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Genetic Mapping of the Camurati-Engelmann Disease Locus to Chromosome 19q13.1-q13.3

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Summary

Camurati-Engelmann disease (CED [MIM 131300]), or progressive diaphyseal dysplasia, is an autosomal dominant sclerosing bone dysplasia characterized by progressive bone formation along the periosteal and endosteal surfaces at the diaphyseal and metaphyseal regions of long bones and cranial hyperostosis, particularly at the skull base. The gene for CED, or its chromosomal localization, has not yet been identified. We performed a genomewide linkage analysis of two unrelated Japanese families with CED, in which a total of 27 members were available for this study; 16 of them were affected with the disease. Two-point linkage analysis revealed a maximum LOD score of 7.41 (recombination fraction .00; penetrance 1.00) for the *D19S918* microsatellite marker locus. Haplotype analysis revealed that all the affected individuals shared a common haplotype observed, in each family, between *D19S881* and *D19S606,* at chromosome 19q13.1-q13.3. These findings, together with a genetic distance among the marker loci, indicate that the CED locus can be assigned to a 15.1-cM segment between *D19S881* and *D19S606*.

Introduction

Camurati-Engelmann disease (CED [MIM 131300]), or progressive diaphyseal dysplasia, is an osteosclerotic dysplasia characterized by symmetrical hyperostosis initiating at the diaphyses of the long tubular bones, usually of the femora or tibiae, and expanding to their metaphyses and to membranous bones. The hyperostosis of the skull base occasionally leads to cranial nerve palsy. The onset of the disease is often during early childhood and almost always at age <30 years. Radiographic abnormalities include diaphyseal cortical thickening, periosteal and endosteal sclerosis of the long bones, and basal skull sclerosis. Patients with CED have severe pain in the legs, muscle weakness, a waddling gait, and easy fatigability and occasionally suffer from systemic manifestations, such as anemia, leukopenia, or hepatosplenomegaly (Crisp and Brenton 1982). No specific laboratory findings for the disease have been identified, although a raised erythrocyte-sedimentation rate, an increased level of serum alkaline phosphatase, increased urinary excretion of hydroxyproline, or a reduced level of hematocrit or hemoglobin have been reported in some patients (Hundley and Wilson 1973; Smith et al. 1977; Crisp and Brenton 1982). Pollack et al. (1989) reported that all the patients examined by them had markedly elevated proportions of OKM1-positive mononuclear cells and that some of the healthy relatives also exhibited the same abnormality. Therapy with a prolonged low dosage of corticosteroid hormone is effective not only in relieving the pain but also in reversing the radiological findings, although the mechanism through which steroids act remains undefined (Minford et al 1981). Reported histopathological findings, although they are controversial, include altered bone cortex, with progressive active bone resorption as well as deposition (Rubin 1964); the absence of osteoclasts in the bone lesions, which recovered after steroid treatment leading to increased bone absorption and secondary remodeling (Allen et al. 1970); and thickening of the blood-vessel wall in the bone (Rubin 1964) and skeletal muscles (Yoshioka et al. 1980; Naveh et al. 1985). Diagnosis is based on these clinical, radiographic, and/or pathological manifestations. More than 100 patients with CED have been reported in the literature, and the prevalence of CED is

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estimated to be <1/1 million (Wynne-Davies et al. 1985). Both sporadic and familial cases have been described. In familial cases, the disease is inherited in an autosomal dominant mode with incomplete penetrance and variable expression (Sparkes and Graham 1972). Here we report the result of a genetic linkage analysis of two unrelated Japanese families with CED.

Subjects and Methods

Families and Patients

Two unrelated Japanese families (families 1 and 2) were analyzed in the present study. A total of 27 members of the two families agreed to participate in the present study, with written informed consent. Of the 27 members, 16 were diagnosed as having CED (fig. 1), on the basis of clinical and radiographic findings—that is, continuous disabling pain in the legs, muscle weakness with waddling gait, diaphyseal cortical fusiform thickening, and periosteal and endosteal sclerosis of the long bones. Detailed clinical data on affected members of family 1 have been reported elsewhere (Makita et al., in press). The proband in family 2 (I-3; see fig. 1) was a 48-year-old woman with complaints of upper- and lower-limb pain, easy fatigability, muscle weakness, and waddling gait. Onset of her disease was at age 3 years. The findings of radiographic survey and 99mTc-MDP bone scintigraphy were consistent with CED. Three of her four children are also affected with CED (fig. 1).

