 2.205 Stopford Building, Oxford Road, Manchester M13 9PT, United Kingdom. E-mail: gwallis@fs1.scg.man.ac.uk

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Common Fragile Sites: G-Band Characteristics within an R-Band

To the Editor:

Common fragile sites are chromosomal loci prone to breakage and rearrangement and are considered to be part of the normal chromosome structure. They are visualized as constrictions, gaps, or breaks on metaphase chromosomes from cells exposed to specific tissue-culture conditions (Sutherland and Richards 1995). Three common fragile sites—FRA3B, FRA7H, and FRA7G—were recently cloned and identified at the molecular level (Boldog et al. 1997; Inoue et al. 1997; Huang et al. 1998; Mishmar et al. 1998). Sequence analysis of these three common fragile sites revealed no CGG or other expanded repeated sequences, such as have been found in rare fragile sites (Sutherland and Richards 1995). DNA sequence analysis of FRA3B, FRA7H, and FRA7G did not reveal any obvious feature that could account for the fragility of these sites. To shed light on the mechanism of fragility, we undertook a new approach and analyzed the available sequences of FRA3B, FRA7H, and FRA7G, for DNA structural characteristics that might be associated with their fragility (Mishmar et al. 1998). The analysis revealed several regions with a potential to form unusual DNA structures, including high flexibility, low stability, and non–B DNA–forming sequences. Thus, these unusual DNA characteristics are possibly intrinsic properties of common fragile sites, which may affect their replication, condensation, and organization and may lead to fragility.

While analyzing the sequences of FRA3B, FRA7H, and FRA7G, we noticed several features that are characteristic of G-bands (Gardiner 1995). The three cloned fragile sites have high (>57%) A/T content, and are all gene poor. FRA3B and FRA7H are rich in LINE sequences. FRA3B and markers proximal to FRA7G were shown to replicate late during S-phase (Selig et al. 1992; Huang et al. 1998; Le Beau et al. 1998). G-bands and R-bands correspond to functional subregions, represented as stained bands, that apparently reveal the basic structural organization of chromosomes. G-bands are characterized as regions with high A/T content that replicate late during S-phase, are insensitive to DNase-I, and are gene poor, Alu poor, and LINE rich. In contrast, the complementary R-bands are regions with high G/C content that replicate early during S-phase, are DNase sensitive, and are gene rich, Alu rich, and LINE poor (Gardiner 1995). Most (76/89 [>85%]) of the common fragile sites, including the cloned sites, map to R-bands (according to our analysis of the Genome Database data). These characteristics suggest that fragile sites