

Localisation of the gene causing diaphyseal dysplasia Camurati-Engelmann to chromosome 19q13

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Revised version received
6 January 2000
Accepted for publication
7 January 2000

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Abstract

Camurati-Engelmann disease, progressive diaphyseal dysplasia, or diaphyseal dysplasia Camurati-Engelmann is a rare, autosomal dominantly inherited bone disease, characterised by progressive cortical expansion and sclerosis mainly affecting the diaphyses of the long bones associated with cranial hyperostosis. The main clinical features are severe pain in the legs, muscular weakness, and a waddling gait. The underlying cause of this condition remains unknown.

In order to localise the disease causing gene, we performed a linkage study in a large Jewish-Iraqi family with 18 affected subjects in four generations. A genome wide search with highly polymorphic markers showed linkage with several markers at chromosome 19q13. A maximum lod score of 4.9 ($\theta=0$) was obtained with markers D19S425 (58.7 cM, 19q13.1) and D19S900 (67.1 cM, 19q13.2). The disease causing gene is located in a candidate region of approximately 32 cM, flanked by markers D19S868 (55.9 cM, 19q13.1) and D19S571 (87.7 cM, 19q13.4).

(J Med Genet 2000;37:245-249)

Keywords: Camurati-Engelmann disease; progressive diaphyseal dysplasia; chromosome 19q13; sclerosing bone dysplasia

Camurati-Engelmann disease (CED, MIM 131300) is a sclerotic bone disorder of unknown cause. This dysplasia was first described by Cockayne in 1920,¹ but Camurati² was the first to suggest its hereditary nature in 1922, describing a father and son, both with painful lower extremities, showing cortical thickening and sclerosis of the diaphyses on x ray examination. In 1929, Engelmann³ reported a single case with muscular wasting and marked bone involvement. Neuhauser *et al*⁴ named this disease progressive diaphyseal dysplasia, emphasising the progressive nature of the hyperostosis and the involvement of the diaphyses. However, Kaftori *et al*⁵ later proposed that the disease should be renamed progressive bone dysplasia since the metaphyses, skull, and even the vertebrae can be affected. Currently, the eponym Camurati-Engelmann disease is the most widely accepted, although recently the International Working Group on Constitutional Disease of Bone suggested

naming this condition diaphyseal dysplasia Camurati-Engelmann.⁶

Besides muscle weakness, claudication, and severe pain in the legs, which affect the majority of CED patients, other observed clinical symptoms are easy fatigability, reduced muscle mass, general weakness, exophthalmos, facial paralysis, hearing difficulties, and loss of vision.⁷ The first symptoms of the disease are mostly detected before the age of 30, but in many cases may be present in the first decade of life.

Routine biochemical parameters of bone and mineral metabolism, such as serum calcium and alkaline phosphatase, are usually normal.⁸ A study by Hernández *et al*⁹ in four CED patients, however, showed increases in several markers of bone turnover, such as bone alkaline phosphatase, serum osteocalcin, and crosslinked N-telopeptides of type I collagen.

Radiologically, CED is characterised by fusiform thickening of the cortex in the diaphyseal portions of the long bones. In some cases the metaphyses are affected,¹⁰⁻¹¹ but the epiphyses are typically spared.¹² The medullary canals may be narrowed as shown in fig 1. The distribution of the lesions in the skeleton is symmetrical. In decreasing order of frequency, the tibia, femur, fibula, humerus, ulna, and radius are affected. Usually, sclerotic changes are found at the base of the skull with occasional involvement of the cranial vault and the facial bones.

There is no specific treatment for CED, but glucocorticoids can alleviate fatigue, limb pain, and muscle weakness. They do not alter the radiological signs such as thickened long bones and have no effect on muscle mass. The effect of the drug is only temporary; when the treatment ends, the symptoms recur.

The prevalence of CED is very low; so far only 170 patients have been described. Multiple family studies⁷⁻¹³⁻¹⁴ have shown the autosomal dominant mode of inheritance of CED although a few isolated patients have been reported.

