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The Gene for Autosomal Dominant Craniometaphyseal Dysplasia Maps to Chromosome 5p and Is Distinct from the Growth Hormone–Receptor Gene

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Summary

Craniometaphyseal dysplasia (CMD) is an osteochondrodysplasia of unknown etiology characterized by hyperostosis and sclerosis of the craniofacial bones associated with abnormal modeling of the metaphyses. Sclerosis of the skull may lead to asymmetry of the mandible, as well as to cranial nerve compression, that finally may result in hearing loss and facial palsy. We have analyzed a large German kindred with autosomal dominant (AD) CMD and found tight linkage between the disorder and microsatellite markers on chromosome 5p (maximum two-point LOD score 4.82; $\theta = 0$). Our results clearly establish the existence of a locus for AD CMD on central chromosome 5p (5p15.2-p14.1). This region overlaps with the mapping interval of the growth hormone–receptor (GHR) gene (5p14-p12), which is known to be involved in the mitogenic activation of osteoblasts. Therefore, we tested the GHR gene as a candidate gene. However, recombination events between the CMD locus and the GHR gene identified in two members of this family clearly exclude this candidate.

Introduction

The term "craniometaphyseal dysplasia" (CMD) dates from 1954, when Jackson and colleagues reviewed disorders of osseous modeling. They used this term to denote a specific syndrome comprising metaphyseal dysplasia with a typical flaring of the metaphyseal ends of the long bones and marked cranial hyperostosis and sclerosis (Jackson et al. 1954). Later it was recognized that CMD may present as a mild autosomal dominant (AD) as well as a more severe autosomal recessive (AR) form (Gorlin et al. 1969).

So far, only ~10 patients with AR CMD (MIM 218400) have been described in the literature (Beighton 1995). However, nearly 100 patients with AD CMD (MIM 123000) have been reported, with many of them being members of a few large kindreds from the United States (Rimoin et al. 1969), South Africa and England (Beighton et al. 1979), Mexico (Carnevale et al. 1983), and Australia (Taylor and Sprague 1989). Recently, we have added a further large AD CMD kindred of German extraction with 24 affected persons in six generations (Tinschert et al., in press).

The nature of the basic defect is unknown in both AR and AD CMD. Therefore, we performed a genomewide search for the CMD locus in a part of the German kindred with AD CMD. We report on tight linkage of the disease locus to markers on chromosome 5p as well as exclusion of the most promising positional candidate—the growth hormone–receptor (GHR) gene.

Subjects and Methods

Family Studies

Observations are based on the nuclear family of a larger German AD CMD kindred (Tinschert et al., in press). We analyzed 25 living members, including 13 affected persons in three generations, after obtaining informed consent from the subjects (see fig. 1). All members were clinically characterized, including radiography of the skull and femora. An individual was determined to be affected if characteristic hyperostosis and sclerosis of the skull was present in conjunction with metaphyseal widening of the distal portions of the femora. There was no case of discordance between skull and long bone involvement. All persons with radiologically detectable changes were also noticed by their typical facial appearance. The final decision about the disease status was made by three of the authors (S.T., E.F., and H.-S.B.). Anticoagulated venous blood samples obtained from the examined individuals were used for direct DNA prepa-