Genotyping and Linkage Analysis

Genomic DNA samples were collected from the 27 available individuals in the two families. Highly polymorphic microsatellite markers were chosen from the Généthon database (Dib et al. 1996), and their corresponding primer sets were synthesized. The forward primer of each set was labeled with fluorescent dye (Cy5; Pharmacia Biotech), and the reverse primer was not labeled. For the chromosomal assignment of the disease locus, we adopted a two-step analysis. In the first step, we performed a genomewide linkage analysis using markers distributed across the genome that were separated by an average distance of ~10 cM. When preliminary data for assignment to a chromosomal region were obtained, we analyzed additional markers to confirm the linkage and to delimit the CED critical region (CEDR) (fig. 1).

PCR amplification was performed in a 12.5-μl reaction mixture containing 50 ng of genomic DNA, 1–3 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.5 U of DNA polymerase (Ampli*Taq* Gold; PE Biosystems), and 12.5 pmol of each primer. PCR conditions consisted of an initial denaturation at 94°C for 10 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 48–58°C for 30 s, and extension at 72°C for 30 s; and final extension at 72°C for 10 min. PCR products were resolved by electrophoresis on 6% polyacrylamide DNA sequencing gel, by use of an automated sequencer (ALFexpress; Pharmacia Biotech). Electrophoretic patterns were analyzed by computer software (Fragment Manager; Pharmacia Biotech), to determine the genotypes of the family members.

Two-point linkage analysis was performed by the computer program MLINK of the FASTLINK software, version 4.0P (Lathrop et al. 1984; Cottingham et al. 1993; Schäffer et al. 1994), under the assumption that CED in the two families is inherited in an autosomal dominant mode, with a disease-gene frequency of .000001 (Wynne-Davies et al. 1985). Equal allele frequencies were used for all marker loci. However, the allele frequency for each of five markers (*D19S422, D19S223, D19S918, D19S902,* and *D19S606*) around the CEDR was calculated in 80 chromosomes from unrelated healthy Japanese individuals.

Results

Since samples were collected earlier from family 1 and since it is larger than family 2, we first undertook a genomewide search in family 1. A maximum two-point LOD score (Z_{max} = 6.20 at recombination fraction [θ] .00) was obtained at the *D19S918* locus when penetrance (p) was assumed to be 1.0. We chose 23 additional markers (16 markers centromeric to and 7 telomeric to *D19S918*) that are located around *D19S918,* to analyze family 1. Haplotype analysis of family 1 demonstrated that all the affected members had a common haplotype between *D19S881* and *D19S606,* including 15 markers (fig. 1). Recombinations had occurred four times in three affected members: one between *D19S422* and *D19S223* in individual II-2, one between *D19S219* and *D19S606* in II-10, and two (double recombination), between *D19S414* and *D19S570* and between *D19S881* and *D19S211,* in III-9 (fig. 1). Thus, most of the region associated with CED lies between *D19S881* and *D19S606.*

We then extended the analysis with the same markers to family 2 and confirmed a linkage of the disease to the same chromosomal region between *D19S881* and *D19S606* (fig. 1). There was one recombination between *D19S606* and *D19S867* in individual II-3 in this family. Results for this family, however, did not allow us to narrow the linked segment.

The two-point Z_{max} calculated for the two families was 7.41, for *D19S918,* at θ = .00 (p = 1.00) (table 1). In light of the genetic distance between the markers used, the CEDR lies in a 15.1-cM segment between *D19S881* and *D19S606,* at 19q13.1-q13.3 (Genome Database [Japan]; Human Genome Resources) (fig. 1).