In order to localise the gene responsible for CED, we performed a genetic linkage study on an Israeli family of Jewish-Iraqi origin. This family was previously ascertained and studied by Naveh *et al*¹³ and is one of the largest families published.

Methods

SUBJECTS

Blood samples were collected from an extended four generation Jewish-Iraqi family. Twenty eight family members were clinically



Figure 1 X ray of the diaphysis of the left femur of an adult CED patient. Fusiform thickening of the cortex is observed in the diaphyseal portion of the femur. The medullary canal is narrowed.

examined and 25 of these were, after signing informed consent, willing to participate in the study by the donation of a blood sample. The diagnosis was made on clinical grounds by the presence of bone pain, a waddling gait, and muscular weakness. In most cases the diagnosis was confirmed by radiological examination. Asymptomatic subjects who did not undergo radiological examination were considered as diagnosis unknown.

GENOTYPING

Genomic DNA was isolated from fresh leucocytes using standard techniques. The Cooperative Human Linkage Centre Human Screening Set (Weber version 6) was used for a genome wide linkage study. This set contains 391 fluorescently labelled, highly polymorphic markers covering the entire human genome with an average spacing of 10 cM. The markers were analysed by use of an automated DNA sequencing apparatus (Applied Biosystems model 377). Extra markers for regions of interest were chosen from the Génethon Genetic Linkage Map.¹⁵ These were analysed using either radioactive isotope labelling or a 5'-IRD label. In the first protocol, radioactive end labelling of one oligonucleotide was performed before PCR using T7 polynucleotide kinase. Amplification products were separated according to size on a 6% polyacrylamide gel and analysed after autoradiography. In the alternative approach, one of the primers is synthesised with an M13 forward or reverse sequence at the 5' end. An IRD labelled (800 nm) M13 primer is included in the PCR reaction and is incorporated from the second cycle, thus labelling the PCR product. Gel electrophoresis and pattern

visualisation were performed using a LI-COR model 4200 DNA analyser (NEN).

LINKAGE ANALYSIS

Two point lod scores were calculated by use of MLINK (version 5.1).¹⁶ The disease frequency was set at 1/1 000 000. An autosomal dominant mode of inheritance was assumed with a disease penetrance of 100%. Allele frequencies were set at 1/n with n being the number of alleles detected.

Results

CLINICAL AND RADIOLOGICAL EXAMINATION OF THE FAMILY MEMBERS

In our current update of this family, we diagnosed 18 subjects as being affected with CED (fig 2). Both sexes were almost equally affected with CED (eight women and 10 men). Their diagnoses were mainly based on the presence of clinical symptoms. The most frequent symptoms were muscle weakness, reduced muscle mass, a waddling gait, exophthalmos, and thickening of the long bones. A few people (II.1, III.4, III.6, and III.9) were asymptomatic but based on the presence of affected descendants they must be obligate carriers. II.1 (currently 64 years old), for example, has no symptoms, while her daughter (III.1) suffers from pain and claudication. III.4, III.6, and III.9 all have a child showing typical symptoms of CED, including a waddling gait, inability to run quickly, and muscle pains. For two of these asymptomatic carriers (III.4 and III.6), radiological examination confirmed the affected status. Because of the presence of clinically asymptomatic gene carriers, all at risk subjects with no clinical symptoms and without radiological survey were assigned an unknown affected status (II.1, III.9, III.14, III.17, III.20, IV.6, and IV.7).

The onset of the disease in this family varied, but was more common in childhood. There is considerable variability in the symptoms and clinical signs of the affected subjects; some patients are asymptomatic while III.15 and III.18, only teenagers, already complain of severe pains and muscle weakness in the lower extremities. Radiological examination showed symmetrical involvement of the long bones.