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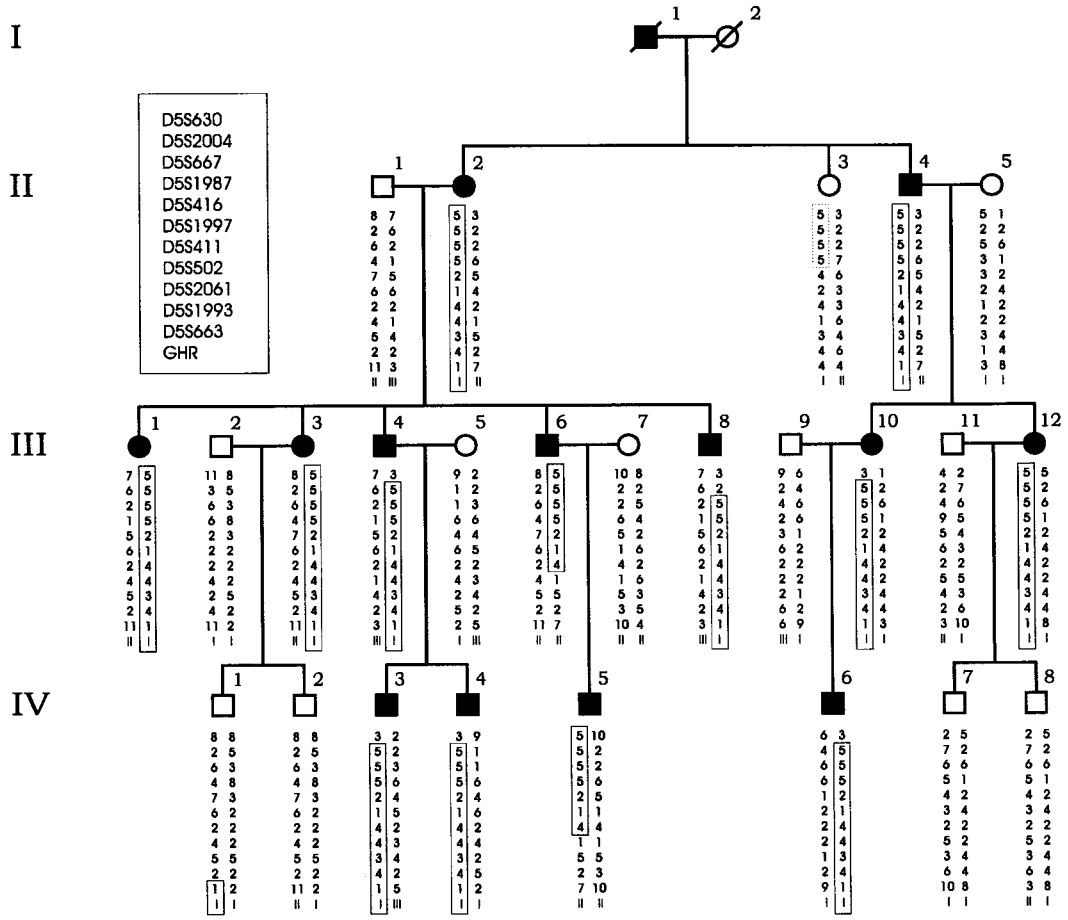

Figure 1 Pedigree of the CMD family, with haplotypes for selected markers on 5p. The panel in the upper-left corner shows the order of markers. Alleles are number coded, and the disease haplotype is shown in the box. The critical crossovers defining the proximal and distal boundaries of the CMD candidate region are shown in generation III, persons 6 and 8, respectively. The haplotypes of individual II-3 could only be tentatively assigned. The broken box suggests a possible recombination event with the disease haplotype (for details, see text).

rations. The affected individual II-2 was also checked for chromosomal abnormalities; however, a normal karyotype was found (resolution: >500 G-bands). All human tissue samples used in this project were obtained with the approval of the institutional review board at the Charité Medical School of the Humboldt University in Berlin.

DNA Analysis

High-molecular-weight genomic DNA was isolated from whole blood lysate with a phenol/chlorophorm extraction followed by isopropanol precipitation. Microsatellites were selected from various genetic maps of the human genome. For the genomewide screen, mainly Cooperative Human Linkage Center markers (Murray et al. 1994) at ~20-cM intervals were used. For the fine mapping, markers from the final Généthon human linkage map were chosen (Dib et al. 1996). Markers were analyzed by PCR and gel electrophoresis according to the recommendations of the manufacturers. The polymorphic intron 9 of the GHR gene was PCR amplified with primers P4 and P5 and was directly sequenced to determine the variable nucleotide positions as described elsewhere (Amselem et al. 1989, 1993). Sequencing reactions were performed using a cycle sequencing kit (Thermosequenase, Amersham/USB) with oligonucleotides TAAAGTTGTAGCTAGTAC-3′ and GAAAATGAAGATCTATG-3′ as sequencing primers for the sense and antisense strands, respectively.