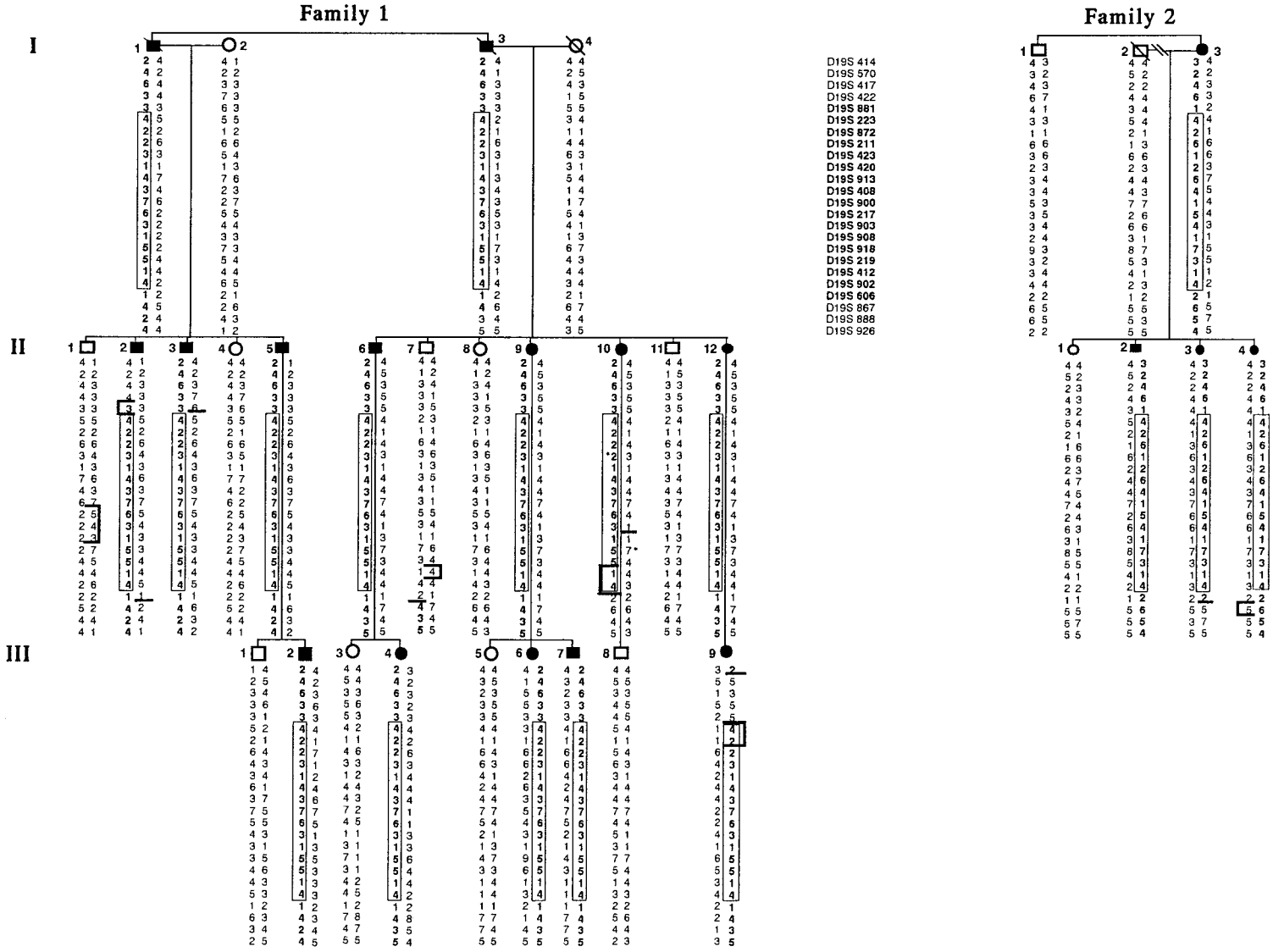

Figure 1 Pedigrees of families 1 and 2, with haplotypes at marker loci on chromosome 19. Blackened squares and circles denote individuals affected with CED; numbers in unblackened boxes indicate putative disease haplotypes; single thick underlines lines indicate definite recombination sites; and thick square brackets indicate recombination sites that could have occurred on either side of the corresponding marker(s). Haplotypes of I-1, I-2, I-3, and I-4 in family 1, as well as haplotype I-2 in family 2, are deduced from those in offspring. An asterisk (*) indicates the allele showing novel microsatellite mutations.