LINKAGE ANALYSIS

Linkage analysis in the Israeli family throughout the whole genome with markers of the Weber set excluded more than 70% of the human genome with lod scores below -2. D19S900 (19q13.2) was the marker most suggestive of linkage. To confirm these initial data and to delineate the candidate region, extra markers from this region were genotyped. The highest lod score of 4.9 ($\theta=0$) was obtained with markers D19S425 and D19S900 (table 1). Four other markers in the same region (D19S416, D19S223, D19S879, and D19S867) also showed a lod score above 4 ($\theta=0$). Besides these, three more markers (D19S876, D19S412, and D19S606) appeared to be free of recombination (table 1, fig 2). Together, these markers indicated a candidate region of approximately 32 cM. The

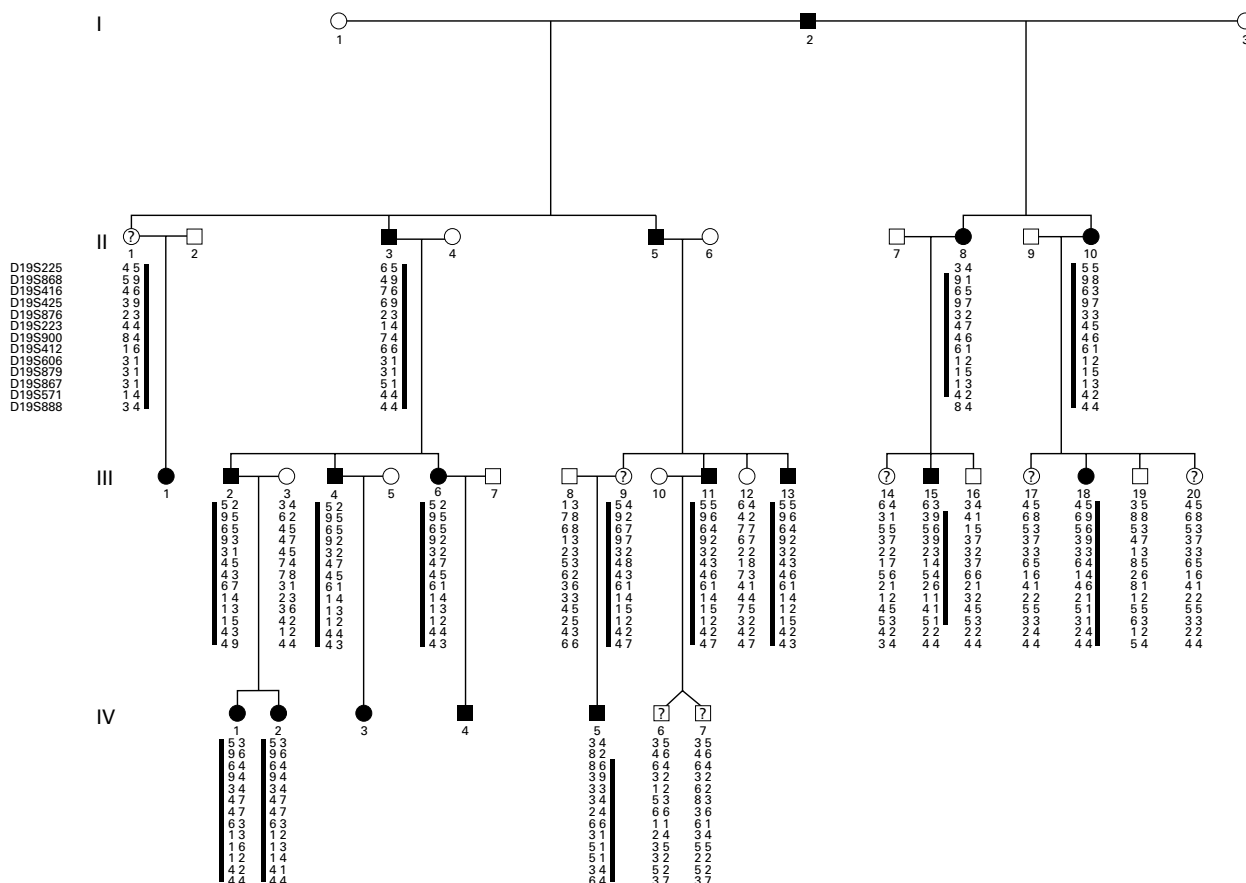


Figure 2 Pedigree reconstruction of the four generation Jewish-Iraqi CED family. Question marks represent subjects with an unknown affected status. Manually constructed haplotypes for the chromosome 19 markers are shown below the symbols. The haplotype consists of alleles from 13 marker loci. The black bars represent the haplotype that cosegregates with the disease.