Linkage Analysis

Two-point linkage analyses were performed using the MLINK program of the FASTLINK package, version 2.1, which uses an improved algorithm for internal calculations but produces identical results as the original LINKAGE package (Lathrop et al. 1985; Cottingham et al. 1993; Schaffer et al. 1994). An AD model of disease inheritance was used, and the disease allele frequency was set equal to 10^{-5}. Equal female and male recombination rates were assumed. Because of the variable ex-

pressivity of the disease phenotype, LOD-score calculations were performed at penetrances of 1.0 and 0.9. The allele frequencies for the markers were calculated from the typing data of the CMD family (Boehnke 1991).

Results

Linkage to Chromosome 5p

We analyzed some 70 microsatellite polymorphisms covering about one half of the human genome until we obtained a significant positive LOD score (Z). Marker GATA7C06 (D5S1470) gave maximum LOD score Z_{max} = 3.14 at a recombination fraction (θ) of 0.06, under the assumption of an unreduced penetrance (table 1). Thereafter, a set of >20 contiguous markers of the 1996 Généthon human linkage map (Dib et al. 1996) known to be linked to D5S1470 according to the Whitehead map (Hudson et al. 1995) were analyzed to perform the fine mapping of the CMD locus. The maximum of all pairwise LOD scores (Z_{max} = 4.82 at θ = 0) was obtained for marker AFMa302wc9 at locus D5S1997 (table 1), because this marker was the most informative from the CMD gene region. When haplotypes were constructed, obligate recombination events were noted with markers at or distal to locus D5S2004 and at or proximal to D5S502 (fig. 1). Thus, the CMD candidate region comprises ~19 cM (fig. 2) spanning the chromosomal region 5p15.2-p14.1 (Chumakov et al. 1995). Calculation of pairwise LOD scores under the assumption of a reduced penetrance of .90 did not significantly change the data (table 1).

Exclusion of the GHR Gene

The GHR gene harbors several polymorphic nucleotides in intron 9 known to form at least seven different haplotypes (Amselem et al. 1989, 1993). We determined these haplotypes and analyzed their segregation in the CMD family. Two obligate recombination events were identified in individuals III-6 and IV-1. Interestingly, these two individuals also exhibit recombinations for marker D5S663 (fig. 1) as well as more proximal markers, including D5S455 (not shown). Analyses of more distal markers including D5S1993, however, revealed only the recombination event in patient III-6 but no longer in subject IV-1. These results place the GHR locus proximal to D5S1993, that is, outside the CMD candidate region, thereby definitely excluding it as a candidate for CMD (fig. 2).

Discussion

We have mapped a gene responsible for AD CMD to chromosome 5p15.2-p14.1 by linkage analysis in a sin-

Table 1

Pairwise Linkage Data

Marker and Penetrance	Recombination Fraction at θ =								
	0	.01	.05	.1	.2	.3	.4	Z_{max}	θ_{max}
D5S2004:									
1.0	−∞	1.84	2.35	2.38	2.05	1.48	.75	2.40	.08
.9	−.74	1.82	2.30	2.31	1.97	1.40	.70	2.34	.07
D5S667:									
1.0	3.97	3.92	3.69	3.38	2.68	1.85	.90	3.97	0
.9	3.91	3.85	3.61	3.29	2.58	1.77	.85	3.91	0
D5S1987:									
1.0	4.10	4.04	3.79	3.46	2.72	1.88	.91	4.10	0
.9	4.01	3.95	3.69	3.36	2.62	1.79	.86	4.01	0
D5S1997:									
1.0	4.82	4.74	4.42	3.99	3.08	2.06	.96	4.82	0
.9	4.61	4.53	4.22	3.81	2.93	1.95	.90	4.61	0
D5S2096:									
1.0	4.78	4.70	4.38	3.96	3.05	2.03	.94	4.78	0
.9	4.58	4.50	4.19	3.78	2.90	1.92	.88	4.58	0
D5S411:									
1.0	4.12	4.06	3.80	3.47	2.73	1.88	.91	4.12	0
.9	4.02	3.96	3.70	3.37	2.63	1.80	.86	4.02	0
D5S502:									
1.0	−∞	1.55	2.00	1.97	1.57	.99	.40	2.02	.07
.9	−1.25	1.43	1.88	1.86	1.48	.93	.37	1.90	.07
D5S1470:									
1.0	−∞	2.74	3.14	3.04	2.48	1.70	.81	3.14	.06
.9	−.49	2.54	2.94	2.86	2.33	1.59	.75	2.94	.06

