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

Katrien Janssens, Ruth Gershoni-Baruch, Els Van Hul, Riva Brik, Nuria Guañabens, Nicola Migone, Leon A Verbruggen, Stuart H Ralston, Maryse Bonduelle, Lionel Van Maldergem, Filip Vanhoozenacker, Wim Van Hul

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 (θ=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 D19S751 (87.7 cM, 19q13.4).

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, but Camurati 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, Engelmann reported a single case with muscular wasting and marked bone involvement. Neuhäuser et al named this disease progressive diaphyseal dysplasia, emphasising the progressive nature of the hyperostosis and the involvement of the diaphyses. However, Kaftori et al 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.

Besides muscle weakness, claudication, and severe pain in the legs, which affect the majority of CED patients, other observed clinical symptoms are easy fatiguability, reduced muscle mass, general weakness, exophthalmos, facial paralysis, hearing difficulties, and loss of vision. 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. Mineral metabolism, such as serum calcium and 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, but the epiphyses are typically spared. 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, sclerosis 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 studies 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 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...
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 Généthon Genetic Linkage Map. 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). 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, II.3, II.8, 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 (θ=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 (θ=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
Camurati-Engelmann disease maps to 19q13

D19S571  D19S867  D19S879  D19S606  D19S412  D19S900  D19S223  D19S876  D19S416  D19S225

we find the conditions with decreased bone density, which usually present as endosteal hyperostoses, van Buchem disease and sclerosteosis, autosomal dominant and a recessive form of autosomal dominant mode of inheritance but some so called skipped generations, in which carriers show no radiological abnormalities, have been described. Sparkes and Graham, 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 family.
(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). NFKBIB encodes the IkBβ 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. Transforming growth factor-β (TGFβ-1) is a multifunctional growth factor in bone with effects on cells of the osteoclast and osteoblast lineage, whereas LTBP-4 is a member of the LTBP-fibrillin family of proteins which are needed for the secretion and folding of TGFβ-1. It is of interest that glucocorticoids are known to regulate the activity of both NfκB and TGFβ-1, which would make NFKBIB, 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.
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