Table 1 Two point lod scores (Z) at recombination fraction (θ) between CED and 13 markers from chromosome 19q13

	Lod score (Z) at recombination fraction (θ)						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D19S225	∞	-0.02	1.15	1.45	1.40	1.05	0.57
D19S868	∞	2.83	3.22	3.12	2.57	1.82	0.95
D19S416	4.83	4.75	4.43	4.00	3.10	2.12	1.08
D19S425	4.92	4.84	4.51	4.09	3.18	2.19	1.13
D19S876	2.30	2.28	2.16	1.96	1.47	0.93	0.41
D19S223	4.74	4.66	4.33	3.91	3.00	2.02	0.97
D19S900	4.90	4.82	4.50	4.08	3.17	2.19	0.13
D19S412	3.02	2.97	2.73	2.41	1.73	1.03	0.40
D19S606	3.28	3.25	3.10	2.86	2.26	1.56	0.79
D19S879	4.36	4.28	3.96	3.54	2.66	1.72	0.78
D19S867	4.37	4.29	3.96	3.54	2.66	1.72	0.76
D19S571	∞	0.47	1.03	1.15	1.04	0.76	0.40
D19S888	∞	-0.10	0.50	0.67	0.67	0.49	0.26

proximal key recombinant can be seen with marker D19S868 (19q13.1) which recombines in IV.5. On the distal side, D19S571 (19q13.4) recombines in III.15 (fig 3).

Discussion

Bone is a dynamic tissue, which is constantly remodelled throughout life by two processes, bone formation and bone resorption. The balance of these processes is under control of both systemic and local factors. Disruption of this balance can cause a diverse spectrum of pathological conditions. At one end of the spectrum, we find the conditions with decreased bone density, of which osteoporosis is the most

frequent. At the other end, there is a set of mainly rare, inherited conditions characterised by increased bone density. CED is an example of these sclerosing bone dysplasias.

The great majority of sclerosing bone dysplasias have not yet been characterised at the molecular level, with the exceptions of carbonic anhydrase II, which causes osteopetrosis with renal tubular acidosis,¹⁷ and of the cathepsin K gene which underlies pyknodysostosis.¹⁸ However, genetic linkage studies in extended pedigrees have recently localised disease causing genes for several of these conditions: An autosomal dominant and a recessive form of osteopetrosis were assigned to 1p21 and 11q13 respectively,^{19, 20} whereas two related conditions, van Buchem disease and sclerosteosis, both presenting as endosteal hyperostoses, were assigned to 17q12-q21.^{21, 22}

Taking into account the progress of the Human Genome Project, the identification of other genes responsible for sclerosing bone dysplasias can be expected soon.

CED presents in most cases with an autosomal dominant mode of inheritance but some so called skipped generations, in which carriers show no radiological abnormalities, have been described.²³ Sparkes and Graham,⁷ for example, presented the case of a severely affected subject with asymptomatic parents and grandparents showing no definite radiological abnormalities. However, six additional cases of CED were discovered in the same