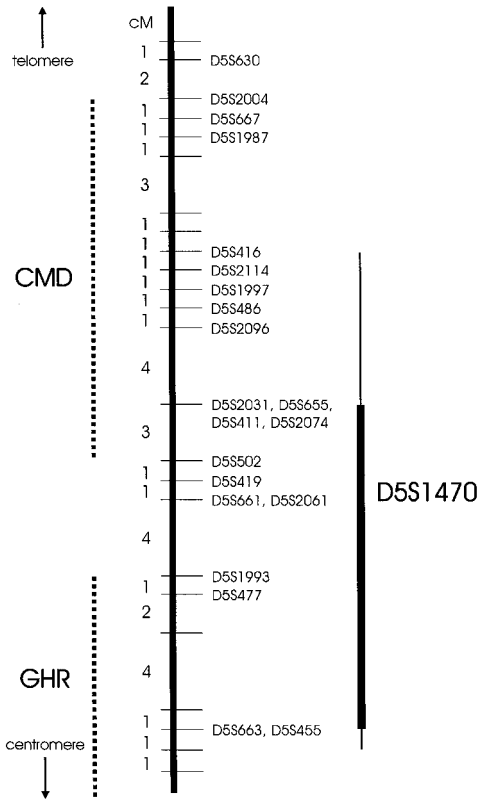

Figure 2 Genetic map of the region containing the CMD locus. Order of markers and approximate genetic distances are taken from the final Généthon linkage map (Dib et al. 1996). Only markers analyzed in the present study are shown. For D5S1470, a new likely location interval was defined in this study (thick bar), which is based on the occurrence of recombination events in the CMD family (not shown).

gle large pedigree of German extraction. The CMD candidate region defined in this study is still ⩽19 cM. Unfortunately, marker D5S2074 was not informative in this family; hence we were unable to decide whether the recombination breakpoint in patient III-6 might be situated distal to this locus, thereby further reducing the candidate region by 3 cM. Furthermore, one might speculate whether the unaffected individual II-3 has inherited a recombined haplotype from her affected father (I-1) with the part distal to D5S416 (~8 cM) originating from the disease chromosome. However, it is not possible to determine whether a recombination occurred or whether the deceased father was uninformative.

The past few years have witnessed a true explosion in the number of identified genes involved in the human skeletal dysplasias, thereby dramatically advancing this particular field (Erlebacher et al. 1995; Francomano et al. 1996). Emerging themes in the molecular analysis of the skeletal dysplasias include the identification of allelic series of disorders and the existence of mutational and genetic heterogeneity in many of these conditions. Since allelic series, that is, different conditions caused by mutations of the same gene, seem to be quite common among bone dysplasias (Francomano et al. 1996), we expected to map the CMD locus to a region already well known to skeletal geneticists. Surprisingly though, no skeletal dysplasia has so far been mapped to chromosome 5p.

Bone is a dynamic tissue in which the processes of resorption and formation are integrated through systemic and local interactions to achieve skeletal remodeling and morphogenesis during growth and development. Three major cell types contribute to the skeleton: chondrocytes, which form cartilage; osteoblasts, which deposit bone matrix; and osteoclasts, which resorb bone. The activity and differentiation of osteoblasts and osteoclasts are closely coordinated during development as bone is formed and during growth and adulthood as bone undergoes continuous remodeling. The latter also links bone turnover to the endocrine homeostasis of calcium and phosphorus, since the mineralized bone matrix serves as the major repository for these ions in the body. Obviously, the complex interactions necessary for normal bone formation may be impaired by a defect of any of the many genes that seem to be involved in the process of bone morphogenesis (Francomano et al. 1996).