Table 1

Two-Point LOD Scores of CED in the Two Families, at Representative Marker Loci at 19q13.1-q13.3, with Representative *p* Values

	LOD Score at θ=					
Locus and *p*	.00	.001	.05	.1	.15	.2
D19S422:						
1.0	−∞	−3.38	.01	.50	.68	.71
.8	−7.13	−3.52	−.17	.31	.49	.54
D19S223:						
1.0	3.60	3.59	3.25	2.88	2.50	2.11
.8	3.13	3.12	2.81	2.48	2.13	1.79
D19S211:						
1.0	5.44	5.44	5.14	4.73	4.27	3.75
.8	5.03	5.03	4.65	4.22	3.77	3.28
D19S913:						
1.0	4.92	4.91	4.65	3.99	3.49	2.97
.8	4.37	4.36	3.95	3.50	3.05	2.58
D19S408:						
1.0	4.22	4.22	4.08	3.81	3.45	3.03
.8	4.09	4.09	3.80	3.46	3.08	2.67
D19S217:						
1.0	5.84	5.84	5.36	4.82	4.24	3.62
.8	5.18	5.18	4.70	4.19	3.65	3.08
D19S918:						
1.0	7.41	7.39	6.77	6.10	5.44	4.67
.8	6.55	6.54	5.96	5.35	4.72	4.06
D19S219:						
1.0	4.25	4.24	3.90	3.52	3.12	2.68
.8	3.94	3.93	3.59	3.23	2.83	2.41
D19S902:						
1.0	2.54	2.54	2.32	2.07	1.80	1.52
.8	2.28	2.27	2.06	1.82	1.58	1.33
D19S606:						
1.0	−∞	.23	1.68	1.71	1.60	1.41
.8	−2.34	−.02	1.43	1.47	1.37	1.21

Discussion

It has been documented that CED is inherited in an autosomal dominant fashion with incomplete penetrance and variable expressivity (Sparkes and Graham 1972). The two families examined in the present study showed complete penetrance, although expressivity was very variable in family 1. In family 1, 5 of the 12 affected members had clinical manifestations milder than those seen in the other 7, and the age at disease onset among these 12 members varied from age 3 years to the late 20s. An 8-year-old boy (III-2) and a 25-year-old man (III-7) in the family did not present any clinical symptoms of CED (Makita et al., in press) but were found to have both periosteal sclerosis of the fibula and the disease-associated haplotype (fig. 1). Thus, penetrance was complete in this family, when an extensive radiographic survey was performed in the two asymptomatic patients. The highly variable expressivity of the disorder may explain why "incomplete penetrance" has been proposed, and why "autosomal recessive inheritance" has been considered in some families (Hundley and Wilson 1973). Clybouw et al. (1994) reported a family in which the mother of a patient with CED had no abnormal clinical findings but showed, on 99mTc-scintigraphy, increased osteoblastic activity in the diaphyseal portions of almost all long bones, and they suggested the necessity of detailed radiographic examinations to diagnose CED. Phenotypes of five affected members of family 1 were reminiscent of Ribbing disease, as suggested by Makita et al. (in press).

The region of 19q13.1-q13.3 defined as the CEDR is a gene-rich region to which several genes, skeletal tissue–derived expressed-sequence tags, or bone dysplasia–related disease loci have been mapped. Among them, the genes for calmodulin 3 (*CALM3*) (Berchtold et al. 1993), zinc-finger protein 36 (*ZFP36*) (Taylor et al. 1991), and transforming growth-factor beta 1 (*TGFB1*) (Fujii et al. 1986) merit comments. *CALM3* encodes a calcium-modulated protein; *ZNF36* is the gene for a zinc-finger protein, and *ZNF36*-deficient mice are born as normal neonates but soon develop a complex syndrome of inflammatory arthritis, dermatitis, cachexia, autoimmunity, and myeloid hyperplasia; and *TGFB1* encodes a member of the TGF-β superfamily. Since a high level of the *TGFB1* message appears in both osteoblasts and osteoclasts of the developing bone, as well as in the periosteal fibroblasts and bone marrow cells, TGFB1 may play an important role in postnatal bone development (Sandberg et al. 1988*a,* 1988*b*). *Tgfb1* knockout (*Tgfb1*−/−) mice show decreased mineral content in the proximal tibial metaphyses—as evidenced in the width of the tibial growth plate and the longitudinal growth rate—as well as various systemic manifestations, although the heterozygous (*Tgfb1* +/−) mice show normal bone development (Geiser et al. 1998). It remains to be seen whether any of these genes, especially *TGFB1,* are causally related to CED.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Genome Database (Japan), http://gdb.jst.go.jp
Human Genome Resources, http://www.ncbi.nlm.nih.gov/genome/guide

Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim (for CED [MIM 131300])

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