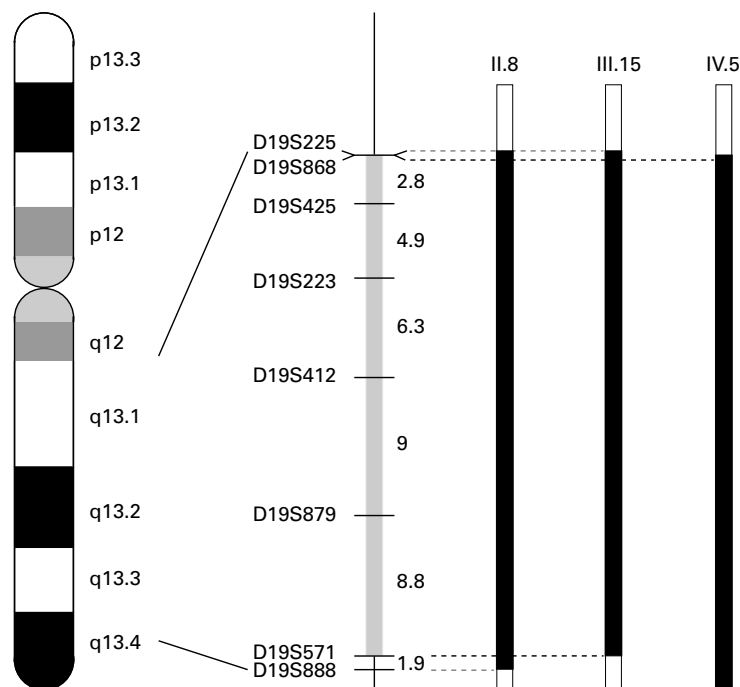


Figure 3 Representation of chromosome 19 with an enlargement of the candidate region 19q13 and its delineation by the key recombinants. The recombination event in IV.5 between D19S868 and the CED gene localises the gene distal to D19S868. The recombination event in III.15 indicates that the CED gene must be located proximal to D19S571.

family rejecting the isolated status of this case. In our family, non-penetrance was observed in several cases based on clinical examination, but, when available, radiological examination allowed us to confirm the affected status. The progression of the disease is highly unpredictable; stages of progression can alternate with quiescent and inactive stages. Onset of the disease seems to be more common in childhood, as is the case in our family.

So far, the cause of CED is unknown. Since CED is a progressive disease, the gene responsible is unlikely to be involved in bone development but rather is most probably involved in the regulation of bone metabolism and bone turnover throughout life. During skeletal growth and ageing, new bone is laid down on the periosteal surfaces of long bones and undergoes a process of maturation to form cancellous bone of the bone cortex. Simultaneously, bone is removed from the endosteal surfaces to create the marrow cavity. Both of these processes appear to be abnormal in CED, where the formation of periosteal bone is disorganised and the removal of endosteal bone is abnormal. In view of this, identification of the disease causing gene in CED will provide new insight into the mechanisms by which normal bone remodelling occurs and may also represent a therapeutic target for the prevention of osteoporosis or the healing of bone fractures.

The candidate region we have identified is large and contains several known genes. Among others, we feel that at least four genes need further consideration. These are "nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, beta" (*NFKB1B* on 19q13.1), human proto-oncogene *BCL3* gene

(19q13.1), transforming growth factor beta-1 (*TGFβ-1* on 19q13.1), and latent transforming growth factor beta binding protein-4 (*LTBP-4* on 19q13.1-19q13.2). *NFKB1B* encodes the IκBβ protein, an inhibitor of NFκB. NFκB regulates the expression of a variety of genes involved in immune and inflammatory responses, but also plays a role in the generation and function of osteoclasts.²⁴ *BCL3* has a similar function.²⁵ Transforming growth factor-β (*TGFβ-1*) is a multifunctional growth factor in bone with effects on cells of the osteoclast and osteoblast lineage,²⁶ whereas *LTBP-4* is a member of the *LTBP*-fibrillin family of proteins which are needed for the secretion and folding of *TGFβ-1*.²⁷ It is of interest that glucocorticoids are known to regulate the activity of both NFκB²⁸ and *TGFβ-1*,²⁹ which would make *NFKB1B*, *BCL3*, *TGFβ-1*, and *LTBP-4* strong positional candidate genes.

In conclusion, the localisation of the disease causing gene for CED presented here will most probably accelerate its characterisation. This might result in a better understanding of the underlying pathogenesis and might contribute to our general knowledge of bone metabolism.

This study was supported by a concerted action grant from the University of Antwerp to W.V.H. K.J. is holder of a predoctoral position with the Flemish FWO (Fonds voor Wetenschappelijk Onderzoek). S.H.R. is supported by the Arthritis Research Campaign ICAC award and a cooperative group grant from the Medical Research Council of the UK.

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