The bone changes seen in CMD, cranial hyperostosis and sclerosis and the abnormal modeling of the metaphyses, have been attributed to a defective osteoclastic activity (Cole and Cohen 1988), but at present this view is merely speculative. For AR CMD, however, this concept may be correct, since two affected children have, respectively, been reported to respond well to therapy with calcitonin (Fanconi et al. 1988) and calcitrol (Key et al. 1988). Moreover, by use of specific monoclonal antibodies, it could be shown in a third patient that osteoclast-like cells derived from the bone marrow lacked expression of the osteoclast-reactive vacuolar proton pump (Yamamoto et al. 1993).

When looking for candidate genes in the AD CMD critical region on chromosome 5p15.2-p14.1, we noticed several genes in the databases that might be involved in the pathological condition. Our prime suspect was the GHR gene (5p14-p12), because of reports on a direct stimulating effect of growth hormone on osteoblasts mediated by its authentic receptor (Barnard et al. 1991; Morel et al. 1993). Because mutations of the GHR gene were already known to cause Laron dwarfism (Amselem et al. 1989, 1993), we suspected yet another allelic series also comprising CMD. Now, having excluded GHR as the faulty gene in this CMD kindred, we are going to turn toward the other candidate genes. An adenylate cyclase was mapped to chromosome 5p15.3-p15.2 (Stengel et al. 1992). An adenylate cyclase–coupled calcitonin receptor is specifically expressed by osteoclasts (Lin et al. 1991). The steroid 5-alpha-reduc-

tase, cytogenetically localized at chromosomal band 5p15 (Jenkins et al. 1991), might cause an endocrinological imbalance, since it is known from osteoporosis. The death-associated protein (DAP1) gene at 5p15.2 could be involved in the programmed cell death of chondrocytes during endochondral bone formation (Feinstein et al. 1995). Finally, the zinc finger protein-4 at 5p14-p13 (Habeebu et al. 1989) may act as an osteoclast-specific transcription factor. A mutation in this gene might impair osteoclastic function. A similar scenario is found in the mouse mutant *mi/mi,* which develops osteopetrosis as a result of a mutation in a putative transcription factor (Hodgkinson et al. 1993). However, by far not all genes situated on chromosome 5p have been identified to date. Thus, the characterization of the culprit may result in the description of a new gene.

To our knowledge, this is the first report of a particular locus responsible for CMD. Interestingly, others have observed a t(12;18)(q13;q12) translocation in a sporadic CMD patient (Yamada et al. 1987), thereby providing a clue to the possible chromosomal location of a second CMD gene. The severity of the clinical manifestation in the child is suggestive of AR CMD. Although in other chondrodysplasias AD and AR conditions have been attributed to the same gene (Vikkula et al. 1995), we assume the putative second CMD locus on chromosomes 12q or 18q to be responsible for the AR form of CMD while the locus on 5p is associated with AD CMD. To date, there is no reason to assume genetic heterogeneity within AD CMD; however, it would be interesting to confirm linkage to 5p in the other large AD CMD kindreds described so far (Rimoin et al. 1969; Beighton et al. 1979; Carnevale et al. 1983; Taylor and Sprague 1989). This, as well as the investigation of additional members of the German kindred, might help to reduce further the critical CMD region on 5p. We also think it would be worthwhile to study families with related conditions such as metaphyseal dysplasia (Pyle disease), craniodiaphyseal dysplasia, and sclerosteosis for involvement of CMD loci. Irrespective of the advances in the classification of skeletal dysplasias, the identification the CMD locus on 5p and its future characterization will provide new diagnostic tools for CMD patients and a first step toward the elucidation of its etiology and thus greater insight into the morphogenesis of the skeleton in general.

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